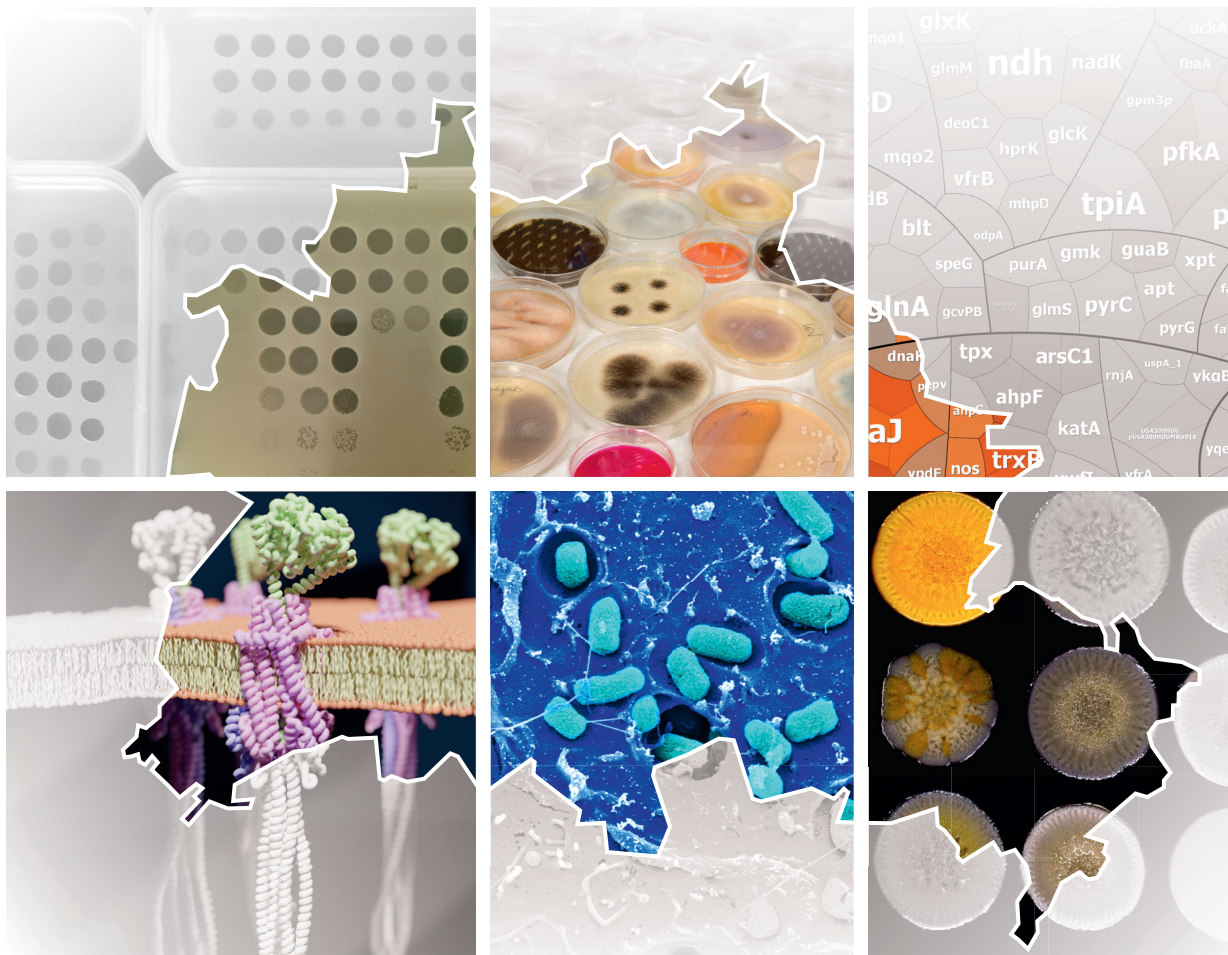




# JAHRESTAGUNG

OF THE ASSOCIATION FOR GENERAL  
AND APPLIED MICROBIOLOGY

# 2026



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## ABSTRACTS



© Saureus-redoxome: J. Bernhardt | Phage-assays: H. Antelmann | Bakterien-Hefe-Pilze: M. Weinhold | Salmonella-infection: M. Rhode | Saureus-colonies:  
D. Berg / Zorya 3D Modell: .F.J.O. Martin (Universität Kopenhagen) | greens87 | Stock.Adobe

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# Plenary Lecture

## Chemical strategies for decoding host–microbe interactions

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Bacteria that chronically colonize the host must adapt to various forms of stress in the host environment. The molecular mechanisms cells use to sense and respond to these environmental signals are crucial for maintaining homeostasis at the host–microbe interface. My lab uses chemical tools to discover proteins, post-translational modifications, and metabolites that shape cellular responses to environmental cues in the gastrointestinal tract. Our primary goal is to understand how bacterial and host cells adapt to oxidative stress, which contributes to the development of severe diseases including gastrointestinal cancers. In this talk, I will present some of our recent work using chemical approaches to uncover redox-regulated pathways that influence cellular physiology at the host–microbe interface.

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## Towards a carbon agnostic fermentation

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Laboratory "Biochemistry and Systembiology of Metabolism"

The development of next-generation bioprocesses relies on genetically stable biocatalysts which can maintain high production yields. Synthetic metabolic rewiring offers a powerful strategy to couple cellular fitness to product formation. Such self-optimizing synthetic growth-coupled bioproduction (GCBP) systems ensure robust product yields and long-term genetic stability.

Conventional anaerobic fermentations, which are the key to the efficient production of ethanol or lactate, depend on ATP generation via glycolytic substrate-level phosphorylation. However, this mode of bioproduction requires a canonical glycolytic architecture and a strict balancing of reduction equivalents and thereby restricts feasible substrate–product combinations.

To break these boundaries, we engineered the *E. coli* NNMini strain. By removing the enzymatic catalyzing the transfer of electrons from NADH to the respiratory chain, we generated an obligatory fermenting strain even under aerobic conditions. In this background, respiratory modules can be deliberately integrated into fermentative metabolism to channel excess electrons to oxygen, allowing fundamentally new and previously inaccessible substrate–product pairings through unbalanced fermentation. When linked to the respiratory chain, these modules also enable the construction of GCBP designs with a strong intrinsic drive for ATP generation, thereby reinforcing flux through the desired production pathway.

By strategically integrating respiratory modules, this platform expands GCBP for the synthesis of oxidized products and opens new design opportunities to achieve fermentative conversion of sustainable substrates.

## Synergy between Enterobacteriaceae and dietary components shapes the competition between prevalent anaerobes of the human gut

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Analyzing the human gut microbiota worldwide has uncovered diverse structures in healthy individuals. Although the specific taxonomic composition varies widely among people, clear signatures defining a healthy gut microbiota are now well established. It is often dominated by the Bacteroidota phylum, with members of the family Bacteroidaceae being common in modern lifestyles and members of the family Prevotellaceae in traditional ones. While these different profiles are well documented across various groups, the underlying mechanisms of microbial competition in the ecosystem remain poorly understood. Moreover, although molecular insights into different Bacteroidaceae members have been gained over the last decade, molecular studies of Prevotellaceae members have only recently begun. After establishing a gene targeting system for *Segatella copri*, the main Prevotellaceae in the human gut, we identified, upon characterizing the RNA landscape, a small RNA required for gut colonization as well as regulatory systems for responding to oxygen stress. In my presentation, I will mainly focus on our efforts to study microbial interactions. Specifically, to explore ecosystem-level interactions, we combined defined gut community models and genetic tools to experimentally investigate the ecological principles that favor either Prevotellaceae or Bacteroidaceae. Testing 94 dietary components in a 21-member community model shows these two families compete for similar nutritional niches. Notably, *S. copri* outcompetes Bacteroidaceae strains when preferred polysaccharides are present. Interestingly, this competitive advantage depends on additional community members, such as Enterobacteriaceae, which work together with dietary components to support *Segatella*'s dominance. Our findings suggest that the dynamic interactions between dietary components and the main community residents influence the dominance of different Bacteroidota families.

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## Keynote Lecture – Special Groups

### Biotransformation

#### K-SG-BT-01

#### Chances and Current Limits of Microbial Electrobiotechnology

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For 25 years, it is known that certain microorganisms can perform a targeted, energy generating direct extracellular electron transfer. Since then, research into microbial extracellular electron transfer has taken many routes and proposed many applications from electric power generation with Microbial Fuel Cells to advancing Bioremediation, finally to steering and enabling new electrochemistry-coupled

reactions in biotechnological processes. The main impact of electrobiotechnological approaches in microbial bioprocesses has been made for methane production, microbial CO<sub>2</sub> fixation, and through the broad effects of modulating the bioprocess redox environment in electrofermentation. Besides this, first examples of applying molecular and synthetic biology to enable entirely new electrobiotechnological production pathways on their way and might open doors to new products.

Where are we today? Which directions proved fruitful and successful? Where are the main technological and biological bottlenecks? Can electrochemistry as a process tool in the toolbox of bioprocess engineering really make a difference in process outcome and performance?

This keynote lecture will try to devise a role to electrobiotechnology.

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## Viruses of microbes

### K-SG-VM/R-01

#### Consequences of host superinfection by rapidly recombining bacteriophages

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Closely related phages are often prevented from infecting the same host by multiple superinfection exclusion systems encoded within phage genomes. However, this is not always the case. For example, within the SPbetavirus phage genus, it has been shown that phi3T can superinfect an SPβ lysogen. Moreover, such double lysogeny can give rise to chimeric phage variants.

Here, we investigate additional pairs of phages belonging to this genus. We compare single and double lysogens and observe marked differences between them, including, in some cases, protection of the host from cell morphology changes. We also detect elevated phage release during double lysogeny and perform direct plaque sequencing to characterize the resulting chimeric phage variants.

We further assess the ability of these chimeric variants to establish stable lysogeny and examine their consequences for host fitness. Our results demonstrate rapid phage evolution through the exchange of large DNA segments, while retaining the capacity to lysogenize the host. This highlights the extreme potential of phage-mediated genetic innovation in host evolution. Ongoing experimental evolution will further elucidate the impact of phage diversity on host adaptation.

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## Microbial pathogenicity

### K-SG-MP-01

#### Chemical biology tools to track the secret life of lipids

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Lipids are essential for numerous cellular functions and signaling processes. However, they are understudied compared to other biological molecules such as proteins mainly due to a lack of appropriate methodology. Compared to proteins, it is very difficult to introduce perturbations such as concentration changes and it is further very challenging to visualize lipids especially in a cellular context. This has mainly to do with both the size and also the chemical diversity of lipids. Chemical biology approaches such as the introduction of photolabile moieties, photocrosslinking- and click chemistry-groups offer the possibility to fill this methodological gap and in particular to address biological questions on the level of individual lipid species.

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## Archaea & Prokaryotic Cell Biology

### K-SG-AR/PCB-01

#### How to make a new cytoskeleton

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Bacteria, like eukaryotes, use conserved cytoskeletal systems for intracellular organization. Recently, we found that in multicellular cyanobacteria, including the model strain *Anabaena* sp. PCC 7120, the canonical ParMR plasmid partitioning system has been evolutionarily and functionally diversified into the CorMR cytoskeletal system, which controls cell shape in multicellular cyanobacteria rather than segregating DNA. Here, we describe a series of coordinated evolutionary adaptations that together transformed a conserved protein system into a new cytoskeleton: (i) chromosomal integration of the ParMR system; (ii) modification of CorM filament polymerization properties, mediated by the acquisition of an N-terminal intrinsically disordered region; and (iii) a key rewiring of cellular localization, whereby CorR lost its DNA-binding domain and instead acquired membrane-binding properties through a novel amphipathic helix. Regulation of this new cytoskeleton required additional innovation, including (iv) the co-option of MinC, a canonical inhibitor of FtsZ polymerization, which acquired an additional function to depolymerize CorM and spatially restrict CorMR filaments away from the poles and the division plane. Finally, an additional layer of posttranslational control through (v) proteolytic degradation of CorM by an as-yet-unidentified protease provides precise control of the dynamic CorMR filament system. In addition, through comparative genomics we found that these changes co-evolved with cyanobacterial multicellularity, highlighting the evolutionary modifications necessary to permit the drastic transition from single-celled to multicellular lifestyles. Together, our findings outline general principles by which new cytoskeletal systems can emerge through the repurposing, modification, and regulatory integration of pre-existing protein systems.

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### K-SG-AR/PCB-02

#### The life cycle of an archaeon with two membranes

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*Ignicoccus hospitalis* is an hyperthermophilic archaeon with an unusual and unique cell architecture as it possess an inner membrane that is separated by a wide inter-membrane compartment from the bounding outer membrane. This raises the question: how does an archaeon with two membranes divide?

In exploring this question, we first analysed the *Ignicoccus* genome for components of the cell division machinery. We identified homologues of the ESCRT-III based division system (CdvA, CdvB, CdvB1, CdvB2, and CdvC) and generated antibodies to these proteins allowing us to visualize the cell division rings by fluorescent expansion microscopy, along with multi-colour membrane staining to visualize the two different membranes of *Ignicoccus*.

Strikingly, our analysis reveals that ESCRT-III based division rings bend and cut the internal membrane without affecting the outer membrane to generate cellular clusters that possess multiple internal cells within a single bounding membrane. This life cycle then restarts when the outer membrane bursts to release the many cells contained within - which acquire a second membrane via a process that appears to resemble membrane blistering. All the other *Ignicoccus* species examined, despite differing in the relatively timing of outer membrane bursting and double membrane reformation, exhibited a similar life cycle.

These data show that the *Ignicoccus* life cycle is very different from that described for other related archaea like *Sulfolobus*, which use a comparable cell division machinery to cut a single bounding membrane, and from *Escherichia coli* which constricts and cuts both membranes when it divides.

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## Environmental microbiology

### K-SG-EM-01

#### The Nitrogen Cycle in Crisis: Greenhouse Gases, Environmental Pollution, and Solutions

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Nitrogen is an important nutrient for all living things and at the same time so-called reactive nitrogen introduced by agricultural fertilizers pollutes the environment, with serious consequences for biodiversity, surface and groundwater quality. This also impacts climate change through nitrous oxide production, one of the most potent greenhouse gases. In this talk I will address the current status of the nitrogen cycle and present research activities that aim at a better understanding of the role of nitrifying microorganisms in bioremediation strategies. A major focus will lie on nitrification inhibition by both synthetic and biological inhibitors, how they can be used to improve nitrogen use efficiency by plants in agriculture, how affect ammonia oxidizing archaea and bacteria on the cellular level and how plants can be screened for the production of novel inhibitors.

## Phage biology

### K-SG-VM/R-02

#### The Anatomy of a Phage: A Functional Dissection of SP $\beta$ in *Bacillus subtilis*

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Temperate bacteriophages are major drivers of bacterial evolution through horizontal gene transfer and lysogenic conversion, yet the mechanisms underlying this transition remain poorly understood. To address this problem, the temperate bacteriophage SP $\beta$  of *Bacillus subtilis* serves as an excellent model to dissect phage–host interactions and the molecular switches controlling this balance.

We are establishing a comprehensive deletion library of the endogenous SP $\beta$  prophage, enabling systematic gene disruption combined with controlled induction of the lytic cycle. Induction is achieved through inactivation of the phage repressor MrpR, which controls the transition between lysogeny and lysis (1). This approach facilitates targeted analysis of phage functions involved in distinct stages of the viral life cycle, including lysis and host infection. Using time-resolved proteomics, we characterized the dynamic protein landscape following MrpR-dependent induction, providing a temporal view of host and phage protein regulation during lysis.

Analysis of selected deletion mutants revealed strains in which lysis was abolished, or the released phages had lost infectivity, identifying key genes essential for the lytic process and virion formation. We are currently characterizing these candidate proteins to elucidate their precise roles in host–phage interactions.

Together, our findings illuminate the genetic and regulatory architecture of SP $\beta$  and establish a versatile experimental framework for systematic functional analysis of temperate phages in *B. subtilis*.

(1) Kohm *et al.* (2023) Nucleic Acids Res. 51(17):9452-9474.

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## Microbiome

### K-SG-MB-01

#### Nomen est omen? Why terminology matters and why synthetic communities can be misleading

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The term Synthetic Community (SynCom) is widely used to describe defined microbial consortia. Primarily, SynComs were employed to study microbial interactions in well-defined settings. However, the term is used inconsistently across disciplines, leading to confusion within and between research fields. As research on microbiomes and on SynComs shifts towards applications, the term SynCom presents challenges in public and regulatory perception. This is partly due to its semantic association with synthetic biology, even though it most often refers to naturally occurring, non-genetically modified microorganisms in host-microbiome systems. Moreover, the term "synthetic" often carries negative connotations. This may lead to concerns and hesitancy

among consumers and stakeholders who may be reluctant to trust "synthetic" microbial communities.

During an international workshop, experts discussed the evolution of the SynCom concept, emphasizing the importance of precise terminology. As an outcome of this workshop, a contextual use of the term was recommended, particularly when communicating with non-specialist audiences or in regulatory frameworks. To balance scientific diligence with public transparency, "Defined Microbial Community" is proposed as a less ambiguous alternative that avoids negative connotations and builds trust in regulatory and consumer acceptance contexts. Adopting clearer terminology will accelerate the responsible implementation of microbiome-based solutions across healthcare, agriculture, food production, and environmental management.

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## Microverse meets MibiNet

### K-MIBI-01

#### Microbial networking via bacterial membrane vesicles

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Extracellular vesicles are produced by species across all domains of life, suggesting that vesiculation is a fundamental principle of living matter. Bacterial membrane vesicles (BVs) carry specific cargos and are therefore considered a unique bacterial secretion pathway. Initially described as outer membrane blebs of Gram-negative bacteria, BVs are now also known to arise from endolysin-triggered cell lysis during phage release. MVs perform diverse functions, including waste disposal, surface remodeling, nutrient acquisition, neutralization of phages and antibiotics, DNA transfer, bacterial killing and immune stimulation or modulation. It is shown that antibiotics can induce BV formation through distinct mechanisms: DNA-damaging agents trigger vesicle formation in lysogenic strains, but not in their phage-devoid counterparts, via SOS-induced cell lysis while  $\beta$ -lactam antibiotics increase vesicle formation in a prophage-independent manner by weakening the peptidoglycan layer and promoting membrane blebbing. More recent studies have shown that BVs also transport small bioactive molecules, including antibiotics, antifungal compounds and toxins, and that many BV functions depend on the selective enrichment of these agents. BVs are essential for solubilizing and transporting hydrophobic molecules, protecting them from degradation, and enabling targeted delivery. BV-mediated secretion allows for quantal secretion, whereby cargo molecules are transported over long distances while remaining highly concentrated. Consequently, fusion of a single BV with a target cell is sufficient to deliver a dose exceeding the minimal effective concentration. This mode of secretion fundamentally differs from classical secretion systems, in which diffusible molecules are diluted with increasing distance from the producing cell, creating a defined boundary beyond which concentrations fall below effective levels.

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### K-MIBI-02

#### Pathogenic beginnings: a fungal-bacterial partnership shifts toward coexistence

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Endosymbioses are dynamic relationships between organisms, which may involve antagonistic phases during their formation. In this study, we used fluidic force microscopy to induce cell-in-cell interactions between the free-living bacterium *Ralstonia pickettii* and an endosymbiont-free strain of the fungus *Rhizopus microsporus*, to investigate the early stages of endosymbiosis. Following implantation into the cytosol, the rapid proliferation of *R. pickettii* compromised host fitness, as evidenced by reduced fungal viability, and triggered immune responses characterized by upregulated expression of stress-related defense genes. Vertical transmission of the bacteria across fungal generations enabled repeated rounds of selective passaging, ultimately resulting in the relaxation of the fungal defense response at the transcriptional level. High-throughput imaging revealed that the propagated system could accommodate higher bacterial loads within viable spores, with a corresponding reduction in fungal growth. The observed physiological changes and comparative fungal transcriptomic profiles indicated adaptive resilience and a shift from antagonism to commensalism. This transition was characterized by attenuated expression of genes involved in cell wall remodeling and reactive oxygen metabolism. Our experimental system illuminates early endosymbiosis and supports the hypothesis that facultative intracellular pathogens may act as stepping stones toward stable endosymbiotic relationships.

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## Fungal biology and biotechnology

### K-SG-FBB-01

#### Unlocking black fungi: from genomes to material conservation and biotechnological innovation

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Black fungi are a polyphyletic group of melanized ascomycetes adapted to extreme environments such as deserts, rocks, and human-made surfaces. Their slow compact growth, and thick melanized cell walls confer exceptional stress tolerance but have long hindered molecular studies and genetic engineering. Consequently, the biology and biotechnological potential of these organisms remained largely unexplored. To overcome these limitations, we selected the rock-inhabiting black fungus *Knufia petricola* as a model. Its genome sequence (12 contigs, ~10,000 genes) enabled the development of a versatile genetic toolbox based on CRISPR/Cas9-mediated editing, achieving up to 100% homologous recombination and supporting multiplex editing of at least six genomic regions. Color-based selection systems targeting pigment genes enable rapid identification of strains with correctly integrated expression cassettes, including simultaneous insertion of optimized genes coding for blue, green, and red fluorescent proteins for co-localization and protein interaction studies. These tools enable functional analyses of traits central to extremotolerance, such as the role of melanin in stress protection, biofilm formation, and material interactions. Furthermore, *K. petricola* has been established as a

eukaryotic expression platform: heterologous genes from bacteria, fungi, and plants can be expressed under constitutive or inducible promoters, including the Tet-on system for tunable gene regulation. Pigment-free strains provide access to metabolic precursors, enabling high-level synthesis of alternative secondary metabolites. Importantly, the optimized transformation protocols were successfully transferred to the Antarctic black fungus *Cryomyces antarcticus*, a cryptoendolithic extremophile and astrobiology model, demonstrating that even the most stress-tolerant fungi can be genetically engineered. Together, the genome sequence and advanced genetic engineering strategies have transformed *K. petricola* from a challenging extremotolerant organism into a tractable model for studying stress adaptation, material interactions and a promising host for biotechnology and synthetic biology approaches. These achievements, combined with emerging black fungal genomes, inform sustainable material protection strategies to mitigate colonization and biodeterioration of exposed surfaces, linking fundamental research with applied solutions for material conservation and industrial biotechnology.

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#### K-SG-FBB-02

##### ***Coprinopsis cinerea* – a model fungus for the Agaricomycotina**

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The dung fungus *Coprinopsis cinerea* is one of the first species that have been shown to fruit in artificial culture (Brefeld 1877). Its ease of cultivation, combined with a rapid sexual development, made the heterothallic species a mycological model for the study of typical mushrooms with a stipe and cap. Heterothallic species need two distinct haploid genomes for mating and subsequent sexual development. Mating between primary mycelia (monokaryons) and sexual development with fruiting body formation and meiotic basidiospore production on secondary mycelia (dikaryons) after mating are controlled by two multi-allelic mating type loci, A and B. The central A locus encodes two types of homeodomain transcription factors (HD1 and HD2), while the modifying B locus encodes pheromones and pheromone receptors. Different alleles at both loci are typically required in *C. cinerea* for mating and further sexual development. However, certain mutations at both loci lead to self-compatibility. The double mutant strain AmutBmut is therefore homothallic and forms fruiting bodies with meiotic basidiospores without any prior mating with a genetically different strain. Genetic functions are readily accessible in the established haploid genome of the strain, using classical genetics with mutagenesis, transformation and omics techniques. To date, over 40 different genes involved in the complex fruiting body development have been characterized. Nevertheless, we are still far from fully understanding the entire process, as hundreds of genes are likely involved. Therefore, for the time being, it makes more sense to focus on smaller, selected aspects. For a fungus that originates from heavily contaminated horse manure, the cleanliness of the developing fruiting bodies and their sexual spores is astonishing. This is investigated in more detail here. The fungus has developed flushing mechanisms with a slightly hydrophobic and slightly viscous fluid that is excreted from the outer hyphal layer of the pileus. Over 100-150 proteins with typical N-terminal signal peptides are secreted into this liquid, as well as other short proteins that are transferred via a non-classical pathway. Proteomics has been used to identify families of enzymes (subtilisins, fungalysins, chitinases, etc.), types of lectins, and cysteine-rich

(manno)proteins with proven and likely antimicrobial, arthropocidal and nematocidal functions.

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## ***Symbiotic interactions***

#### K-SG-SI-01

##### **Metaproteomics: A comprehensive tool to study holobionts**

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Metaproteomics, the study of the molecular physiological phenotypes of communities of (micro)organisms, offers unprecedented insights into in-situ gene expression. Based on the quantification of tens of thousands of peptides and thousands of proteins, we determine physiological interactions, biomasses of partners, and even carbon assimilation pathways. When studying holobionts, we generate high-resolution insights into host and symbiont physiology at the same time, using the same datasets. For generating these insights, a sound data evaluation and interpretation is crucial, building on appropriate statistical analysis techniques. The statistical analysis of metaproteomics datasets is challenging due to missing data, compositionality, and biological and technical variation. Different combinations of data processing steps exist to tackle these challenges, leading to a wide variety of potential analysis options. We have generated a comprehensive, ground truth-based, freely available framework and recommendations for the best statistical approaches for metaproteomics datasets. Here, I will present an overview of metaproteomics analyses, with a focus on statistical data evaluation, and the biological insights generated with metaproteomics in marine chemoautotrophic symbioses, and in terrestrial plant-microbe associations. I will showcase the identification of metabolic networks and communication pathways between host and symbionts, and adaptations of the host system toward different microbes.

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#### K-SG-SI-02

##### **The functional roles of *Endozoicomonas* in marine Cnidaria: friend, foe, or indifferent bystander?**

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*Endozoicomonas* is a widespread bacterial endosymbiont across a diverse range of marine animal hosts, and constitutes the most consistently associated bacterial genus of the phylum Cnidaria (e.g., corals, sea anemones, jellyfish and their relatives). Similar to *Vibrio*, the symbiotic spectrum of *Endozoicomonas* includes a diversity of lifestyles from mutualistic to pathogenic interactions. The role of *Endozoicomonas* in cnidarian holobiont health and functioning, however, remains poorly understood. *Endozoicomonas* exhibit high abundances in the host tissues, a diverse genomic functional potential, and a rapid priming to a host-associated lifestyle through gene expression and proteome changes in response to chemical cues of their coral host. As such, *Endozoicomonas* are widely believed to be key bacterial symbionts of corals. Considering previous reports of parasitic and pathogenic lifestyles of *Endozoicomonadaceae* in bivalves, tunicates, and fish, the prevailing assumption of a mutualistic role of *Endozoicomonas* in cnidarian hosts deserves careful re-evaluation. I here present a new lab model system for the

functional interrogation of the cnidarian-dinoflagellate-*Endozoicomonas* relationship. This unique reduced model system consists of a temperate photosymbiotic anemone lab strain, and cultures of its algal endosymbiont *Philozoon* and *Endozoicomonas*. Most excitingly, the *Endozoicomonas* isolates are not only the first of their kind from sea anemones, but exhibit two different morphotypes and clear genomic differences, suggesting different functional capacities and, potentially, even different lifestyles. This reductionist model approach will facilitate mechanistic work to identify primary metabolic and molecular mechanisms that underpin the role of *Endozoicomonas* with its animal host. Ultimately, this work will permit placing the cnidarian-*Endozoicomonas* association along the symbiotic spectrum, and help address fundamental questions on the ecology and evolution of marine animal-microbe symbiosis.

## Space microbiology

### K-SG-SPM-01

#### Planetary Protection and how to avoid contaminating the Solar System

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Article IX of the Outer Space Treaty states that space exploration must avoid the harmful contamination of celestial bodies and prevent adverse changes to the Earth's biosphere.

Planetary Protection is particularly critical in missions exploring Solar System bodies which could previously or presently be habitable for life (e.g., Mars, Enceladus). In addition to the ethical concerns of introducing Earth-life that could damage any fragile ecosystem, life-detection science goals could be jeopardised with "false positives" if Planetary Protection requirements are not strictly adhered to. Such requirements can include constraints on the total number of spores on landed systems, organic material inventories and probability assessments of unintended impacts on celestial bodies.

Airbus Defence and Space have extensive heritage in the design and build of spacecraft for missions to celestial bodies of biological interest, including the ESA Rosalind Franklin ExoMars Rover, due to be launched to Mars in 2028.

This keynote lecture will introduce the concept of Planetary Protection and outline how it can be successfully implemented during the design, manufacture and launch of spacecraft. In particular, focus will be placed on the current methods of bioburden reduction and microbiology assessments, and the direction that developments need to take for use in future exploration missions.

## Cyanobacteria

### K-SG-CB-01

#### A cross-scale analysis of *Synechococcus* pigment types highlights the key ecological role of Type IV chromatic acclimation in the marine environment

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*Synechococcus* is the second abundant phytoplanktonic organism of the world Ocean. Most *Syn.* cells thriving in open ocean waters possess phycobilisomes comprising both phycoerythrin-I and -II, which bind the green-light (GL) absorbing chromophore phycoerythrobilin (PEB) and blue-light (BL) absorbing chromophore phycourobilin (PUB) [1]. GL-specialists display a low PUB:PEB ratio ( $\approx 0.4$ ), BL-specialists a high ratio ( $\approx 1.6$ ), while other strains can dynamically modulate this ratio, a process known as Type-IV chromatic acclimation (CA4; [2]). CA4 appeared twice during evolution, a first time to confer the CA4 ability to former GL-specialists and a second time to confer it to BL-specialists [3]. Although the two resulting pigment types (CA4-A and CA4-B cells) have a similar PUB:PEB ratio in GL ( $\approx 0.6$ ) and BL ( $\approx 1.6$ ), their ratios differ in intermediate blue-green light, and they display distinct acclimation dynamics after a shift from BL to GL or vice-versa [4]. Competition experiments between a GL-specialist, a BL-specialist and a CA4-able strain showed that while the GL-specialist wins in both low and high GL, the BL-specialist wins in low BL but is outcompeted by the CA4-able strain in high BL [5]. Analyses of *Tara* Oceans metagenomes showed that the CA4 process has a global significance, since about 41.5% of *Syn.* cells along the transect were potentially capable of CA4 [1]. Recent analyses of two 2-year long time series off French coasts further confirmed the key ecological role of CA4 in marine *Syn.* community [Dufour, Faure et al. In prep.]. To better understand the fitness advantage conferred by CA4, we also modeled the main *Syn.* pigment types into the global ocean model Darwin and showed that BL-specialists were restricted to blue, nutrient-poor open ocean waters and GL-specialists to green, coastal and upwelling, nutrient-rich waters, whereas CA4-able cells were more ubiquitous and showed highest abundances in intermediate regions between the two specialists [6].

[1] Grébert T. et al. Proc Natl. Acad. Sci. USA (2018). doi.org/10.1073/pnas.1717069115

[2] Kehoe D.M. et al. Plant Cell Physiol. (2024). doi.org/10.1093/pcp/pcae115

[3] Grébert T. et al. Proc Natl. Acad. Sci. USA (2021). doi.org/10.1073/pnas.2019715118

[4] Dufour L. et al. Front. Microbiol. (2024). doi.org/10.3389/fmicb.2024.1349322

[5] Dufour L. et al. Appl. Environ. Microbiol. (2025). doi.org/10.1128/aem.00087-25

## Synthetic microbiology

### K-SG-SM-01

#### Synthesis of organic acids using filamentous fungi, as exemplified by citric acid

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**Introduction:** *Aspergillus niger* is a filamentous fungi widely used in the biotechnological industry for citric acid production, a key component in food, pharmaceutical, and cosmetic industries. The intracellular biochemical pathways to produce this acid are known, but the key to achieve high secretion of this acid is connected with the secretion system of the fungus, which is dependent on the gene encoding for the citrate exporter in the plasma membrane.

**Goals:** This research investigates and targets the secretion bottleneck to elucidate *Aspergillus niger*'s exceptional capacity for organic acid production.

**Materials & Methods:** Metabolic engineering approaches created strains with altered transport capacities and deregulated gene expression independent of metal availability. Genetic tools, including inducible promoters like p<sub>tet</sub>-on and CRISPR-Cas9 systems, were developed to enable precise, targeted engineering in filamentous fungi such as *Aspergillus niger*. Shake flask and microbioreactor cultivations were combined with analytical tools, including HPLC for titers, qPCR for expression, and microscopy for morphology assessments.

**Results** New engineering techniques, including RNP/Cas9 for efficient gene targeting and integration, enabled precise modifications in *Aspergillus niger*. Promoter decoupling restored citric acid export regardless of manganese presence and promoted branched hyphae formation. CexA activation achieved up to 109 g/L citric acid—fivefold above wild-type levels—while manganese(II) suppressed cexA transcription, blocking citric acid secretion. GsdA downregulation further balanced metabolic fluxes, enhancing acid yields by redirecting carbon from biomass growth.

**Summary:** These insights enhance our understanding of organic acid secretion in filamentous fungi, underscoring the importance of transport processes—particularly the citrate exporter, CexA—as a critical bottleneck and engineering target.

Microorganisms have driven Earth's sulfur cycle since the emergence of life. Yet, the sulfur-cycling capacities of microorganisms and their integration with other element cycles remain incompletely understood. One undiscovered metabolism is the coupling of sulfide oxidation with iron(III) oxide reduction, a ubiquitous environmental process hitherto considered to be strictly abiotic. Here, we present a comprehensive genomic analysis of sulfur metabolism across prokaryotes, and reveal bacteria capable of oxidizing sulfide using extracellular solid-phase iron(III). Based on a phylogenetic framework of over hundred genes involved in dissimilatory transformation of sulfur compounds, we recorded sulfur-cycling capacity in most bacterial and archaeal phyla. Metabolic reconstructions predicted co-occurrence of sulfur compound oxidation and iron(III) oxide respiration in diverse members of 37 prokaryotic phyla. Physiological and transcriptomic evidence demonstrated that a cultivated representative, *Desulfurivibrio alkaliphilus*, grows autotrophically by oxidizing dissolved sulfide or iron monosulfide (FeS) to sulfate with ferrihydrite as an extracellular iron(III) electron acceptor. The biological process outpaced the abiotic process at environmentally relevant sulfide concentrations. These discoveries expand the known diversity of sulfur-cycling microorganisms, unveil a biological mechanism linking sulfur and iron cycling in anoxic environments, and thus highlight the fundamental role of microorganisms in global element cycles.

## Identification & systematics

### K-SG-SMBE-01

#### Decoding Microbial Foraging Strategies: From Genotype to Function Using Fluorescent Probes and Functional Sorting

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In marine ecosystems, identifying the active microorganisms responsible for organic matter degradation remains a major challenge—particularly when microbial communities are functionally redundant, spatially variable, and dynamically responsive to environmental cues. Traditional approaches often fail to link microbial identity to metabolic activity, especially when key substrates such as complex glycans are involved. To address this, we combine culture-independent and culture-based methods to resolve the identity and functional role of active heterotrophs *in situ*. Using fluorescent substrate analogues, we track real-time substrate utilization across diverse marine environments - from surface ocean to sediments - enabling the detection of active populations without prior cultivation. These probes allow us to distinguish between selfish, sharing, and scavenging foraging strategies, revealing that metabolic behaviour is context-dependent rather than fixed. When combined with additional culture-independent methods - FISH, flow cytometry, and proteomics - they reveal which microbes are active and how they forage. To validate our environmental

## Water / Waste water

### K-SG-WW-01

#### Microbial iron oxide respiration coupled to sulfide oxidation

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findings, we integrate activity data with targeted culture-based experiments, testing isolates under controlled conditions to link genotype to phenotype and dissect mechanisms of substrate processing. Together, these approaches transform our ability to classify microbes and their functions in nature.

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## Functional Genomics & Bioinformatics

### K-SG-FGBI-01

#### A model for bacterial colonisation of a urinary catheter: different factors control long-term versus short-term outcomes

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### Introduction

Urinary catheters are used extensively in hospitals and long-term care and they are highly prone to infection. Understanding the pathways by which bacteria colonise a urinary catheter could guide strategies to mitigate infection, but quantitative models for this colonisation process are lacking.

### Goal and Methods

We aim to predict the timescale on which bacteria colonise a urinary catheter, and to understand what the conditions are that facilitate colonisation. To this end we present a mathematical model for bacterial colonisation of a urinary catheter that describes bacteria migrating up the outside surface of the catheter, spreading into the bladder and being swept through the catheter lumen.

### Results and Conclusion

Computer simulations of the model reveal that clinical outcomes for long-term versus short-term catheterisation are controlled by different factors: the rate of urine production by the kidneys as opposed to urethral length, catheter surface properties and bacterial motility. Our work may help explain variable susceptibility to catheter-associated urinary tract infection (CAUTI) among individuals and the mixed success of antimicrobial surface coatings. Our model suggests that for long-term catheterised patients, increasing fluid intake or reducing residual urine volume in the bladder may help prevent infection, while antimicrobial surface coatings are predicted to be effective only for short-term catheterised patients. Therefore, different catheter management strategies could be rationally targeted to long-term vs short-term catheterised patients. In ongoing work, the model is being tested against clinical data for the incidence of bacteriuria in catheterized patients. The model is also being extended to predict bacterial colonization of other medical devices such as breathing tubes.

### Reference:

Bull F, Tavaddod S, Bommer N, Perry M, Brackley CA, Allen RJ (2025) Different factors control long-term versus short-term outcomes for bacterial colonisation of a urinary catheter. *Nature Communications* 16: 3940.

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## Short Lecture – Special Groups

### Biotransformation

#### SL-SG-BT-01

#### Marine microbes powering seawater electrolysis for green hydrogen production: early insights

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Seawater electrolysis offers a promising route to convert clean electricity from offshore wind turbines into sustainable hydrogen (H<sub>2</sub>), facilitating its transport and utilization by end users. However, the technology remains hindered by challenges such as low catalytic activity and the formation of hazardous chlorine (Cl<sub>2</sub>). In contrast, a unique group of microorganisms—electroactive bacteria—are ubiquitously distributed in marine sediments. These microbes are capable of transferring electrons from intracellular donors to extracellular acceptors (e.g., electrodes) and exhibit strong biocatalytic potential. This potential may help overcome current limitations of seawater electrolysis. In particular, the natural halotolerance or halophilicity of marine microbes eliminates the need for desalination or purification of seawater prior to electrolysis, as these organisms are inherently adapted to saline environments. Therefore, within the *Sa/YsAse* project, we are investigating the use of marine microbes as biocatalysts for H<sub>2</sub> generation through seawater electrolysis.

A lab-scale microbial electrolysis cell (MEC) was constructed, comprising a carbon felt working electrode (CF), a counter electrode (porous titanium foil), and an Ag/AgCl reference electrode. Marine sediments and seawater from the SW Baltic Sea, Germany served as inoculum. A sediment–seawater mixture (1:3 v/v) supplemented with acetate (1 g L<sup>-1</sup>) as the carbon source was introduced into the MEC, which was then purged with N<sub>2</sub> for 30 min to establish anoxic conditions. As of now, the working electrode potential was maintained at 0.2 V vs. Ag/AgCl using a potentiostat.

After one month of operation, a dense electroactive biofilm developed on the electrode surface, as verified by cyclic voltammetry and current response profiles. Gas chromatography with a PDD detector confirmed H<sub>2</sub> production, demonstrating that seawater electrolysis can be microbially catalyzed.

These findings provide a proof of concept for the *Sa/YsAse* project, highlighting the potential of marine electroactive consortia for sustainable hydrogen generation. Future work will focus on elucidating the underlying electron transfer mechanisms and characterizing the active microbial communities.

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#### SL-SG-BT-02

## Towards complete carbon utilization: Improved methane yield from formate and hydrogen co-feeding through constitutive formate dehydrogenase expression in *Methanothermobacter*

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Electrochemical CO<sub>2</sub> capture and conversion are redefining how renewable electricity can drive a circular carbon economy. Formate is among the emerging intermediates, despite being a low-energy product. Converting it into methane offers a powerful route to store renewable energy and fully close the carbon loop. Methanogenic archaea can achieve this transformation. However, natural formate disproportionation yields only 25% CH<sub>4</sub>, with most carbon lost as CO<sub>2</sub>. Supplying hydrogen could drive complete reduction, yet paradoxically suppresses formate utilization. A phenomenon that is thought to be linked to genetic regulation of the formate dehydrogenase (Fdh).

We investigated whether constitutive expression of the Fdh gene cluster enhances carbon utilization and process stability in continuously operated bioreactors during combined formate and hydrogen feeding.

Chemostat bioreactors were used to compare the wild-type donor strain *M. thermautotrophicus* Z-245 with the engineered strain *M. thermautotrophicus* ΔH(pFdh), harbouring a plasmid for constitutive Fdh expression. Cultures were subjected to stepwise increases in H<sub>2</sub> supply, and gas evolution was quantified via online mass spectrometry. Dissolved hydrogen concentrations (cH<sub>2</sub>) were determined from gas-liquid mass transfer coefficients and biomass-specific H<sub>2</sub> uptake rates to interpret metabolic responses across hydrogen availabilities.

Constitutive Fdh gene cluster expression markedly enhanced stable carbon conversion from 36% in Z-245 to 76% in ΔH(pFdh). This higher methane productivity required greater hydrogen transfer, which also intensified formate-derived CO<sub>2</sub> stripping, rendering the system CO<sub>2</sub>-limited. With optimized bioreactor operation, even higher utilization is expected. Furthermore, stepwise increasing the cH<sub>2</sub> revealed rapid responses in biomass-specific formate uptake and CH<sub>4</sub> production rates that cannot arise solely from transcriptional regulation, but are likely tied to shifts in intracellular redox balance. Small upsets in biological uptake can quickly raise cH<sub>2</sub>, further repressing formate metabolism and creating a self-reinforcing decrease in system performance. ΔH(pFdh) was significantly less affected by cH<sub>2</sub> changes, potentially caused by differences in Fdh protein abundance, or by intrinsic differences in CO<sub>2</sub> reduction machinery between Z-245 and ΔH. These findings establish ΔH(pFdh) as a promising candidate for formate valorization, and open avenues to further investigate Fdh regulation.

**Introduction:** Conventional batch reactions are commonly used in chemical synthesis but often fall short in terms of efficiency, scalability, and sustainability. Continuous flow systems offer a promising alternative by addressing these limitations, and incorporating electricity can further enhance process control and support sustainable chemical transformations. Nonetheless, the potential of electro-biocatalytic flow systems remains underexplored.

**Goal:** Our goal was to evaluate H<sub>2</sub>-driven flavin-mediated reduction of cyclic enones in continuous flow setup for sustainable chemical synthesis using electricity.

**Method:** This study utilized TsOYE for ene reduction and soluble hydrogenase (SH) for H<sub>2</sub>-driven recycling of FMNH<sub>2</sub>. H<sub>2</sub> was supplied from water electrolysis by a PEM electrolyzer and fed into the flow system through gas-permeable membrane tubing inside a gas addition module. SH was immobilized on Strep-Tactin resin and TsOYE on EziG beads (SH-Tactin, TsOYE-EziG), both packed in a column integrated into the flow system.

**Result:** We demonstrated electro-driven asymmetric reduction of ketoisophorone to levodione in the flow setup. Subsequently, the setup was used to evaluate the reusability of the immobilized enzymes, where it maintained product formation of >99 % even after seven cycles. Finally, we demonstrated scalability of the system by increasing the flow reaction volume, with TTN of SH-Tactin and TsOYE-EziG reaching 3.2 x 10<sup>5</sup> and 2.6 x 10<sup>4</sup>, respectively.

**Conclusion:** In this study, we established a biocatalytic continuous-flow system using electrical energy for chemical synthesis. Apart from integrating a PEM electrolyzer and highly gas-permeable tubing, this study also introduced Strep-Tactin resin and highlighted EziG beads as stable enzyme carriers suitable for applications in a scalable continuous-flow setup. Overall, we made advancements in continuous flow biocatalysis by incorporating H<sub>2</sub> from water electrolysis into the flow system to fuel *in-vitro* flavin-dependent reactions.

## Reference:

Lim, G., Calabrese, D., Wolder, A., Cordero, P. R. F., Rother, D., Mulks, F. F., Paul, C. E., & Lauterbach, L. (2024). *Communications Chemistry*

Klos, N., ... Lim, G., ..., Lauterbach, L., Jupke, A., Leitner, W., Blank, L. M., Klankermayer, J., & Rother, D. (2024). *JACS Au*

Al-Shameri, A., Petrich, M.-C., Junge Puring, K., Apfel, U.-P., Nestl, B. M., & Lauterbach, L.

(2020). *Angewandte Chemie International Edition*

## SL-SG-BT-03

### Integrated electro-biocatalysis for chemical synthesis in flow system

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## SL-SG-BT-04

### Light-Controlled Enzyme Inactivation and Monitoring of Protein Secretion Using Flavin-Binding Fluorescent Proteins

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Flavin-binding fluorescent proteins (FbFPs) are oxygen-independent reporter proteins with promising applications in synthetic biology and optogenetics. In addition to their fluorescent properties, many FbFPs can function as genetically encoded photosensitizers due to their flavin mononucleotide (FMN) chromophore, which enables light-induced generation of reactive oxygen species (ROS).

Here, we explored the versatile functionality of FbFPs, focusing on their use in chromophore-assisted light inactivation (CALI). When irradiated with blue-light, the flavin protein generates ROS, which leads to an efficient light-triggered inactivation of a fused enzyme. This property was exploited *in vivo* to specifically switch off an enzyme within a biosynthetic cascade, thereby controlling the production of the secondary metabolite prodigiosin. Furthermore, *in vitro* experiments demonstrated that FbFP-mediated CALI can efficiently inactivate purified proteins fused to FbFP, highlighting the potential of FbFPs as precise tools for spatiotemporal regulation of enzymatic activity.

Beyond their application in CALI, FbFPs were employed as biosensors to monitor protein localization and secretion. In the cytoplasm, FbFPs bind endogenous FMN and emit cyan-green fluorescence. Secretion via the Sec pathway into the periplasm or extracellular medium can be analyzed by supplementing the medium with the FMN derivative roseoflavin mononucleotide (RoFMN). After Sec-dependent secretion, FbFPs bind RoFMN, resulting in a red-shifted fluorescence signal. This dual-color system enables non-invasive, real-time tracking of intracellular protein production and secretion during bacterial growth. The biosensor was successfully applied in both the Gram-positive bacterium *Corynebacterium glutamicum* and the Gram-negative bacterium *Escherichia coli*, demonstrating its broad applicability for studying protein secretion dynamics and localization in bacteria.

#### SL-SG-BT-05

##### Elucidating inactivation mechanisms to boost total turnover numbers in the Fe(II)/2-oxoglutarate-dependent halogenase AdaV

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Fe(II)/2-oxoglutarate-dependent halogenases (FeDHs) represent a promising class of metalloenzymes for biocatalytic C(sp<sup>3</sup>)-H functionalization and late-stage halogenation of complex biomolecules. However, their broader application in biotechnology remains limited by low total turnover numbers (TTNs), the causes of which are still poorly understood. In this project, we investigate the nucleotide halogenase AdaV, which catalyzes selective chlorination at the 2'-position of dAMP, as a model system to elucidate enzyme inactivation mechanisms and enhance catalytic performance. We systematically explored inactivation pathways through incubation studies, protein mass spectrometry, and stopped-flow UV-visible spectroscopy. Additionally, computationally guided experiments provided further insights and enabled improvement of TTNs beyond previously reported values for wild-type FeDHs. Collectively, this integrated reaction

engineering approach increased TTNs without the need for protein engineering and offers mechanistic understanding and practical guidelines for improving the efficiency of Fe/2OG metalloenzymes.

#### SL-SG-BT-06

##### Biotechnological production of up to 37 g/L N-(Hydroxycinnamoyl) tyramines in *E. coli* catalyzed by recombinant plant enzymes with continuous *in situ* product precipitation

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Hydroxycinnamic acids are formed in large quantities by all plants for the synthesis of lignin. A smaller proportion of these are condensed with amines derived from tyrosine and tryptophan. The resulting hydroxycinnamoyl amides have functions in plant growth, development and defense. They exert additional biological activities which comprise antioxidant, antidiabetic, anti-inflammatory, cytotoxic, anti-melanogenesis and neuroprotective effects. Pharmaceutical and industrial applications are currently limited by the low yield of extraction processes from plants and the costly, fossil based and multi-step processes of chemical synthesis. Latest biotechnological approaches of synthesis reached approximately 0.5 g/L of coumaroyltyramine (CouT) (1.58 mM).

We were aiming to improve current production processes by establishing recombinant expression of the key plant enzymes: 1) 4-coumarate:CoA ligase from *Arabidopsis thaliana* and 2) hydroxycinnamoyl-CoA:tyramine N-(hydroxycinnamoyl)transferase from *Solanum tuberosum* in *Escherichia coli*. The recombinant strain was used for whole-cell catalysis experiments by feeding a hydroxycinnamic acid and tyramine. Test expressions showed temperature sensitivity in regard to quantity and activity of the recombinant enzymes. We observed a substantial lack of stability of the enzymes at elevated temperatures, suggesting that the temperature is a critical parameter to consider in process development. Between the three tested temperatures (16 °C, 22 °C and 28 °C), 16 °C resulted in the highest enzymes stability in *E. coli*.

Oriented towards scaling up the production, we transferred the process to 1 L bioreactor scale. In a temperature adapted bioprocess with 1 mM/h feeding rate, we noticed that the product precipitated due to its low solubility, preventing possible toxicity of these phenolic metabolites on the production host. Raising educt supply rate to 5 mM/h enabled a product accumulation of 37 g/L of feruloyltyramine (FerT) (118.23 mM), 14.4 g/L CouT (50.7 mM), and 14.6 g/L isoferuloyltyramine (46.5 mM) within 10-23 h. The vast majority of the product was obtained as filterable precipitate. Washed filter cake from FerT production **retained** on a 16-40 µm glass filter showed 99 % mass content of FerT. Product identity was confirmed by NMR after recrystallization. The process is robust, cost-effective and scalable paving the way for commercial applications of biotechnologically produced hydroxycinnamic acid tyramines.

#### SL-SG-BT-07

## From Omics to Engineering: Advancing *Phlebia radiata* for Kraft Lignin Valorization

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Kraft lignin (KL) is an abundant, renewable aromatic waste-product from the pulping industry. Its valorization is challenged by the polymer's heterogeneous and recalcitrant structure. The white-rot fungus *Phlebia radiata* combines robust lignin-modifying enzyme (LME) activities with notable phenol tolerance, positioning it as a promising candidate for KL recycling. We developed a KL-valorization workflow to: (1) identify low-molecular-weight products from KL cultures, (2) map extracellular responses that enable KL degradation, (3) link degradation product formation to distinct metabolic activities, and (4) establish molecular tools to transform *P. radiata* directly.

Therefore, *P. radiata* (IBWF-00045) was cultivated with 0.25 % (w/v) KL supplementation; culture supernatants were extracted, fractionated (silica-gel column chromatography), and purified (preparative HPLC) for compound identification (GC-MS). Extracellular LME activities were investigated via ABTS tests and the tolerance of the fungus against rising KL concentrations determined. For transcriptome and secretome analysis an actively growing culture was induced with 0.25 % (w/v) KL. Furthermore, a monokaryotic strain was generated to enable molecular manipulations. Two methods were attempted: protoplast-mediated (PMT) and *Agrobacterium tumefaciens*-mediated transformation (ATMT).

Key findings include (1) Product discovery: Isolation of trans-3,5-dimethoxystilbene from KL cultures. (2) Extracellular response to KL: Laccase activity was highest in cultures grown with 0.75 mM CuSO<sub>4</sub> and 0.25 % KL supplementation. *P. radiata* showed KL tolerance of up to 0.4 % (w/v) under submerged fermentation conditions. (3) Transcriptome and secretome data illustrate KL exposure, to initiate production and secretion of various LMEs are initiated. (4) Transformation: We established a monokaryotic strain, identified suitable selection-markers as well as candidate promoters, and are optimizing PMT vs. ATMT workflows.

The emergence of the degradation product trans-3,5-dimethoxystilbene and early activation of LMEs support a working model in which KL is funneled toward separable, value-added aromatics. In parallel, the developing genetic toolkit enables the creation of reporter and overexpression strains, enhancing secretion profiles and product selectivity, advancing *P. radiata* from a lignin-degradation model organism to an engineerable biocatalyst for KL valorization.

## Phage Biology

### SL-SG-VM/R-01

#### Role of Lsr2-like xenogeneic silencing proteins in actinobacterial antiphage defense

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Besides the existence of infectious phage particles in the environment, a significant portion of bacterial genomes

harbors dormant phage forms known as prophages. As integral genomic components, prophages act as reservoirs of defense mechanisms against competing viruses and other mobile genetic elements. In this study, we investigate whether and how the prophage-encoded Lsr2-like nucleoid-associated protein CgpS confers immunity against phage infection. CgpS functions as a xenogeneic silencer (XS) that is crucial for maintaining the lysogenic state of the cryptic prophage CGP3 in *Corynebacterium glutamicum* ATCC 13032<sup>(1-3)</sup>.

Based on our results, we present the hypothesis that the binding and silencing activity of CgpS impedes infection by the low-GC phage JeanGrey during the DNA replication stage in *C. glutamicum*. Our in vitro and in vivo assays identified specific interaction loci on the phage genome. Further reporter assay confirmed that the binding of CgpS leads to reduced gene expression. Moreover, since CgpS is positively autoregulated, reducing the amount of targeted prophage DNA within the host genome leads to lower levels of this xenogeneic silencing protein. This reduction effectively restores the infectivity of low-GC phages. In addition, transcriptomic analyses indicate that additional genetic elements within the *C. glutamicum* genome contribute to a broader defense network, unveiling a previously unrecognized arsenal of defense pathways.

Overall, our findings provide compelling evidence that prophage-encoded Lsr2-like proteins play a pivotal role in the actinomycetotal antiviral immunity. This work expands our understanding of the functional diversity of xenogeneic silencing proteins, which are widespread across different phyla.

### References:

1. Pfeifer, E. et al (2016) doi: 10.1093/nar/gkw692
2. Pfeifer, E. et al (2019) doi: 10.1016/j.jmb.2019.02.011
3. Wiechert, J. et al (2020) doi: 10.1128/mBio.02273-19

### SL-SG-VM/R-02

#### Structural and Functional Analysis of the Novel ARMADA II Phage Defense System from enterohemorrhagic *Escherichia coli*

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Bacteria have evolved an impressively diverse arsenal of molecular defense systems to avert phage infections. In recent years, many new putative defense systems have been discovered due to their genomic clustering with known defense systems on so-called defense islands. We are investigating a defense island, O172, of the enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 outbreak strain EDL933.[1] O172 harbors Zorya II and Druantia III defense systems, as well as a four-gene operon

that encodes a new putative defense system, termed disARM-related Antiviral Defense Array (ARMADA) II.[2] Using deletion mutants in the EHEC strain EDL933 and phage infection assays, we found that all four components of ARMADA II are essential for phage defense. We recombinantly produced and purified all components and conducted *in vitro* interaction studies, revealing stable complex formation between two pairs of proteins. By using cryogenic electron microscopy, a high-resolution structure of one of these complexes, comprising a methyltransferase and a Snf2-type NTPase/helicase, was elucidated. Complementation studies using a variant that is dysfunctional in complex formation showed that the formation of the macromolecular complex is required for the phage defense mechanism. Additionally, a structure obtained of the co-factor bound state of this protein complex revealed a unique toggling mechanism of the NTPase/helicase in respect to the methyltransferase. Based on the structures, we have started to engineer protein variants, in which putative enzymatic activities or interaction potentials are compromised. Additional structural and structure-guided functional studies are ongoing to unravel the molecular mechanisms underlying ARMADA II-mediated phage defense system.

[1] Li H, van Loi V, Borelli C, Himpich S, Projahn M, Grass LM, Nguyen TPT, Wahl MC, Antelmann H (2025) *bioRxiv*, doi:10.1101/2025.09.22.677730

[2] Bell RT, Gaudin T, Wu Y, Garushyants SK, Sahakyan H, Makarova KS, Wolf YI, Jackson SA, Nobrega FL, Beisel CL, Koonin EV (2025) *bioRxiv*, doi:10.1101/2025.09.15.676423

### SL-SG-VM/R-03

#### Polyphosphate-mediated protection of *Pseudomonas aeruginosa* against antibiotic-induced membrane stress

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The biopolymer polyphosphate (polyP), consisting of long chains of phosphate residues linked via phosphoanhydride bonds, is critical for microbial stress adaptation, host-pathogen interaction, and biofilm formation, although the mechanism by which this structurally simple molecule contributes to these phenotypes remains enigmatic. We demonstrate that polyP is important for bacterial survival during exposure to membrane-targeting antibiotics (MTAs). PolyP-deficient cells ( $\Delta ppkA$ ) are characterized by  $\geq 3$ -log reduced viability upon polymyxin E (PME) treatment, coupled with the accumulation of reactive oxygen species (ROS) levels, suggesting that these cells experience increased oxidative stress. Propidium iodide and Nile red uptake were also increased in PME-treated  $\Delta ppkA$  compared to wild-type (WT) cells. These defects in membrane integrity and fluidity were not caused by ROS and detectable even in anaerobically grown cells. Surface plasmon resonance assays revealed no stable PME-polyP binding, excluding a protective mechanism that is based on direct antibiotic sequestration by polyP. Instead, our data point towards a regulatory role of polyP: RNA-seq analysis of PME-treated WT and  $\Delta ppkA$  revealed profound transcriptional dysregulation in the absence of polyP, including of two-component regulatory systems, cell wall/LPS remodeling genes, fatty acid/phospholipid metabolism genes, and chaperones/heat shock proteins. Notably, absence of polyP triggers hyperactivation of stress regulons yet fails to prevent membrane damage, suggesting a loss of coordination

between regulatory signaling and successful repair. We hypothesize that polyP protects PME-stressed *P. aeruginosa* through a dual mechanism, its chaperone function and a novel mechanism that affects the cell envelope that is based on rapid activation of envelope stress response systems for lipid A remodeling, membrane repair, and surface charge regulation. Our study investigates three key aspects of polyP function in *P. aeruginosa* during MTA stress: (1) the mechanisms by which polyP mitigates ROS-mediated envelope damage during early antibiotic exposure, (2) the role of polyP in regulating envelope-associated stress pathways that preserve membrane stability, and (3) the impact of polyP on membrane lipid composition and remodeling under antibiotic challenge. Together, these findings highlight polyP as a central regulator of membrane resilience and adaptive signaling in *P. aeruginosa*.

### SL-SG-VM/R-04

#### Exploring the relationship between the two-component system CroRS and the second messenger c-di-AMP in regulating $\beta$ -lactam resistance in *Enterococcus faecalis*

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*Enterococcus faecalis*, a major opportunistic pathogen in hospital-acquired infections, exhibits intrinsic resistance to  $\beta$ -lactam antibiotics. This resistance is regulated by the CroRS two-component system, which controls cellular responses to cell wall-targeting antibiotics such as ampicillin. Deletion of *croRS* leads to increased  $\beta$ -lactam susceptibility, reduced growth, enlarged cell size, and a thinner cell wall. However, the specific mechanisms underlying CroRS-dependent  $\beta$ -lactam resistance remained unclear. Here, we identify the second messenger c-di-AMP as a central mediator of  $\beta$ -lactam resistance in *E. faecalis*. Suppressor screening of the  $\Delta croRS$  strain revealed recurrent mutations in c-di-AMP-degrading enzymes. Notably, deletion of a single phosphodiesterase, *pgpH*, was sufficient to fully restore  $\beta$ -lactam resistance in the  $\Delta croRS$  background. Detailed characterization revealed that  $\Delta croRS$  deletion results in abnormally low c-di-AMP concentrations, while the  $\Delta croRS \Delta pgpH$  mutant showed near wild-type levels of c-di-AMP as well as restored cell size and ampicillin resistance. We propose that the low c-di-AMP level impairs the  $\Delta croRS$  strain's ability to regulate turgor pressure, leading to enlarged cell size, cell wall stretching and increased susceptibility to autolysis. Supporting this model, under hyperosmotic conditions,  $\Delta croRS$  cells showed reduced cell size similar to the wild-type. Elevated autolysis activity evidenced that the stretched cell wall in  $\Delta croRS$  cells was highly sensitive to cell wall hydrolases, which could be suppressed by deletion of *atlA*, the major enterococcal autolysin, or by provision of hyperosmotic growth conditions. Importantly, *atlA* deletion or hyperosmotic conditions also restored  $\beta$ -lactam resistance in  $\Delta croRS$  cells, confirming the turgor-dependent nature of cell wall vulnerability. Together, these findings reveal a novel link between c-di-AMP-mediated osmoregulation and  $\beta$ -lactam resistance in *E. faecalis* and provide new insights into the interplay between second messenger signalling, cell envelope integrity, and antibiotic resistance.

### SL-SG-VM/R-05

#### RNAylation of plant proteins by a bacterial ADP-ribosyltransferase

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RNAylation is a recently discovered posttranslational protein modification in which ADP-ribosyltransferases (ARTs) covalently attach 5'-NAD-capped RNA (NAD-RNA) to proteins. To date, only one bacteriophage T4-encoded ART has been described to RNAylate its target proteins in *E. coli*. Since NAD-RNA and ARTs are present in all domains of life, we hypothesize that RNAylation could be widespread in nature.

This study aims to identify novel ARTs with RNAylation ability and investigate the occurrence of RNAylation in biological systems beyond the described interaction of *E. coli* and bacteriophage T4.

Here, we demonstrate that HopU1, an ART from *Pseudomonas syringae*, a phytopathogenic bacterium, can RNAylate its target proteins, among them GRP7 from *Arabidopsis thaliana*, *in vitro*. Our biochemistry and mass spectrometry-based approaches show that HopU1 modifies the same arginine residue of GRP7 in both ADP-ribosylations and RNAylations. Further, we identified several additional RNAylation targets of HopU1 that were known as ADP-ribosylation targets before.

Our investigation of the substrate scope of HopU1 revealed that HopU1 prefers linear NAD-RNAs over NAD-RNAs with secondary structures for RNAylation.

This work establishes HopU1 as the second described ART with RNAylation ability, suggesting that even more ARTs can catalyze RNAylations. Our results suggest the possible occurrence of RNAylation in the interaction between *P. syringae* and *A. thaliana*, which would represent the first described RNAylation in a eukaryotic system. These results indicate that RNAylation could be a widespread phenomenon.

## SL-SG-VM/R-06

### Sibling Team Work – Interplay of a family of regulatory RNAs with an RNA sponge in *Caulobacter crescentus*

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Small regulatory RNAs (sRNAs) contribute significantly to the post-transcriptional control of gene expression in bacteria. sRNAs may influence mRNA translation and stability by engaging in direct base-pairing interactions with target transcripts. In some cases, a given bacterium expresses multiple, seemingly homologous sRNAs which are called sibling sRNAs. The physiological advantage of encoding several copies of nearly identical regulators or how their activity is integrated into established regulatory circuits is generally not well understood.

The  $\alpha$ -proteobacterium *Caulobacter crescentus* encodes four sibling sRNAs of the  $\alpha$ R8 RNA family sharing conserved sequence elements and a characteristic secondary structure. The  $\alpha$ R8 sRNAs are expressed from independent genomic loci under different environmental conditions, including carbon availability and iron deficiency, indicating sibling-specific transcriptional input. To determine the target spectra of each family member we performed comparative transcriptome analyses and identified both shared as well as individual target interactions. Additionally, by performing RIL-seq (RNA interaction by ligation and sequencing) we recovered interactions between the  $\alpha$ R8 sRNAs and the sRNA CrfA. CrfA is induced in response to carbon starvation and acts as a sponge RNA that binds to and facilitates  $\alpha$ R8 turn-over. Using both *in vivo* as well as *in vitro* approaches, we have untangled a complex post-transcriptional network modulating carbon metabolism when *Caulobacter* switches between feast and famine.

## SL-SG-VM/R-07

### Phages carry multiple triggers and blockers against the same bacterial defence system

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Recently, it was established that retrons defend against phages through abortive infection (Abi)1–3. Retron-Sen2 and -Eco9 form tripartite toxin-antitoxin systems where the toxin (RcaT) is inhibited by an antitoxin composed by a cognate reverse transcriptase (RT) bound to its multi-copy single-stranded DNA (msDNA)1. RcaT toxicity is triggered by proteins that perturb or modify the msDNA, which are often carried by phages. But at the same time, phages can also carry anti-retron defence mechanisms that block the activity of RcaT to prevent Abi. We used a recently developed high-throughput reverse genetics method called TAC-TIC (Toxin Activation-Inhibition Conjugation) to screen genome-wide libraries of phages T5 and P1vir for Retron-Sen2 and Eco9 triggers and blockers. Although both retrons can defend against T5, they fail to do so against P1vir. Despite this difference, we identify that both phages carry multiple potential triggers and blockers in their genome. For example, the essential DNA exonuclease of phage T5 triggers both retrons, by degrading msDNA during phage infection. On the other hand, although P1vir encodes triggers for Retrons-Sen2, the retron does not protect against phage P1vir, likely due to the different potent blockers it carries. By evolving T5 to circumvent retron defence, we isolated mutants on specific blockers and triggers we identified by TAC-TIC, showing that the two methods provide complementary results. *In toto*, we propose that the retron msDNA senses incoming phage to activate RcaT-mediated Abi, and that the specificity of retrons is defined by their collective capacity to sense phage functions and to bypass phage blockers.

## References

1. Bobonis, J. *et al.* Bacterial retrons encode phage-defending tripartite toxin-antitoxin systems. *Nature* 1–3 (2022) doi:10.1038/s41586-022-05091-4.

- Gao, L. *et al.* Diverse enzymatic activities mediate antiviral immunity in prokaryotes. *Science* **369**, 1077–1084 (2020).
- Millman, A. *et al.* Bacterial Retrons Function In Anti-Phage Defense. *Cell* **183**, 1551–1561.e12 (2020).

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## SL-SG-VM/R-08

### The PhageExpressionAtlas - a comprehensive transcriptional atlas of phage infections

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Bacteriophages (phages) are bacterial viruses that infect and eventually lyse their bacterial hosts, offering promising alternatives to the rising antimicrobial resistance crisis. However, to fully harness the potential of phages, a detailed understanding of phage-host interactions is essential. For this, **dual RNA sequencing** (dual RNA-seq) has emerged as a powerful tool that simultaneously captures host and phage transcriptomes across infection stages. Previous dual RNA-seq studies have revealed critical insights into host responses and phage takeover strategies. However, such transcriptomic data are often difficult to immediately reuse for custom analyses, in part due to the lack of uniform data processing, storage and interactive visualization tools.

Here, we present the **PhageExpressionAtlas**, the first interactive and visual platform for exploring phage-host interactions on the transcriptome level across infection stages. We developed a unified analysis pipeline to process a diverse set of dual RNA-seq datasets covering model and therapeutic phages as well as clinically relevant ESKAPE pathogens. Users can browse and download datasets and explore phage and host gene expression via customizable heat maps and expression profile plots. A central feature of the atlas is the ability to **classify phage genes** into early, middle, and late infection phases based on pre-defined or user-specified criteria. These classifications can be visualized as expression profiles or in genomic context, facilitating gene function inference and insights into phage genome organization.

Beyond the platform itself, we demonstrate its utility through systematic analysis of the integrated datasets. We show how diverse bacterial hosts mount both conserved and host-specific transcriptional responses to phage attack, and how distinct phages converge on shared transcriptional strategies – particularly in the arms race between defense and anti-defense mechanisms. Furthermore, we use transcriptomic profiles to train classifiers that predict the temporal gene class of phage genes directly from sequence, offering a novel, additional route supporting phage genome annotation.

Altogether, the PhageExpressionAtlas provides an extensible, user-friendly, and openly accessible resource that democratizes transcriptomics-driven discovery for the broader phage research community. The PhageExpressionAtlas is available at [phageexpressionatlas.cs.uni-tuebingen.de](http://phageexpressionatlas.cs.uni-tuebingen.de).

## SL-SG-VM/R-09

### Structural basis of quinone-sensing by the MarR-type repressor MhqR in *Staphylococcus aureus*

**Introduction:** During infection, *Staphylococcus aureus* is exposed to reactive electrophilic species, such as quinones and aldehydes. The MarR-type repressor SaMhqR was previously shown to confer resistance towards quinones and quinone-like antimicrobials [1]. However, the redox-regulatory mechanism of SaMhqR by quinones was unknown. **Goals:** We hypothesized that quinones bind to a conserved ligand-binding pocket of MhqR, leading to its inactivation and derepression of transcription of quinone detoxification genes. **Materials & Methods:** We resolved the crystal structures of apo-SaMhqR and the 2-methylbenzoquinone (MBQ)-bound SaMhqR complex, predicted the structure of DNA-bound SaMhqR by AlphaFold3, and used molecular dynamics (MD) simulations to investigate the structural changes of DNA-bound SaMhqR upon MHQ-binding. Using biochemical and mutational analyses, we studied the roles of amino acid residues involved in quinone-sensing and DNA-binding. **Results:** In the DNA-bound SaMhqR state, S65 and S66 of an allosteric  $\alpha$ 3- $\alpha$ 4 loop adapted a helically wound conformation to elongate helix  $\alpha$ 4 for optimal DNA binding. Key residues for MBQ interaction were identified as F11, F39, E43, and H111, forming the MBQ-binding pocket. MBQ binding prevented the formation of the extended helix  $\alpha$ 4 in the allosteric loop, leading to steric clashes with the DNA. MD simulations revealed an increased intrinsic dynamics in the allosteric loop and the  $\beta$ 1, $\beta$ 2-wing regions after MBQ binding, to prevent DNA binding. Transcriptional analysis validated that F11, F39, and H111 are required for quinone sensing *in vivo*, whereas S65 and S66 of the allosteric loop and D88, K89, V91 and Y92 of the  $\beta$ 1, $\beta$ 2-wing are essential for DNA binding *in vitro* and *in vivo*. **Summary:** Structure-guided modeling and mutational analyses identified a quinone-binding pocket and the mechanism of inactivation of SaMhqR, involving local structural rearrangements of an allosteric loop and a high intrinsic dynamics in the wHTH motif, to prevent DNA interactions. Our results provide novel insights into the redox-sensing mechanism of the conserved SaMhqR repressor, that functions as an important determinant of quinone and antimicrobial resistance in *S. aureus*.

## Reference:

[1] Fritsch VN, Loi VV, Busche T, Sommer A, Tedin K, Nürnberg DJ, Kalinowski J, Bernhardt J, Fulde M, Antelmann H. 2019. The MarR-type repressor MhqR confers quinone and antimicrobial resistance in *Staphylococcus aureus*. *Antioxid Redox Signal*. 31:1235-1252.

## SL-SG-VM/R-10

### The epigenetic regulation of the CfrBI restriction-modification system and its impact on phage restriction

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Restriction modification (R-M) systems are among the most widespread and, due their often plasmid-based nature, easily transmittable systems that bacteria can use to protect themselves from infection by bacteriophages (1). The CfrBI R-M system studied here is a minimal type II R-M system consisting only of the divergently expressed methyltransferase (MT) and restriction endonuclease (RE), while lacking the typical C protein as transcriptional regulator that most type II R-M systems possess (2,3). MT and RE recognize the same DNA sequence, with MT providing protection against RE, which can only act on unmethylated DNA. Interestingly, in the promoter region of the CfrBI R-M system a single CfrBI recognition site is present that has been shown to regulate the expression of the system (4,5). Here, we show how the copy number of plasmids harboring the R-M system, as well as promoter methylation influence the expression dynamics and the protective properties of the R-M system against phages in vivo. While higher plasmid copy numbers lead to higher expression of both MT and RE, the ratio shifts towards higher RE expression. Point mutations that prevent promoter methylation lead to a dramatic shift of the expression ratios towards higher MT expression, highlighting the role of the MT-dependent methylation in repressing its own gene expression. Interestingly, the protection of the R-M system against phage infections is highest in the background of a medium copy plasmid with balanced MT and RE expression, contrary to observations with other R-M systems (6). Metabolic burden in the background of high copy plasmids likely negatively impacts phage restriction. Interestingly, loss of promoter methylation completely abolishes the protective properties of the R-M system, independent of the plasmid background. In summary, the results underline the importance of epigenetic regulation of the expression of the CfrBI R-M system for its protective properties and show how the expression dynamics and phage resistance are influenced by the plasmids carrying the R-M system.

1. Loenen, W.A.M., et al. (2014) *Nucleic Acids Res.* 42: 3–19. doi: 10.1093/nar/gkt990.
2. Kravets, A.N., et al. (1992) *Mol Gen Mikrobiol Virusol.* 7-8: 4–7.
3. Zakharova, M.V., et al. (1993) *Gene.* 129: 77–81. doi: 10.1016/0378-1119(93)90698-3.
4. Beletskaya, I.V., et al. (2000) *Nucleic Acids Res.* 28: 3817–3822. doi: 10.1093/nar/28.19.3817.
5. Zakharova M.V., et al. (2004) *J Mol Biol.* 335: 103–111. doi: 10.1016/j.jmb.2003.09.081.
6. Kirillov A., et al. (2022) *Nucleic Acids Res.* 50: 12355-12368. doi: 10.1093/nar/gkac1124.

## SL-SG-VM/R-11

### Phage Infection Induces Stress-Linked Violacein Biosynthesis in *Janthinobacterium lividum* EIF1

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*Janthinobacterium lividum* produces the purple pigment violacein, a secondary metabolite associated with oxidative and stress responses. The strain EIF1 carries an active prophage, suggesting that prophage dynamics might

influence pigment regulation. The effect of lytic phage infection on this system remains unclear.

We investigated how infection by the lytic phage vB\_JliS-Donnerlittchen affects violacein production and growth in *J. lividum* EIF1 and whether prophage presence might modulate this response.

Cultures were incubated at 25 °C and 30 °C under four conditions: untreated control, conditioned medium, heat-inactivated phage, and active phage infection. Growth and pigment accumulation were quantified, and violacein production was normalized to cell density to assess specific productivity. Parallel samples are being analyzed by qPCR to examine transcriptional responses of the *vioABCDE* operon.

Phage infection reduced host growth while markedly increasing violacein accumulation per biomass. Heat-inactivated phage caused an intermediate response, suggesting that both infection and surface-recognition stress can trigger pigment biosynthesis. Strikingly, although *J. lividum* typically produces little to no violacein at 30 °C, phage-infected cultures synthesized substantial pigment at this normally non-permissive temperature. This unexpected reversal indicates that phage-induced stress can override temperature-dependent repression of violacein biosynthesis. Occasional spontaneous prophage induction was also observed microscopically, hinting that resident prophage activity may further influence stress signaling.

Infection by the lytic phage vB\_JliS-Donnerlittchen induces a stress-linked increase in violacein production in *J. lividum* EIF1, even under conditions that typically suppress pigment formation. Ongoing qPCR analyses and the generation of a CRISPR/Cas9-engineered prophage-free derivative will clarify whether resident prophage dynamics modulate this defensive metabolic response.

## SL-SG-VM/R-12

### Single-cell insights into the dynamic virulence gene expression of *Salmonella enterica*

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During the initial phases of infection, the enteropathogen *Salmonella enterica* uses flagella-mediated motility to reach the intestinal epithelium and to initiate host cell contact. At the infection site, *Salmonella* employs a type III secretion system (T3SS) encoded in the *Salmonella* pathogenicity island 1 (SPI-I), T3SS-1, to secrete several effector proteins into the host cell cytosol that mediate uptake of the bacteria. Expression of these two virulence factors is heterogeneous among the population, and only a fraction of cells displays a motile (flagella<sup>ON</sup>) or invasive (T3SS-1<sup>ON</sup>) phenotype. However, the spatiotemporal expression coordination of flagella and T3SS-1 in individual cells during infection remains elusive. In this study, we use a microfluidic device that allows precise control over the bacterial cultivation to mimic infection conditions and elucidate the temporal dynamics of virulence gene expression of *Salmonella* at the single-cell level. We will use dual fluorescent reporter strains to simultaneously monitor the expression of flagella and T3SS-1 in individual cells. By comparing the expression dynamics between wildtype and selected mutants we aim to disentangle the impact of gene regulatory and environmental components on the expression dynamics of flagellar and

T3SS-1 genes and identify key regulatory interactions that allow *Salmonella* to coordinate virulence gene expression during infection. Microfluidic single-cell analyses under infection-mimicking conditions will provide novel insights on the heterogeneous expression of two essential virulence factors and thereby contribute significantly to the understanding of the regulatory network controlling *Salmonella* virulence.

SNARE-like protein IncA. *Nat Commun* **10**, 2747 (2019).

## Microbial pathogenicity

### SL-SG-MP-01

#### IncA enforces a dynamic, steady-state architecture at *Chlamydia* inclusion - inclusion membrane-contact sites

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Membrane-contact sites (MCS) between the *Chlamydia trachomatis* - containing vacuoles (inclusions) and host endomembranes govern fusion, cargo flow, and organelle avoidance<sup>1-4</sup>. The inclusion protein IncA harboring SNARE-like domains (SLD1/2) binds host SNAREs to drive homotypic inclusion-inclusion fusion and oppose lysosomal fusion<sup>2, 5</sup>. To determine whether IncA's role is transient (fusion-initiating) or persistent (fusion-maintaining), we leveraged a host-directed, auxin-inducible degron (Ctr-AID) to acutely and reversibly remove inclusion-anchored effectors with minute-scale and post-translational control, enabling temporal dissection without polar genetic effects.

Following successful fusion, degradation of membrane-bound IncA triggered progressive vacuole fragmentation, whereas IncA re-expression rapidly restored inclusion fusion. Time-resolved perturbations thus reveal a dynamic equilibrium in which inclusion integrity is not a "set-and-forget" state but an IncA-dependent steady state that demands continuous SNARE-like engagement to counter host membrane-destabilizing forces. By establishing the maintenance phase as both IncA-dependent and swiftly reversible, our data identify a vulnerable window in which disrupting IncA-SNARE interactions could fragment mature inclusions and destabilize the pathogen niche. Beyond IncA, this rapid degradation-rescue framework offers a generalizable strategy to disentangle initiation versus maintenance roles of other MCS components in intracellular pathogenesis.

7. Richards, T.S., Knowlton, A.E. & Grieshaber, S.S. *Chlamydia trachomatis* homotypic inclusion fusion is promoted by host microtubule trafficking. *BMC Microbiology* **13**, 185 (2013).
8. Linton, C. et al. Specialized contact sites regulate the fusion of chlamydial inclusion membranes. *Nat Commun* **15**, 9250 (2024).
9. Elwell, C., Mirrashidi, K. & Engel, J. *Chlamydia* cell biology and pathogenesis. *Nature Reviews Microbiology* **14**, 385-400 (2016).
10. Derré, I., Swiss, R. & Agaisse, H. The Lipid Transfer Protein CERT Interacts with the *Chlamydia* Inclusion Protein IncD and Participates to ER-*Chlamydia* Inclusion Membrane Contact Sites. *PLOS Pathogens* **7**, e1002092 (2011).
11. Cingolani, G. et al. Structural basis for the homotypic fusion of chlamydial inclusions by the

### SL-SG-MP-02

#### Contact points of type 4 secretion system with host facilitated by direct physical interactions of *Helicobacter pylori* outer membrane proteins with T4SS components and host factors

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**Introduction and Goals.** Type 4 secretion systems, for instance the CagT4SS of *Helicobacter pylori*, encode membrane-spanning protein complexes in Gram-negative bacteria which can transport various molecules that modulate the cell response to the bacteria. T4SS have to interact intimately with host components to function in their transport processes. To elucidate mechanisms and new functionalities, we use the CagT4SS as a model, which is an important virulence factor in the human *H. pylori* infection. Recently, structural information on various T4SS has been substantially improved by cryo-EM. However, important structural and functional information is still lacking, in particular on protein interactions between T4SS proteins and other, bacterial and host, proteins. We follow the hypothesis that the bacteria need to establish particular host membrane contact points, with specific properties of the membrane and membrane proteins on both host and bacterial sides, so that the activity of the T4SS can be governed in the optimal manner.

**Methods and Results.** We have been using interaction screens of CagT4SS and non-CagT4SS proteins with host cell surface proteins, followed by biochemical and functional characterization (proteomics, protein purification, biophysical interaction analyses, microscopy etc.), to establish a more precise data collection of intra-bacterial and bacteria-host contacts and contact factors centered around the T4SS. Thereby we hope to improve the knowledge on functional protein-protein interactions at those focal contact points and the subsequent functional/transport processes. We have recently found, as a first addition to the functional CagT4SS protein contacts toolbox, that two autotransporter outer membrane proteins (Hops) directly interact with components of the secretion apparatus [1]. Functionally opposing roles of specific Hops for the T4SS were uncovered. We visualized surface-exposed co-localization of outer membrane proteins with the T4SS. We also identified that bacterial outer membrane proteins share host cell factors in dedicated membrane patches, among them CEACAM and integrins, with T4SS proteins, but we do not know yet how this is temporally and spatially organized. On this basis, we are currently testing expanded families of bacterial outer membrane proteins as functional contact factors for the T4SS and establish their functions and functional redundancies for host-directed transport processes.

**Summary and Conclusions.** In summary, we identified novel interactions between *H. pylori* outer membrane proteins and CagT4SS outer proteins and characterized

them as functionally important for membrane transport processes. In the future, we will expand this membrane contact toolbox of a bacterial T4SS in biochemical and functional detail.

[1] Metz et al., *microLife*, Volume 6, 2025, uqaf027, <https://doi.org/10.1093/femsml/uqaf027>.

#### SL-SG-MP-03

##### Role of membrane contact site (MCS) formation during *Salmonella* infection

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During its intracellular life in host cells, *Salmonella enterica* serovar Typhimurium (STM) relies on the translocation of effector proteins by the type III secretion system (T3SS-2). These effectors manipulate the endosomal system, resulting in the formation of an extended, interconnected network of *Salmonella*-induced filaments (SIF) and the formation of a replication-permissive niche within host cells<sup>1</sup>. Goals: This project studies the formation and dynamics of membrane contact sites (MCS), during STM-induced endosomal remodeling. Additionally, we want to understand how the intracellular microenvironment and establishment of MCS influence the physiological state and fate of STM, and vice versa. Materials & Methods: We deploy SPLIT-GFP-based contact site sensors<sup>2</sup> for live-cell imaging and super-resolution microscopy to evaluate MCS formation during infection, and evaluate the contribution of T3SS-2 and host proteins. Correlative light and electron microscopy (CLEM) are applied to link the physiological state of STM, which we assess using dual-fluorescence reporters, with ultrastructural analyses of the cellular (micro)environment. Results: Our work revealed that endosomal remodeling by STM establishes new MCS between *Salmonella*-modified membranes and various host organelles, including the ER. Deficiencies in this endosomal remodeling process induce alternative phenotypic resistance mechanisms, such as persister formation, resulting in distinct morphological characteristics and microenvironments of intracellular STM. Summary: Ultrastructural features and cellular microenvironments reveal the heterogenous intracellular fate of STM, which is caused by differences in the endosomal remodeling and induction of novel MCS.

<sup>1</sup>Liss and Hensel (2015). Take the tube: remodelling of the endosomal system by intracellular *Salmonella enterica*. *Cellular Microbiology* 17(5), 639–647.

<sup>2</sup>Cali and Brini (2021). Quantification of organelle contact sites by split-GFP-based contact site sensors (SPLICS) in living cells. *Nat Protoc.* 16(11):5287–5308.

#### SL-SG-MP-04

##### *Salmonella* hijacking of host membrane-contact-sites modulates pyroptotic cell death

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In infected cells, *Salmonella enterica* serovar Typhimurium (STM) resides within a cholesterol-rich organelle termed the *Salmonella* Containing Vacuole (SCV). SCV stability is critical for immune evasion, yet the host-lipid trafficking

events that regulate SCV maturation and stability have remained unclear. Here we reveal the endosomal cholesterol transporter Niemann-Pick disease protein (**NPC1**) is a host factor that promotes SCV destabilization, enabling bacterial translocation to the host cytosol which triggers non-canonical inflammasome activation and pyroptotic cell death. In contrast, the secreted STM effectors **SseJ** and **SseL** interfere with ER–SCV contact sites involved in host lipid transport machinery and we provide evidence that these interactions play an important role in modulating inflammasome activation and downstream pyroptotic cell death. We propose that **NPC1**, **SseJ**, and **SseL** form a complex interlinked regulatory axis at organelle contact sites that coordinate subcellular lipid mobility, controlling SCV integrity and inflammatory cell death of infected cells.

#### SL-SG-MP-05

##### Cryo electron tomography reveals the molecular anatomy of bacterial conjugative junctions

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The rapid spread of antibiotic-resistance genes in bacteria is driven largely by conjugation, a unidirectional transfer of single-stranded DNA from donor to recipient cells. Despite decades of genetic and biochemical work, the physical architecture that enables this process has remained elusive. Here we integrate fluorescence imaging, correlative cryo-light microscopy and cryo-electron tomography (cryo-ET) to visualize the molecular architecture of conjugating *Escherichia coli* at nanometer resolution. Cryo-ET of mating pairs reveals tight membrane–membrane junctions and discrete electron-dense structures consistent with type IV secretion systems (T4SS). Sub-tomogram averaging resolves discrete T4SS modules and an outer membrane fusion between mating partners, suggesting a previously underappreciated mechanism for DNA transfer. These findings illuminate the ultrastructure of conjugative junctions and provide key insights into the physical mechanisms underlying T4SS-mediated gene transfer during bacterial conjugation.

#### References.

[1] Couturier, A. et al. Real-time visualisation of the intracellular dynamics of conjugative plasmid transfer. *Nat Commun* 14, 294 (2023).

[2] Goldlust, K. et al. The F pilus serves as a conduit for the DNA during conjugation between physically distant bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 120, e2310842120 (2023).

[3] Costa, T. R. D. et al. Structural and functional diversity of type IV secretion systems. *Nat Rev Microbiol* (2023).

#### SL-SG-MP-06

##### Identification of a *S. aureus* virulence factor that disturbs host cell plasma membrane asymmetry

The Gram-positive bacterium *Staphylococcus aureus* (*S. aureus*) is internalized by non-professional phagocytes (NPPCs) where it initially resides within phagoendosomes, so called bacteria-containing vacuoles (BCVs). During its pathogenesis *S. a.* can escape from BCVs and replicate within the host cytosol, followed by host cell lysis.

The pathogenesis of several bacteria is dependent on host sphingolipids. The most abundant sphingolipid is sphingomyelin (SM) on the extracellular surface of the plasma membrane. It was shown that SM on the luminal leaflet of BCVs is exposed to the cytosolic face of infected phagoendosomes prior to the membrane's catastrophic breakdown. Further, cytosolic SM was shown to target the autophagy machinery to BCVs thereby leading to formation of autophagosomes and eventually autophagolysosomes. Cytosolic SM thus may serve as an early danger signal for host cells infected with bacteria.

To test if SM exposure on the cytosol leaflet of membranes was dependent on secreted virulence factors of *S. aureus*, we treated host cells with sterile culture supernatants of various mutant strains. To detect SM exposure to the cytosol we generated a HeLa reporter cell line expressing YFP-Lysenin<sup>W20A</sup> in the cytosol. The fluorescent marker is an inactive version of a pore-forming toxin from *Eisenia fetida* and specifically binds to SM in the cytosol.

With this setup we identified a *S. aureus* virulence factor which is responsible for SM translocation from the extracellular to the cytosolic leaflet of host cell membranes.

## Quality Management & Food Microbiology

### SL-SG-QMFM-01

#### **Listeria monocytogenes: persistent, sporadic, or repeatedly introduced via raw materials? Prevention strategy for meat and fish processing**

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*Listeria monocytogenes* is a significant pathogen that causes both persistent and sporadic contamination in food businesses. Of particular concern is the repeated introduction of certain hypervirulent genotypes via raw materials from poultry, pork, beef, and fish. These genotypes, such as CC1, CC2, CC4, CC6, CC8, and CC155, pose a significant risk to food safety. This study investigates the prevalence and persistence of hypervirulent *Listeria monocytogenes* genotypes in various food businesses. Environmental samples and raw materials were analyzed for the presence of these genotypes. Molecular biological methods (3-allele typing, wgMLST, WGS, plasmid typing) were used to characterize the genetic characteristics and virulence factors of the isolated strains and to identify epidemiological links. The results show that hypervirulent genotypes of *Listeria monocytogenes* are frequently detectable in raw materials from poultry, pork, beef, and fish. These strains can persist in the processing environment and cause sporadic contamination. Repeated introduction via raw

materials and the formation of biofilms contribute to long-term colonization of the processing environment. The persistence and sporadic contamination by hypervirulent *Listeria monocytogenes* genotypes pose a significant challenge to food safety. Repeated introduction via raw materials and the adaptability of the bacteria make control difficult. Prevention strategies must therefore focus on strict control of raw materials and regular monitoring of the processing environment.

#### **Prevention strategies:**

1. **Raw material control:** Regular testing of raw materials for the presence of hypervirulent *Listeria monocytogenes* genotypes.
2. **Hygiene measures:** Strict separation of raw and processed products and regular cleaning and disinfection of processing equipment.
3. **Environmental monitoring:** Annual environmental testing for *Listeria monocytogenes* in cleaned and disinfected areas.
4. **Staff training:** Raising employee awareness of the importance of hygiene and the dangers posed by *Listeria monocytogenes*.
5. **Use of biocides:** Use of suitable disinfectants that are effective against biofilms.

**Conclusion:** The results underscore the need for targeted prevention strategies to prevent persistence and sporadic contamination by hypervirulent *Listeria monocytogenes* genotypes. Close cooperation between food businesses, research institutions, and regulatory authorities is crucial to ensure food safety.

### SL-SG-QMFM-02

#### **Antimicrobial Photodynamic Inactivation of *Pseudomonas aeruginosa* Biofilm and Persister Cells**

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*Pseudomonas aeruginosa* is a difficult-to-treat human pathogen and remains a priority for which new antimicrobials are needed. Many photosensitizers are tested against planktonic cells, which do not accurately reflect the clinical challenge posed by biofilms. This study investigates the efficacy of the photosensitizer, chlorophyllin, exposed to red light alone and in combination with the cationic permeabilizer polyethylenimine or the activator of mechanosensitive channels butylparaben against *P. aeruginosa* persister cells and biofilms. The broth microdilution checkerboard and colony biofilm methods were used to determine the bactericidal concentrations. Serine hydroxamate was used to induce persister cells via amino acid starvation. Results showed that chlorophyllin effectively eradicated *P. aeruginosa* biofilm cells. The treatment resulted in a 2.5-log<sub>10</sub> reduction at 250 µg/ml, an 8-log<sub>10</sub> reduction at 500 µg/ml, and complete eradication (10-log<sub>10</sub> reduction) at 1000 and 2000 µg/ml. Persister cells exhibited more sensitivity to chlorophyllin/polyethylenimine combinations compared to growing cells, whereas they were less sensitive to chlorophyllin/butylparaben combinations. These findings underscore the antimicrobial effect of chlorophyllin against *P.*

*aeruginosa* biofilms, with potential application against biofilm-related infections.

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### SL-SG-QMFM-03

#### Rapid and specific quantification of viable probiotics and their spores with Flow VIT® enables robust quality control of multi-strain products

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### Introduction

The high interest in beneficial effects of certain bacteria on the human microbiome, if administered alive and in sufficient quantities, make accurate enumeration in probiotic products crucial. Plate count, the current gold standard, is often unreliable and limited. PCR-based technologies do not provide information about bacterial activity. Additionally, bacterial spores cannot be distinguished in morphological status by plate count or PCR. Here, we describe Flow VIT® for species-specific quantification of living bacteria, total cell numbers and differentiation of vegetative cells and spores.

### Methods

Flow VIT® combines fluorescence *in situ* hybridization (FISH) with flow cytometry. Specific rRNA-targeted probes with distinct fluorescent dyes enable simultaneous detection and quantification. A non-specific dye estimates total cell counts. Products with various species and morphological status were pretreated to render cells accessible to the probes while maintaining morphological integrity, then hybridized and counted. Results were validated against cultivation, PCR and microscopic FISH.

### Results

Flow VIT® enables species-specific detection and quantification while determining morphological status. Counts were higher and more reproducible than CFUs suggesting that cultivation underestimates true counts due to VBNC cells and aggregation, especially during sporulation. Plate counts assume each colony originates from a single cell. In contrast, Flow VIT® disperses spore clusters so each cell is counted as a separate event. Analysis time is reduced from days to hours with improved species separation. PCR bias results from assigning rRNA/gene copy numbers to total cell counts causing under- or overestimation particularly in sporulating species and does not report activity. Such bias does not arise with Flow VIT® leading to higher reproducibility.

### Summary

The Flow VIT® protocol avoids weaknesses of conventional methods and supports R&D and quality control of probiotic products including spore-formers. It minimizes bias from hard-to-cultivate or clustered bacteria and nucleic acid extraction/amplification by direct species-specific rRNA labeling and standardized flow cytometry. Flow VIT® resolves biological activity at the single cell level and distinguishes spores vs. vegetative cells, enabling multiplex detection of viable cells and a more realistic picture of living

bacterial populations than conventional methods in complex probiotic matrices.

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### SL-SG-QMFM-04

#### Changes in microbiome of pork minced meat under different MAP packaging and storage conditions

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The project "Zukunftslabor 2030" (Future Lab 2030) aims to develop models to predict the sensory and microbiological quality of minced pork meat by implementing digital twins based on a comprehensive description of meat quality through a variety of analytical methods.

Model productions of pork minced meat were provided by the project partner MRI in Kulmbach. The meat samples were packed under different modified atmospheres (O<sub>2</sub> or N<sub>2</sub>) and stored at various temperatures (2, 10 and 14°C) for up to 17 days. Additionally, the impact of interrupting the cold chain at 2°C for 6 or 12 hours on meat quality was investigated.

The changes in microbiome composition over storage time were analyzed by amplicon sequencing of the V3-V4 region of the 16S rRNA gene. In all analyzed storage variations, the initial diverse meat microbiome developed to a population dominated by a few genera. Higher storage temperatures (10 and 14°C) promote the growth of Gram-negative bacteria such as *Acinetobacter* or enteric bacteria, while during storage at 2°C different lactic acid bacteria or *Brochothrix* become dominant. Different MAP conditions as well as the cold chain interruption do affect the microbiome, but not as pronounced as differences originating from different production batches.

Although storage conditions largely influence which group of bacteria can become dominant, this study highlights the significant impact of the initial microbial load at the beginning of storage which is a key factor for shelf life.

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### SL-SG-QMFM-05

#### Microbial specialists shape ready-to-heat and ready-to-eat convenience products

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Cross-contamination poses a significant challenge in producing multi-component convenience products, which have seen a significant market increase. There is limited research on the microbiological issues in facilities processing diverse raw materials, which are often processed simultaneously, heightening the risk of cross-contamination. A comprehensive understanding of their composition is essential to ensure product safety. This study aims to analyse product samples from various categories of combination convenience products to mitigate microbiological risks and enhance the identification of indicator organisms for better process control.

Bacterial counts, including aerobic mesophilic bacterial count (AMC), *Enterobacteriaceae* (EB), coliforms, lactic acid bacteria (LAB), and presumptive *Bacillus cereus* (pBC), were

determined using ISO reference methods (ISO 4833-2:2013/Cor 1:2014, ISO 21528-1:2017, ISO 7932:2004, ISO 15214:1998). Species identification was performed using 16S rRNA PCR sequencing and MALDI-ToF biotyping. RAPD-PCR was employed to identify recurrent isolable microorganisms.

The microbial diversity of the samples was substantial, with *Paenibacillus tundrae*, *Peribacillus frigotolerans*, and *Leuconostoc mesenteroides* being the dominant species. PA samples, derived from packaging materials and fillers, frequently contained *Pseudomonas weihenstephanensis* and *Pantoea agglomerans*. The microbial composition of the products was strongly shaped by the raw material components, and the products generally contained a high microbial diversity. Notably, some PA samples (filler and packaging materials) harbored a significant bacterial reservoir. Heat treatment and pasteurization did not fully inactivate the microbial flora in the products. During their shelf life, 60.9% of samples exceeded the AMC limit of 6 log<sub>10</sub> CFU/g. The pH of the samples ranged from 4.4 to 6.1, depending on the product group.

Multi-component products exhibit considerable microbial diversity, closely linked to their raw materials. Therefore, it is crucial to prevent cross-contamination and monitor raw materials rigorously. *Paenibacillus*, *Peribacillus*, and lactic acid bacteria have been identified as key organisms requiring monitoring. Additionally, regular monitoring of product-associated samples is recommended, as contamination has been detected in these areas.

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#### SL-SG-QMFM-06

##### **Plasma-processed agents: possibilities and challenges for applying innovative antimicrobial agents like plasma-processed air (PPA) and plasma-treated water (PTW) in food safety contexts**

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The need for innovative technologies and agents in food processing to enhance food safety reasons is high. New microbiological challenges arising from climate change affecting field-based raw materials and water use during processing increase this need. Combined with energy costs of conventional treatments and growing demand for eco-friendly, reduced chemical treatments, new solutions have better prospects for industrial transfer.

The presented research will focus on the manifold application possibilities of non-thermal plasma based antimicrobial agents from the field to the consumer, including plant- and animal-based produce.

Advantages and challenges of these applications from different perspectives, such as food safety, food quality, legal issues and economics, will be discussed.

The outlook will cover next steps, industry requests and directions for future research.

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#### SL-SG-QMFM-07

#### **Water for Cooling and Water Activity Measurements in the Pharmaceutical Production**

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After a severe water damage to a pharmaceutical sterile filling line, it was necessary to repair the damage as quickly as possible and prevent long-term damage (e.g., mold).

A particular problem was that the Pharmaterazzo floor was also wet: It was necessary to determine very quickly how large the affected area was and how to dry the floor. The presentation highlights how microbiologists can help with accident remediation.

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#### SL-SG-QMFM-08

##### **Old Beer, Modern Methods: Analysis of Cellared Berliner Weisse Beer and its Microbiome**

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Berliner Weisse is a sour wheat ale fermented using a mixture of yeasts and lactic acid bacteria. It has been brewed in northern Germany since the 16th century. The brewing process involves a complex fermentation, with a succession of fermentative microorganisms. In the first phase, *Saccharomyces cerevisiae* and lactic acid bacteria dominate. This is followed by a late fermentation phase involving *Brettanomyces bruxellensis*. The complexity of the fermentation is believed to contribute to the typical complex flavor profile of the beer. The traditional processes used to produce Berliner Weisse predate the advent of advanced microbiological or molecular tools to accurately determine the composition of the microbial cultures involved. Here, modern methods were applied to both new and cellared beers from several different breweries to determine the compositions of their microbiomes. Metabarcoding of the 16S and 18S rRNA gene was used to characterize the microbial community. We further aimed to amplify the genomes of the main organisms in the old and new beers to investigate which traits are responsible for the traditional sensory qualities of the beer. Comprehensive beer analysis was performed; this included identification of aroma compounds using GC-MS, quantification of organic acids via HPLC, and measurement of attenuation to provide insight into the behavior and interplay of the microorganisms. It was expected that different breweries would possess distinct strains of the yeast genera *Saccharomyces* and *Brettanomyces* as well as lactic acid bacteria genera *Pediococcus* and *Lactobacillus*. These findings are expected to inform brewers on the traditional characteristics and brewing process of the style and show how modern bacteria and yeast strains differ from their earlier counterparts. Such information may help to reproduce traditional starter cultures as well as authentic Berliner Weisse beers.

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## **Microbial Cell Biology**

#### SL-SG-AR/PCB-01

##### **Lysis-independent bacterial killing by cell wall-targeting antibiotics**

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Antibiotics targeting bacterial cell wall synthesis are generally assumed to induce cell lysis as their core antibacterial mechanism. This process is catalysed by cell wall autolysins that degrade peptidoglycan in an uncontrolled manner upon inhibition of cell wall synthesis. The resulting degradation weakens the cell wall sacculus, ultimately leading to cell lysis. However, lysis alone cannot explain the rapid killing observed in Gram-positive bacteria, which often die much faster than they lyse. To investigate the mechanisms behind this unexpectedly rapid killing, we analysed the mode of action of cell wall antibiotics using microscopic, single-cell techniques. Our experiments with *Bacillus subtilis* and *Staphylococcus aureus* revealed that inhibition of cell wall synthesis triggers depolarisation of the cytoplasmic membrane, which precedes and is independent of the lysis process. Using various fluorescence reporters and cellular assays, we found that depolarisation induced by cell wall antibiotics leads to energy starvation, extensive disturbances in cellular organisation, and production of reactive oxygen species (ROS) that leads to protein and DNA damage. Thus, rather than being a simple lytic process, the bactericidal activity of cell wall-targeting antibiotics is a complex cellular phenomenon that integrates autolysis with severe disturbances and damage triggered by membrane depolarisation.

#### SL-SG-AR/PCB-02

##### The T3SS\* in *Myxococcus xanthus* combines two chaperones with unique substrates to lyse bacterial prey cells

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In soil environments, microorganisms constantly compete for limited nutrients. To secure their resources, selected bacterial species use the strategy of predation. A key model to study bacterial predation is *M. xanthus*, a Gram-negative epibiotic predator with a broad prey spectrum. To feed on other bacteria, *M. xanthus* cells engage in one-on-one cell contact and use a distinct predation machinery: A Tad-like "Kil" system mediates prey killing and an atypical type 3 secretion system ("T3SS\*") is responsible for prey lysis [1]. Compared to typical T3SS, the T3SS\* in *M. xanthus* lacks a needle and outer membrane components, yet it is functional.

Using fluorescence microscopy, we observed that Kil and T3SS\* components colocalize at the predator-prey interface. Nevertheless, it is still unknown how exactly these two systems work together to induce prey cell death. Aiming to understand the secretion mechanism of the incomplete T3SS\*, we investigate the role of class II chaperones. Phenotypic analysis showed that two out of four class II chaperones within the T3SS\* gene cluster are required for prey lysis. Typical class II chaperones bind translocator proteins that build a translocon pore in the target cell membrane. However, homologs to known T3SS translocators are not encoded in the *M. xanthus* genome. We are currently looking into candidate substrates for each chaperone by screening uncharacterized T3SS\* proteins that are essential for prey lysis. Furthermore, we analyze a potential dependency of the Kil system and the newly identified T3SS\* components by using fluorescence microscopy and phenotyping deletion mutants in predator-prey co-cultures.

#### Reference:

1. Thiery, S., Turowski, P., et al., (2022). The predatory soil bacterium *Myxococcus xanthus* combines a Tad- and an atypical type 3-like protein secretion system to kill bacterial cells. *Cell Reports*, 40(11).

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#### SL-SG-AR/PCB-03

##### Structural and Functional Insights into Exopolysaccharide Production in *Sulfolobus acidocaldarius* Biofilms

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Biofilms are microbial communities encased in a matrix of self-produced extracellular polymeric substances (EPS) and represent a key lifestyle and survival strategy of Archaea. Yet, compared to bacterial biofilms, the molecular mechanisms, composition, and dynamics of archaeal biofilms and EPS matrices remain relatively underexplored. *Sulfolobus acidocaldarius*, a thermoacidophilic archaeon of the phylum Thermoproteota (formerly Crenarchaeota) [1], is a genetically tractable model organism suitable for investigating archaeal biofilm formation and EPS architecture.

Previously, we observed that exposure to organic solvents, such as 1-butanol, enhanced *S. acidocaldarius* biofilm formation and altered its EPS composition [2]. 1-Butanol-exposed biofilms exhibited a denser, tower-like architecture, increased amounts of extracellular carbohydrates and proteins, and a greater solvent tolerance than planktonic cells [2]. Transcriptome analysis revealed, among others, a reduced expression of motility and cell envelope genes, and indicated enhanced vesicle formation in response to 1-butanol, highlighting an adaptive stress response.

Exopolysaccharides (PS) are a major component of the EPS matrix alongside proteins and extracellular DNA, suggesting an important role in *Sulfolobus* biofilms. Thus, ongoing studies focus on PS composition, size, and biosynthesis. As detailed PS characterization requires substantial biofilm biomass, a new cultivation method was established, growing biofilms on membranes floating on the surface of liquid medium [3]. EPS components were isolated [4] and quantified, and the monomeric composition of *S. acidocaldarius* PS was determined via acidic hydrolysis, chromatography, and mass spectrometry [3]. PS molecular weight was assessed by size-exclusion chromatography [3]. Consistent with our findings on the 1-butanol response, variations in available carbon sources also affected EPS composition.

Overall, our findings provide new insights into the adaptive strategies of *S. acidocaldarius* biofilms and establish a methodological framework for further analyses, including the biofilm life cycle, the structural elucidation of PS by NMR, and the investigation of its biosynthesis and adaptive degradation.

- [1] Brock et al. (1972). *Arch Mikrobiol* 84, 54-68.

- [2] Benninghoff et al. (2021). *Appl Environm Microbiol* 87:e02988-20.



[3] Kuschmierz et al. (2022). *Front Microbiol* 13:982745.

[4] Jachlewski et al. (2015). *Front Bioeng Biotechnol* 3, 123.

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## SL-SG-AR/PCB-04

### Molecular tinkering and functional conservation of the archaeal Replication Protein A

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Single-stranded DNA-binding proteins (SSBs) are essential in all lifeforms to stabilise and protect exposed single-stranded DNA (ssDNA) during replication and repair. In Bacteria, this role is performed by the homotetrameric SSB; in Eukaryotes and some Archaea, by the heterotrimeric Replication Protein A (RPA), a complex containing multiple oligonucleotide/oligosaccharide-binding folds with distinct DNA-binding properties. Despite its central role in DNA metabolism, the diversity and mechanisms of archaeal RPAs remain largely unexplored. Many archaeal lineages encode atypical monomeric and/or dimeric RPA variants, yet how these minimal architectures bind and protect ssDNA, and whether they preserve the cooperative filament-forming behaviour of canonical RPAs, remain unknown.

Our phylogenomic analyses reveal that heterodimeric RPAs originated before the last common ancestor of Methanosarcinia, followed by lineage-specific domain rearrangements and gene losses. Do these structural innovations compromise RPA function or reflect adaptive optimisation? We address these questions using the *Methanosarcina acetivorans* (Mac) RPAs, which exist as both monomeric (MacRPA1-like) and heterodimeric (MacRPA\_A & MacRPA\_B) forms. AlphaFold models predict that both heterodimers bind ssDNA directionally and assemble into extended nucleoprotein filaments, while the monomeric protein binds ssDNA but cannot form filaments. Both heterodimers were overexpressed recombinantly in *E.coli* and purified. Electrophoretic mobility shift assays confirm strong and cooperative ssDNA binding for both complexes. Biophysical analyses show that the heterodimers oligomerise in solution but respond differently to ssDNA: one dissociates into monomers upon binding, whereas the other remains stably oligomeric, suggesting functional specialisation within the same organism. Crystallisation trials of MacRPA\_A-ssDNA complexes have produced microcrystals and needles; optimisation toward diffraction-quality crystals is ongoing.

Our results demonstrate that archaeal heterodimeric RPAs retain essential ssDNA-binding and filament-assembly functions using a reduced structural toolkit. In contrast, the monomeric form may rely on auxiliary factors or alternative mechanisms for DNA protection. Future structural studies will elucidate how minimal MacRPAs organise and protect ssDNA at the molecular level, aiming to reveal how archaeal RPAs ensure genome stability through minimal yet adaptable architectures.

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## Environmental microbiology

### SL-SG-EM-01

### Seeds of change - Leveraging the seagrass microbiome for ecosystem restoration

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Seagrasses have an evolutionary history on land yet have adapted to a life in the ocean during a turbulent time on our planet. Their microbiomes are distinctly marine, yet also share features with those of terrestrial plants. Seagrass seeds are unique in the marine environment and it is currently unclear how microbiota associated to them influence germination and seedling survival. *Zostera marina* is a prominent habitat-forming seagrass in the Baltic Sea which has faced declines in the last half century due to anthropogenic pressures. Initial efforts to restore lost meadows have shown promising results, yet are time-consuming and rely on clonal propagation which limits genetic diversity of restored populations. We aimed to 1) understand the adaptation of the seed microbiome to environmental change, and 2) to improve seagrass meadow restoration perspectives in the Baltic Sea. We first investigated the composition and variability of the *Z. marina* seed microbiome along a natural salinity gradient. Then, we manipulated the seed and sediment microbiome by sterilization and bacterial inoculation in order to find conditions which maximize seed germination rates and seedling development. We discovered a natural seed microbiome which was remarkably stable across the salinity gradient, featuring putatively opportunistic taxa. Germination was highest in seeds with an intact, natural microbiome compared to surface-sterilized seeds. We identified three strains belonging to *Psychrobacter* and *Pseudomonas* which stimulated germination and may be promising candidates for inoculation of seeds in seagrass meadow restoration. Our findings suggest that seed microbiomes play a critical role in the decisive moment of germination and are thereby tightly interlinked with the past and future of its host species, which is still adapting to the marine environment and the shifting conditions of the Baltic Sea.

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### SL-SG-EM-02

#### Forming a new microbiome: Interactions between aquatic microorganisms and microplastics

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Microplastics have found a way into all corners of the world, accumulating in a broad range of habitats under various environmental conditions. In doing so, microplastics have become a habitat themselves for a diverse community of prokaryotic and eukaryotic microorganisms. When assessing the fate and ecological impact of microplastics, not solely the particles themselves, but also the colonizing microbiome, associated chemicals, and (changing) environmental conditions should be considered. In our studies, we explore how microplastics affect the surrounding microbial communities and how vice versa the colonizing microorganisms might influence the plastic particles. We investigate the microplastic microbiome from strain to community level using cultivation dependent and independent methods, applying high-throughput barcode, metagenome, and whole genome sequencing. Overall, colonization of plastics in the aquatic environment appears to be of an opportunistic nature. The community composition associated with this new human-made material is similar to the one associated with natural materials, with a strong

impact of spatial and seasonal factors. Potentially pathogenic species do colonize microplastics, especially in areas of high anthropogenic pollution, but in similar or even lower abundances than natural particles. Rising water temperatures and ongoing pollution are factors that might increase this transport of potential pathogens. Some microbial taxa, however, thrive particularly on aquatic plastics. Our data indicate that plastic degradation does not play a relevant role in these biofilm communities, but that the interaction with likewise associated pollutants, such as PAHs, or other growth advantages might boost the survival of certain taxa. One of these advantages is likely the formation of photoreactive pigments. These protect the microorganisms against UV stress or enable them to harvest the sun light, while attached to plastics floating on the water surface. Currently, we are screening the genomes of about 40 plastic colonizers from the Great Pacific Garbage Patch for their physiological potential and adaptation strategies. Among them we find several new taxa with interesting traits, that may support new strategies for tackling societal challenges involving pollution and climate change.

### SL-SG-EM-03

#### Electricity generation by anaerobic methanotrophic archaea

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Methane is a potent greenhouse gas that is produced by methanogenic archaea. The amount of emitted methane is controlled by a powerful biological filter where aerobic and anaerobic methane oxidizing microorganisms thrive. While aerobic methanotrophic bacteria are already relatively well understood, the physiology of anaerobic methanotrophic (ANME) archaea is still enigmatic, in part due to the inability to cultivate them axenically.

Here, we cultivated ANME archaea in bioelectrochemical systems with methane as electron donor and an anode as electron acceptor. We observed stable and reproducible methane-dependent electrical current generation at the anode poised at 0V vs. SHE. Up to 97% of the current was derived from methane oxidation. Metagenomic and metatranscriptomic analyses indicated the selective enrichment of ANME archaea from 9% in the inoculum to 95% at the bioanode within 120-days incubation; in addition, meta-omics data pointed towards a role of uncharacterized multi-heme c-type cytochromes in current generation. CryoEM imaging revealed the presence of conspicuous nanowire-like structures whose identity was elucidated using proteomics. These exciting insights elucidate previously unknown physiological properties as well as presenting the first near-axenic culture of ANME archaea key to the microbial methane filter.

### SL-SG-EM-04

#### Methane sink function of grassland soil microbiomes - negative effects of intensive management persist three years after land-use extensification

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Distinct methanotrophic groups maintain the atmospheric CH<sub>4</sub> sink of upland soils and act as a biological filter, oxidizing CH<sub>4</sub> produced by methanogens within the soil. In grasslands, intensive management reduces the CH<sub>4</sub> sink potential. While long-term recovery of methanotrophs is expected, short-term effects of reduced land-use intensity (LUI) in grasslands remain unclear. We investigated the impact of a three-year experimental LUI reduction (no fertilization, no grazing, one mowing event per year) across 45 grassland sites in three pedoclimatic regions in Germany, using 15 historically low-LUI sites as a baseline. After three years, LUI reduction had no significant effect on CH<sub>4</sub> oxidation and production potentials as well as on methanotroph gene abundance (*pmoA*). In contrast, historically low-LUI sites showed higher potential to oxidize atmospheric CH<sub>4</sub> and greater Upland Soil Cluster γ (USCγ) methanotroph abundance, indicating the potential for long-term recovery of the atmospheric CH<sub>4</sub> sink. Decreased methanogen gene abundance (*mcrA*), reduced soil bulk density, and increased soil water content after three years highlight possible mechanisms that can mediate future recovery. A constant *pmoA* to *mcrA* ratio suggests a soil methane filter function that is stable across diverse soils and LUIs.

### SL-SG-EM-05

#### Plant diversity modulates soil microbial functional potential linked to nutrient cycling and stress adaptation

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Drought strongly affects soil microbial communities, reducing metabolic activity and altering composition. As climate change increases drought frequency and duration, understanding microbial resilience becomes crucial. Plant diversity can buffer ecosystems against stress by enhancing functional redundancy and complementarity among microbes.

We investigated whether plant richness influences the abundance and diversity of genes linked to plant growth promotion and drought resistance in soil microbial communities. Bulk soil samples from The Jena Experiment spanning 1–16 plant species were collected from 5 cm depth. Metagenomic libraries were sequenced on an Illumina NextSeq 550 and analyzed with SqueezeMeta v1.7.2 in sequential mode including singletons. Functional and taxonomic annotations were retrieved for predicted open reading frames. Genes related to plant growth promotion and drought response were filtered, and linear mixed-effects models tested effects of sown diversity with plot properties as random intercepts. Multiple testing was corrected using the Benjamini–Hochberg procedure (FDR < 0.05).

Microbial functions showed significant associations with sown diversity. Functions related to antimicrobial biosynthesis and nutrient cycling increased with plant richness, while osmolyte uptake and production decreased.

These results indicate that plant diversity modulates microbial functional potential, fostering versatile soil microbiomes that enhance ecosystem stability under climate variability.

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#### SL-SG-EM-06

##### **Hitchhiking Bacteria: Diverging effects of different tyre wear particles on microbial biofilm composition**

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Tyre wear particles (TWP) represent an emerging form of microplastic pollution in freshwater systems, providing surfaces for microbial colonization while potentially altering community structure. We investigated how TWP size, wear condition, and vehicle type affect microbial biofilm composition. Over four weeks, TWP were incubated in the Rhine River alongside size-matched sediment controls, followed by 16S V4 rRNA amplicon sequencing. Our results reveal pronounced differences in microbial composition between TWP and sediment biofilms, with TWP exhibiting markedly reduced biodiversity. Particle size had a strong effect, as larger TWP further decreased diversity compared to smaller ones. Moreover, *Aquabacterium* and *Ketobacter* were strongly enriched on TWP, suggesting a selective advantage under these conditions. These findings highlight that TWP can substantially modify microbial biofilm communities, emphasizing their potential role in altering freshwater microbial ecosystems. Given the widespread release of TWP, understanding their ecological consequences is essential for assessing their impact on aquatic pollution and ecosystem functioning.

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#### SL-SG-EM-07

##### **Exploring the phyllosphere microbiome in agroecological living labs for climate-resilient agriculture**

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Conventional farming exacerbates climate change causing soil degradation and biodiversity loss. The "AllEcoSys" (Agroecology living labs fostering prosperous and sustainable agri-food systems) project addresses this by investigating agroecological approaches to build sustainable and resilient agricultural systems.

An important part of the project is the study of the critical role of the phyllosphere microbiome, the microbes living on the plant aerial surface, as which indicator of agricultural health and climate resilience. We aim to identify the microbial taxa that mediates plant performance such as nutrient uptake, growth and disease suppression. Since the phyllosphere is highly dynamic and responsive to both farming practices and climate, managing its microbiome is vital for plant health and performance in a changing world.

Through a network of "living labs" from six different European countries, we are studying how different diversification strategies affect microbial communities, soil quality as well as insects and crops. Our methods include amplicon sequencing of phyllosphere microbiome and quantitative PCR of genes related to nutrient cycles. The project anticipates demonstrating that agroecological practices foster beneficial phyllosphere microbes, providing a scientific basis for sustainable farming that enhances plant health, reduce chemical use and strengthen food system climate resilience.

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## Microbiome

#### SL-SG-MB-01

##### **Function-based selection of synthetic communities enables mechanistic microbiome studies**

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Understanding the complex interactions between microbes and their environment requires robust model systems such as synthetic communities (SynComs). We developed a functionally directed approach to generate SynComs by selecting strains that encode key functions identified in metagenomes. This approach enables the rapid construction of SynComs tailored to any ecosystem. To optimize community design, we implemented genome-scale metabolic models, providing in silico evidence for cooperative strain coexistence prior to experimental validation. Using this strategy, we designed multiple host-specific SynComs, including those for the rumen, mouse, and human microbiomes. By weighting functions differentially enriched in diseased versus healthy individuals, we constructed SynComs that capture complex host-microbe interactions. We designed an inflammatory bowel disease SynCom of 10 members that successfully induced colitis in gnotobiotic IL10<sup>-/-</sup> mice, demonstrating the potential of this method to model disease-associated microbiomes. Our study establishes a framework for designing functionally representative SynComs of any microbial ecosystem, facilitating mechanistic study.

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#### SL-SG-MB-02

##### **Network-Guided Discovery of Microbial Keystone Taxa for Simplified Microbial Consortia Assembly**

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## Introduction

Developing simplified microbial consortia holds significant potential for enhancing both environmental and human health. However, advancement in this area has been hindered by the fact that most microbes remain uncultured, either due to unknown specific isolation requirements or their dependence on minimal microbial consortia for successful

establishment. Applying ecological concepts to microbiome research, such as the keystone taxa concept, might help prioritize microorganisms for culturing and integration into representative simplified microbial consortia.

## Goal

To detect candidate microbial keystone taxa based on the integration of metabarcoding and metagenomics data.

## Materials & Methods

Soil samples were taken from Cambisol grasslands of the National Park Hainich, Thuringia, Germany, from 2014 to 2023 ([www.biodiversity-exploratories.de](http://www.biodiversity-exploratories.de)). We used metabarcoding and metagenomic data to map the functional potential of the identified candidates. Co-occurrence networks were inferred using *SpiecEasi* implemented in *NetCoMi*, and node influence was quantified using the Integrated Value of Influence (IVI) by *influential*. The most influential taxa were selected by ranking the IVI scores of the nodes.

## Results

The network analysis result is a list of 74 highly connected nodes that were consistently detected as HUBs across years. From these, the 15 taxa with the highest IVI were selected as high-priority HUBs, mapped to MAGs, and considered as *candidate microbial keystone taxa*. The IVI scores of these *candidates* illustrate how the HUB connections can vary in time while maintaining a central position in the network.

We mapped the 16S rRNA gene sequences of these prioritized candidates and recovered most of them as metagenome-assembled genomes (MAGs) from the same soil samples. Among the best-quality MAGs recovered were an Actinomycetota (MB-A2-108) bacterium, a Verrucomicrobiota (*Candidatus Udaeobacter*), and a Nitrososphaeria (Thermoproteota) archaeon. The Actinomycetota MAG contained carbon-degradation genes (*aceA*, *aceB*, *acs*) that enable the degradation of complex organic compounds and their efficient assimilation through the glyoxylate cycle. The Verrucomicrobiota MAG contained nitrogen- and phosphorus-recycling genes (*ureA*, *ureB*, *ureC*, *pho*-like). The Nitrososphaeria MAG contained nitrification genes (*amoA*, *amoB*, *hao*, *nxrA*) supporting chemolithoautotrophic ammonia oxidation.

These profiles suggest, respectively, a filamentous saprotroph driving carbon turnover, an oligotrophic nutrient recycler maintaining N–P balance, and an ammonia-oxidizing archaeon (AOA) sustaining nitrate supplies. Together, the taxonomic and functional information found in the MAGs support their key role in sustaining microbial network stability and productivity in grasslands.

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Microbial communities perform myriad functions both in natural environments and in engineered systems and medical applications, where communities are designed for a given purpose. Many of these functions are based on the metabolic activity of member species and their interactions. However, bacterial metabolism and the resulting crossfeeding relationships are often inferred from species' characteristics in pure culture or pairwise cocultures. Although these approaches allow detailed insights into potential community function, they neglect the complexities of microbial communities, such as higher-order relationships, competition outcomes and the regulatory influence of environmental factors and other community members. It is becoming clear that community function is highly context-dependent and cannot be deduced simply from the species composition. Here, we employ a combined experimental and simulation approach to shed light on these complex functional changes. We study a mouse gut community along a gradient of complexity - from monocultures to in vitro communities to the mouse gut, to probe how bacteria adapt metabolically to their biotic and abiotic environments.

We used substantially metaproteomic data to analyze realized bacterial niches. We observed that bacteria adapted their carbon source profile and biosynthetic activities when grown in communities versus isolation. Furthermore, bacteria growing in communities dedicated a relatively higher proportion of cellular protein to carbon source acquisition and energy production. Communities themselves also adapted to their growth environment. Strikingly, host-associated communities compared to communities grown in vitro clearly shifted their proteome allocation to carbon source acquisition at the expense of growth and utilized a broader spectrum of carbon sources. We integrated metaproteomic data into metabolic models to further analyse metabolic exchanges between community members. We could observe changing connectivity between bacterial species in different environments. The higher investment into resource acquisition observed in host-associated communities resulted in a large increase in predicted interactions between members. Our findings demonstrate the remarkable adaptability of microbes and microbial communities across environmental contexts. This underscores the critical need to study microbiomes under near-natural conditions and incorporate context-specific data into functional analyses.

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## SL-SG-MB-03

### Context-Dependent Metabolic Adaptation in Microbial Communities: From Monocultures to Complex Ecological Interactions

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## SL-SG-MB-04

### Synthetic Reconstruction of a Short-Lived Vertebrate Gut Microbiome to Study Aging

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The turquoise killifish (*Nothobranchius furzeri*) has emerged as a model for aging research due to its exceptionally short lifespan. Previous studies have shown that the gut microbiome undergoes marked compositional changes during aging and plays a key role in modulating host longevity. Notably, heterochronic microbiota transplantation, from young to old killifish, extends lifespan providing the first evidence that the microbiome can directly influence vertebrate aging. However, the complexity and variability of natural microbiomes make it challenging to disentangle microbial interactions and their effects on host physiology. To address this, synthetic microbial communities offer a tractable and controlled system. This work aims to construct a defined synthetic microbial community that mimics the gut microbiota of young killifish using a bottom-up approach. Culturomics was employed to isolate bacterial strains from the killifish gut, leading to the establishment of a five-member community composed of *Vibrio metschnikovii*, *Aeromonas veronii* (dominant taxa in captivity and the wild, respectively), *Brevibacterium pigmentatum* and *Leucobacter* sp. (*Actinomycetota*, typically enriched in young fish), and *Pseudomonas spirodelae* (*Pseudomonadota*, persistent across host lifespan). When cultivated under artificial conditions, *A. veronii* and *V. metschnikovii* tended to dominate the community. However, under increased oxygenation, *B. pigmentatum* became the prevalent member, indicating that community dynamics strongly depend on environmental conditions. Interaction modeling revealed predominantly negative interactions within the community, with *P. spirodelae* and *B. pigmentatum* displaying the highest connectivity, whereas *V. metschnikovii* showed the fewest interactions. *A. veronii*, mostly interacted negatively to *P. spirodelae* and *B. pigmentatum*. Last, *Leucobacter* sp. showed insufficient growth to reliably assess its ecological role in this community. Our results demonstrate that community structure and dominance patterns are highly sensitive to culture conditions, yet the overall trends mirror in vivo observations, where *V. metschnikovii* and *A. veronii* are key players. Future work will focus on expanding community complexity and optimizing culture parameters to better replicate the intestinal environment of the host, ultimately providing a controlled platform to dissect microbiome-host interactions in aging.

## SL-SG-MB-05

### Aging-Associated Microbiota Dysbiosis Impairs Gut–Lung Axis Immunity Against Influenza A Virus

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## Introduction

Aging is accompanied by marked alterations in the intestinal microbiota, including reduced microbial diversity, impaired metabolic capacity, and chronic low-grade inflammation. Microbial metabolites, particularly short-chain fatty acids (SCFAs), have emerged as key modulators of inflammation and antiviral defense along the gut–lung axis. These

changes increase vulnerability to respiratory viral infections such as influenza A virus (IAV).

## Goals

This study investigated how aging-associated microbial dysbiosis influences susceptibility to IAV infection. We further explored whether SCFA supplementation can restore antiviral responses in the context of cellular senescence.

## Materials & Methods

Using a murine model, we analyzed microbiota composition and metabolic function in young and aged mice in the uninfected state and following IAV infection. Shotgun metagenomics were performed to assess microbial compositions, but also the microbial transcriptional response. Murine lungs were analyzed for transcriptome (RNAseq), cytokine secretion (flow cytometry) and virus replication (plaque assay). *In vitro* studies were conducted with senescent primary human lung fibroblasts to evaluate the effects of the SCFA acetate during IAV infection on virus replication, cytokine secretion and host transcriptome response.

## Results

Aging in mice was associated with pronounced microbial shifts, including a reduced abundance of commensals such as *Akkermansia muciniphila* and *Faecalibaculum rodentium*, together with reduced expression of carbohydrate-fermenting enzymes, and impaired SCFA synthesis. IAV infection further exacerbated these microbial and metabolic alterations in aged animals. *In vitro*, senescent lung fibroblasts showed increased susceptibility to IAV infection compared to non-senescent cells. Acetate supplementation significantly reduced viral replication and inflammatory cytokine expression. Mechanistically, these effects were mediated through activation of GPR41/43 receptors and enhanced gene expression via histone acetylation.

## Summary

Our findings demonstrate that aging-associated microbial dysbiosis diminishes SCFA production, weakening antiviral defense mechanisms along the gut–lung axis. Acetate supplementation restores antiviral responses in senescent cells through receptor-mediated and epigenetic pathways. These results underscore the importance of microbiota-derived metabolites for immune resilience during aging.

## SL-SG-MB-06

### Minimally Invasive Surfacial Biosensors to Diagnose Minor Hydrocarbon Seepage

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The ongoing European Union's quest for energetic independence is inherently dependent on bolstering short- to medium-term exploration of its own energetic resources. The Barents Sea holds a predicted three times more the 1500 million Sm<sup>3</sup> oil equivalents it was estimated to in 1993 (Larsen, Fjæran and Skarpnes 1993), which transformed it in a hub for hydrocarbon (HC) exploration in the last decades. However, standard practices for the prospection of subsurface HC are reliant on seismological and drilling approaches that are harmful to marine macrofauna ecology, highlighting the need for low-impact techniques to diagnose HC presence in the marine subsurface. Here, we illuminate the potential of surficial gravity core sampling and metagenomics in diagnosing subsurface hydrocarbon presence. To do this, we generated 48 short- and long-read metagenomes taken across HC-affected and -non-affected sites across the Barents Sea. This hybrid metagenomics sequencing approach sustained the recovery of highly contiguous assemblies. The presence, taxonomy and functionality of metagenome-assembled genomes (MAGs) across site types were leveraged to infer on their potential in regional biogeochemical cycling. Several metagenomics data levels were clustered at increasing similarity (70-100%) to assess their diagnostic capability, namely proteins and rps3 genes, MAGs and predicted viruses. The predictive power of these across HC-affected and -non-affected sites was then assessed via statistical testing of their mean metagenomic coverages. Diagnostic potential for 3,960 rps3 genes at 99% similarity and 1,058 and 2,046 95% and 99% average nucleotide identity (ANI) MAGs respectively, was assessed as being non-significant. However, a total of ten uncharacterized candidate proteins out of 18,451,924 95% identity clusters and one 100% complete 6.9 kbp virus out of 62,352 (100% ANI) were significantly more abundant in HC-affected sites when compared to HC-non-affected sites. A consensus protein annotation approach deploying Hidden Markov Models (HMMs), BLASTp and predicted protein structure-based function inference uncovered potential functions of seven of these, mostly as transporter and DNA repair and recombination proteins. This work paves the way towards the use of as-yet understudied specific proteins and viruses as biosensors for HC presence in the marine subsurface, potentially circumventing the need for damaging HC prospection techniques.

#### SL-SG-MB-07

##### **Microbial allies: Algal–prokaryotic communities from a natural terrestrial oil seep facilitate oil degradation in freshwater**

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Crude oil can naturally reach the Earth's surface through geological processes, releasing hydrocarbons into marine, freshwater, and terrestrial habitats via fractures in the subsurface. From a microbiological perspective, natural oil seeps are far less studied than anthropogenic oil spills, with most research focusing on marine systems. Oil is generally considered toxic, a few hundred prokaryotes and less algae are known to use oil as a carbon source and degrading it.

We suggest that algal–prokaryotic communities living in and around natural oil seeps are adapted to tolerate and degrade anthropogenic oil pollution in freshwater. The algae likely support oil-degrading prokaryotes within their phykosphere. The association allows both partners to persist under toxic conditions while algae are benefiting from CO<sub>2</sub> released during oil breakdown and oil biodegradation is benefiting from algal produced O<sub>2</sub>. We isolated algal–prokaryotic communities from a natural terrestrial oil seep in Germany and set up three different microcosm conditions, containing a specific algal–prokaryotic consortium of the algae *Euglena* sp., *Euglena* alone and the prokaryotes alone, all treated with and without light oil to test who and how fast they are degrading the oil. Oil biodegradation was assessed by monitoring visual and microscopic changes, pH, cell numbers (DAPI/flow cytometry), determining microbial community composition via 16S rRNA gene sequencing, and measuring CO<sub>2</sub> evolution using reverse stable isotope labelling (RSIL) method. Our results showed that *Euglena* hosts typical known oil degrading prokaryotes in its phykosphere and we observed obvious signs of oil degradation by-eye visual "oil snow" formation and by decrease in pH. *Euglena* was performing most of the oil degradation (15.9 μM CO<sub>2</sub> day<sup>-1</sup>) rather than the prokaryotes (2.8 μM CO<sub>2</sub> day<sup>-1</sup>). *Euglena*-prokaryotic communities degraded oil faster at the beginning of the experiment but finally ended up with a comparable CO<sub>2</sub>-production rate of 15.4 μM day<sup>-1</sup>, like *Euglena* alone.

We demonstrate that understudied natural oil seeps provide a diverse habitat with a large potential of welladapted oil-degrading consortia that could accelerate the degradation of anthropogenic oil spills and contribute to carbon cycling. Algal-prokaryotic interactions appear to be important in creating more stable conditions for both to thrive in toxic oil environments.

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#### SL-SG-MB-08

##### **A division of labor controls the degradation of fucoidans in the ocean**

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Fucoidans—complex polysaccharides produced by brown algae and diatoms—contribute to long-term carbon sequestration due to their resistance to microbial degradation. While individual microbes can break down portions of these polymers, it remains unclear whether complete breakdown is possible in nature and, if so, by what mechanisms. Here we show that fucoidans are degraded through synergistic interactions between specialized bacteria with conserved metabolic functions. Using metabolomic analysis of a reconstructed marine consortium, we uncovered functional guilds of bacteria that target either the sulfated fucose backbone or the side-branches of rare monomers. This division of labor leads to an unexpectedly high number of positive interactions between different degraders that enhanced degradation efficiency up to 97.1%. Despite variation in fucoidan structure across different types of algae, the metabolic functions of degraders remained conserved, enabling quantitative prediction of degradation outcomes based on community composition. Our findings suggest that the environmental turnover of complex macromolecules depends not only on individual metabolic capabilities but also on ecological interactions shaped by substrate architecture. This work provides a mechanistic framework for understanding carbon cycling in the ocean and

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## Fungal biology and biotechnology

### SL-SG-FBB-01

#### Intracellular networking: coupling of mitochondrial energy metabolism with microtubule-dependent mRNA logistics

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Eukaryotic complexity relies on precise intracellular networking, particularly the bidirectional communication between the nucleus and endosymbiont-derived organelles. In highly polarized cells, this presents a unique logistical challenge, requiring specialized mechanisms to couple nuclear gene expression with local metabolic demands. Using the polar-growing fungus *Ustilago maydis* as a model, we discovered a critical link between endosomal long-distance mRNA transport and mitochondrial energy metabolism.

We found that the key RNA-binding protein Rrm4 binds numerous nuclear-encoded mitochondrial mRNAs; consequently, loss of Rrm4 impairs the formation of mitochondrial respiratory chain complexes. By coupling genetic engineering with live-cell imaging of the photoconvertible protein tdEosFP, we demonstrated that mitochondrial localization of the newly synthesized ATP synthase subunit  $\gamma$  (Atp3) is strictly dependent on this long-distance transport. Furthermore, genome-wide transcript analysis revealed that hyphae with defective transport undergo a significant metabolic shift, suggesting a compensatory response to impaired mitochondrial function. To quantify these dynamics spatiotemporally, we are currently implementing genetically encoded ratiometric Matryoshka biosensors for ATP and NADH alongside sophisticated metabolomics.

Overall, we show that in polar cells, such as fungal hyphae or neurons, mRNA logistics post-transcriptionally regulate mitochondrial energy metabolism. These findings reveal a novel layer of host-organelle networking and provide key insights into the maintenance of intracellular microbial associations.

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### SL-SG-FBB-02

#### Two gene clusters are required for Mannosylerythritol lipid biosynthesis in *Sporisorium reilianum*

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Almost all microorganisms produce secondary metabolites to ensure adaptation, survival or nutrition in their corresponding microbiome. Mannosylerythritol lipids (MELs) produced by basidiomycetous fungi are such secondary metabolites and belong to the class of glycolipids. MELs are antibacterial surface-active molecules and involved in plant penetration, cellular movement and biocontrol. Enzymes for MEL biosynthesis are usually encoded in a gene cluster

composed of five genes, which are both necessary and sufficient for the biosynthesis of MELs in many basidiomycetous fungi.

Here, we report that in two closely related biotrophic pathogens – *Sporisorium reilianum* f. sp. *reilianum* (SrS) and *Sporisorium reilianum* SRZ2 – MEL production is achieved through a novel combination of two gene clusters. While the larger cluster is required for production of the disaccharide moiety, for attachment of one acyl chain to the sugar backbone and for export of MELs, the smaller cluster encodes two enzymes for additional acylation of the sugar backbone and is also essential to produce mature MELs. The genes in the smaller cluster appear to be duplications of two of the original genes in the larger cluster. The latter are not expressed in SrS but in related fungi e.g. *Ustilago maydis*. MELs made by SrS contain a distinct acylation pattern and thus exhibit altered physical and chemical properties making them interesting candidates for biotechnological applications.

In summary, our study showcases how duplication and diversification of MEL clusters can lead the evolution of distinct biosurfactants.

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### SL-SG-FBB-03

#### Establishing a basidiomycete chassis for the production of mushroom-derived sesquiterpenoids

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Mushroom-forming basidiomycetes are known as a tremendous source of secondary metabolites like sesquiterpenoids, but their slow growth and low isolation yields limit the access to those valuable compounds. Moreover, chemical synthesis as well as functional expression of the underlying biosynthetic pathways in traditional heterologous expression platforms can be challenging. This is in line with our observation, that multiple interesting sesquiterpenoids are not yet commercially available or only to small amounts for high prices. *Ustilago maydis*, a well-established fungal model and a basidiomycete itself, is a promising alternative production host potentially offering metabolic compatibility and tolerance to metabolites that are toxic to other, farther related microorganisms. Although several ascomycetes are available as fungal production hosts, a basidiomycete chassis is still lacking. We are currently addressing this gap by establishing *U. maydis* as a new production host.

At first, we studied the potential for production of heterologous sesquiterpenes by expanding the repertoire from the first proof-of-principle product  $\alpha$ -cuprenene to other valuable sesquiterpenes like nerolidol or  $\Delta$ -cadinol by expressing the respective fungal sesquiterpene synthases (STSs). In this framework, we successfully tested different previously reported STSs to confirm their functional expression in our chassis and to determine the most effective synthase for heterologous production of the respective sesquiterpene. Now, our focus lies on further enhancing the sesquiterpene yield through metabolic engineering. To facilitate these tremendous genetic modifications, we implemented a modular cloning system for *U. maydis*, that e.g. permits the parallel integration of up to four transcriptional units. Moreover, we expanded our

genetic toolbox with a broad selection of mainly strong, native promoters and for instance already applied one of them to increase the  $\alpha$ -cuprenene yield approximately tenfold.

On the one hand, the new genetic tools currently enable the efficient pursuit of various metabolic engineering strategies by e.g. overexpressing different targets individually as well as in varying combinations. On the other hand, they provide the foundation for the transplantation of entire biosynthesis pathways to enable the heterologous production of more complex and highly valuable fungal sesquiterpenoids in *U. maydis* in the future.

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#### SL-SG-FBB-04

##### **Resolving signal crosstalk: How GPCRs orchestrate plant colonization by the smut fungus *Ustilago maydis***

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Fungal pathogens are an increasing concern for agriculture, ecosystems, and human health, yet molecular mechanisms enabling infection establishment are not well understood. To cause infections, fungi must sense host environments to adapt their physiological responses accordingly. G-protein-coupled receptors (GPCRs) are the largest class of membrane receptors in eukaryotes and play key roles in cellular adaptation to changes in the surroundings. GPCRs are well-understood in humans and are major pharmaceutical targets, whereas fungal GPCRs remain largely unexplored. Understanding their functions and downstream signaling is key to understanding how fungi perceive their environment and cause disease.

Using a structure-based bioinformatic pipeline, we identified 34 GPCRs in the smut fungus *Ustilago maydis*. Based on this analysis, we characterized a new peptide-GPCR system: the Gpe1 receptor and its peptide ligand Pit2. Gpe1 is expressed only during pathogenic development, particularly at early infection stages. Its activation depends on a specific peptide fragment derived from Pit2, a secreted effector encoded by a neighboring gene, processed by host-specific proteases. Functionally, Gpe1/Pit2 signaling releases the cell cycle arrest required for mating and promotes proliferation inside the host, representing a key switch from filamentous growth to proliferation. Interestingly, Gpe1 displays key features known from Ste3-type pheromone receptors, sharing an overall structural architecture and a peptide ligand. Furthermore, evidence suggests that both Pra1 and Pra2 (*U. maydis* pheromone receptors) and Gpe1 interact with the  $\alpha$ -subunit Gpa1 and might converge on similar MAPK signaling pathways. Plant infection in *U. maydis* is therefore orchestrated by the peptide GPCRs Gpe1 and Pra1/2, with Pra1/2 potentially playing an additional role for host colonization besides mating.

Together, Pra1/2 and Gpe1 and potentially another Gpa1-interacting GPCR Glp16 orchestrate a sequential program: establishing cell cycle arrest for mating and filamentous growth, then driving rapid proliferation *in planta*. We aim to identify a general mechanism by which different GPCRs can transduce signals through a shared downstream cascade. These findings will lay the groundwork for a detailed understanding of GPCR-ligand interactions in fungi and improve insight into signal perception in pathogenic basidiomycetes.

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#### SL-SG-FBB-05

##### **Tools to study the fungal side of ectomycorrhizal symbiosis**

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Mycorrhizal symbiosis between fungal microorganism and plants is an important feature in many ecosystems. In boreal and temperate forests, ectomycorrhizal fungi, which remain outside of plants cells, are important as partners for many trees. These fungi are mostly ascomycetes or basidiomycetes that can be generalists or specialists. During symbiosis, the fungi transfer water and minerals to the plants, while the plants transfer carbohydrates to the fungi. Although many aspects of ectomycorrhizal symbiosis have been studied in detail, molecular genetic work and functional characterization of ectomycorrhizal fungal genes remains scarce.

Here, we aim to establish molecular models of ectomycorrhizal symbiosis. Both, the ascomycete *Cenococcum geophilum* and the basidiomycete *Paxillus involutus*, undergo symbiosis with the genetically tractable poplar *Populus x canescens* under laboratory conditions. We show results on the morphology and physiology of various fungal strains to define growth conditions suitable for downstream experiments. These experiments include mycorrhization assays of poplar plants as well as phosphorus-uptake experiments in changing physiological conditions. For genetic analyses we focus on developing a protocol for DNA transformation. We will show data regarding selection markers and strategies for generating suitable vector systems allowing the testing of different promoters for gene expression.

With a robust transformation system, in combination with genetically modified poplar trees, we will be able to unravel the molecular mechanisms underlying ectomycorrhizal symbiosis, which is of major importance for understanding temperate and boreal forest ecosystems.

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#### SL-SG-FBB-06

##### **Phylogenetic analysis of three antifungal peptide families from filamentous fungi discloses them as secondary metabolites and one superfamily**

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Since decades, fungi are leveraged in biotechnology to produce high-value compounds used in multiple economic sectors. Strain and process optimization is based on a comprehensive understanding of the production organism on the cellular and molecular level. Members of three antifungal protein families in fungi, i.e., Antifungal Protein (AFP), Bubble Protein (BP), and *Neosartorya fischeri* Antifungal Protein 2 (NFAP2), consisting of small cysteine-stabilised proteins, were shown to have not only antimicrobial activity, but are also associated with other functions in their hosts, e.g., carbon metabolism, autophagy, or asexual



development. Their impact on biotechnological production is not yet endeavoured.

We have identified a new antifungal bubble protein, AgBP, from *Aspergillus giganteus*, which exerts almost no antimicrobial activity and might serve a different biological purpose. We used NCBI PSI-BLAST to phylogenetically and structurally analyse members of the families and could identify further putative members from the non-redundant protein database: 165 AFPs, 102 BPs, and 219 NFAP2s. Half of them were for the first time assigned to the respective family on InterPro. All members were exclusively identified in fungi. Interestingly, the recently identified members are also present in Basidiomycota, whereas previous members have been found in Ascomycota only.

Using maximum-likelihood methods, resulting phylogenetic tree analyses demonstrate restricted taxonomic distribution within single genera. Furthermore, the comparison of the tertiary structures of all members of the three AFP families clearly separates them from each other and from non-fungal small cysteine-stabilised antifungal proteins.

We hypothesise that the three protein families represent a distinct superfamily of evolutionary related proteins which must be classified as secondary metabolites of ribosomal origin. For brevity, we named this superfamily fAFP (fungal antifungal proteins). Results indicate that the distribution of fAFPs is driven by horizontal gene transfer. Furthermore, we hypothesize that fAFP members probably serve rather different biological roles than merely acting as antimicrobials. Their classification as secondary metabolites in combination with their occurrence among several biotechnologically relevant fungal genera, e.g. *Aspergillus*, *Penicillium*, *Trichoderma*, *Schizophyllum*, etc., emphasizes their potential relevance for genetic and metabolic engineering.

## Symbiotic Interactions

### SL-SG-SI-01

#### Cell wall degrading enzymes facilitate cross-kingdom RNA delivery via fungal extracellular vesicles into plant cell

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Microbial pathogens deliver RNA and protein effectors into host cells to manipulate innate immunity. The fungal plant pathogen *Botrytis cinerea* sends small RNA inside extracellular vesicles (EVs) into plants to induce cross-kingdom RNA interference for promoting host infection. How pathogen EVs pass through the rigid plant cell wall, primarily composed of cellulose, hemicellulose and pectin, is currently not understood. Here, we identified a new role of cell wall degrading enzymes as a key in EV cargo uptake from pathogen into plant cells. Using a GFP reporter system, we discovered that fungal crude EVs but not EVs depleted of external proteins induced cross-kingdom RNA interference. Comparative EV proteome analysis between crude and purified EVs revealed that several plant cell wall degrading enzymes were highly enriched in crude EVs. By fluorescence-staining, transmission-electron microscopy, and molecular genetic approaches, we provide evidence that EV-associated proteins, including pectin degrading enzymes, damage the plant cell wall enabling EV-mediated RNA delivery. We further propose that cell wall degrading enzymes are commonly functional in EV uptake because

these proteins are often associated with EVs released by plant-colonizing bacterial, fungal, and oomycete species. These findings highlight a new role of cell wall degrading enzymes as known hallmarks in microbial pathogenicity.

### SL-SG-SI-02

#### Predatory bacteria influence host fitness via microbiome modulation

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Microorganisms and their hosts together form a metaorganism—a unit on which ecological and evolutionary processes act—which implies that host and microbiome causally affect one another. Consistent with this, host health has been associated with various traits of microbiomes or their members. One such trait is diversity: higher microbiome diversity has often been linked to improved host health, commonly explained by the idea that greater species diversity increases functional capacity and redundancy, which buffer against disturbances. What, then, mediates diversity? According to ecological theory, predators can promote diversity by suppressing dominant prey and thereby freeing niches for other species. Indeed, predators are integral components of microbiomes, including obligate predatory bacteria belonging to the *Bdellovibrio* and like organisms (BALOs). These bacteria prey on Gram-negative bacteria by invading or vampirizing them. In a meta-analysis across multiple host microbiomes, we linked BALO presence to increased microbiome diversity [1]. However, the consequences of such predators for host health have not been analyzed so far.

Using the nematode *Caenorhabditis elegans* as a host and the defined *C. elegans* microbiome CeMbio [2], we show that BALO presence can positively affect worm fitness by increasing lifespan. Based on *C. elegans* microbiome composition, we associate this effect with an increased abundance of vitamin B12-producing bacteria in communities where a broad-prey-range BALO is present. Conversely, when switching to a BALO strain that selectively preys on the dominant B12 producer, we do not observe changes in *C. elegans* fitness.

We followed up by measuring B12 production across all CeMbio strains and testing whether bacterially produced B12 reaches the host using a *C. elegans* reporter strain (Pacdh-1p::gfp). Additionally, we assessed the effect of B12 supplementation on *C. elegans* when supplied with non-B12-producing bacteria. Together, these experiments show that members of CeMbio produce vitamin B12, that B12 accumulates in *C. elegans*, and that it positively affects lifespan. Moreover, metabolomics revealed that adding BALOs to the CeMbio community likely releases cell-bound B12, thereby increasing its availability to the host.

Our results demonstrate that trophic complexity within microbiomes can be an important determinant of host fitness and should be considered in both research and microbiome remediation efforts.

[1] Johnke et al., 2020. *Microb Ecol* 79, 252–257. doi: 10.1007/s00248-019-01395-7

[2] Dirksen et al., 2020. *G3 Genes|Genomes|Genetics* 10, 3025–3039. doi: 10.1534/g3.120.401309

## SL-SG-SI-03

### Metabolic cross-talk: endosymbiont and host glycosomes in the trypanosomatid *Angomonas deanei*

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The trypanosomatid *Angomonas deanei* contains a  $\beta$ -proteobacterial endosymbiont (ES) in its cytosol. An advanced integration level of the endosymbiont is indicated by the observed cell cycle synchronization between host and ES, restricting the number of ES per host cell to a single bacterium. This synchronization is apparently host-controlled and mediated by so-called ETPs (ES-targeted proteins) which are nucleus-encoded proteins that localize to specific sites at/in the ES including its division site. In addition, metabolic integration of host and ES is indicated by highly complementary sets of enzymes encoded by host and ES. Reconstruction of metabolic pathways indicates that the host is supplied with metabolites like heme and riboflavin by the ES which in turn depends on its host to synthesize certain amino acids. Interestingly, the only known nucleus-encoded protein in *A. deanei* resulting from endosymbiotic gene transfer, the OCD (ornithine cyclodeaminase) protein, localizes to specific host organelles - the glycosomes. These specialized peroxisomes contain the enzymes for the first few steps of glycolysis and are tightly associated with the ES. Localized in host glycosomes, the OCD protein converts ornithine to proline which is likely provided to the proline-auxotrophic ES. Hence, host glycosomes apparently became specialized to support ES integration. To further assess the metabolic integration of host and ES and the role of glycosomes in that context, subcellular proteomics was used to establish protein localizations throughout the *A. deanei* cell. The glycosomal sub-proteome was used to identify complementary metabolic pathways and potentially exchanged metabolites. The proteomic analysis was complemented by the definition of the metabolomic profiles of the ES-harboring wildtype *A. deanei* and an aposymbiotic strain (without ES) to determine changes in metabolism caused by the presence/absence of the ES.

## SL-SG-SI-04

### Investigating algal mechanisms governing mutualistic symbioses with fungi

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Mutualistic associations between fungi and algae have shaped our biosphere for millions of years and were instrumental for the colonisation of land by plants. Among green algae, members of the chlorophyte lineage are particularly known for engaging in such partnerships with fungi in the form of lichens. While fungal mechanisms

contributing to these interactions are gradually being uncovered, the corresponding molecular responses in algae remain comparatively uncharacterised.

Here, we show that the model chlorophyte *Chlamydomonas reinhardtii* engages in a mutualistic interaction with the soil-borne fungus *Verticillium dahliae*. When co-cultured in carbon-free medium, *C. reinhardtii* exhibited significantly enhanced photosynthetic efficiency and elevated photosynthetically active biomass estimated from total chlorophyll fluorescence. Quantification of fungal and algal biomass with quantitative real-time PCR confirmed that both partners consistently showed increased growth in co-culture, when compared with axenic controls. Moreover, co-cultures were characterised by physical cell-cell contact. These observations were consistent across multiple *C. reinhardtii* strains. Remarkably, the mutualistic interaction was established even in the presence of acetate as external carbon source or in the absence of light, suggesting that the exchange of photosynthates is not the sole basis of the interaction and that additional physiological or metabolic mechanisms are involved.

Together, our results demonstrate that *C. reinhardtii*-*V. dahliae* co-cultures can serve as a tractable experimental framework for studying fungal-algal mutualism, providing a foundation for dissecting algal molecular pathways involved in establishing and maintaining beneficial fungal associations.

## Space Microbiology

### SL-SG-SPM-01

#### Microbial Archaeologies: Art, Science, and the Speculative Lives of Archaea

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This body of artistic and scientific work explores *Archaea* as both material and conceptual collaborators through a series of interconnected art-science projects. Combining microbiological protocols with sculptures, performance, and speculative narrative, the work investigates microbial resilience as a means to question boundaries between life and matter, human and non-human timescales, and terrestrial and cosmic environments. At the core of the projects lies a commitment to science communication—positioning art as a Trojan horse for public engagement with science and the unseen microbial world.

The interconnected art-science projects *Performing Beyond the Human* and *Red Perseverance* are developed through the collaboration between artist Anna Steward and microbiologist Robert Reichelt at the German Archaea Centre in Regensburg. During a year-long residency, Steward developed *Performing Beyond the Human*, an embodied exploration of coexistence and failure in interspecies collaboration. *H. salinarum* was cultivated in both laboratory and performative settings, attempting to host the archaea on the artist's body and embracing inevitable failure as an integral part of the process. These experiments

culminated in salt-encrusted agar sculptures that preserve microbial traces as material witnesses to persistence and decay. This inquiry directly led to the current project, *Red Perserverance*, which explores how halophilic archaea and other microorganisms might survive Martian-analogue conditions, extending the work into the field of astrobiology.

Together, these projects propose an expanded understanding of art, microbiology, and astrobiology as intertwined practices—where laboratory procedures become performative gestures, and microbial resilience invites new ways of thinking about embodiment, failure, and life's potential beyond Earth, as well as the fragile conditions that sustain it on our own planet.

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#### SL-SG-SPM-02

##### **Stress Survival of Methanogenic Archaea and their Habitability Potential under Enceladus-like Conditions: Implications for Astrobiology, Biotechnology, and Planetary Protection**

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Saturn's moon Enceladus has emerged as a leading astrobiology target after Cassini observations found its plume material to be rich in water vapor, dissolved salts, molecular hydrogen, CO<sub>2</sub>, and organic molecules. These findings point to a subsurface alkaline ocean in contact with a rocky core, where ongoing hydrothermal activity may supply redox gradients and nutrients necessary for life. Among microbial metabolisms of astrobiological interest, hydrogenotrophic methanogenesis is especially compelling for Enceladus, as it relies directly on H<sub>2</sub> and CO<sub>2</sub>, compounds abundantly detected in the plume. Methanogenic archaea are further recognized for their exceptional tolerance to environmental extremes such as low temperatures, fluctuating salinity, and alkaline pH, making them promising model systems for exploring the limits of life in planetary ocean worlds. In this study, survival and activity of three reference methanogens (*Methanococcoides burtonii*, *Methanosarcina soligelidi*, *Methanobacterium subterraneum*) are being investigated under Enceladus ocean analog conditions: carbonate-buffered minimal medium (Mg<sup>2+</sup>, Ca<sup>2+</sup>, NH<sub>4</sub><sup>+</sup>, Na<sub>2</sub>S), pH 8, salinity 0.5-2% (w/v), and incubation at 4-12 °C. Both hydrogenotrophic and methylotrophic growth strategies are represented, and the headspace gas composition reflects plausible geochemical substrates for icy moon metabolism. Survival and metabolic activity are quantified using dilution-to-extinction MPN assays. Experimental designs include fractional factorial matrices to efficiently map temperature and salinity effects, with internal blanks and positive controls. Complementary stress survival experiments (freeze-thaw and desiccation) benchmark planetary protection concerns and validate the sensitivity and reproducibility of the methodology. The study is ongoing, with expanded series currently underway to systematically define environmental boundaries for methanogenic life under simulated Enceladus ocean conditions. Together, these complementary assays establish a robust methodological framework for evaluating microbial persistence and activity in astrobiologically relevant settings. The findings are directly relevant to planetary protection by informing risk assessments of microbial contamination and suggest broader biotechnological applications for stress-resilient methanogens in terrestrial systems.

## Cyanobacteria

#### SL-SG-CB-01

##### **Flexible tetrapyrrole metabolism for photosynthetic efficiency**

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The main photosynthetic pigment of oxygenic phototrophs, chlorophyll (Chl), is synthesized together with heme and other tetrapyrroles via a common, branched pathway. Their accumulation is strongly dependent on the availability of light and macronutrients, and it is mainly regulated at the formation of early precursors and at the branch point of Chl and heme synthesis<sup>6,7</sup>. In cyanobacteria that harvest light using heme-derived phycocyanobilins, the control of the pathway is particularly dominant at the Chl-heme branching. At this biosynthetic step, the magnesium chelatase and ferrochelatase enzymes are competing for the protoporphyrin IX substrate delivered by protoporphyrin oxidase (PPO). To gain insight to the regulation of this key biosynthetic step, we searched for interacting partners of PPO and Gun4, a regulatory subunit of magnesium chelatase. Our results demonstrated the effect of nitrogen availability on the regulation of the branch point, and further resolved the co-regulation of tetrapyrrole biosynthesis with the biogenesis of photosystems. Reverse genetics coupled laboratory evolution of *Synechocystis* sp. PCC 6803 showed that in cells with lower amounts of photosystems complexes, heme production was upregulated that consequently increased the relative amount of cytochrome *b6f* in the membranes. The triggers and consequences of the re-organization of photosynthetic complex stoichiometry are discussed.

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#### SL-SG-CB-02

##### **Thylakoid Na<sup>+</sup>/H<sup>+</sup> Antiport is Essential for Cyanobacterial Energy Homeostasis**

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Conversion of light into usable energy during photosynthesis involves forming an electrochemical gradient across the thylakoid to drive ATP synthesis, the proton motive force (pmf). In eukaryotes, the pmf has well characterized roles in activation of photoprotective processes. However, the regulation of the thylakoid pmf in cyanobacteria is poorly understood and thylakoid ion transporters known in plants are not found in cyanobacterial genomes. The Na<sup>+</sup>/H<sup>+</sup> antiporter NhaS3 has previously been localized to the thylakoid membrane in *Synechocystis* sp. PCC 6803, but no successful knockout has been generated in either *Synechocystis* or *Synechococcus elongatus* PCC 7942, leading to its characterization as essential but providing minimal information regarding its physiological role. Using heterotrophic growth selection, a fully segregated *nhaS3* knockout was generated. While *nhaS3* remains photoautotrophic, the mutant has severe growth impairment and light-dependent glucose sensitivity. Measurements of cytosolic pH show an alkalization in *nhaS3*, confirming the expected directionality of Na<sup>+</sup> movement into the thylakoid lumen and H<sup>+</sup> release by NhaS3. The disrupted cytosolic pH is expected to decrease the availability of CO<sub>2</sub>, which is reflected in a significant increase in carboxysomes in *nhaS3* and upregulation of proteins involved in 2-phosphoglycolate detoxification due to Rubisco oxygenation reactions. From phylogenetic analysis, NhaS3 is exclusive to cyanobacteria

and universally present in every cyanobacterial genome. The requirement of thylakoid Na<sup>+</sup> transport in cyanobacteria has apparently been lost in eukaryotic photoautotrophs, providing an avenue to understand the evolution of photosynthetic energy storage, plastid evolution, and the unique role of thylakoid mediated cellular pH homeostasis in cyanobacteria.

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### SL-SG-CB-03

#### Modeling Trade-offs and Growth Strategies in Cyanobacteria

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Cyanobacteria exhibit remarkable metabolic and ecological diversity and thrive in almost all ecosystems despite differences in light, nutrient availability, and environmental context. We argue that metabolic diversity and the capacity of cyanobacteria for adaptation can be studied in terms of trade-offs that shape cellular resource allocation and growth strategies. We present a modeling framework for cyanobacterial metabolism and growth that integrates these trade-offs using principles of cellular optimality. Building on coarse-grained resource allocation models, we describe how cells adjust their proteome and metabolism under changing environmental conditions. The resulting models can reproduce known "growth laws" and suggest distinct physiological strategies. Two aspects of cyanobacterial physiology are of particular interest: (i) trade-offs associated with rapid phototrophic growth, including the allocation of protein resources between photosynthetic, metabolic, and storage functions; and (ii) photo-heterotrophic interactions within microbial communities, where exudation of organic carbon and nutrient exchange give rise to emergent ecological interactions. Both examples illustrate how cyanobacterial strategies can be viewed in terms of a "cellular economy" constrained by limited resources and optimality principles. Our aim is to link mechanistic models of metabolism with ecological theory, providing a framework to interpret and predict cyanobacterial diversity across environments.

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### SL-SG-CB-04

#### Glycogen-deficiency response during light-dark cycle in the cyanobacterium *Synechocystis* sp. PCC 6803

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Phototrophic organisms convert light energy into chemical energy. Shorter absences of light can be compensated via rerouting of energy flux. Longer periods of darkness require a carbon storage to provide energy. Eukaryotic Archaeplastida typically use starch for carbon storage, while cyanobacteria, the evolutionary ancestors of plastids, use glycogen.

The ménage-à-trois hypothesis explains how a cyanobacterium could have evolved into a plastid and proposes the linkage of carbon and energy metabolism between the eukaryotic host and cyanobacterial endosymbiont as the main driver for the establishment of initial endosymbiosis. The loss of glycogen as a storage compound is a likely first step in this. We hypothesize that the detailed analysis of a glycogen-deficient cyanobacterium provides insights into the mechanism enabling endosymbiosis.

Here, we studied the model cyanobacterium *Synechocystis* sp. PCC 6803. The accumulation of glycogen was abolished via knockout of the *glgC* gene, preventing the expression of the ADP-glucose pyrophosphorylase that forms the glycogen precursor molecule ADP-glucose. Two independent knockout mutant lines were characterized in a microfluidic environment. The single-cell resolution allowed linking the reduced growth of glycogen-deficient mutants under diurnal conditions to a longer lag phase once illumination resumed.

Metabolomic analysis revealed strongly reduced levels of ATP at night compared to the wildtype. Transcript levels of proteins involved in ATP-dependent processes, such as DNA-duplication and cell division were downregulated highlighting the effects of the insufficient energy supply during period of darkness without glycogen. Metabolites of the oxidative-pentose-phosphate-pathway, needed in photosynthesis and the Calvin-Benson-Bassham cycle, and other pathways were depleted as one compensatory mechanism to generate ATP. Alternative carbon compounds, like GDP-hexose and UDP-lactose, accumulated during the illumination period.

Our results show that the cells enter a maintenance-mode, deprioritizing growth and cell division. Growth arrest and production of hexose-phosphates are important prerequisites for the establishment of endosymbiosis as uncontrolled growth of an endosymbiont is detrimental due to spatial constraints, while the supply of carbon compounds benefits the host. Thus, a glycogen-deficient cyanobacterium serves as the ideal basis for further genetic modification to turn into an invaluable endosymbiont.

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### SL-SG-CB-05

#### Establishment of cell polarity in cyanobacterial phototaxis

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Many photosynthetic cyanobacteria are motile and can adjust their cellular motility in response to light stimuli, thus seeking out favorable light conditions. Movement towards or away from directional light sources – termed positive and negative phototaxis, respectively – is facilitated by the coordinated activity of type IV pili (T4P). In the spherical model cyanobacterium *Synechocystis* sp. PCC 6803, incident light is diffracted into a sharp focal spot at the distal side of the cell, providing a means to sense light direction. We hypothesize, that sensing of this focal spot sets off a signaling cascade that promotes asymmetric T4P activity. Presumably cell polarity is established via dynamic protein-protein interactions at the base of the T4P complex, including

the T4P motor ATPases PilB and PilT, the cyanobacterial Hfq homolog and the filament forming coiled-coil protein HmpF, which is conserved in cyanobacteria and crucial for T4P assembly and function. We used confocal microscopy, single-cell tracking and radially-resolved fluorescence intensity measurement to probe spatiotemporal dynamics of crucial T4P proteins during motility. We could show that HmpF oscillates between opposing cell poles in *Synechocystis* with proper localization depending on the chemotaxis-like Tax3 system and PilB. Notably, HmpF oscillations correlated with the cell division axis of *Synechocystis*. Furthermore, we could show that HmpF, like PilB and Hfq, accumulates at the leading cell pole during cellular movement and phototaxis. To understand the molecular function of HmpF and in particular its N-terminal OB-fold, we performed crosslinking immunopurification and could show nucleic acid binding, presumably to RNA molecules. Overall, we provide evidence that HmpF is a Tax3-associated key player in establishing cell polarity during directional motility in *Synechocystis*, and might act as an underlying scaffold to orchestrate polar T4P activation.

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Nature employs a limited and conservative set of amino acids to synthesize proteins. The ability to genetically encode an extended set of building blocks can be used in diverse applications, including approaches to study and control protein function as well as to design novel therapeutics. Non-natural amino acids (NAA) are co-translationally incorporated into proteins by orthogonal pairs consisting of aminoacyl-tRNA synthetase and cognate tRNA. However, the current repertoire can neither display the full natural diversity of NAAs and is especially limited for backbone modifications. Excitingly, we now succeeded in reverse engineering a protein ligase into new-to-nature tRNA synthetases that can load tRNA either with  $\beta$ -amino acids ( $\beta$ aa) or expand the genetic code by novel non-natural  $\alpha$ -amino acids including advanced glycation endproducts.

## Synthetic microbiology

### SL-SG-SM-01

#### Genetically Programmable Integration of a Reversibly Taggable Amino Acid Into Peptides

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A variety of chemical biology and pharmaceutical applications, such as targeted drug delivery, fluorescent labeling, and controlled release, depend on the site-selective functionalization of bioactive peptides. However, current methods rely on externally supplied substrates and irreversible conjugation reactions.

Here, we present an integrated microbial system that can biosynthesize and site-specifically incorporate a reactive amino acid into customized peptides. Guided by the active site of naturally selective enzymes, we rationally engineered a model peptide synthetase to change the substrate specificity from its native substrate to a cognate reactive amino acid with only five mutations. We demonstrate that this selectivity switch is generalizable and show the feasibility of site-specific integration using an antibiotic peptide synthetase as an example. By combining the heterologous production of the reactive amino acid with the engineered peptide synthetase *in vivo*, we present an integrated system for producing customized reactive peptides. Importantly, an engineered reactive peptide analog retained its bioactivity. This work introduces a generalizable strategy for the *de novo* biosynthesis of reversibly taggable peptides, expanding the interface between biosynthetic pathway engineering and bioorthogonal chemistry.

### SL-SG-SM-03

#### Identification of a membrane-bound protein complex that fully drives microbial catabolism

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Synthetic biology aims to redesign microbial metabolism, often with the goal of synthesizing valuable compounds. The necessary energy derives from external substrates that are converted by a network of catabolic pathways, often resulting in a significant proportion of unwanted side products.

Here, we introduce a novel bioenergetic system in which catabolism is represented by a single multimeric membrane-bound protein supercomplex that harnesses the redox potential difference between an external electron donor (hydrogen gas) and an external electron acceptor to generate a *proton motive force* and subsequently ATP. Incorporating such a system into a production organism could eliminate the need for maintenance and regulatory control of any other catabolic reaction.

The protein supercomplex originates from the strictly anaerobic bacterium *Dehalococcoides mccartyi* strain CBDB1, which naturally conserves energy by respiring with halogenated pollutants. In *Dehalococcoides*, this process is the only known energy-conserving process. The presence of 32 paralogous operons within the small 1.4 Mbp genome highlights the evolutionary dependence of the metabolism on this process.

We have identified the seven subunits of the supercomplex and characterized their roles in electrogenic proton extrusion. Here, we describe these findings along with our progress towards heterologous expression of the subunits. This work lays the foundation for developing minimal, self-sufficient bioenergetic modules that could advance sustainable biotechnologies beyond plant-based systems.

References:

### SL-SG-SM-02

#### Repurposing a protein ligase into an orthogonal tRNA synthetase for co-translational backbone expansion

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Hellmold N, Eberwein M, Phan MHT, Kümmel S, Einsle O, Deobald D, Adrian L (2023) *Dehalococcoides mccartyi* strain CBDB1 takes up protons from the cytoplasm to reductively dehalogenate organohalides indicating a new modus of proton motive force generation, *Front. Microbiol.* 14, 1305108, <https://doi.org/10.3389/fmicb.2023.1305108>

Adrian L, Sawers RG, Deobald D (2025) Organohalide respiration in *Dehalococcoides* strains represents a novel mode of proton motive force generation, *Adv. Microb. Physiol.* 86, 141–173, <https://doi.org/10.1016/bs.ampbs.2024.12.001>

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#### SL-SG-SM-04

##### Leveraging orthogonality to escape the limitations of bioproduction

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Engineered microbes offer exciting new opportunities for the sustainable production of value-added chemicals from cheap feedstocks without the need for high temperatures, pressure, or toxic solvents. However, the metabolic integration of synthetic pathways can be challenging. One major concern is the competition of natural and synthetic pathways for the same core metabolites. So far, these conflicts have been approached by spatial or temporal separation of synthetic metabolism. Orthogonal pathways offer a third possibility for metabolic separation – one on the biochemical level. Pathways proceeding via non-natural intermediates lower the cross-talk between synthetic and natural metabolism, and thus the overall burden on the cell. Here, we discuss the benefits of such systems, as well as the use of non-natural cofactors to achieve further separation of synthetic and natural metabolism.

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#### SL-SG-SM-05

##### Metabolic changes in CRISPRi strains reveal insights into metabolic robustness and provides engineering target

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CRISPR interference (CRISPRi) can be used to perturb gene expression. Here, we created a CRISPRi library in *Escherichia coli* (*E. coli*) targeting all 1,515 genes in the iML1515 metabolic model and measured the metabolome response. Downregulation of gene expression reduces enzyme abundance, which can then lead to substrate accumulation. With this we were able to measure metabolites which are normally maintained at low levels. Metabolite accumulations were confirmed by LC-MS/MS and putative reference spectra were generated for 102 metabolites that previously lacked MS<sup>2</sup> spectra, expanding current metabolomics reference databases. Our findings highlight that low metabolite pools contribute to metabolic robustness by enabling substrate level buffering, minimizing competitive inhibition, and suppressing unwanted side reactions. Conversely, low metabolite pools can constrain flux through heterologous or engineered pathways. The CRISPRi library helps us to identify those bottlenecks and use this for metabolic engineering. Furthermore, we measured the exometabolome of the *E. coli* CRISPRi library. This provides insights into transport mechanisms and

metabolic overflow. Together, the results provide a map of metabolic engineering targets, expand the measurable metabolome and reveal principles of metabolic robustness.

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#### SL-SG-SM-06

##### Rapid identification of superior endogenous signal peptides for heterologous protein secretion by *Corynebacterium glutamicum* through modular cloning and automation

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The recombinant protein market has significantly expanded in recent years and is now a multi-billion dollar industry. Secretory protein production by microbial hosts simplifies product recovery, increases yield as well as purity while reducing production costs and is therefore preferred over intracellular protein production. However, efficient secretion of heterologous proteins by bacteria requires identification of optimal signal peptides (SPs) for each protein of interest (POI), a step that often limits process development. Therefore, our goal was to establish an easy and rapid system to identify superior SPs for the secretion of POIs.

Using *Corynebacterium glutamicum* as a model host, we established a modular cloning system enabling rapid assembly of expression plasmids for secretory protein production. By screening a library of 30 individually cloned endogenous SPs with a fungal cutinase as POI, we demonstrated that several native *C. glutamicum* SPs achieved substantially higher secretion levels than the widely used *Bacillus subtilis* NprE reference SP.

In addition, to further accelerate SP discovery, we developed a one-pot approach in which *C. glutamicum* was directly transformed with a single modular cloning reaction mixture containing the coding sequences for all 30 SPs, the POI and a suitable receiving vector. Combined with the AutoBioTech1 high-throughput automated platform for cultivation, harvesting, and protein quantification, this strategy enabled the rapid screening of several hundred clones in parallel. Superior SPs were rapidly identified not only for cutinase but also for four polyethylene terephthalate hydrolases (PETases). Using this strategy, we improved cutinase secretion by ~3-fold and reduced the optimal SP identification time to one week.

This streamlined workflow significantly reduces time and cost for selecting effective SPs and can be used to not only identify improved SPs for protein secretion, but also to screen large libraries of SPs in different organisms which can expand our understanding on SP function and help improve computational tools for the prediction of an optimized SP for the secretion of a particular POI and in the organism of choice.

1. Rosch, T. M.; Tenhaef, J.; Stoltmann, T.; Redeker, T.; Kösters, D.; Hollmann, N.; Krumbach, K.; Wiechert, W.; Bott, M.; Matamouros, S.; Marienhagen, J.; Noack, S., AutoBioTech - A versatile biofoundry for automated strain engineering. *ACS Synth Biol* 2024, 13 (7), 2227-2237.

## SL-SG-WW-01

### Role of endolithic microorganisms in nitrate turnover within the Ammer river catchment (SW Germany)

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The application of nitrogen fertiliser is the main cause of NO<sub>3</sub><sup>-</sup> contamination in groundwater. Concentrations above 50 mg/L have frequently been observed in the recharge zone of the Ammer River catchment in south-west Germany. However, the anoxic groundwater in the confined part of the aquifer usually contains around 1 mg/L of NO<sub>3</sub><sup>-</sup>. Our recent studies demonstrated the vital role of the denitrifying microbial community in the groundwater NO<sub>3</sub><sup>-</sup> turnover. However, it remains unclear i) which electron donors these microorganisms use in oligotrophic environments and ii) whether they can grow within the porous rock matrix or only in the fracture network.

To answer these questions, we carried out exploratory drilling in the transitional zone of the catchment and retrieved limestone cores. Rock cores were used to determine potential electron donors, analyse the endolithic microbial community and enrich key denitrifying microorganisms. Additionally, microbial trapping devices filled with carbonate rock were installed in the groundwater well and incubated in anoxic conditions for seven years to enrich the endemic denitrifiers.

The microbial community identified at different depths consisted of microorganisms capable of coupling NO<sub>3</sub><sup>-</sup> reduction with oxidation of Fe (*Gallionella*), reduced S (*Thiobacillus*), H<sub>2</sub> (*Hydrogenophaga*), CH<sub>4</sub> (*Candidatus Methyloirabilis*), and OM (*Pseudomonas*). Geochemical analysis revealed the presence of pyrite (FeS<sub>2</sub>) (4.1±1.4 mg/g rock) and CH<sub>4</sub> (up to 14.3 ppm) in rock samples, and H<sub>2</sub> (up to 13.5 ppm) in groundwater samples. Finally, autotrophic microorganisms that can couple the reduction of NO<sub>3</sub><sup>-</sup> to the oxidation Fe<sup>2+</sup>, H<sub>2</sub>, CH<sub>4</sub> and S<sub>2</sub>O<sub>3</sub><sup>2-</sup> and which potentially play an important role in the nitrogen cycle of groundwater, were enriched.

However, the location of the denitrification hotspot remains unknown. To address this issue, we have designed diffusion cells containing synthetic rock discs that have a mineral composition and porosity similar to that of the rock matrix found in the aquifer. These cells will be used to determine whether microorganisms enriched from the aquifer can colonise the porous, FeS<sub>2</sub>-containing synthetic rock matrix and use minerals as electron donors for NO<sub>3</sub><sup>-</sup> reduction.

## SL-SG-WW-02

### Lab-scale and *in-situ* trials of hydrogen and methane driven chemolithoautotrophic nitrate reduction as a potential nitrate mitigation strategy for shallow porous drinking water aquifers

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Nitrate pollution in groundwater is a major challenge for drinking water production across Europe. Avoiding a nitrate overload requires sustainable management schemes especially for shallow aquifers. However, their attenuation potential against nitrate is often limited. High nitrate levels persist in many aquifers even long after reducing nitrogen inputs. Especially for otherwise oligotrophic shallow groundwater systems, innovative and cost-effective technologies to eliminate nitrate loading and to ensure a sustainable supply of drinking and utility water are urgently needed. In this project we are implementing a novel approach for nitrate removal from shallow groundwater. Via the injection of reduced gaseous electron donors (H<sub>2</sub> & CH<sub>4</sub>), we aim to stimulate autotrophic denitrification directly upstream of burdened drinking water wells. This has the advantage of avoiding an enrichment of heterotrophic aquifer microbiota, some of which can be of hygienic drinking water concern.

In lab scale microcosms, we have tested a range of *in-situ* conditions and aquifer materials. We successfully enriched a stable hydrogen-driven community from impacted groundwater sediments and characterized metabolic processes and temperature-dependent reaction kinetics. We were also able to inoculate non-denitrifying aquifer material with a pre-enriched stable bioreactor community, demonstrating that bioaugmentation can successfully (re-)establish chemolithoautotrophic denitrification in aquifer sediments. In a mesoscale pilot plant flume system, methane-driven nitrate reduction was indeed observed. Microbiome and process data suggested at least to distinct zones and mechanisms of methane-dependent nitrate elimination to be active along the flow path, only one of them suggesting N-DAMO. We isolated and further characterized stimulated microbial populations in lab microcosms, additionally identifying a methane-driven nitrate reduction under microoxic conditions. In a concluding proof-of-concept field experiment, we are currently testing our novel nitrate remediation strategy upstream of an abandoned drinking water well. Overall, harnessing the intrinsic potential of autochthonous autotrophic microorganisms of groundwater systems and using gaseous electron donors that can be sourced from renewable energies may offer a local but sustainable solution to notorious nitrate problems in ground and drinking water supply.

## SL-SG-WW-03

### Integrating Stable Isotope Labeling and Quantum-Chemical Raman Simulations to Elucidate Carbon Assimilation in the Chemo-Organo-Autotrophic lifestyle of the Anaerobic Naphthalene-Degrading Culture N47

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The enrichment culture N47 is a sulphate-reducing, naphthalene-degrading culture that exhibits an unusual chemo-organo-autotrophic lifestyle, combining organic substrate oxidation with CO<sub>2</sub> fixation.

Building on earlier work by Heker et al 2025, this study aimed to elucidate the central carbon metabolism of N47 by combining isotopic labelling, Raman microspectroscopy and cavity ring down spectroscopy. Cultures were grown with varying proportions of <sup>13</sup>C-labeled naphthalene and bicarbonate to trace carbon assimilation from both sources. To interpret the Raman spectral data, I developed a computational framework based on quantum mechanical simulations of <sup>13</sup>C-labeling patterns in phenylalanine. These simulations allowed prediction of Raman band shifts associated with different isotopic labelling patterns and guided the design of an analysis pipeline for quantifying <sup>13</sup>C incorporation in single cells.

To validate the approach, I applied the same workflow to reference organisms with known metabolic pathways grown on <sup>13</sup>C-labeled substrates. The results demonstrate that N47 actively assimilates inorganic carbon from bicarbonate, supporting its proposed chemo-organo-autotrophic metabolism. Although the quantitative Raman analysis is still being refined, theoretical predictions and preliminary data indicate that the method will enable detailed, non-destructive insights into carbon fluxes in microbial systems. This integrative approach bridges isotope labeling, spectroscopy, and quantum chemical modelling to better understand microbial carbon utilization in complex anaerobic communities.

#### SL-SG-WW-04

##### **Methanogenic Archaea as Drivers of Microbiologically Influenced Corrosion: Mechanistic Insights, Mitigation, and Simulation in Energy Infrastructure and Marine Settings**

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**Microbiologically influenced corrosion (MIC)** poses a significant threat to metallic infrastructure across sectors—from energy and marine environments to cultural heritage conservation and emerging technologies such as underground hydrogen storage (UHS). **Methanogenic archaea** have emerged as key contributors to corrosion under anaerobic conditions, capable of directly interacting with metal surfaces via extracellular electron transfer.

This presentation provides an overview of our recent work on identifying, characterizing, and mitigating MIC caused by methanogens. We focus on the genetic differentiation of methanogenic strains, highlighting a novel class of **[Ni/Fe]-hydrogenases** identified exclusively in corrosive methanogens to date. These enzymes may serve as molecular markers for MIC risk assessment due to their unique sequence and functional properties.

Additionally, we explore strain-specific differences in hydrogenase glycosylation and correlate these with observed variations in corrosion severity, biofilm formation, microbial surface interactions, and potentially enzyme stability. These

findings suggest glycosylation may play a previously underappreciated role in MIC dynamics.

To mitigate MIC, we investigate **polyoxometalate-based (POM) coatings**—originally developed for protecting stone-based artifacts—which we now apply to metal surfaces. These multifunctional coatings effectively inhibit biofilm formation and microbial activity, offering a promising strategy for corrosion control.

Furthermore, we have developed customized test systems that **simulate realistic environmental conditions**, including high-pressure settings and dynamic flow regimes with varying velocities. These platforms allow for controlled evaluation of microbial corrosion under conditions relevant to **underground hydrogen storage** and **marine environments**, where salinity, pressure, and microbial activity interact.

Our findings demonstrate that integrating molecular diagnostics, surface engineering, and advanced simulation platforms provides new insights into MIC mechanisms and opens avenues for predictive diagnostics and sustainable corrosion control strategies in industrial applications.

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#### SL-SG-WW-05

##### **Abundance and activity of ammonia-oxidizing bacteria in the hypersaline waters of the Dead Sea**

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The Dead Sea is a hypersaline terminal desert lake with a salinity of ca. 350 g L<sup>-1</sup> total dissolved salts. Its unique salt composition consisting of high Mg<sup>2+</sup> (ca. 2M) and Ca<sup>2+</sup> (0.5 M) renders it inhospitable to most organisms. Nevertheless, despite its name the Lake is not bare of life. Microorganisms have been described in the lake already in the 1930s and its waters harbor ca. 5×10<sup>4</sup> cells. The lake is oxygenated to the bottom (300 m) and has extremely high NH<sub>4</sub><sup>+</sup> concentration reaching ca. 600 μM, making it a potential habitat for ammonia oxidizing bacteria. In contrast, it was suggested that the energy yield of ammonia and nitrite oxidation is not sufficiently high to support the energetic costs of osmoregulation required to survive in the lake. A decade ago, with the discovery of underwater freshwater springs in the Dead Sea, ammonia and nitrate oxidation were measured in the lake's sediment in and around the springs. Those measurements pointed toward the presence of halophilic ammonia and nitrite oxidizing bacteria. Based on those results, we asked whether such organisms are present in the water column of the lake. Specifically, of interest are commamox bacteria who by combining ammonia and nitrite oxidation in one cell, benefit from a higher energetic yield. Analyses of metagenomic reads from the lake, revealed the low abundance presence of *amoA/B/C*, *hao* and *nrxA/B* genes, distantly related to *Nitrosomonas*, *Nitrospira*, and *Nitrolancea*. Further qPCR analyses with primers targeting all unique genes involved in ammonia and nitrite oxidation are in progress. To measure the process directly we further conducted incubation experiments with and without 15NH<sub>4</sub> in pure Dead Sea water as well as in dilution series down to 40% of the lake's salinity. Preliminary results from these experiment point to the process taking place in lake water diluted to ca. 70 % percent but not in pure Dead Sea water nor in higher dilutions. Overall, our results so far, point to the presence of ammonia oxidizing bacteria in the Dead Sea



water suggesting that these organisms may use an energetically efficient osmoregulatory process to compensate for the low energetic yield of ammonia and nitrite oxidation.

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## SL-SG-WW-06

### Quantifying photosynthesis associated methane production by phytoplankton and the suppression of its contribution to CO<sub>2</sub> sequestration

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Not long ago, biological methanogenesis was considered to be restricted to Archaea in anoxic conditions. However, it was recently demonstrated that methane (CH<sub>4</sub>) could also be produced in oxic conditions, a term coined as Oxidic Methane Production (OMP). Furthermore, it was demonstrated that Cyanobacteria and some eukaryotic algae produce methane (CH<sub>4</sub>) in oxygen (super)saturated environments as a by-product of photosynthesis, termed as Photosynthesis-associated methane production (PAMP). Phytoplankton, consisting of Cyanobacteria and eukaryotic planktonic algae, are ubiquitous, being found in nearly all aquatic environments on Earth, as well as several terrestrial ones. Accordingly, though on a local scale their contribution to the global methane budget may seem minor, on a global scale it is likely significant. Phytoplankton form massive blooms, that are enhanced in frequency and intensity due to global warming and anthropogenic eutrophication. Thus, their contribution to OMP and global CH<sub>4</sub> emissions could attain even higher magnitudes. However, PAMP is currently poorly quantified and the factors driving variation in methane emission are unknown.

To fill in this knowledge gap, we are quantifying PAMP for major representatives across the phytoplankton phylogenetic tree and are calculating the ratio of fixed CO<sub>2</sub> which is later released as CH<sub>4</sub> across various conditions. The latter was termed Methane Production Quotient (MPQ). We have set different phytoplankton cultures and measured both CH<sub>4</sub> and CO<sub>2</sub> fixation/production using membrane inlet mass spectrometry (MIMS) and gas chromatography. We have found that MPQs for Cyanobacteria ranged between 0.16% and 1.4%, while for eukaryotic algae we have found values on the lower range (0.0004%<sup>(1)</sup> - 0.19%). Furthermore, we have found the MPQ calculated for our own culture of *T. erythraeum* measured using the MIMS (0.28%) was similar to that obtained in a parallel study (0.32%) using Cavity Ring-Down Spectroscopy gas analyser, validating both methods. Quantifying MPQ across major representatives across the phytoplankton phylogenetic tree, will give a comprehensive view on the contribution of phytoplankton to OMP. This will allow the grouping of different species/groups according to their CH<sub>4</sub> emission potential and the consideration of their impacts according to their bloom potential in ever more eutrophicated water systems.

1. Rao Y et al., Commun Earth Environ. 2024 Nov 12;5(1):695.

## Identification & systematics

### SL-SG-SMBE-01

#### Chemical composition of the cell membrane, a valuable, well tried and tested aspect in the classification of organisms

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The cell membrane is a universal and essential feature of all known cellular forms of life. Genomics indicate that between 25% and 50% of the genes in a genome encode for compounds that are either integrated into the cell membrane or are directly attached to it. A fundamental aspect of cell membranes is the presence of a class of compounds that are referred to as "lipids". Lipids are a diverse range of compounds largely defined by their hydrophobicity. It is their diversity that makes them more challenging to study because no one single method can provide all the information needed to characterise all the lipids in a cell. Essentially divided into two chemical groups, either those derived from fatty acids or those derived from isoprenoids they play an indispensable role in maintaining membrane structure and function. Studies over the past 60 years have shown unambiguously that there is a clear link between cellular lipid composition and cellular evolution. It is important to remember that although a genome encodes all enzymes involved in lipid synthesis, simply knowing the enzymes involved does not allow one to make a detailed qualitative or quantitative study of cellular lipids *in vivo*. Despite the wealth of data available on lipid distribution and diversity a number of problems can be identified in the literature. These include inaccurate data, partial data sets, difficulties in interpretation of the data and preconceived concepts as to whether one data set is superior to another. Detailed and accurate studies on lipid composition are far from complete and based on past knowledge provide essential information on the functioning and evolution of the cell membrane. Combining that knowledge with information from enzymology and genomics is central to understanding the origin and subsequent evolution of the cell membrane

### SL-SG-SMBE-02

#### Combined molecular detection and cultivation of an abundant and uncultured Verrucomicrobiota from the Baltic Sea

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Cultivating yet uncultured but environmentally abundant microorganisms remains a major challenge in microbiology. To address this, we developed and applied a combined molecular and cultivation-based approach aimed at isolating a *Verrucomicrobiota* bacterium, known as "*Spartobacteria baltica*", from brackish Baltic Sea water. This organism remains elusive to cultivation, despite being very abundant in this environment. Supported by metagenomics and abundance data, our strategy integrates rapid molecular identification with diverse cultivation approaches to enhance the recovery of the target organism.

In the first step, we designed, optimized, and validated a specific primer system that enables the sensitive and precise detection of "*Spartobacteria baltica*" via PCR in complex environmental samples. This allowed a rapid screening of enrichment and isolation experiments for the presence of the target organism. In parallel, we explored multiple cultivation strategies, including *ex situ* enrichment experiments with different carbon sources, inoculation with fractionated samples, and incubation under varying temperature conditions. Additionally, we performed *in situ* cultivation experiments in the Baltic Sea using isolation chips (iChips) to mimic natural environmental conditions and promote the growth of otherwise unculturable taxa.

The newly established primer system provided a reliable tool for the detection of "*Spartobacteria baltica*" and represents a methodological advance. Among the cultivation approaches tested, the *in situ* strategy proved to be the most promising. "*Spartobacteria baltica*" was detected in one single iChip well, whereas no detection was achieved in any *ex situ* experiment.

Although a pure culture could not yet be obtained, the successful growth *in situ* marks an important methodological step toward the targeted isolation of "*Spartobacteria baltica*". Future work will focus on further optimizing the *in situ* cultivation and re-isolation procedures to obtain a pure culture and unravel the ecological role of "*Spartobacteria baltica*" in the Baltic Sea ecosystem.

### SL-SG-SMBE-03

#### Better together: Cross-phyta mutualistic symbionts coevolved into diverse multicellular photosynthetic consortia

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Green sulfur bacteria frequently dominate the microbial communities that develop at illuminated oxygen-sulfide chemoclines of stratified lakes. Here, they are often found in multicellular symbiotic associations with motile Betaproteobacteria forming photosynthetic consortia. Several morphotypes have been described globally based solely on microscopy. Most are distinguished by green or brown pigmentation due to the epibionts production of bacteriochlorophyll *c* or *e* and respective carotenoids, or based on the number, size, and shape of each Chlorobi. Only one variant has been cultured to date, "*Chlorochromatium aggregatum*", containing epibiotic *Chlorobium chlorochromatii* bound to *Ca. Symbiobacter mobilis* as the motile central-rod. However, 16S rRNA analysis of natural consortia had indicated that other morphotypes likely contain related species of *Chlorobium* and *Commomonadaceae*.

To determine the genomic variability of phototrophic consortia, we examined natural populations of up to six morphotypes occurring in the chemocline of Lake Dagow, Germany. Combining microscopy-paired fluorescence-activated single-cell sorting (FACS) and single-cell sequencing with long-read environmental metagenomics, we

reconstructed complete genomes of epibionts and their central rods. This paired approach allowed us to disentangle phototrophic consortia relationships, with phylogenomics identifying epibiont taxa matching morphologies observed. Comparing uncultured central-rods to their specific epibionts revealed coevolution patterns of both symbionts. Novel circularized genomes further supported the discovery of analogous symbiosis genes in different consortia types, with metatranscriptomics confirming the relevance of such species in the microbial community. Taken together, we broaden our understanding of the evolution and genetic basis of bacterial multicellular interactions, by defining new photosynthetic consortia and their role in microbial ecosystems.

#### SL-SG-SMBE-04

##### Reconstructing the tree of life with reduced amino acid alphabets

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For decades, recoding amino acid sequences into reduced alphabets has been a very successful tactic in various bioinformatics applications, such as improving the speed and sensitivity of sequence searches, protein functional assignments, and predicting structural features. In phylogenetics, two types of alphabets are mainly used: the so-called Dayhoff alphabets, and saturation bin alphabets. The latter address biased tree estimation resulting from substitution saturation by aiming to maximize the rate of substitutions within bins, based on an amino acid replacement matrix. Another recoding approach that targets alignment compositional heterogeneity, called minmax chi-squared bins, has remained mostly unused. Saturation recoding has helped resolve some of the trickiest nodes in the tree of life, like the origin of Eukaryotes within Archaea.

Here, I will introduce a series of variant and new approaches in using reduced amino acid alphabets for phylogenetics. I first created an implementation of the SR saturation bin algorithm that will produce a custom set of alphabets (2-19 states) for any amino acid replacement matrix, plus a helper script for obtaining the corresponding profile mixture models. Then, I tested the applicability of minmax chi-squared bins in large-scale concatenations for microbial phylogenomics. Finally, I explored ways to integrate protein structural information in reduced alphabets. I demonstrate the utility of these approaches, planned for an upcoming release of my WhereDoGGo phylogenomics pipeline ([github.com/MEDEAlab/WhereDoGGo](https://github.com/MEDEAlab/WhereDoGGo)) through a number of case studies on prokaryotic lineages with unstable taxonomic placement.

#### SL-SG-SMBE-05

##### From reef to relic: what ancient DNA reveals about historical European flat oyster *Ostrea edulis* associated microbes

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The European flat oyster *Ostrea edulis* once formed extensive reef ecosystems across Europe and shaped coastal biodiversity, culture and economies. Despite its former abundance, the species experienced a dramatic decline and became locally extinct in the Wadden Sea by 1930 (1). The full extent and underlying causes of this collapse remain unresolved. We examined whether pathogen pressure may have contributed to historical mass mortality events, as disease continues to pose a significant threat to modern oyster populations. Oyster shells spanning over 6000 years and representing several European regions were analysed using ancient DNA approaches to trace when certain pathogens may have first appeared and to compare patterns across locations. DNA was extracted from shell powder, sequenced using high-throughput shotgun sequencing, and subjected to metagenomic taxonomic profiling to detect microbial DNA preserved within the shell matrix with a focus on potential pathogens. Initial results revealed the presence of *Vibrio* species belonging to the virulent *Splendidus* clade in multiple specimens. Members of this clade are particularly known for causing disease in marine invertebrates such as shrimp, corals, and molluscs. Certain *Vibrio* species are influenced by warming seas and are relevant for both shellfish aquaculture and human health, as they continue to cause summer mass mortalities in oysters and also lead to severe human infections (2). Therefore, this work underlines the importance of integrating microbial and environmental perspectives when assessing ocean health and the resilience of coastal ecosystems. Reconstructing past pathogen dynamics provides valuable context for present-day restoration efforts and can inform marine disease management.

#### References:

1. Hayer S, Bick A, Brandt A, Ewers-Saucedo C, Fiege D, Fütting S, *et al.* Coming and going – Historical distributions of the European oyster *Ostrea edulis* Linnaeus, 1758 and the introduced slipper limpet *Crepidula fornicata* Linnaeus, 1758 in the North Sea. *PLoS ONE*. 2019;14(10):e0224249.
2. Sampaio A, Silva V, Poeta P, Aonofriesei F. *Vibriosp.*: Life strategies, ecology, and risks in a changing environment. *Diversity*. 2022;14(2):97.

#### SL-SG-SMBE-06

##### Plasmid domestication in the plant-associated *Pantoea*

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Plasmids play an important role in prokaryote evolution as they drive genetic innovation and ecological adaptation. While typically known for mediating horizontal gene transfer, some plasmids evolved a stable inheritance within their host lineage. Such domesticated plasmids constitute an integral component of the host pangenome, where they contribute to long-term evolutionary trajectories. We investigated the evolutionary fate of two large plasmids (LPP-1 and LPP-2) in the plant-associated genus *Pantoea*. Our analyses reveal that each plasmid originated from a single ancestral acquisition event: LPP-1 in the ancestor of plant-associated *Pantoea*, and LPP-2 in the ancestor of plant growth-promoting species. Both plasmids are now vertically inherited. Additionally, we show that LPP-1 replication is synchronized with that of the chromosome. Comparative

genomics shows that both plasmids encode core gene families, enriched in transcriptional regulators, whose chromosomal or plasmid localization varies among species. This pattern suggests a gradual integration of both plasmids into the host regulatory networks, linking plasmid domestication to the evolution of transcriptional control in *Pantoea*. Phylogenomic analyses show a deep evolutionary split between plasmid and chromosomal core genes, reflecting limited gene exchange between replicons. We further infer that plasmid acquisition introduced genetic redundancy, followed by lineage-specific waves of gene loss, leading to streamlined plasmids of different sizes—especially reduced in *Pantoea* species known as plant pathogens. Together, our findings demonstrate that the domestication of LPP-1 and LPP-2 shaped species divergence and likely ecological specialization in *Pantoea*, providing a model for how plasmids can transition from mobile elements to permanent, adaptive replicons in bacterial genomes.

## Functional Genomics & Bioinformatics

### SL-SG-FGBI-01

#### Elucidating Functional Dark Matter through Independent Component Analysis

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Annotating protein sequences according to their biological functions is one of the key steps in understanding microbial diversity, metabolism, and evolutionary histories. However, even in the best-studied prokaryotic genomes, not all proteins can be characterized by classical *in vivo*, *in vitro*, and/or *in silico* methods – a challenge rapidly growing alongside the advent of next-generation sequencing technologies and their enormous extension of 'omics' data in public databases. These so-called hypothetical proteins (HPs) represent a huge knowledge gap and hidden potential for biotechnological and biomedical applications. Opportunities for leveraging the available 'Big Data' have recently proliferated with the use of artificial intelligence<sup>1</sup>.

Independent Component Analysis (ICA) has proven to be a powerful unsupervised machine learning method for decomposing transcriptomic data across different experimental conditions into independently modulated gene sets (so-called "i-modulons"), thereby revealing underlying regulatory structures that are associated with functional context<sup>2</sup>. Previous work in *Escherichia coli* K-12 demonstrated that ICA captures biologically meaningful transcriptional modules and can aid in the successful annotation of HPs in this strain, confirmed *via* experimental feedback loops<sup>3</sup>. Building on these studies, we here use ICA to study a comprehensive *Escherichia* pan-genome, integrating expression-derived i-modulons with strain-specific genomic variation, thereby identifying core and accessory regulatory modules to characterize HPs beyond strain K-12. We further assess whether functional annotations can be transferred across phylogenetic scales, from species within *Escherichia* (*E. coli* and *Shigella*), to related genera (*Salmonella*), and even across distant phyla (Bacillota). This

effort established ICA as a framework to assist in functionally annotating HPs and thus systematically elucidating functional dark matter.

### References

1. Arden, Z. et al. Elucidating the functional roles of prokaryotic proteins using big data and artificial intelligence, FEMS Microbiology Reviews, Vol 47, Issue 1, Jan 2023.
2. Sastry, A.V. et al. The *Escherichia coli* transcriptome mostly consists of independently regulated modules. Nat Commun 10, 5536, 2019.
3. Chakraborty, S. et al. Deciphering the proteome of *Escherichia coli* K-12: Integrating transcriptomics and machine learning to annotate hypothetical proteins. Comput Struct Biotechnol J. 2025.

### SL-SG-FGBI-02

#### BakRep – A searchable large-scale web repository for bacterial genomes, characterizations and metadata

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### Introduction

Bacteria represent fascinating research subjects across numerous disciplines, and whole-genome sequencing (WGS) has become the cornerstone of modern microbiology. The rise of affordable sequencing technologies has dramatically accelerated bacterial WGS, introducing new challenges related to data accessibility, computational demands, heterogeneous analysis workflows, and overall scientific usability. Hence, the vast amount of available bacterial genomic data and information itself has become a severe challenge.

### Objectives

To address these issues, Blackwell et al. [1] released a uniformly processed dataset of 661,405 bacterial genome assemblies. Building on this foundation, we characterized and annotated all genomes, developed a web-accessible bacterial data repository integrating genomic, taxonomic information as well as metadata. To that regard, we expanded and refined the dataset by computing assembly statistics, performing a robust taxonomic classification using the Genome Taxonomy Database [2], conducting multilocus sequence typing (MLST), and annotating all assemblies with Bakta [3].

### Results

In collaboration with the global AllTheBacteria [4] initiative, we processed an additional 1,932,812 assemblies, bringing the total number of characterized genomes to 2,438,386. All results are uploaded to a cloud storage, and made publicly accessible and searchable via the BakRep repository: <https://bakrep.computational.bio>. By providing a consistently processed and comprehensively annotated dataset, BakRep addresses major challenges in microbiological research, including data integration, standardization, and practical usability. This large-scale yet easily accessible genome repository enables researchers across diverse disciplines to utilize its extensive genomic collection as a foundation for

focused analyses. With its intuitive interface and rich dataset, BakRep represents a valuable resource for the scientific community, supporting a broad range of comparative and clinical studies and driving new advances in microbial genomics.

## References

1. <https://doi.org/10.1371/journal.pbio.3001421>
2. <https://doi.org/10.1093/bioinformatics/btz848>
3. <https://doi.org/10.1099/mgen.0.000685>
4. <https://doi.org/10.1101/2024.03.08.584059>

## SL-SG-FGBI-03

### From sponges to the sea – Creating functional understanding and sustainable utilization of marine microbiomes in the age of AI

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With advances in sequencing technologies, the amount of available sequence data has increased exponentially. This led to a recent paradigm shift from exotic, expensive expeditions to the computational analysis of already available data. Nowadays, genetic information about most environments is just one click away, though to varying degrees. The challenges lie in meaningful interpretation and exploitation to achieve a functional understanding of biological interactions and to extract promising putative biocatalysts. In traditional enzyme discovery sequence alignment and structure prediction are used to identify targets for heterologous expression. Machine learning (ML) algorithms now enable us to pinpoint specific enzyme characteristics (pH optimum, temperature tolerance, ...) and select relevant candidates for biotechnological applications before cloning.

We have compiled several thousand metagenome-assembled genomes (MAGs) from marine sponges, extreme habitats, and other marine biofilms to investigate their functional repertoire and identify enzymes with relevant properties for biotechnological applications. We focused here on enzymes for the degradation of natural (chitin, collagen, cellulose, keratin, fucose) and non-natural polymers (plastics) as well as carbon-fixing enzymes. To conduct this work, we created an enzyme discovery pipeline starting with Hidden Markov Model profiling of MAGs, dereplication using CD-HIT, similarity network analyses (EFI-EST), and categorizing the hits utilizing different ML tools (ThermoProt, TemBERTure, EpHod) with respect to specific characteristics before finally cloning the candidates into a suit of different expression vectors for *Escherichia coli* using FX cloning. This pipeline enabled us to discover a marine genus of plastic-degrading microbes, of which all members contain putative polyethylene terephthalate (PET) degrading enzymes. Further, we functionally expressed an archaeal xylanase from a Red Sea brine pool and identified and cloned RubisCOs, chitinases, collagenases and others from sponge microbiomes.

## SL-SG-FGBI-04

### Application of the iModulon-approach for microbial proteomics – A case study on unraveling complex multi-parametric proteomic data by independent component analysis

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Large scale proteome data sets harbor immense amount of valuable biological information and, in theory, allow for the understanding of microbial physiology, adaptation to stress conditions, and host-pathogen interactions. However, data analysis and interpretation are often not straightforward, especially when investigating proteomic data sets recorded from multi-parametric experimental set-ups (e.g., multiple mutants, growth phases, and experimental conditions). Recently, the iModulon-approach was introduced for transcriptomics to identify co-regulated genes in an unbiased manner (Sastray *et al.*, 2019; doi: 10.1038/s41467-019-13483-w). The approach was shown to be powerful for a wide range of bacteria and could detect so-called regulon-associated i-modulons.

We demonstrate that this approach is also applicable to identify groups of proteins with similar profiles across samples (Busch *et al.*, 2025; doi: 10.1101/2025.09.04.674172). The effect of ClpX deficiency in the opportunistic pathogen *Staphylococcus aureus* was assessed during infection-relevant stress conditions. The protease ClpXP is crucial for general and targeted proteolysis and thus for protein homeostasis. Mass spectrometry-based proteome profiles of *S. aureus* HG001 wild-type, an isogenic  $\Delta clpX$  mutant, and a complemented strain were recorded in data-independent acquisition mode under control conditions as well as iron and oxygen limitation in the exponential and stationary growth phase. Classic pair-wise statistics of the sample conditions revealed specific ClpX- and stress-dependent changes, which are difficult to interpret in combination. To unravel the different influences, an iModulon-based analysis was performed, which resulted in 32 independent components. Overall, the study identified a set of 24 robust stress-independent but ClpX-dependent modulated proteins, and additionally stress-dependent influences were unraveled by the iModulon-approach. These analyses revealed a role of ClpX in e.g., cell division and prophage activation as well as ClpX-dependent stress-specific effects, such as reduced induction of the heme uptake system under iron limitation and a dampened Rex-mediated response to oxygen limitation. The results are in line with an observed attenuation of the  $\Delta clpX$  mutant in infections and drastically reduced intracellular replication of the mutant. However, high intracellular persistence of the  $\Delta clpX$  mutant highlights the complex role of ClpX for bacterial fitness and virulence.

## SL-SG-FGBI-05

### Mining Natures sequence diversity reveals non-explored lignin-degrading laccase

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Lignin is one of the most abundant biopolymers on Earth and a defining component of plant cell walls. Its highly recalcitrant nature makes its degradation into smaller aromatic units challenging. In nature, lignin depolymerisation and its mineralisation is carried out by wood-decaying fungi and bacteria. These organisms secrete a wide range of enzymes, including laccases which oxidise a variety of compounds. Despite being widely distributed in plants, fungi and bacteria, with similar 3D structures and highly conserved copper binding sites, bacterial laccases have been largely overlooked (Panwar et al. 2023; Guan et al. 2018; Xu et al. 2018). As functional characterisation is oftentimes the major bottleneck, we develop a toolbox for yeast surface display to experimentally screen laccase activity in a high-throughput manner. This work reveals an unexplored laccase from metagenomic data, identified through comparative genomics and structure-based comparisons. We benchmarked a metagenome derived laccase against the small laccase from *Amycolaptosis* sp. (slacAmyco). slacAmyco has been successfully used for delignification. We then through HPLC-MS validated the activity of the metagenome derived laccase and an evolved version of the slacAmyco on Kraft lignin. Our results showed an increase of aromatic monomers and oligomers, showing the potential hidden in bacterial laccases for the depolymerisation of lignin. This advances our understanding of the microbial repertoire of lignin turnover and implements yeast surface display as a whole-cell biocatalyst, thereby broadening the enzymatic toolkit for carbon cycling and industrial biotechnology.

Panwar, V., Lzaod, S., & Dutta, T. (2023). Thermostable bacterial laccase: catalytic properties and its application in biotransformation of emerging pollutants. *ACS omega*, 8(38), 34710-34719.

Guan, Z. B., Luo, Q., Wang, H. R., Chen, Y., & Liao, X. R. (2018). Bacterial laccases: promising biological green tools for industrial applications. *Cellular and Molecular Life Sciences*, 75(19), 3569-3592.

Xu, R., Zhang, K., Liu, P., Han, H., Zhao, S., Kakade, A., ... & Li, X. (2018). Lignin depolymerization and utilization by bacteria. *Bioresource technology*, 269, 557-566.

## SL-SG-FGBI-06

### The disinfectant tolerome of *Escherichia coli*

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The ever increasing global use of disinfectants bears the risk of selecting for tolerant bacteria that are able to tolerate disinfection. The genetic determinants and molecular mechanisms of disinfectant tolerance are not well understood. Knowledge of the genetic determinants that influence the tolerance to disinfectants - the disinfectant tolerome - could facilitate the understanding of the molecular mechanisms and enable genetic surveillance. Here, we performed a whole-genome CRISPR-interference screen to identify genes that change the survival of *E. coli* when exposed to lethal concentrations of eight different, widely used disinfectants. Using complementary data- and hypothesis-driven approaches we identify a subset of genes which are then further investigated experimentally. We delineate the disinfectant tolerome, consisting of hundreds of genes that alter the ability of *E. coli* to survive lethal disinfectant stress. In addition to canonical stress resistance genes that are important for the survival of multiple disinfectants, we find a number of genes involved in metabolism and cell wall synthesis to be multi-tolerance determinants. Importantly, we identify opposite-effect genes that, while conveying tolerance against one disinfectant, sensitize *E. coli* to other disinfectants. These findings could pave the way for genetic surveillance of disinfectant tolerance in hygiene sensitive environments and inspire intervention strategies that prevent the emergence of genetic disinfectant tolerance

## SL-SG-FGBI-07

### Transcriptome regulation by DksA, Mfd, and RapA in *Escherichia coli* using end-enriched RNA sequencing

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RNA synthesis carried out by RNA polymerases (RNAP) is an essential first step of gene expression. Its regulation is crucial for adapting to changing environments and for switching between growth phases. While transcription regulation (e.g., by sigma factors) is well-characterized, the specific roles of other associated factors, such as RNAP-binding transcription factor DksA, transcription-repair coupling factor Mfd, and RNAP-recycling factor RapA, are less well understood. Furthermore, quantifying the contributions of RNAP pausing and termination to setting mRNA expression levels across different genes and operons remains a challenge. Our toolkit of high-resolution nascent- and total-RNA sequencing, including Rend- and 3' end-seq, enables us to assess RNA expression levels, map transcription unit architecture with differential 5'- or 3'-end usage, and determine RNAP occupancy during transcriptional pausing and termination. With this, we previously quantified the proportion of nascent, mature, and decaying RNAs and found that the *Escherichia coli* transcriptome harbors a substantial amount of decaying transcripts, limiting the functional portion of the transcriptome with respect to translation (Herzel et al. NAR 2022).

Here, we apply this approach to transcriptome regulation by DksA, Mfd, and RapA in exponential and early stationary phases. To this end, we deleted each gene using the  $\lambda$ -Red recombination system, probed strain growth in defined minimal and rich media under varying cultivation conditions, and performed Rend-seq on these deletion and respective control strains in exponential and early stationary phases ( $OD_{600} \approx 0.3$  and  $1.7$  in LB, respectively). Consistent with DksA's role as a ppGpp-coordinated RNAP regulator, the  $\Delta dksA$  mutant displayed about a two-fold slower growth in minimal medium. More subtle effects were observed for  $\Delta rapA$  and  $\Delta mfd$  in both minimal and defined media. Differential gene expression analysis (absolute  $\log_2$ -fold change  $> 1$ , FDR  $< 0.01$ ) shows alterations of overall RNA expression for 2 and 8% of genes in  $\Delta dksA$ , 5 and 2% in  $\Delta rapA$ , and 1 and 2.5% in  $\Delta mfd$  in exponential and early stationary phase, respectively. In some instances, differential expression is linked to 5'-3' RNA gradients, suggesting altered Rho-dependent termination, or the use of different transcription start sites and terminators. This prompts future mechanistic analyses of these protein factors and their quantitative contributions to regulating transcription output.

## Microverse meets MibiNet

### SL-MIBI-01

#### Parasitic *Cand. Gracilibacteria* (Candidate Phyla Radiation) from groundwater exhibit a unique host infection strategy

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The Candidate Phyla Radiation (CPR) superphylum comprises an estimated 25% of all bacterial diversity. The strongly reduced genomes of its members suggest primarily host-dependent lifestyles. However, cultivation-based, mechanistic insights into host-CPR interactions have so far been restricted to the *Cand. Saccharimonadia* lineage. We employed a recently obtained stable enrichment culture of a member of *Cand. Gracilibacteria* (BD1-5) from oligotrophic groundwater to (i) elucidate its growth dynamics and mode of host infection, and (ii) identify the genetic traits underlying its putative parasitic lifestyle. The culture was grown under anoxic, nitrate-reducing conditions with hydrogen or acetate, resulting in a co-enrichment of *Cand. Gracilibacteria* strain FG1 and its host *Azonexus* sp., together accounting for more than 99% of the community. Growth experiments and scanning electron microscopy showed that *Cand. Gracilibacteria* strain FG1 lives epibiotically on its host and divides with a minimum generation time of less than four hours, forming extensive host-attached cell stacks. Live microscopy coupled to microfluidics under anoxic conditions revealed that these cell stacks play a key role in the infection process with a successive transfer of the apical cells to new host cells, enabling infection spread to multiple new host cells within less than ten hours. Infection by *Cand. Gracilibacteria* appeared to suppress host cell division, while

live/dead staining suggested that the infected host cells remained metabolically active, possibly allowing the parasite to exploit host resources for its own proliferation. Absence of complete pathways for amino acid biosynthesis and central carbon metabolism in the genome of *Cand. Gracilibacteria* strain FG1 indicated strong host dependence. Its genome also shared multiple features of a parasitic lifestyle with *Cand. Gracilibacteria* and *Cand. Absconditabacteria*, including type II and type IV secretion systems or pili, toxin formation, and enzymes mediating host cell wall and membrane penetration. Our findings demonstrate a novel, cell-stack mediated mode of host infection by *Cand. Gracilibacteria*, which is in strong contrast to the previously reported infection mode of other CPR representatives that involves free-living stages. Complementing the existing, rare cultivation-based studies of CPR members, our results highlight the remarkable diversity of the CPR in terms of cell morphology, lifestyle, and infection strategy.

### SL-MIBI-02

#### Microplastic particles and plastic additives promote biofilm formation, virulence and antibiotic resistance in *Vibrio cholerae*

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Plastic waste is ubiquitous in ecosystems and poses an increasing ecological challenge with unpredictable consequences. Microplastics, i.e. particles smaller than 5 mm, serve as a surface for microorganisms to form biofilms, the so-called "plastisphere", which favors certain species and can thus disturb the ecological balance. The colonization of microplastics by pathogenic bacteria, e.g. the cholera-inducing *Vibrio cholerae*, poses a clinical threat as these bacteria-carrying microplastic particles (MP) can enter the food chain and biofilm-associated bacteria also display increased antibiotic resistance.

To understand the mechanisms underlying biofilm formation of *V. cholerae* on MP, we employed confocal microscopy with automated image analysis, including a microfluidic chip model for live monitoring of biofilm formation and investigation of factors impacting biofilm integrity and an intestine-on-chip model as well as droplet digital PCR.

Our results show a robust formation of biofilms by *V. cholerae* on MP which enhances the attachment of MP to the epithelium in the intestine-on-chip. Considering that the continuous degradation of plastic debris in the environment causes leaching of chemical plastic additives such as bisphenol A (BPA), we further investigated the effects of BPA on the formation of these microplastic associated biofilms. Here, we observed that BPA triggers *V. cholerae* to form thicker and more enduring biofilms on MP. Applying transcriptome analysis, we identified a distinct response of *V. cholerae* to the presence of BPA, such as increased

resistance to  $\beta$ -lactam antibiotics, suggesting that BPA may be a key contributor to the negative impacts of biofilms associated with microplastics.

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#### SL-MIBI-03

##### Algal-fungal symbioses and their molecular foundations

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The transition of plants to land over 450 million years ago was aided by mutualistic relationships with fungi. Since then, plants have coevolved with fungal microbes, resulting in a continuum of interactions between extant plant and fungal species, ranging from mutualism to parasitism. Fungal colonization across this diversity of interaction types elicits the activation of complex plant host regulatory programs to accommodate or resist invading fungi and promote symbiosis or prevent colonization. Phylogenomic analyses have revealed the presence of key molecular components for symbioses in the closest algal relatives of land plants and even distantly related chlorophytic algae. While Chlorophytes are known to interact with fungi spanning a behavioral continuum, the best-described examples of fungal-algal interactions are mutualistic lichen symbioses. Since lichens are difficult study systems for functional analyses and other types of algal-fungal interactions have mostly been disregarded, the molecular basis of many of algal-fungal interactions remains largely unexplored. We previously established an experimental system in which highly divergent algal lineages engage in differential interactions with a soil-dwelling fungus. Using a combination of genetic, genomic and physiological techniques, our future research will reveal which key molecular components of algal signalling pathways govern such interactions with fungi.

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#### SL-MIBI-04

##### Cross-kingdom interaction study identifies a novel $\beta$ -lactone from *Ustilago* as a potent inhibitor of itaconate metabolism in *Pseudomonas*

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Itaconic acid (ITA) is a universal metabolite across kingdoms, acting as a key regulator in mammalian immune response and plant metabolism, and serving as an antimicrobial compound by targeting essential bacterial enzymes. This defensive role has driven the evolution of pathogens such as *Yersinia pestis* and *Pseudomonas aeruginosa*, which have developed mechanisms to detoxify and exploit ITA as a carbon source. Interestingly, certain plant-pathogenic fungi, such as *Ustilago*, convert ITA into the derivatives 2-hydroxyparaconate (2-HP) and itatartarate (ITT). The ITA degradation operon of *P. aeruginosa* includes genes not directly involved in degradation but essential for tolerance to these fungal-specific derivatives.

We aim to elucidate the ecological function of these derivatives and their impact on bacterial metabolism and tolerance. By introducing the ITA degradation operon (PA0878-0886) from *P. aeruginosa* into the non-pathogenic model strain *Pseudomonas putida* KT2440, we pinpointed Rdo<sub>PA</sub> (PA0880) as a key tolerance factor. Interestingly, we found homologs in the ITA synthesis clusters of *Ustilago maydis* and *Aspergillus terreus*, and complementation and

biochemical assays confirmed that these evolutionarily distant enzymes share lactonase activity. The substrate, a previously overlooked  $\beta$ -lactone isomer of the  $\gamma$ -lactone 2-HP, was identified and structurally confirmed via 2D-NMR, which we designated Ustilactone (UL). The production and purification of both lactones were optimized, yielding gram-scale quantities of UL and 2-HP at ~89% and ~92% purity, respectively. Inhibition studies revealed that 2-HP has almost no antimicrobial effect (IC<sub>50</sub> >100 mM), while UL potently inhibits ITA metabolism in *Pseudomonas* (IC<sub>50</sub>  $\approx$  250  $\mu$ M). Co-expression with the lactonase rdo<sub>PA</sub>, which catalyzes the ring-opening of UL to the less harmful compound ITT, increases the IC<sub>50</sub> by over 90-fold. Combined with the finding that UL inhibits ITA degradation, this catalytic activity clarifies its physiological relevance in *Pseudomonas*.

Future studies will address the molecular targets of UL, building on adaptive evolution experiments that identified a mutation in an acyl-CoA thioesterase associated with increased tolerance. We hypothesize that this enzyme plays a so-far unknown role in ITA metabolism and may be directly inhibited by UL. These findings highlight a fungal-bacterial arms race with novel inhibition and tolerance strategies beyond human-pathogen interactions.

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## Workshop of Applied Sciences

#### SL-HAW-01

##### Deep insight in microbiomes of technical habitats by metaproteomics

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In the last two decades, metaproteomic has been established as powerful tool in microbial ecology. Whereas most metaproteomic studies focus on microbiomes of humans, animals or environmental samples, metaproteomics of technical habitats is underrepresented although it can provide deep insights into microbiomes and delivers evidence for optimization of biotechnical processes.

The presentation will highlight exciting results from agricultural biogas plant, UASB reactors for anaerobic wastewater treatment, bioreactors for bio-methanation and industrial biofilters. The presentation also underlines the necessity to combine metaproteomics with metagenomics and metabolomics to exploit its full potential in microbiome research.

Metaproteomics is a quickly developing technology. Recent advances enable the detection or enrichment of newly synthesized proteins by labeling with stably isotopes (protein-SIP) or by labeling with non-canonical amino acids and subsequent click chemistry, respectively. Both approaches provide access to functions of low-abundant microbes in complex microbial communities.



## SL-HAW-02

### Why seek far afield when the good is close by - The Microbiome of Things

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Microbiologists often search for their research subjects in remote environments such as Arctic soils, deserts, deep-sea habitats, or volcanic regions. Yet, much like natural ecosystems, the artificial surfaces of everyday man-made objects also host microbial communities of remarkable structural and functional diversity. According to the Microbiome of Things concept [1], these artificial products are not necessarily dominated by human-associated microbiomes; instead, they may harbor unique microbial populations shaped by specific—and often extreme—environmental selection pressures. Such pressures promote adaptive mechanisms including biofilm formation, sporulation, and pigmentation.

A deeper understanding of the structure and function of these microbial communities is not only of fundamental microbiological interest but also carries important practical implications for human health and well-being, giving it substantial economic relevance.

This presentation highlights the structural and functional microbial ecology of several artificial domestic devices, such as kitchen sponges [2,3] and household washing machines [4,5], and explores the extreme conditions that shape their microbiomes. For example, washing machines constitute highly dynamic environments due to strong fluctuations in moisture, temperature, nutrient availability, surfactants, and disinfectants. Microbial communities inside these appliances develop resilient, often conspicuously colored biofilms whose composition varies by location within the machine. These biofilms are believed to contribute to machine and laundry malodor and may even harbor and transmit pathogenic microbes during washing. Consequently, effective biofilm removal in washing machines is of great hygienic and commercial importance [6].

[1] Satari et al. (2023), *Microorganisms* 11(6):1507; [2] Jacksch et al. (2022), *Microorganisms* 8(5):736; [3] Brandau et al. (2023), *Arch Microbiol.* 204(7):363; [4] Jacksch et al. (2019), *Microorganisms* 8(1):30; [5] Jacksch et al. (2021), *Microorganisms* 9(5):905; [6] Jacksch et al. (2025), *J Microbiol Methods* 235:107154

## SL-HAW-03

### Understanding microbial key-players in biogas technology

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Anaerobic digestion AD is a well established technology worldwide, mainly to reduce and stabilize waste and wastewater. Biogas is produced via microbial metabolism, by which organic matter is degraded in a series of sequential and parallel steps by Bacteria and Archaea in a trophic cascade. Biogas is still considered as an emerging technology for renewable energy production worldwide, with many countries relying on its implication to achieve climate goals, such the European Commission's aim to reduce greenhouse gas emissions by 55% by 2030 or even the

energy neutral operation of wastewater treatment plants WWTPs until 2045. Many biogas digestors are often running well below their potential production capabilities due to different reasons. Understanding the needs of the microbial key-players during AD could help to optimize the microbiological interaction and metabolism and to avoid process disturbances and failure.

Metagenomics and metatranscriptomics in combination with knowledge about operational conditions of lab- or full scale biogas digestors (organic loading rate, type of substrate, pH shifts, biogas amount and composition, process disturbances) or addition of co-substrates or materials, supporting direct interspecies electron transfer could be applied to get a deeper insight into the microbial community composition (T. Jeske & Gallert 2021, S. Dykma & C. Gallert 2022). Also shifts or changing metabolic pathways could be elucidated e.g. during thermophilic digestion of biowaste, syntrophic acetate oxidation replaces acetoclastic methanogenesis (Dykma et al. 2020).

Both, omics-technologies and enrichment cultivation of the complex anaerobic population will reveal insight into a stable core community which are common for biogas digestors treating similar organic residues but will deliver also diverse or novel uncultured e.g. syntrophic bacteria. In environmental microbiology, modern and classical methods should be used to make reliable statements about metabolic capacity, microbial complexity, and ultimately ecosystem services. Understanding the microbial reactions and co-occurrence means influencing the technical process of AD.

Dykma et al. *Microbiome* (2020) 8:105

Jeske & Gallert *Bioengineering* (2021) 8(12), 197;

Dykma & Gallert *Environ Microbiol Rep* (2022) 14(4), 664–678

## SL-HAW-04

### Mechanoenzymatic Process Development for Disaccharide Synthesis – Bridging Biocatalysis and Process Intensification

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**Introduction** Disaccharides such as cellobiose, laminaribiose or sophorose serve as structural, functional, or prebiotic molecules, but their enzymatic production in aqueous systems often suffers from low yields due to thermodynamic and hydrolytic limitations. Mechanoenzymatic approaches offer an emerging alternative by coupling nearly solvent-free biocatalysis with mechanical energy input, thereby promoting synthetic reactions under mild yet intensified conditions.

**Goals** The project aims to develop a versatile mechanoenzymatic platform for the synthesis of tailor-made disaccharides using a temperable oscillating ball mill. Core objectives are (i) to understand enzyme stability and activity under mechanical stress, (ii) to optimize reaction parameters (frequency, milling time, temperature, enzyme loading), and (iii) to establish structure–function correlations between mechanical energy input and glycosidic bond formation.

**Materials & Methods** Commercially available  $\beta$ -glucosidases were tested in terms of stability, overall productivity and product spectrum under mechanoenzymatic conditions. The process was optimized in the ball mill in the ml-scale by variation of temperature, frequency, ball size, temperature, enzyme addition, substrate concentration, liquid content and rasing cycles. Products were analyzed by HPLC-RI and HPLC-MS, and NMR. In order to evaluate the performance of the process, the results are compared directly with classic in-solution reactions.

**Results** Mechanochemical activation significantly enhanced disaccharide synthesis compared to aqueous reference reactions, with higher yields and productivities for  $\beta$ -1,4- and  $\beta$ -1,3-linked glucose dimers. Process parameters influenced regioselectivity and reaction kinetics, enabling controlled formation of target linkages.

**Summary** This work demonstrates how biotechnological research at Universities of Applied Sciences can bridge enzyme technology with process engineering. The mechano-enzymatic approach integrates biocatalysis, green chemistry, and materials science, complementing the applied research environment at Kaiserslautern University of Applied Sciences. Together with complementary expertise in protein analytics (Prof. Peter Groß) and extremophile biotechnology (Dr. Michael Lakatos), this project contributes to the development of innovative, sustainable bioprocesses within the STAMP research focus "Sustainable Materials, Products and Processes".

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#### SL-HAW-05

##### **Skin microbiome and probiotic cosmetics – new approaches to dermatological diseases**

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Atopic dermatitis (AD) is a widespread chronic inflammatory skin disease whose pathogenesis is closely linked to dysbiosis of the skin microbiome. Furthermore, clinical studies have shown a link between the presence of *Staphylococcus aureus* and the severity of AD. We conducted three studies. First, in a randomized, double-blind study, the efficacy of synbiotic baths – consisting of a defined mixture of six viable lactic acid bacteria (LAB) and prebiotics – was investigated over a period of 14 days in comparison to baths containing only prebiotics and placebo baths. A total of 22 AD patients were divided into three groups: synbiotics (n = 7), prebiotics (n = 8), and placebo (n = 7). Disease activity was assessed using the SCORAD score, patient questionnaires, and 16S rRNA amplicon sequencing before and after treatment. In a second study, an optimized synbiotic bath was applied to additional patients with AD. Finally, in a third study, an ointment with the synbiotic recipe of the second study was used to test a further application form. The results of all three studies showed a significant reduction in the SCORAD score with daily use of synbiotic treatments over a course of 14 to 21 days. Particularly noteworthy is the improvement in eczema, pruritus, and skin dryness as well as the decrease of *S. aureus* by about 83% and enrichment of LAB in the skin microbiome after baths. This study highlights the potential of synbiotic skin applications as an innovative therapeutic approach for the relief of AD and the targeted modulation of the skin microbiome.

#### SL-HAW-06

##### **Tiny Predators, Big Impact: Harnessing Amoebae for Eco-Friendly Crop Protection**

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Fungal crop diseases cause massive agricultural losses worldwide, amounting to billions of euros each year. Although chemical fungicides remain the dominant means of control, their extensive use raises growing ecological and health concerns. Biological control agents are already a valuable alternative, but are limited to only few bioprocessable (micro-)organisms. We are currently addressing this challenge by developing the fungivorous amoeba *Protostelium aurantium* into a biological alternative to conventional fungicides, aiming to reduce or replace harmful chemical treatments in crop protection.

*P. aurantium* is leaf-dwelling micropredator that feeds on a wide variety of ascomycetous and basidiomycetous fungi, including fungal plant pathogens. However, any future application in field trials will require a large set of biotechnological and bioprocess developments. We have previously focused on establishing methods for cultivation, large-scale production, formulation, and long-term storage of amoeba cysts, as well as assessing their efficacy against major plant-pathogenic fungi *in vitro* and *in planta*.

In the first project phase, we successfully developed cultivation techniques in liquid cultures and bioreactors, achieving high cell densities under controlled conditions in a dual cultivation approach based on a feeding yeast and subsequent inoculation of the amoeba. Collaboration with e-nema GmbH provides the technological infrastructure for upscaling and pilot-scale production, enabling first field trials at a small scale. Initial results demonstrate that the amoeba maintains viability as drought-resistant cysts even after long-term storage and can suppress disease development in two distinct plant fungal pathogens.

This interdisciplinary collaboration between academia and industry combines fundamental biological research with applied biotechnology to enable a novel and sustainable approach in biological plant protection. *P. aurantium* may thus represent an environmentally friendly alternative to chemical fungicides in certain crops.

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## **Industry meets Academia**

#### SL-IMA-01

##### **Leveraging AI to learn evolutionary patterns across bacteria**

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The advent of deep learning architectures in genomics has revolutionised biology, e.g. AlphaFold for protein folding. Biological language models, like ESM, show intriguing emergent properties (such as state-of-the-art variant eCect prediction), provide the foundations for a growing number of tasks in molecular biology, and allow the design of new biomolecules.

I will showcase the potential of such approaches for bacterial genomics by presenting two models we recently developed:

1) A convolutional neural network called GeneBac that operates directly on the gene nucleotide sequence enabling accurate antimicrobial resistance and gene expression prediction, further boosted by transfer learning. 2) A foundational genome language model called Bacformer, which can extract common representations from bacterial genomes across broad evolutionary distances. The model exhibits emergent properties (such as knowledge of phylogenetic relationships and protein-protein interactions) and has impactful applications in synthetic biology (by designing new genomes) and ecology (by predicting bacterial lifestyle).

Together, these approaches demonstrate how exploiting the latest advances in AI can uncover fundamental principles of bacterial evolution. This will pave the way for clinical applications, such as antimicrobial diagnostics, as well as serve as a discovery engine for bacterial genomics.

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## SL-IMA-02

### Accelerating Cell & Gene Therapy Development Through Artificial Intelligence at Roche

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Roche is leveraging Artificial Intelligence (AI) to overcome the extreme biological and manufacturing complexities inherent in Cell and Gene Therapy. By integrating AI as a "research co-pilot," Roche aims to bridge the gap between biological hypotheses and clinical reality, ensuring these life-changing therapies reach patients more efficiently and at a sustainable cost to society.

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## Short Lectures

### *Environmental microbiology and ecology*

#### SL-EME-01

##### When interactions change: Resolving temporal dynamics of marine bacterial interactions

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Within Earth's manifold microbiomes, microbial communities both shape and are shaped by their environment through the exchange of molecules. Individual bacterial cells interact via metabolites, signaling molecules, and other bioactive compounds that can impact growth. Through these interactions, microbes collectively modify their environment, altering nutrient availability, and local conditions. These environmental changes, in turn, can shift the nature of microbial interactions, creating a dynamic feedback between community activity and environmental state. Yet, we lack a mechanistic framework that links microbial growth,

environmental modification, and the resulting shifts in interaction dynamics captured across diverse species and conditions. So far, most studies characterized interactions only from endpoint outcomes, overlooking how transient feedbacks accumulate over time.

To gain insights into these dynamic feedbacks, we implemented the kChip, a high-throughput microdroplet platform that enables highly combinatorial and time-resolved phenotyping. This setup allows us to quantify how environmental modification and strain-specific interaction motifs feed back on interspecies outcomes across 30 carbon sources. By tracking the dynamics of simple marine bacterial communities under diverse metabolic conditions, we reveal how interactions change over time within and across environments.

We find that bacterial interactions are inherently dynamic, shifting between beneficial, competitive, and neutral over ecological timescales. These dynamic shifts follow distinct and quantifiable trajectories, exhibiting characteristic temporal patterns that differ across environmental contexts. Across 450 tested conditions, we systematically quantify these dynamic trajectories, revealing how specific combinations of interaction motifs give rise to reproducible co-culture dynamics. Linking these data to a consumer-resource model uncovers systematic differences in the prevalence and temporal progression of interaction types on simple versus complex substrates.

Together, our findings highlight the importance of quantifying the temporal dynamics of interactions and disentangling how environmental modifications affect species relationships. Our work provides a foundation for the mechanistic dissection of interactions and a deeper understanding of how interaction dynamics in microbial communities scale up to community-level processes.

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#### SL-EME-02

##### Predicting Antifouling Paint Particle Contamination based on 16S rRNA Gene Sequencing Data using Random Forest-Based Machine Learning

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Antifouling paints often contain biocides designed to inhibit biological growth, and antifouling paint particles (APPs) have been previously shown to affect microbial communities in sediment. When painted surfaces wear, APPs end up in sediment. Thus, not only are these microplastic pollutants widespread in coastal sediments but they also have the potential for significant microbial disruption. Given the importance of sediment microorganisms for sediment biogeochemical processes, APP pollution is highly important and in urgent need of better monitoring. Yet typical methods for monitoring APP presence can be specialised and challenging and thus, alternative methods using simple, standardised and universal approaches, such as 16S rRNA amplicon sequencing, would be highly valuable. This study used a field-based mesocosm approach incubating sediment with APPs in mesh chambers anchored in sediment for 60 days to train a random forest-based (supervised) machine learning model able to predict APPs presence in sediment based on changes in the 16S microbial community data. The model correctly predicted 100% APP-presence samples and 83.3% APP-absence samples in the incubation testset. To determine real-world applicability of the model, samples from

14 different sites along the Baltic Sea coastline and Warnow estuary in NE Germany were collected and APP-presence was pre-determined using SEM-EDX spectroscopy. Remarkably, despite being trained on a relatively small amount of samples (n=57) from just 1 location at 1 time of year, across these 14 different geographic locations and at a different time of year, the model correctly assigned APP-absence status to all APP-absent sites, and correctly assigned 3 of 5 APP-contaminated sites as having APP contamination. As such, these results confirm it is possible to predict APPs in sediment based on the microbial community, and the "PaintSed" model serves as a proof-of-concept for the further development of machine learning-based predictive tools for environmental monitoring using a small scale targeted experimental mesocosm approach.

### SL-EME-03

#### Beyond culturing: Tracking key species in environmental microbiomes using FISH-FACS and proteomics

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In ecology the adaptation of a microbial community to environmental condition is often described in a comprehensive and generalized manner. However, to understand interactions, functional roles, and environmental adaptability of key microbial clades in a community, we need detailed insights into yet uncultured bacteria within microbiomes. Metaproteomics is an essential approach to investigate microbial metabolic activity in diverse environments. Still, functional analysis of specific microorganisms is often underexplored because of the protein inference problem, caused by sequence homologies among closely related species. This challenge limits our understanding of the role of particular microbes in complex environmental samples.

We have developed a workflow that combines fluorescence in situ hybridization (FISH) and fluorescence-activated cell sorting (FACS) with mass spectrometry-based proteomics to analyse proteins from uncultured bacteria directly from environmental samples. We demonstrate that this workflow allows for reproducible protein identification and quantification after FISH and FACS thereby enhancing sensitivity and coverage on proteome level for targeted bacterial clades. The FISH-FACS-proteomics workflow not only addresses a major methodological gap but also provides a broad application for directly enriching and analysing yet uncultured microbes from their habitat.

### SL-EME-04

#### The fate of DMSP, DMS and DMSO in the Bay of Bengal under anthropogenic influence

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The oceans exhibit positive and negative effects on the earth's climate. While they absorb ~30% of the atmospheric CO<sub>2</sub>, they also emit large amounts of the climate-active gas dimethyl sulfide (DMS). This main source of biogenic sulfur in the atmosphere represents a major component of the global sulfur cycle and is known to enhance the earth's albedo through cloud formation. Its precursor, dimethylsulfoniopropionate (DMSP), is produced as an osmolyte by phytoplankton, which is mainly converted microbiologically to DMS or further oxidized to dimethyl sulfoxide (DMSO). Although the metabolic pathways of natural DMSP/DMS cycling are well known, the role of airborne pollution on these processes is rarely understood. To investigate the effect of these anthropogenic influences on the oceanic organosulfur cycling, we analysed samples from the Bay of Bengal (BoB), collected on RV SONNE during the BIOCAT cruise (SO305). The BoB is surrounded by countries with rapidly growing populations and economics, accompanied by land-based atmospheric pollution. This anthropogenic input is transported to the BoB during the NE monsoon and is combined with pollutants from a major shipping route in the south. Thus, the BoB appears to be a perfect location to understand the complex interactions of organosulfur cycling via air-sea exchange. We therefore analysed microbial communities from different depths along a south-to-north transect in the BoB and related the community structures to concentrations of DMSP, DMS and DMSP within the water column. We further followed the fate of organosulfur from the production of DMSP by phytoplankton over its conversion to DMS and finally to DMSO by using a metagenomic approach. Additionally, onboard incubation experiments were carried out using BoB surface water spiked with pollutants such as aerosol particles and scrubber water from ship exhausts to observe their impact on the microbial communities and especially the production of DMS. By analysing how airborne pollution affects microbial communities in the surface water will help to identify how their metabolism changes the amount of biologically produced trace gases emitted from the ocean back into the atmosphere.

### SL-EME-05

#### From biofilms to infection: Investigation on anthrax infection cycles in rainforest ecosystems using a BSL-3 leaf-model

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*Bacillus cereus* biovar *anthracis* (*Bcbva*) is an emerging pathogen of the *Bacillus cereus* group, that carries the virulence plasmids of classical *Bacillus anthracis* and causes an anthrax-like disease in diverse wildlife species of African rainforests. Although human infections have not been confirmed, exposure is likely through hunting and consumption of "bushmeat", as indicated by seroprevalence studies in local populations.

The transmission routes of *Bcbva* remain largely unknown. While *B. anthracis* typically infects animals via ingestion or inhalation of spores from contaminated soil, arboreal monkey species are also affected by *Bcbva*, suggesting alternative infection pathways. Carrion flies, in which *Bcbva* has been detected, may act as vectors, transferring bacteria and spores from carcasses to leaves and fruits in the canopy, which are then consumed by monkeys.

This led us to hypothesize that biofilms on leaves and fruits may play a possible role in transmission by providing a reservoir for *Bcbva*. Using an *ex vivo* leaf model under BSL-3 conditions, we demonstrated that *Bcbva* can persist and replicate within biofilms on leaf surfaces. Confocal laser scanning and electron microscopy revealed distinct biofilm structures, while colony-forming unit enumeration and qPCR confirmed bacterial proliferation and sporulation. Ongoing analyses aim to characterize biofilm components and matrix by proteomics and fluorescent labeling.

Our findings suggest a novel ecological niche for *Bcbva*, highlighting the potential role of biofilm-associated proliferation on leaves in maintaining anthrax infection cycles in African rainforests.

## SL-EME-06

### Microbiome profiling in airports: Establishing a baseline for future pathogen control strategies in aviation-related indoor environments

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The potential of disease transmission exists in public transportation vehicles, such as trains, buses and airplanes, as passengers usually spend extended time periods in them with limited mobility. With over 9 billion passengers annually, air travel can, therefore, act as a vector for pathogen spread and the transmission of diseases over long distances. Disease transmission can happen via airborne pathogens or highly-touched surfaces, either in the airplane cabin or at the stay at the airport in terminal areas during travels. Documented cases of transmission of pathogens such as SARS-CoV-2 or Influenza have already suggested the risks of infection while traveling. Within the frame of this study, we investigate the temporal dynamics of the airport microbiome to imply mitigation strategies in order to reduce the microbial load within aviation interiors. For this purpose, microbiological sampling campaigns are currently taking place at a German airport. As part of these campaigns, air sampling is being conducted within the airport terminal buildings, using cultivation-based methods followed by microbial identification, for example, by next-generation sequencing. Furthermore, highly-touched surfaces by passengers, including toilet door handles or armrests in waiting areas of the terminals, are swabbed and subsequently tested by fast-detection methods such as loop-mediated isothermal amplification for the occurrence of specific microorganisms, for instance, human pathogens such as the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) organisms. Using environmental microbes that were identified during previous samplings, such as *Pseudomonas stutzeri*, we are investigating their stability and survival on different airplane cabin material samples provided by AIRBUS, as well as antimicrobial surfaces. Also, antimicrobial coatings that can be applied to various materials are being tested, especially in regard to their longitudinal impact on the survival of the organisms. We will present preliminary findings from these tests, highlighting the molecular mechanisms by which these antimicrobial tactics influence microbial stress responses in the organisms. This will be analyzed through diverse approaches, including transcriptomic profiling, as well as complementary molecular microbiology assays.

## SL-EME-07

### Ectomycorrhiza: Response on metal and salt stress

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The mutualistic symbiosis ectomycorrhiza is formed by fungi with roots of shrubs and trees. Especially in times of climate change and forest dieback, it is important for plant health in disturbed areas.

Here, we focused on ectomycorrhiza of the basidiomycete *Tricholoma vaccinum* with spruce (*Picea abies*) using different metals and seepage water from a former uranium mining site in Ronneburg, Thuringia, Germany. We used these treatments to mimic metal and salt stress of contaminated habitats, and to investigate effects on the exchange of nutrients and signals between both partners. This study was based on growth experiments comparing pure culture of the fungus, mycorrhiza and metal and salt stressed fungus and mycorrhiza, expression analyses using RNAseq experiments and qPCR, the analyses of volatiles by SPME fibers and GC-MS, and microscopy.

Reduced growth of mycorrhiza was observed in cultivation on metal containing media. Further effects on volatile and phytohormone production, on glutathione S-transferases are visible, but also on cell wall and surface proteins like hydrophobins. Gene expression changes indicate a reduced plant defense, a rearranged secondary metabolite production, an upregulated water supply and energy production, as well as an increased detoxification in metal and salt stress conditions.

Ectomycorrhiza and interacting organisms in the rhizosphere can be strongly affected by metal and salt stress. This has to be considered in reforestation strategies with mycorrhizal fungi to select fungal strains showing abilities to manage different stresses occurring in the habitat, e.g., using adapted strains to metals.

## SL-EME-08

### Cultivation-based detection of a novel high-GC *Nitrospira* derived from the Argentinian Copahue volcano area

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Nitrification is an essential process within the global nitrogen cycle and also occurs under extreme conditions, such as in geothermal environments. The nitrite-oxidizing group *Nitrospira* represents key nitrifiers in these systems, as several species inhabit hot springs worldwide.

The aim of this study was to enrich and to genetically and physiologically characterize nitrite oxidizing bacteria (NOB) originating from the acidic geothermal area of Copahue.

Recently, the *Chloroflexota*-affiliated bacterium *Nitrolancea copahuensis* was successfully isolated from this site (Spieck et al., 2020), but other NOB have not yet been identified. While the most ubiquitous and diverse *Nitrospira* lineages I, II, and IV are well represented by both cultivated species and environmental MAGs, cultures and genomes of lineages V to VIII are still scarce, highlighting the importance of both cultivation-based and molecular approaches.

In this cultivation-based study, two novel moderately thermophilic *Nitrospira* strains were obtained from the geothermal field Las Máquinas in the Neuquén Province, Argentina. We used temperature stress at the lower and upper growth limits (28°C and 50°C) in combination with manual cell separation to selectively enrich a distinct novel *Nitrospira* species.

Strain Vd2 belongs to the *N. bockiana* lineage V, whereas the second enrichment (E2OT) represents the novel taxonomic lineage VIII, which was only recently introduced by Kop et al. (2025) together with strains from Kamchatka (Kam-Ns4a) and Garga hot springs (Ga3a). The vibrioid morphology of *Ca. N. neuquenica* E2OT is strikingly different from all described, twisted rod-shaped *Nitrospira*. The recent discovery of quorum-sensing genes outside the *Nitrospira* lineage II was confirmed for both Argentinian cultures, which had not been detected in lineage VIII before, and showed distinct branching patterns of AHL synthases. Notably, the genome GC contents of strains Vd2 and E2OT are 60.6% and 69.4%, respectively. The latter is the highest observed for *Nitrospira* to date. All *Nitrospira* strains with a high GC content (>60%) grow well at elevated temperatures (≥37 °C), suggesting that this trait may contribute to their thermostability.

Our study expands knowledge of the taxonomic and genomic diversity of moderately thermophilic *Nitrospira* and provides genomic data for understudied lineages within the *Nitrospirales*.

Spieck et al. 2020. Front. Microbiol. 11, 1522

Kop et al. 2025. ISME J. wraf151

## SL-EME-09

### Effects of Top-Down and Bottom-Up Manipulation on the Microbial Fungal Communities and Food Web in an Agricultural Soil

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Agricultural soils host complex food webs with multiple interacting trophic levels driving carbon and nitrogen cycling. The lowest trophic level, comprising saprotrophic fungi and bacteria, is regulated by resource availability (bottom-up) and predation pressure from higher trophic levels such as nematodes and protozoa. Using a microcosm experiment with agricultural soil, we investigated how fungivorous nematodes and carbon (C)-resource addition affect fungal community structure and the microbial food web. Respiration

data showed that C addition significantly increased respiration, whereas the introduction of fungivorous nematodes reversed this effect, reducing respiration despite C enrichment. ITS amplicon and qPCR analyses revealed that C-addition enhanced fungal abundance with minimal changes in composition, while nematodes nearly eliminated fungi, significantly lowering fungal  $\alpha$ -diversity. Metatranscriptomic data confirmed that bacteria dominated (≈90%), while fungi represented only ≈2% of the total community. Overall, the fungal community forms a small but highly active trophic channel that is strongly regulated by both top-down predation and bottom-up resource availability. This contrasts with the large but less responsive bacterial channel and highlights the crucial fungal role in rapid plant litter decomposition.

## SL-EME-10

### How microorganisms meddle with the cycle of non-essential (radio)metals

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The importance of radiometals and their ecology is often underestimated by society. They are not only a challenge in the decommissioning of nuclear power plants, but uranium and thorium also often occur together with industrially important metals such as rare earth elements in ores. Together with their decay products, these elements are also released into the environment through natural weathering processes and, to a much greater extent, through anthropogenic activities such as mining and the use of phosphate fertilizers. This releases them into the atmosphere, soil, and water, where they pose chemical and radiological risks to ecosystems and human health.

For this reason, our research focuses on the behavior of radiometals in the environment in interaction with bacteria and fungi, as well as on molecular processes that lead to their uptake into cells or binding to biomolecules. These studies aim to improve our understanding of how (radio)metals affect biological systems and to support the development of innovative remediation strategies and improved radiometal-based cancer therapies.

To achieve these goals, we apply state-of-the-art molecular and microbiological, microscopic, spectroscopic, and analytical techniques. Our studies on radiometal interactions with microorganisms range from investigating uranium binding to magnetotactic bacteria as well as the use of uranium by microorganisms as a terminal electron acceptor leading to U(VI) reduction. Furthermore, we demonstrated the incorporation of radionuclides into native functional enzymes. Radiometals are therefore ecologically and socially relevant elements with promising applications.

## SL-EME-11

### Sulfonolipid Biosynthesis in Soil and Gut Bacteroidota

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## Introduction

Sulfobacins are the sulfonated analogs of ceramides and represent bioactive bacterial sulfonolipids that initiate multicellularity colony formation of unicellular protists, modulate the human immune system and are essential for gliding motility. The specific effect depends on the number and position of hydroxylations and desaturations of the core structure. Sulfobacins can constitute up to 30% of membrane lipids in the gliding bacterium *Flavobacterium johnsoniae* and have been verified in a few members of the Bacteroidota. Despite their ecological and host-modulatory roles in gut and soil environments, the key enzymes for sulfobacin biosynthesis and the distribution of the pathway genes remain unknown.

## Goals

Here, we aimed to identify the genes required for sulfobacin biosynthesis and to assess the genetic potential for sulfobacin production across the Bacteroidota.

## Material & Methods

Comparative genomics of ceramide and sulfobacin producers was performed to identify putative sulfobacin biosynthesis genes. This approach highlighted a putative sulfobacin synthase predicted to catalyze N-acylation of the sulfonated sphinganine precursor (capnine) and two monooxygenases possibly mediating distinct hydroxylation steps. These genes were deleted in *F. johnsoniae* using a markerless deletion strategy. Regulation and cellular localization of the biosynthetic machinery were examined via fluorescently tagged variants of the enzymes. Orthologues were surveyed across publicly available Bacteroidota genomes to evaluate pathway distribution. Lipidomics was used to confirm compositional and structural changes in sulfobacin species.

## Results

Deletion of the predicted sulfobacin synthase or either monooxygenase resulted in a gliding-defective phenotype. Lipidomics validated the N-acylation function of the synthase, as its loss caused accumulation of capnine and complete absence of sulfobacins. The sulfobacin profile of *F. johnsoniae* wildtype remained stable irrespective of growth medium. Orthologue screening revealed that the biosynthetic gene set is present in several Bacteroidota taxa present in the gut and soil. Comparative lipid profiling of representative strains showed that the sulfobacin species composition produced by soil bacterium *F. johnsoniae* closely resemble those of the gut bacterium *Alistipes putredinis*.

## Summary

We identified a previously unknown sulfobacin biosynthesis gene and its distribution across gut and soil Bacteroidota strains.

### SL-EME-12

#### Glycine betaine and cobalamin: How two currencies are connected on the ocean's metabolic marketplace

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In the ocean, primary productivity of microalgae can be limited by the availability of cobalamin (vitamin B<sub>12</sub>, Cbl). Globally distributed, bloom-forming algae like *Gephyrocapsa huxleyi* (former *Emiliania huxleyi*) are predicted Cbl auxotrophs since they require Cbl as co-factor for methionine biosynthesis. They rely on exogenous Cbl provided by associated heterotrophic bacteria like *Phaeobacter inhibens* and other *Rhodobacterales*, that can *de novo* biosynthesize Cbl. Cobalt is the central ion of the corrin ring of Cbl and its scarcity in ocean water can limit Cbl biosynthesis. In exchange for Cbl, algae feed organic compounds to the bacteria. Recently, glycine betaine (trimethylglycine, GB), a compatible solute produced by many algae, was proposed to be a key metabolite mediating algal-bacterial interactions. GB catabolism in the *Rhodobacterales* and other abundant marine pelagic bacteria involves a recently reported modular Cbl-dependent demethylase. However, it remains elusive how Cbl-dependent GB demethylation and limited cobalt availability affect the Cbl production in these bacteria and thus algal-bacterial cross-feeding.

Using RNA sequencing of *P. inhibens* grown on GB, we show simultaneously high expression of genes encoding Cbl-dependent and independent GB demethylases. Growth experiments with deletion mutants revealed that *P. inhibens* switches between these two systems depending on the availability of cobalt. With GB we measured a significantly increased Cbl production in comparison to the control with glucose. Limited cobalt availability decreased the Cbl production. Cbl limitation reduced the growth of the alga *Gephyrocapsa huxleyi*, in monocultures which could be restored by co-cultivation with *P. inhibens*. Here, we establish a functional connection between GB and Cbl, two key metabolic currencies exchanged during algal-bacterial cross-feeding, involving genes that are widespread in members of the *Rhodobacterales* and other abundant marine alphaproteobacterial orders.

### SL-EME-13

#### From Field to Fork: Wastewater irrigation promotes Antibiotic Resistance Genes (ARGs), Mobile Genetic Elements (MGEs), and modifies Bacterial Communities in Soil, Cilantro Rhizosphere, and Phyllosphere

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The One Health concept recognizes the interconnectivity between human, animal, and environmental health, emphasizing that the emergence and dissemination of antimicrobial resistance (AMR) are a shared challenge across these domains. In this context, the environment plays a critical role as a reservoir and transmission route for antibiotic-resistant bacteria and resistance genes, linking anthropogenic activities, such as agricultural practices, to potential public health risks.

In the Mezquital Valley (Mexico), agricultural fields have been irrigated with untreated wastewater from Mexico City for over a century. The commissioning of the Atotonilco wastewater treatment plant (WWTP) shifted irrigation practices toward treated effluent, which likely contains lower levels of nutrients and pollutants but potentially higher proportions of multidrug-resistant bacteria. Although the implementation of wastewater treatment provides clear benefits to human and environmental health, these advantages could still be compromised by the accumulation and dissemination of resistance genes.

The present work assessed the abundance and diversity of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in cilantro fields irrigated with WWTP effluent or spring water. It also examined the effects of irrigation water type on the bacterial community composition in soil, cilantro rhizosphere, and phyllosphere using qPCR and 16S rRNA gene amplicon sequencing. Irrigation water quality emerged as the primary factor shaping ARG and MGE profiles. Specifically, the relative abundance of sulfonamide, quinolone, and tetracycline resistance genes, along with integrons and IncP-1 plasmids, significantly increased across all compartments of cilantro fields irrigated with effluent. Moreover, bacterial community composition in soil, rhizosphere, and phyllosphere was strongly influenced by the type of irrigation water. In-depth 16S rRNA gene amplicon analysis showed that ASVs affiliated with *Acinetobacter* and *Enterobacteriaceae* were enriched in the cilantro phyllosphere irrigated with WWTP effluent. Overall, our findings underscore the importance of irrigation water quality and highlight the connectivity between environmental microbiomes and the human gut microbiome through produce

#### SL-EME-14

##### Identification of metabolic niches and their association to the persistence of antibiotic resistant bacteria in wastewater

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Wastewater treatment plants (WWTP) are hotspots for the development and spread of antimicrobial resistance. The aim of this study is to understand the ecological mechanisms, which allow antimicrobial resistant bacteria (ARB) to persist in the environment. More specifically, we aim to identify metabolic niches that can select for and against ARB. A collection of 62 *E. coli* strains previously isolated, sequenced and phenotypically characterized from different WWTPs with different levels of resistance to a variety of antimicrobials was assembled. The isolates have a wide range of susceptibility to the antimicrobials. Next, genome scale metabolic models (GEMs) were constructed, and the growth of the strains was simulated in the presence of 298 different carbon sources. GEMs identified 40 carbon sources that can

be utilized for growth only by a portion of all the isolates. Furthermore, the growth rates of the isolates were measured in the presence of 3 carbon sources to verify the model predictions. The prediction accuracy of the GEMs was 93% in the case of D-malate. A range of WWTP isolates were identified which use D-malate as carbon source and are susceptible to specific antibiotics. In contrast, antibiotic-resistant WWTP isolates were identified that did use sucrose as carbon source instead of D-malate. Modelling the competition between isolates under different conditions suggests that adding a carbon source to a bacterial community under specific conditions could exclude resistant bacteria from a complex microbial community. This was confirmed by competition experiments with 10 selected *E. coli* isolates in minimal medium. A publicly available dataset of ~20000 *E. coli* genomes was interrogated for antibiotic resistance genes and for genes that enable the utilization of specific metabolites.[SF1] Our analysis revealed that bacteria of the pathogenic sequence type 131 cannot utilize D-malate. Our data suggest that adding a carbon source to a bacterial community can shift the selection advantage against resistant bacteria. If this strategy is confirmed experimentally in complex microbial communities, this concept could be applied to reduce the number of antibiotic resistant *E. coli* in wastewater or in the human gut.

#### SL-EME-15

##### Community structures of methanotrophs and methanogens are affected differently by small-scale heterogeneities in semipermanently flooded kettle holes

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The methane emissions of an environment are mediated by the interplay of microbial groups referred to as methanotrophs and methanogens. Flooded kettle holes (KHs) are potentially significant sources of methane, but provide complex habitats for microbes. These small depressions, typically classified as wetlands, are often situated within agricultural fields. Many experience dry-wet cycles, with drought periods getting prolonged due to climate change. Resulting variation in inundation zones, lateral topsoil influx and carbonate accumulation through groundwater influx create horizontal and vertical heterogeneities, which we hypothesize to influence the abundance and community structure of methanogens and methanotrophs.

We sampled three recently dry-fallen KHs to a depth of 1 m from edge to center, capturing a gradient of inundation history and soil horizons. Total abundances of methanogens and canonical methanotrophs were significantly different between KHs. While total and relative abundances of canonical methanotrophs were highest in lower mineral horizons, the horizon-based abundance patterns of methanogens and the anaerobic methanotroph *Ca. Methanoperedens* varied between KHs and were not necessarily related to horizon pedogenesis. Atmospheric methanotrophs were detected at low abundances in only a few samples, indicating a low relevance of this process in KHs. In contrast to methanotrophs, neither horizon nor sampling site significantly affected methanogen abundances. Since soil horizons impacted significantly on the general methanogen community composition ( $R^2 = 0.45$ ) and



explained more variance than soil depth ( $R^2 = 0.19$ ), it is likely that water table fluctuations in combination with horizon properties select for potentially oxygen-tolerant taxa (*Methanosarcina*, *Methanobacterium*) in these horizons. Generally, the results for methanogens were similar to the general prokaryotic community, although site was not significant for methanogens. The methanotroph community exhibited greater diversity among KHs and was more affected by horizontal (site:  $R^2 = 0.36$ ) than vertical heterogeneities (horizon:  $R^2 = 0.28$ , soil depth:  $R^2 = 0.11$ ).

Therefore, our results indicate that abundance patterns of methanogens, aerobic and anaerobic methanotrophs and thus methane production and consumption cannot easily be generalized across KHs, but the small-scale heterogeneities within KHs shape their community structures.

## SL-EME-16

### Linking microbial community dynamics to methane emissions in a rewetted peatland

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Rewetting drained peatlands is a key climate mitigation strategy to curb greenhouse gas emissions and restore their long-term carbon (C) sink function. However, while rewetting reduces CO<sub>2</sub> emissions, rewetted fens may initially remain net C sources due to elevated methane (CH<sub>4</sub>) release. This early-stage increase in CH<sub>4</sub> fluxes is often attributed to the faster recovery of methanogenesis relative to methane oxidation, yet ecosystem-scale studies linking CH<sub>4</sub> dynamics to microbial community processes remain rare.

Here, we combine 10 years (2015 - 2024) of continuous eddy covariance measurements with quantitative PCR of functional genes for methanogen (*mcrA*) and methane-oxidizing community (*pmoA*) and 16S rRNA amplicon sequencing to investigate the microbial drivers of CH<sub>4</sub> emissions in a rewetted temperate fen in northern Germany. Preliminary analyses reveal that mean CH<sub>4</sub> fluxes (within a 4-week window around microbial sampling) are strongly and positively correlated with methanogen abundance (*mcrA*;  $R^2 = 0.967$ ,  $p = 0.003$ ), indicating that microbial production capacity is a key determinant of variability in CH<sub>4</sub> fluxes. A pronounced drought in 2018 coincided with a sharp decline in the *mcrA:pmoA* ratio, suggesting a drought-induced shift favoring methane oxidation potential.

Vertical profiling shows that the most active CH<sub>4</sub>-cycling biogeochemical processes occur in the upper 0 - 20 cm peat layer. *Methanotrichaceae* consistently dominate across years, suggesting acetate-driven methanogenesis as the prevailing pathway under rewetted conditions. Following the 2018 drought, total methanogen abundance declined but gradually recovered, accompanied by increasing relative contributions of *Methanocellaceae* and *Methanofastidiosaceae*, indicating diversification toward hydrogenotrophic and syntrophic methanogenesis. Minor yet stable fractions of *Methanomassiliicoccaceae* and

*Methanoregulaeaceae* reflect a resilient, functionally redundant community structure. These findings highlight that CH<sub>4</sub> emissions from rewetted peatlands are not solely controlled by abiotic drivers such as temperature and water level but are fundamentally shaped by long-term microbial community dynamics and functional potential.

Our integrative, decade-long dataset reveals one of the first ecosystem-scale linkages between CH<sub>4</sub> fluxes and microbial functional traits in a rewetted fen, offering new insights into how microbial succession governs the transition from CH<sub>4</sub> source to sink in restored peatlands.

## SL-EME-17

### Validation of a 3D-Printed Lysimeter for Controlled Soil Experiments

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The investigation of microbial processes in soils is largely influenced by soil moisture and oxygen concentration. Current approaches to investigate these processes vary significantly. On one hand, experiments are conducted in simple containers without sensors or controlled environmental conditions. On the other hand, expensive equipment, such as lysimeters, is often used, which frequently does not allow for oxygen regulation in the soil and is usually only available in large sizes (>3 L).

The aim of the developed experimental setup is to provide a controlled and reproducible environment for studying microbial soil processes using an open-source, cost-effective, and scalable system.

The experimental setup is entirely fabricated using 3D printing, with Polyethylene Terephthalate Glycol (PETG) for structural components and Thermoplastic Polyurethane (TPU) for the seals. A central control unit (Raspberry Pi), together with attached sensors for measuring soil moisture and oxygen concentration, allows precise regulation of these parameters using a peristaltic or membrane pump. The functionality of the system, as well as the correct adjustment of environmental parameters, is validated through biological experiments and additional functional tests.

The developed setup represents a cost-effective (< 1,000 €) alternative to conventional laboratory systems, with construction data freely available as open-source. The chosen scale of the system (soil volume ~0.5 L) allows realistic experiments to be conducted without requiring large amounts of soil and provides the option to parallelize multiple systems. Furthermore, extending the sample chamber enables the simulation of different soil depths. The setup is suitable for a wide range of research applications, including the study of bioremediation processes, nutrient cycles, microbial behavior under variable environmental conditions, as well as applications in agricultural and plant sciences.

## SL-EME-18

### Quantifying cell-specific CO<sub>2</sub> fixation reveals active chemolithoautotrophs in oxic groundwater

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Elevated abundances of putative chemolithoautotrophs and high rates of primary production in groundwaters implicate dark CO<sub>2</sub> fixation as an integral component of subsurface trophic webs. Active key players of this process and their quantitative contributions, however, are still uncertain. Here we aim to determine active CO<sub>2</sub>-fixers on the single cell level to assess the role of different putatively chemolithoautotrophic taxa in a groundwater microbiome obtained from shallow carbonate rock aquifers.

Microcosms with oxic and anoxic groundwater from two wells accessing different lithologies were supplemented with <sup>13</sup>C-labeled bicarbonate to track CO<sub>2</sub>-derived carbon uptake, and were incubated for only 24 h to retain the native microbial composition. <sup>13</sup>C-incorporation in cells of taxonomic groups of interest was analyzed using nano-scale secondary ion mass spectrometry (NanoSIMS) combined with fluorescence in situ hybridization (FISH). Parallel metagenomics and -proteomics analyses were conducted to provide metabolic context on putative chemolithoautotrophs, and complementary <sup>14</sup>CO<sub>2</sub> incubations were used to determine bulk CO<sub>2</sub> fixation rates.

In incubations of oxic groundwater, <sup>13</sup>C-labeled cells of Nitrospira and Thermodesulfobionia (Nitrospirota) as well as Nitrosomonadaceae (Pseudomonadota) were detected via NanoSIMS, indicating active CO<sub>2</sub> fixation by these taxa. These groups constituted 4.7% of the total community and 28.7% of putative autotrophs (based on metagenomic coverage), collectively accounting for a bulk fixation rate of 0.0816 ± 0.0345 µg C per liter and day. Metagenomic and proteomic data indicated that Nitrospira and Nitrosomonadaceae oxidized reduced nitrogen compounds to drive CO<sub>2</sub> fixation via the Wood–Ljungdahl (WL), reverse tricarboxylic acid (rTCA), and Calvin–Benson–Bassham (CBB) cycles, whereas Thermodesulfobionia oxidized reduced sulfur compounds while employing the WL pathway.

In contrast, in incubations of anoxic groundwater, no cells exceeding natural abundance levels of <sup>13</sup>C were detected, which coincided with lower abundance of putatively autotrophic metagenome-assembled genomes (MAGs) as well as lower bulk CO<sub>2</sub> fixation rates of 0.0427 ± 0.0161 µg C per liter and day.

Together, these results reveal a limited but active subset of chemolithoautotrophic taxa driving CO<sub>2</sub> fixation in oxic groundwater, and the NanoSIMS data provides quantitative constraints on their ecological contribution to subsurface carbon cycling.

## SL-EME-19

### Systematically quantifying the spectrum of bacterial defenses against protist predation

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**Introduction:** Single-cell eukaryotes (protists) impose a major predation pressure on bacterial populations, strongly shaping bacterial evolution and diversity. The molecular mechanisms underlying predation resistance however remain largely unexplored due to a lack of large-scale quantitative assays.

**Goal:** Here, we developed an arrayed assay to precisely quantify predation resistance to the model protist *Dictyostelium discoideum* by timelapse microscopy. By leveraging robotics, the assay can be scaled up to screen hundreds of bacterial isolates in parallel.

**Results and Conclusion:** We showcase our assay in two ways: first, on a global collection of 553 diverse *E. coli* isolates, and second, on a custom collection of 300 soil isolates, the latter we assembled and characterized in-house. Among the *E. coli* isolates, while the majority were susceptible, we nonetheless observed up to 4-fold variation in predation rates and an apparent trade-off between predation resistance and growth rate. To enhance the predation resistance phenotype in *E. coli*, we are exploring genome-wide overexpression libraries. Among the collection of soil isolates, we observed that several novel *Paraburkholderia* species were completely resistant to predation and were carried into the normally sterile amoeba developmental cycle. Additionally, the benefits of our assay's arrayed format, which physically separates prey from predator, was highlighted by the discovery of several *Pseudomonas* isolates that secreted yet uncharacterized small molecules that killed *D. discoideum* over a distance. Overall, our platform combines large-scale functional genomics with the quantitative assays that are key to characterization of the spectrum of predation resistance mechanisms employed by bacteria.

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## SL-EME-20

### Highly-related phages infecting the marine *Sulfitobacter* sp. M53 engage in superinfection inhibition and mediate the formation of virocells with distinct transcriptomic states

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Viruses of prokaryotes are key modulators of microbial communities and biogeochemical cycles, due to their ability to lyse cells, mediate horizontal gene transfer and induce metabolic changes in the host. The latter can be so significant, that the virocell (the infected cell) has been recognized as a transcriptomic and metabolic state distinct

from the noninfected cell. Most of our knowledge about virocells comes from infection mediated by a single virus. However, recent studies revealed that co-infections with two or more viruses are common in the environment. When two viruses meet in the same cell, they can engage in various types of interactions. This suggests that also the co-infected virocell represents a state distinct from the singly-infected virocell. To test this hypothesis, we challenged the M53 strain of the marine, often particle- and microalgae-associated, *Sulfitobacter* genus with either one of the ICBM16 and ICBM18 phages, or with both of them. ICBM16 and ICBM18 are highly related dsDNA tailed phages, sharing an intergenomic identity of 97.5% along 83.5% of their genomes. Using Fluorescence *in situ* Hybridization mediated detection of phages at single cell level, we showed that, hybrid ICBM16/ICBM18 virocells were formed in co-infection cultures. Compared to singly-infected virocells, the genome replication and gene transcription of ICBM18 was impaired in hybrid-virocells, indicating that the two phages interacted competitively, similar to superinfection inhibition. Starting with the phage genome replication stage, in both singly-infected and hybrid-virocells the host gene expression suffered a major shut-down affecting most metabolic and cellular processes. The shutdown was more pronounced in ICBM16 virocells than in ICBM18 or hybrid-virocells. While most of the downregulated genes were shared in common between the three types of virocells, each had uniquely up- and downregulated genes. Therefore, infection with highly-related phages resulted in the formation of virocells with distinct transcriptional, and potentially, metabolic landscapes. Furthermore, co-infection at the single-cell level resulted in transcriptionally unique virocells, even if the two phages were highly-related at genomic level. Our work suggests that, in the environment, phage genomic microdiversity and co-infections contribute to the single-cell level metabolic heterogeneity of microbial populations, especially in high cell density environments that favor co-infections.

## SL-EME-21

### Stalk-mediated attachment enhances organic carbon uptake in diatom-associated bacteria

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Physicochemical interactions between marine bacteria and phytoplankton often require a prolonged spatial proximity between organisms. Yet, direct observations of bacterial strategies to maintain this proximity in dilute ocean waters (where cell-to-cell distances are typically large) are currently limited. Here we describe an unusual association mode, where bacteria use polar stalks to attach to diatoms, a behavior primarily seen on inorganic surfaces. Stalked bacteria were discovered in the natural microbiome of the diatom *Thalassiosira pseudonana* (strain CCMP 3367) representing up to 20% of the bacterial community and belonging to a new species of the genus *Maricaulis*. Their presence did not affect diatom growth, indicating a neutral interaction. Despite being infrequently reported, analysis of existing microbiome datasets revealed that *Maricaulaceae* family members are most abundant in phytoplankton-associated environments (making up to 30% of bacterial

communities), suggesting they are more prevalent colonizers of phytoplankton microbiomes than previously believed.

Given the apparent ecological success of stalked morphologies, our objective was to determine the advantage conferred by *Maricaulis* in its association with phytoplankton. Using the *Thalassiosira-Maricaulis* co-culture as a model system, we combined <sup>13</sup>C-bicarbonate incubations with nanoscale secondary ion mass spectrometry (nanoSIMS) to test whether stalk-mediated attachment enhances uptake of diatom-derived organic carbon (DDOC). To control for attachment to non-living material, we compared bacteria attached to diatoms with those attached to extracellular algal debris. We observed initial <sup>13</sup>C enrichment in diatoms, followed by enrichment in attached bacteria, indicating uptake of DDOC. Stalks showed earlier and higher <sup>13</sup>C enrichment than the bacterial cell body, suggesting they serve as the primary site of carbon acquisition. Despite the increased distance between the bacterial cell body and the diatom surface in stalked cells, we found no significant difference in <sup>13</sup>C enrichment between the cell bodies of stalked and non-stalked diatom-attached bacteria. This suggests that stalk-mediated attachment does not limit access to DDOC and may instead facilitate efficient transfer from stalk to cell body. In summary, our findings suggest that stalk-mediated attachment enhances the acquisition of DDOC, providing a selective advantage for stalked bacteria in phytoplankton-associated microbiomes.

## SL-EME-22

### Microbial Geoprofiling: Unlocking Forensic Insights through Environmental Microbiome Analysis

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This study investigates the potential of soil microbial communities as forensic indicators for determining the geographic origin of soil samples. The research explores whether bacterial profiles vary sufficiently between locations to serve as a distinctive "microbial fingerprint" for geolocation — a concept known as *microbial geoprofiling*. Such an approach could provide forensic investigators with an innovative biological tool for linking individuals or objects to specific environments.

Two sequencing strategies were applied. Whole-genome shotgun sequencing using the Oxford Nanopore Rapid Barcoding Kit offered a broad overview of microbial diversity but did not reveal strong differentiation among the six soil environments analyzed. Nonetheless, significant variation was observed across soil depths, where topsoil (layer A) displayed distinct microbial communities compared to deeper layers (B and C).

In contrast, targeted 16S rRNA gene sequencing using the Native Barcoding Kit produced clearer distinctions among the six soil types at the phylum level, with statistical analyses confirming these differences. Interestingly, genus-level comparisons provided limited additional resolution, suggesting that phylum-level profiling may be sufficient for effective soil discrimination, simplifying potential forensic applications.

Soil samples were collected in collaboration with the International Center for Biosaline Agriculture (ICBA) from six representative environments in Dubai: industrial areas (A and B), marine/coastal zones, desert, agricultural land, and

human-activity sites. The results highlight that microbial geoprofiling can differentiate soils from distinct environments within the same geographic region, demonstrating its promising potential for forensic soil analysis and environmental trace evidence examination.

## SL-EME-23

### Accumulation of Ancient but Labile Dissolved Organic Matter in the Dead Sea

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The global reservoir of dissolved organic carbon (DOC) equals the amount of carbon in the atmosphere, yet the mechanisms allowing its long-term persistence in aquatic systems remain poorly resolved. The hypersaline Dead Sea, ten times saltier than seawater, offers an extreme model for testing how environmental constraints affect organic matter cycling and microbial utilization.

We analyzed dissolved organic matter (DOM) from the Dead Sea, its tributary springs, and surrounding aquifers using radiocarbon dating and ultrahigh-resolution Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). We found exceptionally high DOC concentrations (~2000 µM) in the Dead Sea, roughly 50-fold higher than in the deep ocean, with radiocarbon ages of ~10,000 years.

DOC concentration, DOM molecular composition and microbial communities were heterogeneously distributed in the water column contradicting the concept of a homogenous lake, and suggesting a link between the DOM and microbial community structure.

Dead Sea DOM was molecularly almost undistinguishable from submarine and coastal springs (99% overlap in molecular formula composition), indicating that the DOM originates mainly from groundwater and sedimentary inputs rather than in situ production. Despite its old radiocarbon age, molecular indices revealed features typical of biologically labile material. In incubation experiments with a productive North Sea microbial community, Dead Sea DOM was readily consumed. DOC decreased by 15% and 800 molecular formulas were lost within 3 years, demonstrating that it remains biologically degradable under non-extreme conditions.

Accordingly, the long-term accumulation of DOC in the Dead Sea is not due to intrinsic molecular recalcitrance but to environmental inhibition of microbial degradation caused by extreme salinity and ionic toxicity. The findings redefine the concept of "refractory" DOM as a relative property, determined by local environmental and ecological constraints rather than chemical stability alone.

Under scenarios such as the proposed Red–Dead Sea conveyance project, dilution of the Dead Sea could stimulate microbial activity rapidly remineralizing millennia-old organic carbon, potentially releasing large amounts of CO<sub>2</sub>.

Understanding how extreme physicochemical conditions modulate microbial degradation of DOM is essential for predicting carbon fluxes in saline inland waters under changing climatic and hydrological regimes.

## SL-EME-24

### Impact of extreme hypersalinity on stress resistance and sulfide-oxidation potential of phototrophs in coastal microbial mats

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Although hyperosmotic stress is often compared to desiccation stress, there is currently no systematic understanding how similar or distinct the physiological adaptations of desiccation tolerant and halophilic microorganisms are. Biofilm-like microbial communities forming around photoautotrophic cyanobacteria are known from both, hypersaline (e.g. coastal microbial mats) and desiccation prone habitats (e.g. biological soil crusts). While some stressors like UV irradiation and heat are shared, others like hypersaline water and the physical absence of water are fundamentally different. Here we report on the distinctive aspects of hypersalinity stress and how they are reflected in the functional potential of halophilic cyanobacteria.

Our previous study of salt-crust covered microbial mats at the coast of Oman<sup>1</sup> showed that the mats turned largely anoxic upon oxygenic photosynthesis inhibition during salt saturation. However, phototrophic sulfide oxidation persisted despite the lack of known sulfide-oxidizing taxa in the microbial community data. In order to identify the sulfide-oxidizing microorganisms and investigate genetic signatures of hypersalinity stress tolerance, we generated metagenome-assembled genomes (MAGs) from six mat patches, each sectioned into five layers. The phototrophic sulfide oxidation capability was found in nearly all cyanobacteria MAGs, as well as in *Rhodovibrio* MAGs (Alphaproteobacteria) and in some *Chloroflexota*.

To differentiate genetic signatures of hypersalinity and desiccation stress, we compared the cyanobacteria MAGs from this study to genomes from desiccation-prone environments, using marine cyanobacteria genomes as a control group. Compared to desiccation-tolerant cyanobacteria, genomes of halophilic cyanobacteria were characterized by lack of certain potassium transporters and catalase genes and presence of sulfide-oxidation genes and additional osmolyte transporter subunits. Our findings indicate that oxidative stress plays a central role in desiccation-prone environments while it is alleviated by inhibition of oxygen production and sulfidic conditions in hypersaline microbial mats. At the same time, sulfide serves as an alternative electron donor for hypersaline cyanobacteria. Unlike soil crust cyanobacteria during desiccation, they can stay active and power their metabolism, including constant osmoregulation, through sulfide oxidation.

<sup>1</sup> Meier, D. V. et al. Appl. Environ. Microbiol. 87, e0069821 (2021) [doi.org/10.1128/AEM.0069821](https://doi.org/10.1128/AEM.0069821)

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## SL-EME-25

### Land use affects the chemical and ecological quality of groundwater within drinking water catchments

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Groundwater is our most important resource for clean drinking water. Due to the high population density in European countries many water suppliers are forced to collect groundwater not only in natural landscapes such as forests but also by bank filtration or in agricultural or urban landscapes. Hence, the land use type on the surface might potentially affect the groundwater quality. Here, we studied to which extent land use types affect the quality of groundwater with respect to microbiological and chemical parameters and classified the impacts. We took water samples from monitoring wells in three different areas of the drinking water production from the city of Düsseldorf. The sampling areas were classified according to the land use type and the hydrology: bank filtration from river Rhine, conventional agriculture, and urban areas. The chemical analysis of the water samples with LC-MS/MS indicated a very good correlation between the land use type and the sum of typically expected chemicals from the respective land use. We used a whole set of ~ 117 indicators for this purpose. Chemical markers for conventional agriculture were commonly applied pesticides such as chloridazon metabolites and atrazine, urban areas were indicated by the presence of drugs, and indicators for river water were micropollutants such as diclofenac that passed waste water treatment plants. The beta-diversity of the microbial community composition was determined with 16S rRNA gene amplicon sequencing and Permanova analysis and showed a correlation with both the chemical classification and the land use type. Prokaryotic class ratios were identified that are potentially applicable as indicators for the different types of land use. Further microbiological data such as ATP-content, cell density, and assimilable organic carbon were used for calculating a microbiological index (BAE-index) which identified some groundwater monitoring wells as disturbed groundwater systems. We conclude that the microbial communities of the investigated groundwater bodies were clearly affected by the land use on the surface and that the land use types can be directly determined by the chemical patterns of micropollutants. The discovered chemical and biological characteristics constitute an important step towards developing an ecological assessment scheme of groundwater in the future.

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## SL-EME-26

### What is the global abundance of anaerobic methane-oxidizing archaea in seafloor sediments?

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The ocean subsurface is home to a large number of microbial organisms that span a vast range of metabolic functions, including highly specialized anaerobic metabolisms that operate at minimal energy supply. The absolute abundance of these functional groups is important to understand, both for the rate of biogeochemical cycles as well as for the ecology of subsurface microbial ecosystems.

Here, I present an estimate for the global abundance of methane-oxidizing archaea in seafloor sediments using an order-of-magnitude approach based on geospatial mapping and geochemistry datasets. Other than previous estimates, which often assume a power-law decay of microbial biomass with depth and cannot resolve individual taxa, this approach uses the spatial distribution of redox gradients to constrain the abundance of specific functional groups.

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## SL-EME-27

### From mineral to microbe: Tracking lanthanide mobilization and utilization in a lanthanide-dependent methylotroph

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The lanthanides (elements 57-71, [La-Lu], Lns; singular [Ln]) occur in poorly bioavailable minerals in nature. As metal cofactors in alcohol dehydrogenases, Lns are important in microbial carbon cycling, especially in C<sub>1</sub>-metabolism, and are likely involved in broader microbial physiology. Most Ln-dependent metabolism studies use soluble Ln chlorides, which poorly mimic nature. We performed an in-depth characterization of selected Ln minerals and one alloy (apatite, bastnaesite, gadolinite, loparite, monazite, xenotime, ferrocium) that differed by type (carbonate, oxide, silicate, phosphate) and Ln content. Methanol oxidation in *Beijerinckia* bacterium RH AL1 depends on light Lns (La - Nd). We used this strain to investigate Ln mobilization, uptake, and discrimination via transcriptomics, advanced electron microscopy (EM), and comprehensive analytics. Besides Ln minerals and ferrocium, we tested lanthanide oxides (La<sub>2</sub>O<sub>3</sub>, Nd<sub>2</sub>O<sub>3</sub>) and lanthanum phosphate (LaPO<sub>4</sub>). Strain RH AL1 could utilize all supplied Ln sources. Advanced EM confirmed selective uptake and periplasmic accumulation, although the Ln content of the source material was partially low (0.7% in the case of apatite) and skewed towards non-utilizable Lns (gadolinite and xenotime contain 50.1 and 95.8% heavy Lns, respectively). We propose that periplasmic Ln accumulation contributes to Ln homeostasis. Transcriptomics revealed that the type of Ln source significantly influenced the dynamics of gene expression. Up to 53% of the encoded genes were differentially expressed, and this effect was not limited to genes known to be involved in Ln utilization but extended to many aspects of cellular metabolism. Network analysis identified modules of genes that selectively responded depending on the Ln source, suggesting that genes linked to different modes of Ln mobilization, discrimination, and uptake are co-expressed and co-regulated. Adaptations to low Ln bioavailability, various types of Ln sources, and Ln mobilization turn Ln-utilizing microbes into enablers, making Lns more accessible in the environment, potentially transforming them into a shared good for microbial communities. Likewise, Ln-utilizing microbes are a treasure chest for developing strategies for the sustainable use of Lns as key resources of the 21st century.

# Host-pathogen interactions

## SL-HPI-01

### Hypochlorous Acid (HOCl)-Derived N-Chloramines in Cell Envelopes of Bacteria and Monocytes

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Neutrophils, highly specialized in phagocytosis orchestrate a coordinated attack on engulfed bacteria, combining lytic compounds with oxidants, including the highly toxic hypochlorous acid (HOCl). In host-pathogen interactions, HOCl will naturally target either the bacterial envelope or the phagolysosomal membrane. Here, we examined the occurrence of N-chloramines in the cell envelopes of bacteria and monocytes, their biological activity, and neutralization.

Using a chemical probe for N-chloramines we have shown that HOCl forms N-chloramines with the membrane lipids" amino residues found in the head groups of some lipids in *Escherichia coli* and monocyte membranes. Besides lipids, cell envelopes of Gram-negative bacteria contain lipopolysaccharides (LPS). LPS is localized in the outer leaflet of the outer membrane covering the bacterial surface and can be modified with free amine containing groups, potentially prone to chlorination. We have shown that HOCl indeed forms N-chloramines in LPS molecules of living cells. N-chlorinated model membranes composed of phosphatidylethanolamine, the major membrane lipid in *E. coli* exhibited oxidative activity towards the redox-sensitive protein roGFP2, suggesting that cell envelope N-chloramines might perturbate the cellular thiol homeostasis. While relatively stable *in vitro*, they are significantly less stable in living cells indicating both, active detoxification as well as high reactivity with other cellular components. To unravel the role of the membrane composition for HOCl susceptibility, we manipulated *E. coli* membrane lipids and analyzed accumulation of N-chloramines. We propose that, like protein N-chloramines, N-chloramines in the bacterial cell envelope are involved in inflammation by modulating host immune cells and contributing to bacterial killing.

## SL-HPI-02

### Detection and effects of microbial N-chloramines and thiol oxidation during host pathogen interaction

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In humans, neutrophils represent the initial line of defense against bacterial pathogens. Invading bacteria are killed by neutrophils by a process called phagocytosis, during which bacteria are exposed to oxidative substances. One of these substances is the highly toxic hypochlorous acid (HOCl), which is generated by Myeloperoxidase (MPO) in the

phagolysosome. In the phagolysosome HOCl can react to the even more toxic taurine-N-chloramine (TauCl) or monochloramine (NH<sub>2</sub>Cl). Both are involved in pathogen clearance. In proteins, HOCl, TauCl and NH<sub>2</sub>Cl primarily oxidizes thiol groups but can also react with free amines to form protein N-chloramines. However, which of these three chlorinating species reacts to which extent, and how these reactions contribute to bacterial killing remains unknown.

To better understand the specific roles of different chlorinating species, we compared their reactivity in inducing N-chlorination and thiol oxidation. Our data demonstrate that HOCl, TauCl, and NH<sub>2</sub>Cl exhibit distinct and sometimes opposing chlorination and oxidation efficiencies toward thiols and lysines. While TauCl and NH<sub>2</sub>Cl exhibit higher toxicity to *E. coli* compared to HOCl, TauCl induces lower intracellular thiol oxidation and produces hardly any intracellular N-chloramines. Kinetic analyses *in vitro* further reveal that HOCl reacts fastest with roGFP2 protein thiols, followed by NH<sub>2</sub>Cl and TauCl.

Despite the clear effect of N-chlorination on protein function, there are currently no strategies to study the extent of N-chloramines *in vivo*. We have recently shown that protein N-chlorination can be detected *in vitro* using the DANSyl derivative DANSyl sulfinic acid (DANSO<sub>2</sub>H). To advance insights into the role of N-chlorination in host-pathogen interactions, we aim to harness this chemistry in combination with isotopic labeling to establish a robust, quantifiable labeling protocol to detect N-chloramines *in vitro* and *in vivo*. The labeling degree was quantified through in-gel fluorescence measurements of the DANSyl products and confirmed by mass spectrometry analysis.

## SL-HPI-03

### The T6SS/T1F secretion/adhesion system of the human pathogen *Yersinia enterocolitica* is under the control of a two-component system

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Bacterial secretion systems play a crucial role in the virulence and competition of many bacterial pathogens. Among these, the type VI secretion system (T6SS) functions as a contractile molecular nanomachine, delivering toxic effector proteins into eukaryotic or prokaryotic target cells. The T6SS is comprised of 13 highly conserved core components, typically organized in gene clusters. The human pathogen *Yersinia enterocolitica* harbors a special type of T6SS cluster that also incorporates genes for type I fimbriae (T1F), structures typically involved in surface adhesion. To determine the biological role and function of the T6SS/T1F cluster, we characterized the expression and activation of the T6SS/T1F cluster by live cell microscopy of bacteria expressing a functional fluorescently labeled core component of the T6SS sheath. A genetic screen revealed that the T6SS/T1F cluster is activated by a previously uncharacterized two-component system (TCS). Proteomics analysis demonstrated an upregulation and activation of the T6SS and, surprisingly, additional proteins involved in attachment and type III secretion, which are encoded across the genome. Conversely, motility and chemotaxis-related proteins were strongly downregulated. Our results suggest a lifestyle switch from a motile, non-virulent state to a sessile and virulent state.

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## SL-HPI-04

### Structure function analysis of *Yersinia enterocolitica* YopQ

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## Introduction

The type III secretion system (T3SS) effector YopQ/YopK plays an important role in macrophages infected with pathogenic *Yersinia* strains by regulating T3SS effector translocation and inhibition of T3SS-triggered inflammasome activation.

## Goals

We aimed to characterize the structure-function relationship of YopQ/YopK concerning its secretion, translocation, binding to the T3SS translocation pore and regulation of effector translocation in human macrophages.

## Materials & Methods

Small-angle X-ray scattering, X-ray crystallography and AlphaFold2 were employed for structural analysis. Truncated YopQ constructs, Western blot and SDS-Gel electrophoresis were employed for secretion and translocation assays (into target cells) and confocal immunofluorescence microscopy for localisation studies in host cells.

## Results

X-ray and AlphaFold structures of *Y. enterocolitica* YopQ showed homology to cysteine proteases using Foldseek and revealed motifs that could represent the catalytic triad Cys-His-Asn/Asp characteristic for cysteine proteases. The N-terminal amino acids 1-25 of YopQ were disordered and proved to be sufficient for T3SS-mediated secretion and translocation of heterologous proteins as well as for binding to the T3SS translocation pore from within target cells. However, only full length YopQ could regulate T3SS-mediated translocation.

## Summary

Our study demonstrates that the disordered N-terminus of YopQ has multiple functions mediating secretion/translocation and localisation within target cells. The known activities of YopQ within cells such as regulation of effector translocation depend on the complete protein and might be associated with cysteine protease activity.

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## SL-HPI-05

### Tropism in the gastrointestinal tract – organoids as host model

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In the gastrointestinal tract, the epithelial lining acts as physical and immunological barrier between the microorganisms of the gut and the body. We use adult stem cell-derived organoids to analyze the molecular basis of innate immune signaling in the epithelium and to study infections of the gastrointestinal tract. We generated a biobank of human and murine organoids covering 6 sites from stomach to colon. RNA-sequencing showed that the tissue identity is conserved in the adult stem cells. Using this biobank of organoids, we analyze the molecular mechanism of tropisms of different infections, including *H. pylori*, Enteropathogenic *E. coli* (EPEC), or SARS-CoV-2. In the currently running EU consortium TOP-GUT, we characterize the O-glycosylation of proteins in organoids of the human GI tract, which likely plays a role in bacterial binding to target host cells.

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## SL-HPI-06

### Jasmonic acid shapes the rhizomicrobiome assembly and functions in rice under drought stress

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Similar to human gut enterotypes, the soil microbiome can help maintain plant fitness and resilience under environmental challenges. Using shotgun metagenomics along with molecular and physiological assays, we evaluated whether the rhizomicrobiome is essential for plant resilience during drought. We also examined how jasmonic acid (JA) might influence rhizomicrobiome structure and function using rice mutants with different jasmonate profiles: i) jasmonic acid oxidase (*jao*), which lacks JA breakdown; ii) allene oxide cyclase (*aoc*), a JA precursor mutant; and iii) *coronatine insensitivity1* (*coi*), a JA receptor mutant. The presence of the rhizomicrobiome significantly increased JA signaling genes, including *OsJAZ1* and *OsJAZ4*, and enhanced soil moisture retention during drought in all genotypes except the *aoc* mutant. Although all mutants were grown in soils with identical physicochemical properties, the composition of the rhizomicrobiome was influenced by the rice jasmonate profile. The archaeal phylum *Nitrososphaerota* was notably enriched in the *aoc* mutant. Similarly, two bacterial phyla, *Chloroflexota* and *Actinomycetota*, were more abundant in mutants with impaired jasmonate synthesis or perception (*aoc* and *coi* mutants). These changes in taxonomy were linked to functional differences. The rhizomicrobiome of the *aoc* mutant was enriched with genes related to glycolysis, oxidative phosphorylation, methane metabolism, and fructose and mannose metabolism. By contrast, the *jao* mutant, which accumulates higher JA levels, showed enhanced root growth in the presence of the soil microbiome in two-week-old seedlings. Functional analysis of the soil metagenomes further revealed higher levels of genes involved in flavonoid and carotenoid biosynthesis in older wild-type and *jao* seedlings compared with mutants lacking JA synthesis (*aoc*) or perception (*coi*). These results highlight a crucial role for JA in shaping rhizomicrobiome structure and function, thereby supporting drought resilience in rice.

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## SL-HPI-07

### The sucrose metabolism of the maize pathogen *Ustilago maydis* shows a surprising complexity likely reflecting niche adaptations

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Sucrose is a key carbon source in nature, serving as major transport sugar in plants and as a ubiquitous substrate for microbes. The biotrophic fungus *Ustilago maydis*, a maize pathogen, feeds on sucrose during saprotrophic and biotrophic life stages. Despite its importance, sucrose metabolism in *U. maydis* is poorly characterized.

Based on existing literature and predictions, a high affinity highly specific sucrose transporter (Srt1) and two putative invertases, secreted Suc2 and intracellular Suc1 are predicted key players. To disentangle the sucrose metabolism, we generated deletion mutants of all candidates and conducted complementation assays in *Saccharomyces cerevisiae* to verify protein function. Strains lacking all three proteins, Srt1, Suc1 and Suc2, did still grow saprotrophically on sucrose, suggesting the presence of additional proteins for metabolization of the disaccharide. The yeast complementation assays revealed that the fungal sucrose toolbox contains additional two intracellular invertases, derived from maltose metabolism. Surprisingly, instead of the known high-affinity sucrose transporter Srt1, *U. maydis* utilizes Agt1 for sucrose import during saprotrophic growth, a transporter that is mainly employed for maltose in *S. cerevisiae*. Infection assays with the deletions of *srt1* and *agt1* double deletion strains revealed strongly diminished symptoms, indicating the importance of intracellular sucrose hydrolysis during infection. We currently investigate the role of Suc2, that may constitute a dual targeting invertase, not only present as secreted but also as intracellular variant – and a potential tool to regulate different metabolizer strategies. The complex sucrose metabolism employed by *U. maydis* might reflect the competition between the plant immune system and the infection strategy of the fungus, highlighting how the fungus adapts its metabolic tools to overcome host defenses and establish successful infection.

#### SL-HPI-08

##### Transmission, toxins and competition: what drives virulence evolution in spore forming parasites?

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Why do some parasites cause a great deal of harm to their hosts, whereas others only cause mild symptoms? Often, we expect the harm parasites cause to their hosts, or their virulence, to optimise at an intermediate level. Too low virulence can result in missed transmission opportunities, whereas too high virulence can mean that the host and with it parasite die before transmission has occurred. There are, however, exceptions to this general expectation. Spore formers, for example, can sit and wait in the environment for new transmission opportunities, potentially reducing the costs of higher virulence. The exact circumstances under which high virulence in spore formers is likely to evolve, however, is hotly debated, with some mathematical models predicting that it is tightly linked to certain specific aspects of parasite ecology and biology. These include 1) whether the parasite is transmitted via shedding from alive or dead hosts; 2) whether the parasite produces toxins and 3) whether the parasite is in a single strain or multi-strain infection. We carried out an evolution experiment manipulating all three factors in a model system, using *Caenorhabditis elegans* as a host and *Purpureocillium lilacinum* as a parasite. Toxin producers evolved higher virulence regardless of the

selection regime, whereas whether parasites were transmitted via shedding from alive hosts or upon death only impacted virulence evolution when the fungus was in multi-strain infections. These results suggest that parasite ecology and trade-offs beyond those between virulence, transmission and recovery rate determine optimal virulence in spore formers.

#### SL-HPI-09

##### Reprogramming of bacterial virulence in *Simkania negevensis* by lysine acetylation

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Pathogenic bacteria utilize a broad range of virulence factors to infect host cells. One particular family are CE-clan protease related virulence factors. They are known to mainly target the ubiquitylation machinery acting as deubiquitinases (DUBs) or ubiquitin-like proteases (ULP). Others show acetyltransferase (AcT) activity with the *Chlamydia* effector ChlDUB1 even being reported to exhibit a dual catalytic activity combining DUB and AcT activity <sup>1</sup>. However, the molecular determinants for their preferred catalytic activities remain largely elusive and their substrate range is mainly unexplored. The *Chlamydia*-like intracellular pathogen *Simkania negevensis* encodes for five CE-clan members SnCE1-5 out of which only SnCE1 is active as ULP, targeting the small ubiquitin-like modifier (SUMO) <sup>2</sup>. SnCE1 shows homology to ChlDUB1 suggesting a similar dual specificity.

By following a combined approach using tools from biochemistry, structural and synthetic biology, we performed in depth characterization of SnCE1 to elucidate its enzymatic activity on a molecular level <sup>3</sup>. Besides the known deSUMOylase activity, we report intrinsic AcT activity for SnCE1 and identified multiple acetylation sites. Analytical size-exclusion chromatography studies shows a shift of SnCE1 between its monomeric and tetrameric form, depending on its acetylation state. By site-specifically incorporating acetyllysine using the genetic code expansion concept (GCEC) we were able to show that acetylation at K231 impairs SnCE1 tetramer formation stabilizing the monomeric form. Crystal structures of SnCE1 in its monomeric and tetrameric state as well as in complex with SUMO1 provide a structural basis for regulation of SnCE1 by lysine acetylation. Furthermore, in cellular studies using HEK293 cells we observe that acetylation-dependent autoproteolysis of SnCE1 releases its transmembrane region containing N-terminus. Loss of the N-terminus results in an altered subcellular localization, shifting from an ER-localized state to a more cytosolic distribution. Interestingly, transiently transfected cells with SnCE1 show a drastically changed mitochondrial morphology, suggesting SnCE1 to target mitochondrial proteins.



To this end, we provide a model for regulation of SnCE1-mediated virulence by lysine acetylation modulating autoprolytic processing and its subcellular distribution in the host cell.

<sup>1</sup> Pruneda et al., *Mol. Cell*, 2016

<sup>2</sup> Boll et al., *Nat. Commun.*, 2023

<sup>[3]</sup> Schmöcker et al., *Nat. Commun.* (in revision)

## Computational microbiology

### SL-CM-01

#### Different protein language models uncover 23.21% of the functional dark matter across 16 million open reading frames recovered from thousands of environmental metagenomes

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Using alignment-based functional annotation, 40-80% of open reading frames (ORFs) remain unclassified or hypothetical. In this study, we integrated 4 protein language models (pLMs) into 16 models using different datasets and training approaches to assess their alignment-free functional annotation. To this end, we used the Structural Classification of Proteins (SCOP), a subset of the Pfam database. As our genome set, we used 23,336 high-quality metagenome-assembled genomes recovered from 3,802 metagenomes, spanning diverse environmental samples and encompassing Bacteria and Archaea. Over 16 million ORFs were extracted and dereplicated into 2.4 million orthologous groups (orthogroups). From these orthogroups, we annotated 7.3 million representative ORFs using Pfam, KEGG, COG, and nr\_NCBI databases, with 80.65% of the orthogroups classified as hypothetical or unknown. After filtering, 401,384 annotated ORFs were embedded using four pre-trained pLMs (DLM-LSTM, MT-LSTM, ProtBert-BFD, and ProtT5-XL-U50) and integrated into a hierarchical multi-label classification framework. Our framework relies on 1,436 SCOP-Pfam families mapped via the Local Classifier per Parent Node approach, with pLMs evaluated for statistical significance using hypothesis tests. Hierarchical classification improved the performance of smaller models by an average of 1.85% in terms of sample-average F1-score, making them as robust as more complex models for prediction. While accuracy varied across models, each model's effectiveness depended on which specific protein family it was classified. From the 2.4 million orthogroups, we identified 31,905 unique orthogroups with Pfams using

alignment-based techniques (HMMER and RPS-BLAST). With a high level of reliability (families with F1-score > 0.9 in validation sets and probability estimate > 0.9), we identified 453,261 additional orthogroups (1,022,589 ORFs) containing 1,158 protein families, including plastic-degrading enzymes such as subtilases and prolyl oligopeptidases. Therefore, our pLM alignment-free approach identified 14.2 times more orthogroups than alignment-based methods. These orthogroups spanned 93 bacterial phyla (e.g., Patenscibacteria, Verrucomicrobiota) and 10 archaeal phyla (e.g., Asgardarchaeota). Our findings emphasise that the long road towards uncovering all protein functions in natural habitats may encompass pLMs with unique architectures and datasets, thereby providing new insights across different fields of microbiology.

### SL-CM-02

#### Baktfold - Ultra-sensitive functional annotation of hypothetical proteins in bacteria

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### Introduction

The functional annotation of bacterial genomes has undergone tremendous progress over recent years. Due to the availability of several rapid, fully-automated and easy-to-use command line software tools like Prokka, Dfast and Bakta, the annotation of bacterial genomes has become a standard and routine task. However, the functional description of many protein sequences still remains challenging. In parallel, the size of the so-called microbial dark matter increases with each metagenome project. As a result, too many protein sequences still remain as so-called hypothetical proteins after applying state-of-the-art bacterial genome annotation software pipelines. Meanwhile, the advent of artificial intelligence and protein language models revolutionized the in-silico prediction of protein sequence structures, and novel algorithms and software tools allow for structure-based protein searches outperforming the sensitivity of sequence alignment-based approaches.

### Objectives

We applied novel in-silico sequence-to-structure prediction models to predict the 3D structures of hypothetical proteins. Resulting structures are transformed into a 1D structure grammar allowing for rapid searches against high quality annotation databases and thus, reducing the fraction of protein sequences without functional annotation.

### Results

Here, we introduce Baktfold, a new command line software tool for the ultra-sensitive but taxon-independent fast annotation of bacterial protein sequences. Baktfold conducts a hierarchical protein structure-based search against several structure databases. Protein sequences are transformed into a 1D structure grammar via ProstT5 and subsequently searched against structure databases via FoldSeek. Improved CDS annotation results from Bakta are finally exported in GFF3 and INSDC-compliant flat files as well as comprehensive JSON files facilitating automated downstream analysis 100% interoperable with Bakta. We assessed Baktfold's performance in terms of wallclock runtime and functional annotation of hypothetical proteins from various sources including isolates and metagenomic-assembled genomes.

## Conclusion

Bakfold significantly increases the rate of functionally annotated protein sequences, and thus is an important improvement over existing genome annotation pipelines. The software is implemented in Python 3 and runs on MacOS and Linux systems. It is freely available under a GPLv3 license at <https://github.com/gbouras13/bakfold>.

## SL-CM-03

### Automated curation of metagenomic bins and estimation of binning success using *itBins*

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Most prokaryotic genomes in public databases are reconstructed from metagenomes and thus frequently a compendium of multiple contiguous sequences (contigs) assembled from shotgun sequencing data. Although binning algorithms for assigning contigs to metagenome assembled genomes (MAGs) are manifold and continuously improving, binning errors, i.e. the incorrect assignment of a contig to a MAG, can propagate through various databases and confound taxonomic, metabolic, and/or evolutionary analyses. Here we present *itBins*, a fully automated python-based software that enables ultra-fast curation of metagenomic bins harnessing %GC, coverage, and taxonomy information. When applied to the low, medium, and high complexity data of the CAMI I challenge<sup>1</sup>, *itBins* produced higher F<sub>1</sub> scores compared to MDMcleaner<sup>2</sup> and Rosella<sup>3</sup>. Compared to manual curation via uBin<sup>4</sup>, *itBins* performed similarly well across all three complexity levels of the CAMI I dataset. With an average speed of 61 milliseconds (ms) per bin, *itBins* outperformed all other curation tools by at least three orders of magnitude when input data was accordingly available (%GC, coverage, taxonomy), and was similarly fast when input data preparation was included in the processing time. Application to 64 real-world metagenomes from highly complex river mesocosms resulted in 259 medium-quality and 19 high-quality bins curated by *itBins*, while other automated curation tools failed in generating output at all or in reasonable runtime. Finally, *itBins* also utilizes marker genes to determine the overall binning success for individual metagenomes, providing a crucial benchmark for the user to estimate the ecological relevance of their reconstructed data. The herein introduced software *itBins* is broadly applicable to any type of metagenome data, integrates well with other software like DASTool, and enables swift and reliable curation of genomes from metagenomes along with estimation of the overall binning success.

<sup>1</sup> Sczyrba et al. Critical Assessment of Metagenome Interpretation—a benchmark of metagenomics software. *Nat Methods* (2017).

<sup>2</sup> Vollmers et al. How clear is our current view on microbial dark matter? (Re-)assessing public MAG & SAG datasets with MDMcleaner. *Nucleic Acids Research* (2022).

<sup>3</sup> [github.com/rhysnewell/rosella](https://github.com/rhysnewell/rosella)

<sup>4</sup> Bornemann et al. uBin: A manual refining tool for genomes from metagenomes. *Environ Microbiol.* (2023).

## SL-CM-04

### Predatory bacteria in human gut microbiomes

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*Bdellovibrio* and like organisms (BALOs) are small, fast-swimming, obligate predatory bacteria that kill Gram-negative bacteria. BALOs exhibit invasive and vampirizing modes of predation and likely affect ecosystems by increasing species diversity, as do macroscopic predators [1].

We recently compared genomes and predation modes of BALO strains and described novel taxa, increasing the so far described diversity of BALOs from the surroundings of animal hosts [2]. In contrast, predatory bacteria have been reported only rarely in human microbiomes. This is usually attributed to the fact that BALOs do not seem to tolerate anaerobic conditions present in the human gut.

Here, we re-analyzed a large-scale dataset from the Human Microbiome Compendium of 168,000 consistently processed gut microbiome samples from 482 studies [3] and found BALOs in samples from over 100 studies, suggesting BALO presence in 2.5% of all samples. Interestingly, we did not find BALOs in microbiome samples from Germany, but did find them in samples from other European countries and across all continents globally. We also observed increased microbial alpha-diversity in samples that contained BALO reads and a positive relationship between alpha-diversity and health state.

We therefore hypothesized that BALOs survive under physiological gut conditions (i.e., body temperature, low pH, low oxygen concentration). In agreement with this, we found that multiple *Bdellovibrio* strains are active at 37°C and a pH of 6.5. Additionally, nitrate reductases present in multiple BALO genomes indicate potential anaerobic respiration capabilities.

Finally, we propose that BALOs could thrive especially under inflamed conditions when external electron acceptors are available. This is in agreement with their prey range, which largely consists of facultative anaerobes often associated with dysbiosis. We therefore anticipate a vital role of BALOs in human microbiome health.

[1] Johnke2020 - *Bdellovibrio* and Like Organisms Are Predictors of Microbiome Diversity in Distinct Host Groups.

[2] Maher2025 Comparative analysis of novel *Pseudobdellovibrionaceae* genera and species yields insights into the genomics and evolution of bacterial predation mode. <https://doi.org/10.1101/2025.02.19.638989> (accepted: ISME Communications)

[3] Abdill2025 Integration of 168,000 samples reveals global

## SL-CM-05

### Mechanistic Study of Protein Translation for Revealing Resource Utilization Dependent on Cellular Growth of *Escherichia coli*

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In this study, a mechanistic ODE-based model was developed to describe translation dynamics in *Escherichia coli* across a wide range of growth rates. The model incorporates key molecular components involved in translation initiation, elongation, and termination, together with tRNA charging, with particular focus on the roles of ribosome abundance, initiation factors, and elongation factors.

Simulated results show good consistency with experimental observations for global cellular parameters such as growth rate, translation frequency ( $k_{tr}$ ), and active ribosome fraction. A notable finding is that, despite the declining pool of free ribosomes at higher growth rates, the cell maintains an increasing surplus of free translation factors relative to available ribosomes, which facilitates rapid ribosome turnover and sustains high translation efficiency. Furthermore, translation elongation velocity can be finely tuned by incorporating the inhibitory effect of (p)ppGpp, modeled through the stoichiometric ratio between RelA and SpoT, which improves alignment between simulated and experimental values.

In addition, the predicted tRNA charging scenario provides a distinct perspective on the stringent response, offering hypotheses that merit further experimental validation. A future framework for systematic parameterization is proposed, involving sensitivity analysis and iterative refinement of key kinetic parameters. These results provide a quantitative foundation for exploring translation regulation and resource allocation in bacterial growth.

## SL-CM-06

### Modeling Phototrophic Growth: Advancing Genome-Scale Reconstructions of Cyanobacterial Metabolism for Green Biotechnology

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Cyanobacteria play a central role in global biogeochemical carbon cycles and are a promising chassis for sustainable biotechnological production. Genome-scale metabolic models provide a powerful quantitative framework to evaluate and predict microbial physiology. Yet the modeling of phototrophic metabolism presents unique challenges, as light, rather than organic substrates, serves as the primary energy source.

To address these challenges, we recently developed a novel quantitative approach that extends conventional flux balance analysis (FBA) by explicitly integrating light absorption, quantum yield, and photodamage. Our approach allows the simulation of light-limited cyanobacterial growth under defined conditions. The model was evaluated using high-quality experimental growth data from *Synechocystis sp.* PCC 6803, assessing its ability to predict photosynthetic oxygen evolution, energy cofactor usage (ATP/NADPH ratio), and maximal growth yields.

Beyond fundamental insight, the reconstruction provides a versatile platform for applications in green biotechnology. It enables the quantitative assessment of biosynthetic production pathways, prediction of maximal product yields, and analysis of metabolic trade-offs during heterologous compound synthesis. Furthermore, the model can be incorporated into bioreactor-scale simulations to account for light gradients, gas exchange, and other cultivation parameters.

In summary, our approach advances the quantitative modeling of cyanobacterial metabolism and provides a computationally efficient approach for the rational design and optimization of cyanobacterial bioprocesses.

[1] Höper R, Komkova D, Zavřel T, Steuer R. A quantitative description of light-limited cyanobacterial growth using flux balance analysis. *PLoS Comput Biol.* 2024 Aug 5;20(8):e1012280. doi: 10.1371/journal.pcbi.1012280.

[2] Steuer R. Fast-growing phototrophic microorganisms and the productivity of phototrophic cultures. *Biotechnol Bioeng.* 2022 Aug;119(8):2261-2267. doi: 10.1002/bit.28123.

[3] Rodrigues JS, Kovács L, Lukeš M, Höper R, Steuer R, Červený J, Lindberg P, Zavřel T. Characterizing isoprene production in cyanobacteria - Insights into the effects of light, temperature, and isoprene on *Synechocystis sp.* PCC 6803. *Bioresour Technol.* 2023 Jul;380:129068. doi: 10.1016/j.biortech.2023.129068.

## SL-CM-07

### Decoding *Helicobacter pylori* Resistance: Machine Learning-Enhanced Prediction of Antibiotic Susceptibility using Whole Genome Sequencing

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## Introduction

*Helicobacter pylori* is a significant risk factor for gastric cancer, peptic ulcer disease, and MALT lymphoma. Rising antibiotic resistance complicate treatment strategies. While nucleotide sequence-based assays accurately predict

resistance to clarithromycin and levofloxacin, metronidazole resistance remains difficult to detect due to its polygenic nature and the high genomic variability *H. pylori*.

## Goals

This study aimed to develop machine learning models able to predict antibiotic resistance in *H. pylori* using whole-genome sequencing data in order to improve performance over conventional (non-machine learning, single polymorphisms) molecular assays and identify novel candidate genetic markers contributing to resistance.

## Materials & Methods

A collection of 533 *H. pylori* clinical isolates was characterized by combining whole-genome sequencing and phenotypic susceptibility testing. A sliding-window approach and SHAP-based importance scoring were used for feature selection to identify biologically relevant mutations. Machine learning models were trained on genomic variants to predict resistance phenotypes.

## Results

Machine learning models predicted antibiotic resistance accurately, with metronidazole showing the largest improvement over conventional molecular methods. Feature selection identified 32 relevant variant sets, 11 of which significantly enhanced model F1-scores while combining 2-4 sets yielded 53 synergistic combinations. Independent validation confirmed 16 models with F1-scores above 0.65. The best-performing feedforward neural network improved metronidazole resistance prediction accuracy by an average of 14% compared with conventional detection applied to the same collection.

## Conclusion

Machine learning models for predicting antibiotic resistance can enable more accurate susceptibility-guided therapies for the eradication of *H. pylori*. By improving accuracy, these models could reduce treatment failure and support antibiotic stewardship. Novel genetic determinants of metronidazole resistance in *H. pylori* identified in this study provide new hypotheses about the underlying biological mechanisms of this complex multifactorial trait. Furthermore, the machine learning framework developed here can be easily transferred to other clinically relevant gastrointestinal pathogens.

### SL-CM-08

#### StrainRegistry: A FAIR deposition system for microbial strains

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When researchers isolate and describe large sets of strains from microbiome studies, deposition of these isolates in public culture collections not only is in the interest of open science but is also a prerequisite for formally naming the many new taxa that are typically discovered. The deposition of such large numbers of strains poses significant challenges not only for the submitters, who need to keep track of strain statuses, but also for the culture collections, which lack the capacity to process these sizable collections simultaneously.

To address these challenges, the DSMZ has developed a bulk submission procedure for strain collections that streamlines the deposition of large sets of strains and provides StrainInfo DOIs early in the process. The StrainInfo DOI can be used in publications and database submissions and thus greatly enhance the traceability of strain data.

The procedure is now implemented in a new deposition management system called StrainRegistry, an extension of the existing StrainInfo database (<https://straininfo.dsmz.de/>). StrainRegistry visualizes the deposition status of each strain and facilitates communication between the depositors and the responsible culture collection. Researchers can register strains intended for deposition, including associated metadata and submission information, ensuring that FAIR strain data is available from the outset. While the data will initially be kept private, it can be directly submitted to one or more participating culture collections for deposition. After acceptance, the strain data can easily be integrated into StrainInfo and other databases.

### SL-CM-09

#### A publicly accessible *Enterococcus* genome database: integration of genome assembly and annotation, phylogenetic typing and visualization

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## Introduction

Enterococci are commensal bacteria commonly found in the gastrointestinal tracts of humans, animals, and insects. However, they are also opportunistic pathogens, with multidrug-resistant *Enterococcus faecalis* and *E. faecium* being leading causes of nosocomial infections. The clinical significance and genomic diversity of the genus create a need for a standardized and scalable platform to support global epidemiological monitoring and comparative genomics.

## Goals

We aim to facilitate standardized genomic analyses and genotyping of *Enterococcus* spp. at a large scale by providing a publicly accessible database equipped with powerful analysis and visualization tools. A key feature is the integration of core-genome multilocus sequence typing (cgMLST) schemes to support robust phylogenetic placement and strain typing.

## Materials & Methods

The database for *Enterococcus* was newly established on the EnteroBase platform (<https://enterobase.dsmz.de>). It automatically collects, assembles, and annotates *Enterococcus* short-read sequencing data from public repositories using a standardized pipeline based on open-source tools. Genus-wide and species-specific cgMLST schemes were developed using high-quality reference data, and their performance was validated across thousands of genomes. Hierarchical single-linkage clustering (HierCC) allows analysis of population structures at various levels of genetic relatedness. The platform also supports cgMLST- and SNP-based phylogenetic trees, with custom genome subset selection and user data upload.

## Results

Here, we present a public database within EnteroBase, which currently holds over 53,000 uniformly assembled and annotated *Enterococcus* genomes spanning more than 60 species. The cgMLST schemes and HierCC provide high-resolution typing and support investigations of both local transmission and global diversity. Users can explore interactive phylogenetic trees, upload their own data for rapid typing, and contextualize it within a curated, quality-controlled dataset.

## Summary

This new *Enterococcus* database offers an integrated solution for genome analysis, phylogenetic typing, and visualization. By providing quality-controlled assemblies with standardized annotation and validated cgMLST schemes, the platform facilitates comparative genomics, epidemiological monitoring, and transmission tracking. It is freely accessible and designed to evolve with community input and new data.

# Biotechnology

## SL-BT-01

### Environmental Topology Modulates *E. coli* Antibiotic Response in Microfluidic Microecologies

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Traditional studies on bacterial antibiotic response often rely on homogeneous environments that poorly reflect the spatial complexity of real-world microecologies. In this study, we developed an agarose-based PDMS microfluidic platform to explore how environmental topology influences *Escherichia coli* behavior under ciprofloxacin exposure. We introduced silica particles to mimic heterogeneous microenvironments and monitored cell behavior using time-lapse microscopy.

Ciprofloxacin (0.016 µg/ml) was applied to both homogeneous and heterogeneous surfaces, and single-cell data was collected over 420 minutes. Morphological parameters (length, width, area) and motility characteristics (travel distance, directionality) were extracted from the image sequences. Comparative analyses revealed that cells in heterogeneous environments exhibited distinct morphodynamic profiles and movement patterns compared to those in homogeneous conditions.

Principal component analysis demonstrated that the antibiotic-exposed populations formed separable clusters, with environmental topology being a key determinant of phenotypic variation. Our results underscore that spatial structure significantly modulates antibiotic efficacy, offering new insights into bacterial survival strategies and drug resistance dynamics.

This study highlights the value of integrating topographic complexity into microfluidic models to bridge the gap between in vitro assays and physiological conditions, providing a more predictive platform for antimicrobial research.

## SL-BT-02

### Antimicrobial and biofilm inhibition potentials in microalgae-bacteria community metagenomes – Insights and possible candidates for aquaculture health management

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Aquacultures contribute to a significant extend to global food production considering marine and other aquatic organisms. Pathogenic bacteria and accompanying diseases however pose a serious threat for aquacultures. Common treatment of aquacultures usually involves antibiotics to combat this threat. This practice leads up to unwanted chemicals entering the food chain as well as increasing resistances against antibiotics in bacterial communities. These resistances are also attributed to the formation of biofilms by many bacteria, further complicating the use of antibiotics. Approaching the formation of biofilms to combat bacterial resistances and reduce or replace the use of traditional antibiotics thus seems promising. The realm of microalgae-bacterial communities promises valuable resources for this goal, as they have been found to produce a variety of substances to fight of pathogenic bacteria. In this study, a set of microalgae-bacterial communities (*Tetraselmis chui*, *Isochrysis galbana*, *Nannochloropsis salina*, *Chlorella vulgaris*, *Isochrysis spec.*, *Scenedesmus acuminatus*) has been tested for their ability to inhibit the formation and functionality of biofilms formed by fish pathogens (e.g. *Aeromonas salmonicida*, *Edwardsiella anguillarum*, *Pseudomonas aeruginosa*). The supernatant of the *Tetraselmis chui* culture showed the highest relative activity of 76%. In the most prosing six microalagae-bacteria metagenomic datasets we identified more than 2500 genes coding for proteins with potential biofilm inhibiting activity. Our results summarized the high potential and capacity of microalgae-bacterial microbiomes for sustainable aquaculture systems.

## SL-BT-03

### Exploring the social amoeba *D. discoideum* for natural product discovery and biotechnological applications

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In addition to its remarkable life cycle, the social amoeba *Dictyostelium discoideum* is of particular interest for natural product research and biotechnology due to the wealth of biosynthetic pathways encoded in the genome. There are 11 terpene synthase (TPS) and 45 potential polyketide synthase (PKS) genes annotated so far. However, numerous of the

corresponding products and their physiological or ecological roles are still elusive. It has been demonstrated that polyketide-derived compounds such as DIF-1 and dictyodenes play a role in differentiation processes or spore dormancy, respectively. Although many structures of the amoebal sesqui- and diterpenes have been already identified by *in vitro* studies, their biological function remained to be investigated. Such volatile compounds are proposed to mediate insect behavior or are involved in differentiation during the developmental cycle.

In this study, we aimed to explore still elusive terpene products from *D. discoideum*, investigate their function in an ecological context and evaluate the potential of social amoeba for the biotechnological production of sesqui- and diterpenes. Extrachromosomal expression of different terpene synthase genes in amoeba combined with bioreactor-based fermentation led to the overproduction of corresponding terpenes during vegetative growth, facilitating their structure elucidation and detailed physiological characterization. Subsequent NMR analysis identified a novel sesquiterpene that was further investigated for biological activity. Due to the phagocytic feeding behavior of *D. discoideum* we additionally investigated terpene formation on bacterial biomass to evaluate possible intensifications of bacterial processes. Small scale cultivations on *Escherichia coli* biomass revealed the production of diterpene  $\beta$ -pinacene with an increased space-time-yield compared to media utilization. This work deepens our understanding of amoebal chemical communication and sheds light on possible biotechnological applications in the context of biomass valorization.

#### SL-BT-04

##### **Novel and efficient CRISPR/Cas systems for precise genome editing of industrially relevant microorganisms**

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CRISPR/Cas has revolutionized genome editing and enables the precise genetic modification of various single-celled organisms in biotechnology. However, the commercial use of the well-characterized nucleases is severely hindered due to patent and licensing uncertainties.

To overcome these limitations, we have identified novel Cas nucleases with little or no homology to database Cas proteins in an elaborate metagenomics sequencing analysis of rationally selected environmental samples. Among others, several potential class 2 type V CRISPR systems suitable for genome editing resulted from this metagenome screening of over 3 tera base pairs in sequencing data. In order to evaluate the genome editing activity of our *in silico* identified sequences, we carried out experiments in *E. coli* as the classical prokaryotic model organism. We were able to show that our novel Cas nucleases, which are termed BMC, can be programmed to efficiently and precisely introduce a genomic DNA double-strand break. Using an additional bacterial non-homologous end joining (NHEJ) system, we then introduced gene-specific INDEL mutations with different BMC nucleases. Furthermore, one chosen BMC system in combination with the  $\lambda$ /Red recombinase system enabled efficient homology-directed repair (HDR) with provided DNA repair fragments in *E. coli*. We have proven that we were indeed able to identify functional and novel Cas proteins.

To also evaluate the activity of our BMC nucleases in a eukaryotic organism, we have adapted one selected system to genetically modify various *Saccharomyces* laboratory strains and diploid commercial wine starter cultures in a targeted and efficient manner. This utilized the cell's own ability to repair introduced DNA double-strand breaks by HDR. We have even established a powerful single-plasmid system for a winemaking yeast for which no CRISPR system has been described so far. All the results of our genome editing studies have shown that our proprietary CRISPR systems are valuable tools for the genetic modification of prokaryotic and eukaryotic organisms.

#### SL-BT-05

##### **Split inteins for generating combinatorial non-ribosomal peptide libraries**

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Antibiotics such as Penicillin, Vancomycin, Daptomycin, and Colistin are natural products produced by complex biosynthetic pathways involving non-ribosomal peptide synthetases (NRPS). These enzymes are able to incorporate structural features like cyclizations, acyl chains, D- or other non-proteinogenic amino acids or N-methylations, which confer favorable drug-like properties like high affinity, target selectivity, proteolytic stability, and cell permeability<sup>1</sup>.

Despite longstanding efforts to engineer NRPS, modified peptides were often only produced in very low amounts or only small changes in the peptide backbone could be achieved. However, recent breakthroughs in megasynthetase engineering, such as the XU and XUT concepts, have renewed interest in using these systems for compound library generation<sup>2,3</sup>.

In this study, we introduce inteins as a novel molecular tool for NRPS engineering<sup>4</sup>. Using the xenotetrapeptide synthetase as a model system, we demonstrate the robust and efficient generation of recombinant NRPS from up to three different natural systems. We have created a library of more than 200 new-to-nature peptides that can be biocatalytically produced in *E. coli* using combinatorial transformation of only 21 plasmids.

In contrast to synthetic macrocycle libraries, such genetically encoded compound libraries make the production and screening of natural product-like macrocycles easier and more broadly accessible to the research community. We are currently applying this library in various target-based and bioactivity screens to explore its potential as an entirely new source of peptides.

#### References

- 1 Süssmuth, R. D. & Mainz, A. Nonribosomal Peptide Synthesis-Principles and Prospects. *Angew Chem Int Ed Engl* **56**, 3770-3821 (2017). <https://doi.org/10.1002/anie.201609079>
- 2 Bozhüyük, K. A. J. *et al.* De novo design and engineering of non-ribosomal peptide synthetases. *Nat Chem* **10**, 275-281 (2018). <https://doi.org/10.1038/nchem.2890>

3 Bozhüyük, K. A. J. *et al.* Evolution-inspired engineering of nonribosomal peptide synthetases. *Science* **383**, eadg4320 (2024). <https://doi.org/10.1126/science.adg4320>

4 Gonschorek, P. *et al.* Split inteins for generating combinatorial non-ribosomal peptide libraries. *bioRxiv* (2025). <https://doi.org/10.1101/2025.10.02.680031>

## SL-BT-06

### Multi-omics strategies reveal new structural scaffolds and enrich the diversity in the nylonase family

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The various types of the polyamide (PA) polymer, e.g. PA6, PA6.6, Kevlar® allow a wide range of applications that is reflects in an increasing annual world demand<sup>1</sup>. Corresponding to the established plastics-active database PAZy, currently 65 PA oligomer hydrolases are listed<sup>2</sup>. The NylA,B,C hydrolases from *Paenarthrobacter ureafaciens* have been extensively researched and are active on oligomers<sup>3</sup>. Current research in the field of enzymatic PA degradation is mainly based on the identification of new NylC homologs or the engineering of already known NylC variants<sup>4,5</sup>. Until now, no microorganisms have been identified with the capability of full degradation of a high molecular weight polymer. Here, we report the isolation of a novel consortium able to degrade and assimilate PA6. The consortium derives from the salt marshes of the Elbe estuary the interface between land and sea. Initial enrichment cultures were established using different polyamide substrates as the sole carbon source, led to the isolation of a diverse microbiological community. The SEM-analysis revealed that the microbiota are attached to polyamide substrates as PA6-powder or PA-tights. Using an enrichment approach combined with metagenomics, transcriptomics, and proteomics, we identified 25 putative nylon oligomer active enzymes within a complex microbial community. 8 genes were linked to a novel *Vibrio* species isolate, while sixteen were associated with a new *Marinomonas* species. Notably, 7 of the putative nylon oligomer active enzymes were located within a conserved genomic cluster and were affiliated with *Marinomonas* sp. UHHAW02. Heterologous expression and preliminary biochemical assays confirmed that 4 of the novel enzymes are active on diverse model substrates and nylon oligomers up to heptamers. Most of the functionally validated enzymes were found to be secreted. Additionally, the identified enzymes possess a novel scaffold and are not related to NylC or other known homologs. This study provides significant insights into the enzymatic mechanisms involved in the degradation of PA-polymers by bacterial consortia. The discovery and analysis of PA-degrading enzymes are an important step for mitigating polyamide pollution and advancing enzymatic recycling solutions.

1. MacLeod, *et al.* (2021)
2. Buchholz, *et al.* (2022)
3. Negoro, *et al.* (2000)
4. Bell, *et al.* (2024)
5. Pérez-García, *et al.* (2025)

## SL-BT-07

### In-situ resource utilization and biomining of regolith by filamentous fungi - Exploring fungi to help establish resource-efficient space mission

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With the prospect of human colonization on the Moon and Mars, sustainable space missions are gaining increasing importance. Filamentous fungi are powerful microorganisms with high potential for biotechnological applications due to their metabolic versatility, low resource demands, and tolerance to extreme conditions. These traits make them ideal candidates for in-situ resource utilization (ISRU) in space. Previous work demonstrated the suitability of *Penicillium simplicissimum* for biomining European Astronaut Centre Lunar Regolith Simulant (EAC-1A), highlighting the promise of fungi for mining both in space and on Earth.

However, their properties present both opportunities and risks, as they can interact with and corrode even copper surfaces. This should be taken into account in future missions to minimize spacecraft damage while enabling sustainable mining, as the regolith on the Moon and Mars is rich in ores, and resupply missions remain costly.

This study explores both aspects. First, we examined the interactions between *Aspergillus* species and metals under varying gravity conditions. The results showed that the corrosive potential of *Aspergillus* changed under simulated microgravity and differed among metal alloys, which were corroded to varying extents.

Second, *P. simplicissimum* and two *Aspergillus* species were compared with environmental fungi isolated from Moon analogue sites in Germany to assess their biomining efficiency. This approach identified promising candidates for future biomining. Moreover, we analyzed metabolic pathways and upregulated genes in high-performing strains to optimize and scale the process. Our data demonstrated fungal growth under simulated microgravity and in the presence of Lunar Highland Simulant 1 (LHS-1), as well as the extraction of minerals and metals. Analysis of precipitated metals and ICP-MS of the leachate confirmed the dissolution of several metals.

Overall, fungal bioleaching was clearly demonstrated, though affected by multiple factors that require further study. Although bacterial biomining is well established, our results demonstrate that *Aspergillus* and other filamentous fungi are equally promising for ISRU applications in space and sustainable mining on Earth.

## SL-BT-08

### Microbial ethylene glycol production with *Corynebacterium glutamicum* from xylose

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Ethylene glycol (EG) is an important and versatile platform chemical widely used across various industries, for example as an antifreeze agent in the automotive and aviation sectors, and as a key intermediate in polymer production. With the global demand for EG steadily increasing, current production processes remain heavily dependent on fossil-based resources. Microbial EG production from inexpensive and renewable carbon sources, such as D-xylose, therefore represents a more sustainable and environmentally friendly alternative. The C5 sugar D-xylose is the second most abundant constituent of lignocellulosic biomass, which is generated as waste by the agricultural, pulp, and paper industries<sup>1</sup>.

Among the various metabolic routes known for EG biosynthesis, the Dahms pathway enables the conversion of D-xylose to EG. In this non-phosphorylative pathway, D-xylose is initially oxidized to D-xylonolactone, which is subsequently hydrolyzed to D-xylonate and then dehydrated to form 2,3-deoxyxylonate. The latter is cleaved into pyruvate and glycolaldehyde, with glycolaldehyde being further reduced to ethylene glycol. Sharing the first three reaction steps, the Dahms pathway is closely related to the Weimberg pathway, which instead leads to the tricarboxylic acid (TCA) cycle intermediate  $\alpha$ -ketoglutarate<sup>2</sup>. Since our engineered *Corynebacterium glutamicum* strain is capable of converting D-xylose to D-xylonate via an endogenous dehydrogenase, three additional heterologous genes were functionally expressed to complete the Dahms pathway *in vivo*. With a limited number of further genetic modifications, the resulting strain was able to accumulate up to 45 mM EG from 100 mM D-xylose.

This study investigates the conversion of D-xylose to ethylene glycol via the Dahms pathway in *C. glutamicum*, with the objective of establishing a biotechnological production process that minimizes both production costs and environmental impact.

1. Labib, M. *et al.* Metabolic and process engineering for microbial production of protocatechuate with *Corynebacterium glutamicum*. *Biotechnol. Bioeng.* **118**, 4414–4427 (2021).
2. Brüsseler, C., Späth, A., Sokolowsky, S. & Marienhagen, J. Alone at last! – Heterologous expression of a single gene is sufficient for establishing the five-step Weimberg pathway in *Corynebacterium glutamicum*. *Metab. Eng. Commun.* **9**, e00090 (2019).

#### SL-BT-09

##### **Sweet Solutions: Engineering new-to-nature pathways in *Corynebacterium glutamicum* for the production of high-value sugars**

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Carbohydrates are valuable molecules due to their diverse applications. Monosaccharides such as galactose serve as low-calorie sweeteners, while their derivatives have broad industrial applications. Traditional production of these molecules faces challenges in efficiency and sustainability. Microbial fermentation offers a sustainable alternative, with metabolic engineering enhancing biosynthesis and yield. By

leveraging renewable feedstocks, bio-based production reduces fossil fuel reliance and carbon emissions, making microorganisms a valuable tool for the sustainable production of high-value sugars and their derivatives.

With this motivation, the current study aims to develop engineered *Corynebacterium glutamicum* strains with innovative pathways as a platform for the production of high-value natural sweeteners. By rewiring the carbohydrate metabolism in *C. glutamicum*, solutions for the production of specific carbohydrates such as galactose and lactose were developed. Traditional glycolysis was abolished by deleting core genes in favor of accumulation of glucose 6-phosphate, the key precursor for the products in this study. While most microbial products are derived downstream of, or directly from, glycolysis, these strains serve as a platform for the biosynthesis of upstream metabolites. By introducing heterologous genes, we established two new-to-nature pathways, achieving up to  $13.3 \pm 3.3$  mM galactose. Furthermore, growth on non-native substrates was successfully enabled, characterized and improved using adaptive laboratory evolution experiments.

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## **Infection biology**

#### SL-IB-01

##### **D-gluconate is the primary sugar acid that fuels *Salmonella* expansion**

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Monosaccharides fuel *Salmonella* Typhimurium colonization in the gut. Among these, sugar acids constitute an underexplored class of carbon sources, as they are largely absent from the diet and primarily generated through host-induced oxidative stress. Here, we systematically dissected the degradation pathways for D-glucose- and D-galactose-derived sugar acids in *S. Typhimurium*. Our results identify D-gluconate as the dominant sugar acid supporting *S. Typhimurium* invasion in the streptomycin-pretreated mouse model. In ecological invasion experiments, *S. Typhimurium* mult mutants unable to metabolize D-glucose-derived sugar acids permitted a diluted wild-type population to rapidly re-establish gut-luminal loads, reaching comparable levels as early as day 1 post-infection. During prolonged infection, however, the ability to utilize D-glucose-derived sugar acids became increasingly advantageous under conditions of sustained gut inflammation. These findings highlight D-glucose-derived sugar acids as a key metabolic niche that facilitates *Salmonella* persistence in the inflamed gut. By contrast, D-galactose-derived sugar acids played little to no role in colonization. Notably, in a milder infection model - where colonization resistance was reduced by intraperitoneal LPS administration, resulting in limited inflammation and oxidative stress - no significant fitness defects were observed for either D-glucose- or D-galactose-derived sugar acid mutants. Together, this demonstrates that sugar acid utilization by *S. Typhimurium* is context-dependent and tightly linked to the host's inflammatory state, whether induced by antibiotics in acute or by the pathogen itself in chronic infection models.

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#### SL-IB-02

##### **Inside-out: Remodeling of gonococcal microcolonies due to changing pili interaction network**



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*Neisseria gonorrhoeae*, the causative agent of sexually transmitted gonorrhea, rapidly aggregates from single cells to microcolonies with thousands of cells. This process is facilitated by its type 4 pili (T4P) interactions. Driven by a constant tug-of-war of their T4P, bacteria remain motile within the microcolony and form an interactive network in which pili interactions of single cells govern the properties of the entire microcolony.

During colony development, chemical gradients form within the bacterial microcolony. In this study, we investigate how these gradients influence the regulation of the T4P interactive network and changes here lead to a global reconstruction of the entire microcolony and the dispersal of cells.

To do so, we combine flow chamber-based microscopy and single-cell tracking with theoretical modeling, which allows the simulation and prediction of bacterial microcolony behaviour. In addition, we apply a direct cysteine-maleimide-based pili visualization protocol to investigate the piliation level of single cells via fluorescent microscopy.

We show that after several hours of microcolony maturation, the inner motility-restricted core can become motile again. Like in a jet, highly motile cells flow from the center to the periphery and cause an eversion of the entire colony inside out. During this process, large amounts of living and dead cells are expelled from the colony's center and shed into the environment. We argue that an oxygen gradient could result in fewer interactions between the cells at the center of the colony. Indeed, direct visualization of T4P shows that piliation is decreased in response to oxygen limitation. These reduced interactions lead to an increase in motility, which expands as a shell from the colony center outwards. Theoretical modeling predicts that when the thickness of the outer shell with strongly interacting cells falls below a critical value, capillary instability arises that initiates the rapid, colony-wide eversion. The simulated ratio of outer and inner shells at the point of eversion is mirrored by the experiments. Simulations further predict that increasing the cellular interactions should prevent the eversion. This, as well, can be experimentally confirmed.

Overall, this study shows how gradients arising during the development of the microcolony lead to the regulatory changes in the active pili interaction network, which in turn triggers global colony remodeling and the dispersal of cells.

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#### SL-IB-03

##### Unraveling the sugar-dependent repression mechanism of PrfA in *Listeria monocytogenes*

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*Listeria monocytogenes* is a Gram-positive saprophyte with the ability to transition to a human pathogen upon ingestion of contaminated food. In at-risk groups, such as immunocompromised individuals and pregnant women, listeriosis can cause systemic and neonatal infections,

respectively. Expression of virulence genes needed during infection is controlled by the transcriptional activator PrfA. Constitutive production of PrfA entails a fitness disadvantage for the bacterium hence, its activity has to be tightly regulated. Although not fully understood, one mechanism is the repression of PrfA in the presence of sugars such as glucose and cellobiose. In an attempt to identify factors involved in this mechanism, a suppressor screen was performed. While no such factors were found, 15 novel hyperactive PrfA variants (PrfA\*) were discovered. Promoter and enzyme activity assays revealed that sugar-dependent repression was reduced in PrfA\* strains. Moreover, PrfA\* strains displayed varying dependency to the known PrfA cofactor glutathione. Further experiments will unveil the impact of PrfA\* variants on the virulence potential of *L. monocytogenes*.

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#### SL-IB-04

##### The potential of altering the subgingival microbiome using oxidative stress to prevent periodontitis

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Severe periodontal disease, affecting 743 million people worldwide, is a major cause of preliminary tooth loss. It's caused by inflammation of the gum and bone tissue, leading to their regression. During the last years a correlation between periodontal disease and Alzheimer's, cardiovascular effects, and pancreatic cancer were suspected.

The disease is associated with an unhealthy, dysbiotic oral microbiome that replaces the healthy, symbiotic one. Commensal bacteria like actinomycetes and streptococci form a biofilm on the teeth, initially symbiotic. This biofilm allows intermediate colonizers like *Fusobacterium* to adhere upon gum inflammation. During this phase, the tissue turns hypoxic enabling Gram-negative anaerobic bacteria to colonize the biofilm. These late colonizers including *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* then lead to severe periodontitis. *P. gingivalis* further produces proteolytic exoenzymes (gingipains) and proinflammatory cytokines, causing gum regression.

Current treatment methods, like biofilm scaling and root planing, remove the biofilm but still leave the niche for bacteria to recolonize, leading to recurrence of the disease. Therefore, new treatment methods are needed to effectively remove the biofilm and restore a healthy oral microbiome.

To test new compounds and treatment methods, an *in vitro* model was established. An oxygen-releasing hydrogel was tested, reducing pathogenic *P. gingivalis* while commensal bacteria like *S. oralis* still grew. The gel also increased human cell proliferation, helping to close periodontal pockets. These experiments suggest oxygen-releasing hydrogels as potent therapeutic approaches to regain a symbiotic subgingival microbiome and treat periodontal disease after mechanical removal of the biofilm. In an advanced biofilm model containing ten bacterial species, we observed that

hydrogen peroxide at nanomolar concentrations transformed the biofilm into a more symbiotic state. This transformation was achieved by stimulating the growth of streptococci and reducing the abundance of *A. naeslundii* within the biofilm, which reduced ultimately the amount of *P. gingivalis*. Future work aims to optimize oxygen release in the hydrogel to inhibit the growth of pathogenic bacteria and exploit bacterial communication during biofilm formation for disease prevention and treatment.

## SL-IB-05

### Heterogeneity within *Staphylococcus aureus* small colony variants

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*Staphylococcus aureus* small colony variants (SCVs) are associated with chronic and recurrent infections due to their slow growth, reduced virulence, and antibiotic tolerance [1]. While SCVs are often considered a uniform subpopulation, clinical treatment failures suggest that their behavior is more complex [2]. We hypothesized that SCVs exhibit within-population heterogeneity that contributes to their persistence and variable treatment responses.

In this study, clinical *S. aureus* strains (wild-type and both stable and transient SCV phenotypes) were characterized using single-cell time-lapse microscopy, growth kinetics analyses, switching assays, antibiotic exposure studies, and genomic profiling. Our data show that SCV populations are heterogeneous, with distinct colony types displaying different growth patterns. Stable SCVs showed slow, consistent growth rates and harbored SNPs in genes involved in menadione or shikimate metabolic pathways. Transient SCVs displayed prolonged lag-phases but restored colony size approaching wild-type levels after 48 h of growth. Phenotypic switching demonstrated complex growth-phase dependence with variable frequencies across the OD<sub>600</sub> range 0.01-0.1, independent of simple density correlation. Single-cell analysis demonstrated marked variation in growth rates and morphological characteristics, indicating functional heterogeneity within clonally-derived SCV populations.

These data suggest that SCV populations are phenotypically heterogeneous "meta-populations" comprising multiple stable and switching subpopulations. Understanding this heterogeneity is critical for interpreting clinical SCV behavior and designing effective treatment strategies for persistent *S. aureus* infections.

## References:

1. Kahl B.C., Becker K., Löffler B. (2016). Clin Microbiol Rev. 29(2): 401-427.
2. Tuchscher L., Bischoff M., Lattar S.M., et al. (2015). EMBO Mol Med. 7(2):129-142.

## SL-IB-06

### Exploring the functions of the sRNA *IsrR* within the iron-regulatory network of *Staphylococcus aureus*

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Small regulatory RNAs (sRNAs) are important components of gene regulatory networks. Pathogenic bacteria are exposed to changing nutrient conditions in the different host niches, which not only trigger metabolic adaptations but also modulate the production of virulence factors. The availability of iron and other trace metals is severely restricted by mammalian hosts. Bacteria, in turn, possess various adaptation mechanisms, such as iron acquisition systems, which are controlled by the global regulator Fur (ferric-uptake regulator) and induced upon iron limitation. In various bacteria, this includes the expression of Fur-regulated sRNAs, such as RyhB in *E. coli*, which specifically inhibit the synthesis of non-essential iron-containing proteins. In the human pathogen *Staphylococcus aureus*, a functionally analogous sRNA, *IsrR* (iron sparing response regulator), was recently identified [1,2]. Subsequently, studies were conducted to identify *IsrR* target genes and gain insights into the pathophysiological role of this sRNA.

First, we characterized the global *IsrR* targetome by combining proteomics-based identification of proteins affected by the presence of *IsrR* with an *in silico* target prediction approach [3]. In this way, we identified 21 potential targets, which included several Fe-S cluster- or heme-containing proteins, and demonstrated that *IsrR* negatively affects the expression of TCA cycle and heme biosynthesis genes as well as genes involved in the oxidative stress response. Other studies have identified 18 mRNAs as direct targets of *IsrR* [4,5,6].

We noticed that *isrR* expression is positively associated with  $\alpha$ -hemolysin levels and hemolysis activity of *S. aureus*. A global secretome analysis revealed that *IsrR* affects the levels of several SaeR-dependent virulence factors and is most likely involved in the activation of the Sae system [7]. In addition, protein abundances of heme uptake and siderophore-mediated iron uptake systems were increased in the presence of *IsrR*.

In summary, in addition to its role in modulating the central metabolism and oxidative stress response, *IsrR* also establishes a link between the iron limitation response and virulence of *S. aureus*.

- [1] Mäder et al. (2016) PLoS Genet. [2] Coronel-Tellez et al. (2022) Nucleic Acids Res. [3] Ganske et al. (2024) Front Microbiol. [4] Barrault et al. (2024) Nucleic Acids Res. [5] Rios-Delgado et al. (2025) Nucleic Acids Res. [6] Rios-Delgado et al. (2025) mBio. [7] Busch et al. (2025) J Proteome Res.

## SL-IB-07

### Comparison of the evolution of resistance of *Pseudomonas aeruginosa* in antimicrobial peptides or random peptide mixtures

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Antibiotic resistance poses a threat to public health [1], driving incentives to discover new antimicrobials. Antimicrobial Peptides (AMPs) are small cationic peptides that are present in virtually all multicellular organisms. Bacterial resistance to AMPs has been shown to evolve at a slower rate than to conventional antibiotics [2]. This might be due to the fast-acting membrane disruption caused by AMPs, as well as the fact that AMPs are often released by host organisms as a cocktail of peptides with different targets [3]. This inspired the design of Random Peptides Mixtures (RPMs) consisting in a mixture of polypeptides made of random combinations of cationic and hydrophobic amino acids, to which a lipid residue can be added to increase hydrophobicity.

However, whether resistance towards RPMs evolves is unknown. We studied whether RMPs selected for resistance in *Pseudomonas aeruginosa* PA14, and to what extent, in comparison to AMPs. To do so, we performed in vitro experimental evolution of PA14 in the presence of either single AMPs or RPMs. We used a whole-genome resequencing approach to of PA14 in order to identify mutations having emerged. We studied how they affected bacterial fitness in general, and how were they linked to MIC-fold changes towards several antimicrobials of our panel.

We found that while some mutations emerge spontaneously, some do emerge in response to the selective pressure exerted by antimicrobials, and can confer PA14 a higher resistance towards them. We also found that these mutations affected different parameters of the bacterial fitness which resulted in faster or slower growth. The magnitude of resistance evolution was lower in RPMs compared to AMPs. Our results might offer useful insight for the design of antibacterial drugs.

1. Antimicrobial Resistance C. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 2022;399(10325):629-55.

2. Spohn R, Daruka L, Lazar V, Martins A, Vidovics F, Grezal G, et al. Integrated evolutionary analysis reveals antimicrobial peptides with limited resistance. *Nat Commun*. 2019;10(1):4538.

3. Yu G, Baeder DY, Regoes RR, Rolff J. Combination Effects of Antimicrobial Peptides. *Antimicrob Agents Chemother*. 2016;60(3):1717-24.

## SL-IB-08

### Metabolic adaptation of *Acinetobacter baumannii* to the human host: kynurenine degradation and its role in host adaptation

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**Introduction:** *Acinetobacter baumannii* is an opportunistic human pathogen responsible for nosocomial infections. The emerging antibiotic resistances but also its viable but not culturable (VBNC) state have contributed to its success (1). Another factor contributing to the adaptation to the human host is its broad metabolic versatility. *A. baumannii* uses different amino acids, fatty acids, polycyclic compounds and aromatic compounds, that are abundant in the human host, as carbon and energy source (2). The later include compounds derived from amino acid metabolism of the host, such as kynurenine. Previous studies led to the identification of a gene cluster encoding the kynurenine degradation pathway (3).

**Goals:** Elucidation of the role of the kynurenine degradation pathway in pathogenesis and adaptation to the human host.

**Material & Methods:** Mutant studies, growth studies, antibiotic resistance, *Galleria mellonella* and mouse infection studies.

**Results:** Mutant studies revealed that kynurenine is metabolized by a kynureninase to anthranilate which is further oxidized by an anthranilate dioxygenase to catechol followed by conversion to acetyl-CoA and succinyl-CoA via the  $\beta$ -ketoadipate pathway. Deletion of a potential regulatory gene of the *kyn* gene cluster, *kynR*, abolished growth with kynurenine, indicating that *kynR* encodes a transcriptional activator. A  $\Delta$ *kyn* mutant was significantly impaired in mice infection, indicating a role of kynurenine degradation in host adaptation. Transcriptomics revealed that in addition to the kynurenine degradation pathway genes many other genes were also highly upregulated in the presence of kynurenine, such as genes related to the degradation of malonate and resistance factors. This suggests that kynurenine serves as general signal for host adaptation.

**Summary:** Here we report on the role of kynurenine degradation of *A. baumannii* in pathogenesis and host adaptation.

[1] König, P., Wilhelm, A., Schaudinn, C., Poehlein, A., Daniel, R., Widera, M., Averhoff, B., Müller, V. (2023) *mBio* 14:e02139-23

[2] Breisch, J., Huber, L. S., Kraczy, P., Hubloher, J., Averhoff, B. (2022) *Env. Microbiol. Rep.* 14(1):170-178

[3] Djahanschiri, B., Venanzio, G. D., Distel, J. S., Breisch, J., Dieckmann, M. A., Goesmann, A., Averhoff, B., Göttig, S., Wilharm, G., Feldman, M. F., Ebersberger, I. (2022). *PLoS Genet.*, 18(6): e1010020.

## SL-IB-09

### The intracellular role of flagella in the pathogenesis of typhoidal *Salmonella*

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*Salmonella enterica* is a well-studied, prevalent foodborne pathogen and is classified into non-typhoidal (NTS) and typhoidal serovars (TS) based on the clinical outcome in humans. Both TS and NTS employ a variety of virulence factors, including flagella, adhesive structures, and the *Salmonella* pathogenicity island 1 (SPI1)-T3SS, to efficiently invade epithelial host cells. It is widely recognized that NTS, such as *S. Typhimurium* (STM), suppress flagella gene expression immediately after host cell invasion and remain non-motile in the early stages of infection. In contrast, human-restricted TS *S. Paratyphi A* (SPA), exhibit intracellular flagella-mediated motility and upregulation of flagella gene expression in human epithelial cells. Despite the observed variations in the pathogenesis of TS and NTS, the underlying mechanisms of intracellular flagella gene expression and the fate of flagella during host cell invasion remain largely unknown.

Here, we show that intracellular flagella-mediated motility is a shared feature among TS. We further demonstrate that TS are motile within the *Salmonella*-containing vacuole (SCV) shortly after invasion. Notably, flagella-mediated motility in TS facilitates exit from the SCV into the host cell cytosol, underscoring a specialized intracellular strategy employed by human-restricted *Salmonella* serovars. In addition, we find that both TS and NTS retain flagella that were assembled prior to host cell invasion. While SPA uses these preassembled flagella for intracellular motility within both the SCV and the cytosol, flagella of STM are progressively disassembled and degraded over time.

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## Anaerobic metabolism

### SL-AM-01

#### The electron transport system of *Methanosarcina mazei*: Structural and mechanistic investigation of the energy-converting hydrogenase Ech

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#### Introduction:

Class II methanogenic archaea conserve energy through a membrane-bound electron transport system, similar to the well-known aerobic respiratory chain. This system uses different redox cofactors and protein complexes, one of them being the energy-converting hydrogenase (Ech). Ech shares a common evolutionary ancestor with respiratory complex I. The reaction catalysed by this complex is known, however, structural and mechanistic understanding and its interplay with the other complexes is lacking so far.

#### Goals:

We aim to elucidate the energy conservation mechanisms employed by the methanogenic electron transport system. Due to its simplicity compared to the aerobic respiratory chain complexes and the evolutionary conservation of similar complexes across all domains of life, we would like to infer a general theory of how biological electron transport systems work and evolved.

#### Methods:

We cloned genes encoding subunits of the Ech complex from *Methanosarcina mazei* Gö1 (*M. mazei*) fused to a terminal Twin-Strep-tag into an expression vector for transformation of *M. mazei*. We then employed affinity chromatography and gel filtration to natively pull-down and purify the full Ech complex from detergent-solubilized membranes under anaerobic conditions. Next, we vitrified cryo-EM grids to determine its protein structure using cryo-EM single-particle analysis. Furthermore, we plan to determine catalytically relevant conformations of Ech by vitrifying the protein in copolymer-nanodiscs and native vesicles under turnover conditions.

#### Results:

We successfully purified catalytically active Ech solubilized in LMNG and DDM. The pull-down showed no co-purification of other complexes. Cryo-EM grids of Ech were vitrified and showed good particle distribution.

#### Summary:

Many anaerobic microorganisms like *M. mazei* possess membrane-bound electron transport systems closely resembling the aerobic respiratory chain, which are evolutionary more ancient and offer a possibility to study underlying energy conservation principles. We employ cryo-EM to investigate the catalytic mechanism of the *M. mazei* Ech complex in different membrane environments.

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### SL-AM-02

#### Pathways of glycine or serine degradation in the thermophilic acetogen *Thermacetogenium phaeum*

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The thermophilic, acetogenic bacterium *Thermacetogenium phaeum* is known for utilizing acetate via a reversed Wood-Ljungdahl pathway (WLP), when grown with a methanogenic partner in syntrophic culture. Beyond acetate, *T. phaeum* has a broad metabolic versatility. Amino acids, such as glycine and serine, are among the good substrates for *T. phaeum*, although this organism lacks glycine reductase, a key enzyme in glycine degradation in the mesophilic anaerobe *Peptoclostridium acidaminophilum*.

To study amino acid metabolism in this thermophilic anaerobe, amino acids or non-amino acid compounds were supplied as substrates to axenic or syntrophic cultures of *T. phaeum*. Growth experiments were carried out to observe growth yield, substrate degradation and metabolite accumulation.

Glycine and serine were degraded to acetate in both axenic and syntrophic cultures. The produced acetate accumulated and could not be further utilized, indicating that inhibitors might have been produced, leading to the inactivation of the acetate oxidation pathway.

Furthermore, comparative proteome analysis was performed to demonstrate the differences in abundance of relevant enzymes under various incubation conditions. In addition, in vitro enzyme assays were conducted to investigate the activity of serine- and glycine-converting enzymes.

Based on proteome analysis and enzyme assays, two serine-converting enzyme systems were found: serine dehydratase and the combination of glycine hydroxymethyltransferase and glycine cleavage system, which were expressed and activated specifically for utilizing amino acids. Among these enzymes, serine dehydratase was the most active one in enzyme assays, despite the low abundance in the proteome. A modified thermophilic degradation pathway linking glycine and serine degradation to the WLP is proposed.

## SL-AM-03

### Microbial Electrosynthesis Reshapes the Physiology of *Clostridium ljungdahlii*

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Microbial electrosynthesis (MES) enables microorganisms to use electric energy (electrons) to produce valuable compounds. MES from CO<sub>2</sub> relies on anaerobic, electroautotrophic microorganisms that take up electrons from the cathode for CO<sub>2</sub> fixation, primarily producing acetate via the Wood-Ljungdahl pathway (WLP)<sup>1</sup>. While similar to gas fermentation, the key difference is the direct transfer of electrons instead of H<sub>2</sub> gas. However, the performance of acetogenic microorganisms in MES is lower, with optical densities (OD) less than 0.4, compared to much higher OD in gas fermentation. MES is usually performed in H-type bioreactors, where a cation exchange membrane separates the anode and cathode compartments. This membrane is essential to prevent O<sub>2</sub> generated from the anodic water-splitting reaction from reaching the cathode.

To clarify and identify these stress factors, we studied the MES and gas fermentation performance of the model organism for both processes, *Clostridium ljungdahlii*, grown in H<sub>2</sub>/CO<sub>2</sub> autotrophic gas fermenters and H-type electrobioreactors. We compared growth and acetogenic activity under both conditions, using transcriptome and proteome profiling to gain physiological insights. Additionally, we used transmission electron microscopy to gain a deeper understanding of cellular responses to the different conditions, particularly membrane integrity. We found that the presumed similar physiology between both conditions, as they both rely on H<sub>2</sub>/CO<sub>2</sub>, is not accurate, and that cells grown under MES are different.

Our data indicate a metabolic shift during MES, along with changes in cellular redox state and energetics.

The contribution will present the results of this comparative analysis, highlighting changes in the expression of the WLP, increased activity of genes involved in microcompartment formation, and the expression of an alternative ATP-generating pathway as a stress response during MES.

**Acknowledgments:** The authors acknowledge support from the Deutsche Forschungsgemeinschaft (priority program SPP2240).

## References:

1. Nevin KP, Woodard TL, Franks AE, Summers ZM, Lovley DR. Microbial Electrosynthesis: Feeding Microbes Electricity To Convert Carbon Dioxide and Water to Multicarbon Extracellular Organic Compounds. Colwell RR, ed. *MBio*. 2010;1(2).
2. Abdollahi M, Al Sbei S, Rosenbaum MA, Harnisch F. The oxygen dilemma: The challenge of the anode reaction for microbial electrosynthesis from CO<sub>2</sub>. *Front Microbiol*. 2022;13:947550.

## SL-AM-04

### Recombinant isobutyrate production with the acetogen *Eubacterium callanderi* Marburg

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The usage of fossil fuels is contributing to the generation and emission of greenhouse gases which are important drivers of climate change. For example, isobutyrate is currently synthesized via an acid-catalyzed Koch carbonylation process, which requires fossil-based propylene as precursor. To achieve sustainable production of platform chemicals such as isobutyrate without relying on raw materials derived from fossil fuels, alternative strategies are required.

Acetogens as *Clostridium luticellarii* and *Eubacterium callanderi* can use C1 molecules such as CO, CO<sub>2</sub> + H<sub>2</sub> and, methanol as substrates and convert these to acetyl-CoA via the Wood-Ljungdahl pathway. Acetyl-CoA is here the central metabolic intermediate and is converted to acetate via acetyl-phosphate to obtain ATP by the action of an acetate kinase. Some acetogens such as *C. luticellarii* and *E. callanderi* can elongate acetyl-CoA towards butyrate and hexanoate via reverse  $\beta$ -oxidation. In *C. luticellarii* isobutyrate is presumably produced by the action of a special enzyme called isobutyryl-CoA-mutase (Icm) which uses butyryl-CoA as substrate. The large subunit (IcmA) catalyzes the isomerization, and the small subunit (IcmB) is responsible for the binding of the cofactor vitamin B12. Both genes were cloned in the plasmid pJIR751\_Pfd\_icm under the control of a constitutive promoter and introduced in *E. callanderi*. Isobutyrate production was absent during heterotrophic growth of *E. callanderi* [pJIR751\_Pfd\_icm]. This was most likely due to the lack of an adenosyltransferase (ATR) which activates cob(II)alamin and a G-protein metallochaperone (Mea) which loads the active cofactor to the ICM, which were not expressed in *E. callanderi* [pJIR751\_Pfd\_icm]. Respective genes were subcloned to obtain the plasmids pJIR751\_Pfd\_icm\_Mea and pJIR751\_Pfd\_icm\_Mea\_ATR and to construct the

corresponding strains *E. callanderi* [pJIR751\_Pfd\_1cm\_Mea\_ATR] and *E. callanderi* [pJIR751\_Pfd\_1cm\_Mea]. Heterotrophic and autotrophic growth experiments are currently performed and analyzed in terms of growth and production kinetics. Additionally, the influence of altered concentrations of the inactive form of the vitamin B12 cofactor, cyanocobalamin and the activated form adenosylcobalamin is investigated.

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#### SL-AM-05

##### Metabolic engineering of the acetogenic bacterium *Thermoanaerobacter kivui* to produce lactate

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**Introduction:** The thermophilic acetogenic bacterium *Thermoanaerobacter kivui* grows on different substrates like glucose, pyruvate, formate, CO and H<sub>2</sub> + CO<sub>2</sub> while producing acetate as only end product. Even though it is an ideal organism for industrial purposes because of its thermophilic nature, its resistance to CO and the available genetic system, its product spectrum is limited. To broaden the product spectrum, we developed a production system for valuable products and chose, as proof of principle, the production of lactate.

**Goals:** To genetically engineer *T. kivui* to produce lactate.

**Materials & Methods:** Generation of mutant, growth experiments, cell suspension experiments and enzyme assays.

**Results:** The gene coding for a lactate dehydrogenase (LDH) of the thermophilic acetogenic bacterium *M. thermoacetica* was cloned into a suicide plasmid and integrated into the genome of *T. kivui*. In cytoplasm LDH activity could be measured with lactate:NAD<sup>+</sup> and NADH:pyruvate oxidoreductase activities of 2.4 and 4.0 U/mg, respectively. The LDH integration mutant was able to produce lactate during growth on glucose. Highest lactate production (45 mM) was seen using 100 mM of glucose with a ratio of lactate/acetate of 0.5. Resting cells of the mutant were able to produce up to 15 mM of lactate with 1-15 mM of acetate, depending on the substrate. Interestingly, the mutant was not only able to produce lactate but could also grow on lactate alone, producing acetate as sole end product in a lactate/acetate ratio of 1.4.

**Summary:** We were able to introduce LDH from *Moorella thermoacetica* into the genome of *T. kivui*, enabling it to not only produce lactate but also utilize it.

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#### SL-AM-06

##### Biochemical and functional characterizations of the O-demethylase system from the methanogenic archaeon *Methermicoccus shengliensis*

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Methanogens are known to use small methylated compounds like methanol or methylamines as substrates for methane production. However, a few years ago it was shown that the methanogen *Methermicoccus shengliensis* is capable of releasing methane from methoxylated aromatic compounds (MACs) [1]. As the monomeric building block of lignin, MACs are ubiquitous in nature. The methyltransferase system MtoABCD was found to catalyze MAC demethylation and transfers the methyl group to tetrahydromethanopterin rather than Coenzyme M, which differs from previously characterized catabolic methyltransferase systems [2]. The *mto* operon contains two copies of the substrate methyltransferase (*mtoB1* and *mtoB2*), and each of the paralogs likely has different substrate preferences. To better understand the function of these paralogs, we cultivated *M. shengliensis* on a selection of MACs and used RT-qPCR to evaluate the differential expression of the *mtoB1* and *mtoB2* genes in response to growth on diverse MACs. Our measurements confirm differential regulation of these genes in response to different MACs, and the kinetic parameters for both paralogs will be determined in subsequent activity assays. Furthermore, we are studying the interaction between the different Mto components. We could already demonstrate that the reductive activator protein (MtoD) of the corrinoid protein (MtoC) binds to MtoC using size exclusion chromatography (SEC). However, no interaction between MtoD and the MtoB2C2 complex was observed, implying that MtoC undergoes reductive activation by MtoD before forming the catalytically active MtoB2C2 complex. The MtoCD complex will be analyzed structurally using cryo-electron microscopy. A similar approach was applied to study the interaction of the second methyltransferase MtoA with MtoC, and to verify if the methylated state of MtoC's cobalamin cofactor affects this interaction. Our SEC data suggest that the binding affinity of MtoA and MtoC decreases if the cobalamin cofactor of MtoC is in the non-methylated state. In conclusion, this project sheds light on the substrate versatility of the MtoB1 and MtoB2 enzymes, as well as elucidates transient protein-protein interactions involved with the functionality of corrinoid-dependent methyltransferase systems, exemplified by the MtoABCD system from *M. shengliensis*.

[1] Mayumi *et al.* (2016) *Science* 354: 222

[2] Kurth *et al.* (2021) *ISME J* 15: 3549

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#### SL-AM-07

##### Structural and biochemical characterization of the group IV nitrogenase homolog from *Endomicrobium proavitum*

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Bioavailable nitrogen sources are essential for all life. However, converting molecular dinitrogen (N<sub>2</sub>) to bioavailable ammonia (NH<sub>3</sub>) is energy (ATP)-intensive and catalyzed by the complex metalloenzyme nitrogenase. Thus, nitrogenases play a critical role in the biological nitrogen cycle. All nitrogenase enzymes are highly reactive and reduce a whole range of small substrates. One section of the large nitrogenase tree are nitrogen-fixation-like (Nfl) enzymes, which are structural homologs of nitrogenases with diverse functions<sup>1</sup>. The Nfl-enzymes encompasses ancient metalloproteins involved in archaeal methanogenesis (CfbCD, F430 biosynthesis), bacteriochlorophyll synthesis (COR/DPOR, reducing carbon-carbon double bonds of the

tetrapyrrole rings) and recently it was discovered that they are involved in sulfur scavenging (MarHDK, reducing carbon-sulfur bonds)<sup>2-4</sup>.

The Nfl-enzymes are gaining increasing attention due to the fact that they catalyze novel reduction reactions, that many family members are still uncharacterized and that their functions are still an enigma. One particular intriguing candidate is the group IV nitrogenase homolog from *Endomicrobium proavitum* that has been shown to reduce N<sub>2</sub> to NH<sub>3</sub> *in vivo*<sup>5</sup>. To illuminate this surprising finding, we set out to comprehensively characterize this enzyme structurally and biochemically.

We solved a 2.77 Å cryo-electron microscopy structure of this enzyme and determined the metal content of its nitrogenase-related metal cofactors using ICP-MS. We showed that this enzyme is able to reduce C<sub>2</sub>H<sub>2</sub> to C<sub>2</sub>H<sub>4</sub> and H<sup>+</sup> to H<sub>2</sub> and exhibits low activity in reducing C<sub>3</sub>H<sub>8</sub>OS to C<sub>2</sub>H<sub>4</sub>. Our exciting findings provide new insights into the inner workings of Nfl-enzymes as well as *bona fide* nitrogenases.

1 Raymond, J., *et al.* (2004) The Natural History of Nitrogen Fixation, *Molecular Biology and Evolution*, Volume 21, Issue 3, Pages 541–554.

2 Moser, J., Layer, G. (2019) Biosynthesis of Bacteriochlorophyll and Coenzyme F430. In: Hu, Y. (eds) Metalloproteins. *Methods in Molecular Biology*, vol 1876. Humana Press, New York, NY.

3 Justin A. North *et al.* (2020) A nitrogenase-like enzyme system catalyzes methionine, ethylene, and methane biogenesis. *Science* 369, 1094-1098.

4 Lago-Maciel, A. *et al.* (2025) Methylthio-alkane reductases use nitrogenase metallocusters for carbon–sulfur bond cleavage. *Nat Catal* 8, 1086–1099.

5 Zheng, H., *et al.* (2016), *Endomicrobium proavitum* gen. nov. sp. nov. *Environ Microbiol*, 18: 191-204.

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## SL-AM-08

### SAM- and cobalamin-dependent conversion of estrogens into androgens

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Estrogens belong to the family of steroid hormones, which are derived from cholesterol. They are essential for the metabolism, physiology, development and regulatory functions of female mammals, including humans. The major natural occurring estrogen, 17β-estradiol, is composed of a tetracyclic core ring structure with a phenolic A ring, making it a highly stable molecule with low water solubility (1.5 mg L<sup>-1</sup>). In addition to naturally occurring estrogens such as 17β-estradiol and estrone, estrogens are also produced synthetically, for example in the form of 17α-ethinylestradiol, a component of birth control pill. The stability and low water solubility of these estrogenic compounds present a significant challenge in terms of microbial degradation. Their persistence and elevated concentrations in the environment

has shown to cause adverse effects on aquatic life, e.g. feminization of male fish, and to increase the risk of cancer in humans. Therefore, the World Health Organization has classified estrogens as type I carcinogens.<sup>1</sup>

Biodegradation is considered the main process for the complete mineralization of estrogenic compounds into carbon dioxide. Metabolite analysis revealed that in the denitrifying β-proteobacterium *Denitratisoma* sp. strain DHT3, estrogens are initially converted to androgens via C10 methylation and dearomatization of the phenolic A ring.<sup>2</sup> This energetically challenging reaction is putatively catalyzed by an unusual S-adenosyl-L-methionine- (SAM-) and cobalamin-dependent estrogen methyltransferase (Emt) complex encoded by the *emtABCD* genes.<sup>3</sup>

In this study, we report the enrichment and biochemical characterization of this novel EmtABCD methyltransferase complex from the denitrifying model organism *Denitratisoma oestradiolicum*. Furthermore, we used cryo-electron microscopy to obtain structural and mechanistic insights.

<sup>1</sup> Adeel M. *et al.*, *Environment International* (2017)

<sup>2</sup> Wang P.-H. *et al.*, *PNAS* (2020)

<sup>3</sup> Jacoby C. *et al.*, *mbio* (2020)

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## SL-AM-09

### Beyond the pH comfort zone: Potential of *Ca.*

### *Methanoperedens* to adapt and stay active at low pH

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Members of the archaeal genus *Candidatus Methanoperedens* are able to oxidise methane under anoxic conditions while utilising available electron acceptors such as nitrate. The most advanced laboratory enrichment cultures of *Ca. Methanoperedens* perform methane oxidation at near-neutral pH. However, amplicon data from acidic peatlands show the presence of *Methanoperedens* also in acidic conditions with pH 4-5. Based on the presumed environmental diversity of the genus *Ca. Methanoperedens*, we investigated whether the enrichment culture of *Ca. Methanoperedens* Vercelli Strain 1 is able to adapt to a stepwise decrease in pH in the cultivation bioreactor. Bioreactor batch activity assays utilising <sup>13</sup>C-CH<sub>4</sub> in the headspace were used to compare methane oxidation rates at individual pH checkpoints on the gradient from 7.25 to <6. The enrichment culture adapted to pH decrease, as evidenced by sustained oxidation activity. However, this energy harvesting did not translate into an increase in cell numbers. Analyses of archaeal membrane-spanning lipids revealed that acidic conditions did not alter the composition of the cell membrane. We hypothesize that cells of *Ca. Methanoperedens* remain metabolically active at sub-optimal pH values, allocating the harvested energy to maintenance rather than growth. Such observation can be used to predict the ability of important anaerobic methane oxidisers to colonize distinct environmental niches and to predict their occurrence across a wide range of potential habitats.

## SL-AR/PCB-01

### Influence of the surrounding environment on the swimming motility of *Pseudomonas putida*

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*Pseudomonas putida* is a lophotrichous flagellated bacterium, which lives in the soil. In this heterogeneous porous structure, parameters like the viscosity of the fluid, the pore size and the fluid shear flow massively influence *P. putida*'s swimming motility [1,2]. The flagella of *P. putida* are powered by the two molecular motors MotAB and MotCD leading to three swimming modes [2,3]. When the flagellar bundle rotates counterclockwise it forms the push mode and when it rotates clockwise it either pulls the cell body (pull mode) or wraps around it (wrap mode).

Here, we performed fluorescence microscopy experiments to visualize the swimming modes of *P. putida* wild-type cells and stator knockout mutants swimming in uniform fluids with different viscosities and two agar concentrations as a heterogeneous network. We could show that the two molecular stators have distinct functions in different environments. In aqueous environments, the  $\Delta\text{motAB}$  mutant swims more than four times slower than the wild-type and is unable to form the wrapped mode, demonstrating that the MotAB stator is essential under low viscosity conditions. In contrast, the  $\Delta\text{motCD}$  mutant swims comparably to the wild-type cells in fluids but spreads slower in semisolid agar. It turns out that the MotCD stator prevents the cells from forming sessile clusters and thereby promotes the spreading [1].

Furthermore, we found a higher tendency to form the wrapped mode in higher viscosities and denser structures. This observation supports the hypothesis that the wrapped mode is beneficial for escaping from mechanical traps and navigating in confined spaces. For wild-type cells additional experiments with fluid shear flow were performed. Under shear flow conditions wrapped mode was less often observed probably due to stabilization of the pull mode decreasing the transition rate towards the wrapped mode [2].

Together, these results reveal how *P. putida* dynamically regulates its stator composition and motility modes to adapt to mechanical load and spatial structure. Besides environmental conditions, also the length and mechanical properties of the hook are expected to play a role for the wrapped mode formation, which we are currently investigating based on different mutant cell lines.

[1] Pfeifer V. et al. (2022) *Environ. Microbiol.* 24: 1234–1247.

[2] Muraveva V. et al. (2025) *arXiv*:2510.21548.

[3] Hintsche M. et al. (2017) *Proc. Natl. Acad. Sci. USA* 114: 12955–12960.

## SL-AR/PCB-02

### Forespore centering: a novel step during late *Bacillus subtilis* sporulation?

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Under stress, *Bacilli* can differentiate into metabolically dormant endospores. Sporulating cells divide asymmetrically into a forespore (FS) and mother cell (MC), followed by engulfment of the FS into the MC.

Using automated fluorescence time-lapse microscopy of sporulating *Bacillus subtilis* cells on hydrogel pads, we illustrate that the initially polar position of the engulfed FS within the MC is not fixed. The FS eventually detaches from the MC pole and moves towards the MC's center. While indirect evidence for such a "centering" motion has been abundant for different *Bacilli*, this distinct morphological step is generally not represented in textbooks or reviews of sporulation.

We show that FS centering within the MC depends on key transcriptional regulators exclusively active after engulfment within the MC, and correlates with changes in organization of the MC chromosome. Additionally, we observe that improper FS centering is associated with spontaneous loss of refractivity of the spores either during or even after sporulation.

We propose that FS centering represents an additional developmentally regulated step of the sporulation program that is tightly linked to proper spore morphogenesis.

## SL-AR/PCB-03

### A multidomain response regulator connects the Frz chemosensory system with the polarity module of *Myxococcus xanthus* to regulate motility

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Cell polarity is a fundamental feature of eukaryotic and prokaryotic cells, enabling asymmetric localization of proteins and regulating essential processes such as motility and cell division. In *Myxococcus xanthus*, a model organism for studying bacterial social behavior and development, cell polarity is tightly regulated by a complex network of proteins, including the polarity module and the Frz chemosensory system. While the molecular mechanisms underlying the establishment and reversal of cell polarity have been extensively studied, the direct link between the Frz system and the polarity module remains elusive. This study focuses on PglH, a response regulator identified through proximity labeling experiments as a potential mediator between the Frz system and the polarity module. Bioinformatic analysis reveals that PglH possesses an interesting domain architecture, including a response regulatory domain, a PATAN/GAP domain, and a tetratricopeptide repeat domain, suggesting roles in signal transduction and protein-protein interactions. Phenotypic characterization of a *pglH* deletion mutant shows hyperreversing behavior and defects in both Type IVa pili-dependent and gliding motility, indicating PglH's involvement in regulating cell polarity reversals. Fluorescence microscopy reveals that PglH dynamically



localizes to the lagging cell pole and relocates upon polarity reversal, further supporting its role in polarity regulation. Additionally, PglH influences the localization of MglA and MglB, the two central proteins of cell polarity, suggesting a mechanism by which PglH contributes to the exclusion of MglA from the lagging pole and stimulating lagging pole MglB localization. Mass spectrometry-based proximity labeling revealed that important proteins involved in cell polarity, Frz system and motility localize in proximity of PglH, depending on the Frz signaling intensity. Finally, AlphaFold3-Multimer suggests that PglH could directly interact with MglA and FrzX, thereby bridging the gap between polarity module and Frz system. These findings provide new insights into the molecular mechanisms governing cell polarity and motility in *M. xanthus*. Future studies aim to elucidate the precise molecular interactions and regulatory roles of PglH in this complex signaling network.

#### SL-AR/PCB-04

##### Coordination of DNA replication restart proteins at the single molecule level

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The replication machinery must overcome obstacles during DNA synthesis, including restart of stalled or collapsed forks. DNA helicase PriA is essential for replication restart in a wide range of bacteria, ensuring quick reassembly after obstacle resolution. PriA tightly interacts with SSB tetramers via the last 35 amino acids of SSB, and it has been proposed that PriA accumulates at replication forks facilitating restart via proximity effect [1]. Prior work on *in vivo* dynamics of PriA employed ectopic expression, but PriA is naturally present at low abundance. We generated a strain encoding mNeonGreen-PriA under its native promoter using CRISPR integration [2]. Epifluorescence microscopy indicated that PriA localization at native level leads to foci formation in a minority of cells during exponential growth, dependent on the C-terminus of SSB, and predominant nucleoid-staining. Single molecule tracking (SMT) supports the idea that that besides recruitment via SSB, PriA shows a DNA-scanning mode that enables a target-search. SMT further revealed major differences in the dynamics of SSB, DNA helicase DnaC and further restart proteins DnaI and DnaD, contradicting a simple proximity effects. Replication stress induced by HPUra (polymerase inhibition) or Mitomycin C (DNA crosslinks leading to fork stalling) showed major changes in replication fork-bound and freely diffusing SSB fractions indicating the generation of large ssDNA regions, while PriA showed different changes between bound, DNA-scanning and freely diffusing populations, including dissociation from chromosomes during crosslink repair. DnaC exhibited minor changes, suggesting that SSB alterations arise by continued DnaC helicase activity while polymerases are blocked. Our data indicate that replication restart has to deal with unaltered helicase activity during stalled polymerase function, and PriA that needs to coordinate regaining of coordinated fork progression.

[1] **Lecointe, F., et al. (2007).** "Anticipating chromosomal replication fork arrest: SSB targets repair DNA helicases to active forks." *EMBO J* 26(19): 4239-4251

[2] **Matthews, L. A. and L. A. Simmons (2022).** "The *Bacillus subtilis* PriA Winged Helix Domain Is Critical for Surviving DNA Damage." *Journal of Bacteriology* 204(3): e00539-00521

#### SL-AR/PCB-05

##### Haloarchaea and their viruses in hypersaline ecosystems

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Extremophilic microorganisms are fascinating study objects due to their capability to adapt to the most extreme environments on this planet. Hypersaline environments, such as salt lakes, salt evaporation ponds or solar salterns, are the natural habitat of haloarchaea. Only at these specific places on earth, it is possible to find haloarchaea and haloarchaeal viruses in high abundance and diversity<sup>1</sup>. This study aimed to isolate and identify haloarchaea and their viruses from two distinct salt pans in Italy, from Trapani (Sicily) and Margherita di Savoia (Barletta-Andria-Trapani). Salt water and crystal samples were used as a starting material for the isolation. Here, we present the identified archaea found in the salt pans as well as new virus isolates. In addition to the laboratory approach for the identification of archaea and virus species, a metagenomic analysis of the salt water samples was conducted. The isolated viruses and archaea have been characterized further by, e.g. infection assays and microscopy. Further research with these virus-host pairs will potentially gain new insights into virus-host interactions of archaea and their viruses. Besides, the comparison between the laboratory isolation approach and the metagenomic study underlines the importance to complement laboratory work with bioinformatic methods to prevent a loss of information due to sampling bias. The obtained knowledge of this study will enhance our understanding of the archaeal virosphere and thus contribute greatly to our understanding of viruses in ecology.

#### References

<sup>1</sup> Kuiper, B. P., Schöntag, A. M. C., Oksanen, H. M., Daum, B., & Quax, T. E. F. (2024). Archaeal virus entry and egress. *MicroLife*, 5.

#### SL-AR/PCB-06

##### Pervasive Nucleotide Tandem Repeats Drive Genomic Plasticity and Adaptive Evolution in Methanogens

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Methane emissions are largely caused by methanogenic archaea (methanogens), a phylogenetically diverse group inhabiting numerous anoxic ecosystems. Despite their central role in global carbon cycling, the genomic mechanisms underlying their ecological adaptability and resilience remain largely unexplored. Here we show that, across all prokaryotic lineages, methanogens of the cosmopolitan *Methanosarcina* and the rumen-associated *Methanobrevibacter* genera harbor pervasive nucleotide tandem repeats (TRs) in their genomes, showing TR densities of  $3.1 \pm 0.7$  TRs/100kb and  $2.8 \pm 0.8$  TRs/100kb, respectively. These AT-rich, perfect head-to-tail arrays occur predominantly in intergenic and regulatory regions and have

arisen recently and independently. When in open reading frames, TRs often introduce intrinsically disordered regions in 62.7% of TR-proteins versus only 11.9% of all proteins. These TR-proteins are often predicted to be extracellular or associated with the cell wall ( $p < 1.0e-23$ ). A clustering of methanogen proteomes revealed that TRs of homologous proteins had a similar amino acid composition, and TRs were particularly associated with protein families with nucleic acid binding domains. The organization and composition of methanogen TRs closely parallel those of TRs found in Borgs, which are giant extrachromosomal elements associated with methane-oxidizing archaea. This suggests that TRs may represent an unrecognized and conserved mechanism of genome modulation among archaea and associated elements linked to the methane cycle. Comparative genomics revealed very strong sequence variability in TR composition even among closely related isolates. Laboratory experiments and long read sequencing of the model methanogen *Methanosarcina mazei* Gö1 revealed rapid evolution of several TR regions and even without selection pressure, we observed evolutionary changes in TR unit number in up to 0.5% of the TR-harboring reads. Moreover, several intergenic TR regions were transcribed, suggesting potential regulatory RNA functions. Together, our findings uncover TRs as an intrinsic and dynamically evolving genomic feature that promotes plasticity and adaptive evolution in methanogens.

#### SL-AR/PCB-07

##### Mapping genome organization and DNA-binding proteins in the archaeon *Haloferax volcanii* using super-resolution microscopy

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Archaea, the third domain of life alongside bacteria and eukaryotes, share features with both: like bacteria, they possess circular chromosomes and lack a nucleus, yet they utilize eukaryotic-like transcription and replication machinery.

Among Haloarchaea, *Haloferax volcanii* has emerged as a genetically and biochemically tractable model organism (Pohlschroder et al., 2025). Recent advances in single-molecule imaging of *H. volcanii* (Schrage et al., 2025; Turkowyd et al., 2020) now enable nanoscale-resolution studies in this archaeon.

As a polyploid archaeon with 6–12 chromosomal copies per cell, *H. volcanii* poses unique questions regarding genome organization and regulation. It remains unclear whether all chromosomal copies are equally replicated and transcribed, and how mechanisms like transertion and transcription–translation coupling—well-studied in bacteria (Sanamrad et al., 2014; Spahn et al., 2025; Stracy & Kapanidis, 2017)—contribute to nucleoid architecture in archaea. While nucleoid compaction has been observed, the molecular basis and functional implications of this compaction remain unclear (Delmas et al., 2013).

Here, we use single-molecule localization microscopy (SMLM) to super-resolve the spatial organization of DNA and DNA-binding proteins in *H. volcanii* below the diffraction limit. We establish two complementary DNA imaging methods in fixed cells: (1) DNA-PAINT to visualize the entire genome, and (2) dSTORM imaging of newly replicated DNA via EdU labeling and click chemistry. Proteins are visualized using photoconvertible fluorescent proteins.

This integrated imaging approach reveals, for the first time, distinct and structured nanoscale genome organization in *H. volcanii*, along with its interplay with the transcription machinery. By using antibiotic treatments to halt specific cellular processes, our findings provide new insights into genome regulation, chromosomal architecture, and transcriptional organization in this polyploid archaeon under different cellular conditions and environmental cues.

#### SL-AR/PCB-08

##### *Methanopulvis terrae* gen. nov., sp. nov., a member of the order *Methanomassiliicoccales* isolated from a millipede

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*Methanomassiliicoccales* is one of four methanogenic orders commonly represented in the digestive tract of animals. However, only two species, *Methanomassiliicoccus luminyensis* (*Methanomassiliicoccaceae*) and *Methanomethylophilus alvi* (*Methanomethylophilaceae*), both isolated from the human gut, have been brought into culture. Here, we describe the first members of this order from the intestinal tract of arthropods. Strain Am95 was isolated from the gut of the millipede *Anadenobolus molinicornis*. Based on phylogenomic analysis, it represents a hitherto uncultured genus-level lineage in the family *Methanomassiliicoccaceae*, for which the name *Methanopulvis terrae* gen. nov., sp. nov. is proposed. Like all members of *Methanomassiliicoccales*, the strain Am95 is an obligately methyl-reducing hydrogenotrophic methanogen that utilizes methanol and methylamines as substrates. Cells are true cocci with a diameter of 0.2–3.0 µm and lack the typical autofluorescence of cofactor F420. The cells are non-motile, but cell appendages were apparent in electron micrographs of ultrathin sections. Growth in mineral medium is slow but strongly stimulated by acetate and yeast extract. Highest growth rates (0.065 h<sup>-1</sup>) were observed at 42 °C and pH 7.0. Genome size (2.49 Mbp) and GC content (61.5 mol%) are in the range of other members of *Methanomassiliicoccales*.

#### SL-AR/PCB-09

##### Small protein mediated transport of Trimethylamine in *Methanosarcina mazei*

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Small proteins are increasingly recognized as key players in the regulation and diversification of cellular processes. In *Methanosarcina mazei*, two such proteins, MttS (49 aa) and MttQ (97 aa), form part of a tripartite membrane complex together with the drug/metabolite exporter (DME) family protein MttP. In combination, these three subunits constitute the MttPQS complex, the first experimentally validated trimethylamine (TMA) transporter in archaea.

Using a combination of bioinformatic, biochemical, and genetic approaches, we show that MttP tightly interacts with newly identified protein MttS and MttQ to form a stable

oligomeric complex embedded in the cytoplasmic membrane. Heterologous expression of the MttPQS operon in *Escherichia coli* conferred the ability to import TMA, providing the first experimental evidence that MttP functions as an TMA importer rather than an drug/metabolite export protein, thereby reversing the canonical directionality of DME transporters. Deletion of *mttS* in *M. mazei* resulted in markedly reduced growth on TMA, underscoring its essential role in substrate recognition and uptake.

Since, TMA and its oxidation product TMAO are linked to cardiovascular disease, understanding archaeal TMA metabolism is of growing interest. Methanogenic gut symbionts that consume TMA could thus offer protective benefits to their hosts. In this context, the MttPQS complex exemplifies an evolutionary innovation that allows methanogenic archaea to expand their metabolic repertoire and thrive in methylamine-rich environments, providing new insights into small protein mediated regulation and archaeal ecology.

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## Microbial communities

### SL-MC-01

#### Mapping siderophore and bacteriocin interactions of *Staphylococcus epidermidis* with nasal bacteria

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### Introduction

Staphylococci and corynebacteria are key members of the human nose and skin microbiomes. They engage in various interactions, including those governed by siderophores and bacteriocins. *Staphylococcus epidermidis* is a frequent colonizer of human nose with important roles in health and infection. Despite its relevance, it is unclear how iron limitation and siderophore production shapes interactions of *S. epidermidis* with other bacteria. Furthermore, many strains of *S. epidermidis* are known to produce bacteriocins, including epilancins that can kill other staphylococci as well as corynebacteria. How the production of these antibacterial compounds affects social interactions of *S. epidermidis* and shape nasal bacteria communities has not been elucidated yet.

### Goals

We investigated the types of interactions, *S. epidermidis* engages in with nasal bacteria, focusing on the role of iron limitation. Furthermore, we explored the impact of epilancin production on these interactions with the goal to understand the ecological role of these bacteriocins in the nasal microbiome.

### Materials & Methods

We determined the ability of a range of nasal isolates to grow in iron-limiting conditions as well as siderophore production and consumption capabilities. We then mapped their interactions with *S. epidermidis* under iron-limiting conditions to evaluate the role of siderophores in microbial social relations. We further assessed the antibacterial activity of staphylococcal epilancin producers against the nasal isolates.

## Results

Interaction mapping revealed that *S. epidermidis* engages in various types of interactions with nasal bacteria. We found that fast-growing species of the genera *Klebsiella*, *Corynebacterium* and *Staphylococcus* were cheated on by *S. epidermidis*. Some of these strains defended against this at least in part by production of enterobactin-type siderophores. We frequently observed cheating of slow-growing, non-siderophore-producing staphylococci and corynebacteria against *S. epidermidis*, while mutualistic interactions were restricted to few staphylococci including *Staphylococcus aureus*. Epilancin producers were found to differ significantly in inhibiting activity and primarily targeted species observed to engage in cheating against *S. epidermidis*.

## Summary

Siderophores and epilancins shape the interactions of *S. epidermidis* with other bacteria commonly found in skin and nose microbiomes. This may also affect proliferation of *S. aureus*.

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### SL-MC-02

#### Winning the Iron Race: Ferritin is essential for survival of *Corynebacterium glutamicum* under iron limiting conditions in microbial communities

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Introduction: Iron (Fe) is an essential co-factor for many bacterial enzymes involved in processes like DNA synthesis or gene regulation [1]. Since Fe<sup>2+</sup> causes oxidative stress through the Fenton reaction, bacteria must keep the concentration of free Fe<sup>2+</sup> in the cell low. Therefore, many bacteria employ iron storage proteins (ISPs) such as ferritin (Ftn), bacterioferritin (Bfr), or Dps (DNA-binding proteins from starved cells), to oxidize and store iron in the non-toxic Fe<sup>3+</sup> state [1].

Objectives: Our goal is to elucidate the importance of ISPs for competitive iron acquisition, cytoplasmic iron levels, and oxidative stress responses using *Corynebacterium glutamicum* as a model system. For this purpose wild type (WT) and ISP-deficient mutants [2] were compared with various methods.

Materials & Methods: For competition experiments, WT and ISP-deficient strains were fluorescently tagged with mVenus or E2-Crimson and co-cultivated in glucose minimal medium. After serial transfers, strain ratios were determined by flow cytometry. Additionally, growth dynamics of the WT and the  $\Delta ftn$  mutant were monitored in a  $\mu$ -fluidic device. Labile iron levels were compared using a novel "Matryoshka"-style ferrous iron sensor [3]. The responses and sensitivity to oxidative stress were assessed by RNA-Seq and H<sub>2</sub>O<sub>2</sub> diffusion assays.

Results and Summary: In serial co-culture experiments, the  $\Delta ftn$  and  $\Delta ftn\Delta dps$  mutants were rapidly outcompeted by the WT under iron limitation.  $\mu$ -fluidic time-lapse imaging confirmed a reduced growth rate of the  $\Delta ftn$  mutant under these conditions. Preliminary measurements with a ferrous iron sensor further suggested an increased labile iron pool in the  $\Delta ftn$  or  $\Delta ftn\Delta dps$  mutant compared to the WT. RNA-Seq analysis revealed that cells lacking Ftn respond to oxidative

stress by downregulating the expression of iron uptake systems via the master regulator DtxR [4]. Consistently, inhibition zone assays demonstrated that loss of ISPs increased the sensitivity to oxidative stress. Together, these findings demonstrate the critical role of ISPs in competitive fitness under iron limitation and oxidative stress resistance, both of which are crucial for survival in natural habitats.

[1] Andrews et al. (2003) FEMS Microbiol. Rev. 27: 215-237

[2] Küberl et al. (2016) PNAS 113: 4806–4811

[3] Papadopoulos et al. (2025) bioRxiv  
<https://doi.org/10.1101/2025.07.16.665043>

[4] Wennerhold and Bott (2006) J. Bacteriol. 188: 2907–2918

### SL-MC-03

#### Spatiotemporal gene expression and multilayer formation in *Stenotrophomonas maltophilia* mixed species biofilms

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#### Introduction:

Pathogenic multispecies biofilms play a crucial role in the persistence and virulence of several bacterial infections, complicating treatment methods due to their strong resistance to antimicrobial therapies. The bacteria *Stenotrophomonas maltophilia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are widely known for their substantial role in pathogenicity of infected lungs.

#### Goals:

This study employs advanced live cell imaging and quantification techniques to enhance our understanding of the time-dependent and spatial dynamics involved in the formation of these complex biofilm communities and to explore the localization and timing of gene expression within the different layers of a biofilm.

#### Methods:

Using high-resolution lattice light sheet microscopy combined with the quantification tool Biofilm Q we were able to visualize and quantify the development stages and architectural organization of single and dual species biofilms composed of *S. maltophilia*, *S. aureus* and *P. aeruginosa* over 24 hours. Spatiotemporal gene expression could be visualized for specific genes with fluorescent reporter fusion constructs.

#### Results:

This approach enabled the detailed observation of initial adhesion, microcolony formation, maturation, and dispersion phases in real-time. In dual species biofilms we observed

layer formation with *S. maltophilia* dominating the bottom layers of the biofilms. *S. maltophilia* also exhibited spatiotemporal gene expression for selected genes involved in antibiotic resistance, virulence and respiration.

#### Conclusions:

The data obtained in this study offer novel insights into the sequential and spatial organization of gene expression in multispecies biofilms and highlight the variable genetic responses triggered by inter-species interactions and environmental pressures. These findings improve our understanding of biofilm physiology and pathogenesis, providing potential targets for therapeutic strategies and disruption of biofilm development.

### SL-MC-04

#### Toolkit for investigating phenotypic heterogeneity in multidrug-resistant *Klebsiella pneumoniae*

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Molecular studies of *Klebsiella pneumoniae* are limited by the prevalence of multidrug-resistant (MDR) strains, which are resistant to commonly used antibiotic selection markers, rendering standard molecular tools ineffective. Here, we developed a set of plasmids enabling simultaneous visualization and single-cell gene expression analysis in MDR *K. pneumoniae* using alternative antibiotic markers.

Using this system, we observed pronounced phenotypic heterogeneity during *in vitro* biofilm formation, with two distinct populations: biofilm-associated cells at the base and large, clonal microcolonies on the top. Proteomic analysis revealed that microcolony growth correlates with elevated expression of RpoS-regulated stress-response genes. Confocal microscopy with our dual-fluorophore system confirmed higher RpoS expression in microcolony cells compared to the rest of the biofilm. Consistent with this, an *rpoS* knockout mutant failed to form mature microcolonies. We further identified type 3 fimbriae and capsule as contributors to microcolony formation.

Importantly, our imaging toolkit is applicable for visualizing *K. pneumoniae* cells in *ex vivo* systems, such as precision-cut lung slices.

Overall, this toolkit provides a robust approach for studying phenotypic heterogeneity in MDR *K. pneumoniae in vitro*, *ex vivo* and potentially *in vivo*.

### SL-MC-05

#### Deep metaproteome analysis of microbiomes using the MPA-Cloud showcased by microbiomes studies from the human gut

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Metaproteomics provides comprehensive insights into microbiomes, which is beneficial for studying human health, disease, and environmental processes. Advances in mass

spectrometry have markedly increased the depth of analysis, enabling the detection/identification of thousands of proteins and providing detailed functional and taxonomic profiles. However, the large data volume and complexity of multi-species communities pose major challenges for data processing. To address these, we developed the MPA-Cloud, a cloud-based successor of the MetaProteomeAnalyzer. It provides scalable computation, a user-friendly web interface, and reliable data management through RESTful services and an integrated NEO4J-database backend. The platform supports reproducible, large-scale analysis of metaproteomics data directly in the browser.

The usability of the MPA-Cloud is demonstrated through a meta-study of human gut microbiomes from patients with inflammatory bowel disease (IBD) analyzed with different experimental meta proteomics workflows. Over 600 fecal samples were reanalyzed, supported by knowledge graph-based interpretation. We identified 59 protein groups primarily varying due to disease rather than technical variation; among them, Alpha-1-acid glycoprotein, which was newly reported for IBD. Validation across independent datasets confirmed 53 of these groups, with 23 proteins, mainly linked to neutrophil vesicles significantly associated with remission during treatment. Comparative analyses in non-alcoholic steatohepatitis, diabetes, and colorectal cancer highlighted the value of biomarker panels, as single markers lacked disease specificity.

Our work demonstrates how metaproteomics empowers the discovery and validation of disease-specific biomarker candidates. The MPA-Cloud accelerates and standardizes metaproteomic data evaluation, supporting integrative microbiome research on a global scale.

## SL-MC-06

### Stable Microbial Assemblages on Aquatic Macrophytes Reflect Generalist Phyllosphere Taxa

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Shallow coastal habitats support diverse macrophyte communities and are characterized by steep abiotic gradients that can strongly influence the leaf surface epimicrobiome, known as the phyllosphere. To investigate the effects of salinity and host species on phyllosphere microbial composition, we sampled the leaf surfaces of eelgrass (*Zostera marina*) across a salinity gradient of 6–15 PSU, alongside Eurasian water milfoil (*Myriophyllum spicatum*), muskgrass (*Chara* spp.), and sago pondweed (*Stuckenia pectinata*) in the brackish Baltic Sea during the summer of 2022. Microbial communities were profiled using 16S and 18S rRNA gene amplicon sequencing. As hypothesized, the phyllosphere microbiome was distinct from that of the

surrounding seawater, indicating the presence of a host-associated microbial signature. Bacterial taxa commonly associated with the phyllosphere included *Loktanelia*, *Pseudorhodobacter*, the methylophilic genus *Methylotenera*, unclassified *Synechococcales*, and members of *Rhodobacteriaceae*. Among protists, *Picochlorum* was consistently detected across all macrophyte hosts, while *Cocconeis*, *Cyclotella*, *Mondou*, and unclassified *Bacillariophyceae* were present on all hosts except *Chara* spp. Both salinity and host species significantly influenced phyllosphere microbial communities, primarily by altering the relative abundance of core taxa. However, only 4–11% of taxa were uniquely associated with a specific salinity level or macrophyte species. Our findings demonstrate that aquatic macrophyte phyllospheres harbor a characteristic and relatively stable microbiota. The limited number of taxa exclusive to particular salinities or hosts suggests that dominant phyllosphere microbes are broadly adapted to the general conditions of leaf surfaces in aquatic environments. This implies that the presence of the macrophyte itself exerts a stronger influence on microbial community structure than variations in salinity or host identity. For seagrass meadow management, this stability suggests that the phyllosphere microbiome may be resilient to moderate salinity fluctuations.

## SL-MC-07

### The Methane Makers Within Us: Defining the Ecological Niches of the Three Major *Methanobrevibacter* Species in the Human Gastrointestinal Tract

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Methane (CH<sub>4</sub>) production is not limited to ruminant animals; the human gastrointestinal tract also hosts methanogenic archaea capable of generating methane, though at lower levels. Roughly 40% of individuals can be classified as high methane emitters (HE; CH<sub>4</sub> > 4 ppm). These individuals possess a distinct gut microbiome characterized by a predominance of one archaeal genus, namely *Methanobrevibacter*. The composition of this HE microbiome is influenced by diet and has been associated with gastrointestinal disturbances, including constipation and inflammatory bowel disease.

We hypothesized that *Methanobrevibacter* species rely on specific bacterial partners to sustain growth and activity. Such cooperative interactions, together with dietary inputs and overall host health, likely determine the establishment and function of methanogenic consortia in the gut. To explore these dynamics, fecal samples from 80 participants (40 HE and 40 low emitters, LE) were subjected to an integrative multi-omics analysis combining metagenomics, metatranscriptomics, and culturomics.

High emitters displayed a significantly greater abundance of archaeal taxa than low emitters ( $q = 1.43 \times 10^{-6}$ ). While both groups were dominated by *Methanobrevibacter*, the species composition varied markedly. In HEs, *M. smithii* and *M. intestini* prevailed, whereas *M. sp900766745* was most abundant in LEs. Each of these methanogens showed distinct associations with bacterial partners, forming specific interaction networks unique to the respective gut environment. Transcriptional profiling revealed enhanced activity of methanogenic archaea in high emitters, particularly for genes involved in methanogenesis, the incomplete tricarboxylic acid (TCA) cycle, and gluconeogenesis. These

functional patterns were further validated through pure culture experiments with isolated *Methanobrevibacter* strains, which expressed the same genes observed in the metatranscriptomic data of stool samples.

Together, these results provide new insights into the ecological and functional complexity of human gut methanogens. Differences in species composition, gene expression, and syntrophic networks in high and low emitters emphasize the specialized roles of *Methanobrevibacter* species in methane metabolism. These findings underscore the central contribution of methanogens to gut microbial ecology and potential strategies for targeted modulation of methane production and host intestinal health.

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#### SL-MC-08

##### **ISOTRACE-WWT: Isotope Tracing of Carbon and Oxygen in Microbial Communities of Wastewater Treatment Systems**

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Wastewater treatment plants (WWTPs) play a crucial role in safeguarding aquatic ecosystems and drinking water resources by employing physical, chemical, and biological processes to remove contaminants. As they treat influent from sewage systems, industrial facilities, and agricultural runoff, WWTPs function as complex engineered ecosystems in which diverse microbial communities drive organic matter degradation and nutrient turnover through tightly coupled biogeochemical processes. Yet, the composition of these microbial consortia, the identity of key taxa, and the environmental drivers shaping their activity remain insufficiently understood. Because carbon (C) and oxygen (O) cycling are central to pollutant degradation, the ISOTRACE-WWT project seeks to elucidate microbial contributions to C and O transformations along the treatment cascade of a large-scale WWTP (Nürnberg, Germany). In this project, both biofilm and planktonic fractions will be characterized using shotgun metagenomic sequencing, providing insights into microbial diversity and metabolic potential. The molecular data will be integrated with stable isotope analyses of dissolved inorganic, dissolved organic, and particulate C pools ( $\delta^{13}\text{C}_{\text{DOC,DIC,POC}}$ ), as well as dissolved O ( $\delta^{18}\text{O}_{\text{DO}}$ ), to trace microbial processing of C substrates and O utilization. Complementary nutrient, hydrochemical, and physicochemical parameters will further outline microbial-isotopic interactions across treatment stages. This interdisciplinary approach aims to unravel the microbial mechanisms driving C turnover, respiration, and O dynamics in wastewater treatment systems, offering new perspectives on microbial controls over engineered biogeochemical cycles.

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#### SL-MC-09

##### **Qualitative and quantitative molecular investigations of bacterial community composition in domestic washing machines categorized as smelling by their users**

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**Introduction:** Washing machines contain significant amounts of microorganisms with negative implications for laundry and machine hygiene [1]. The causes of laundry and machine malodor and the role of microbes in this process remain unclear [2]. We applied Oxford Nanopore-based full-length 16S rRNA gene sequencing and quantitative PCR to characterize bacterial communities from detergent drawers, door seals, and sumps of washing machines categorized as "smelly" (n=8) or "non-smelly" (n=8) by users at Furtwangen University.

**Goals:** To qualitatively and quantitatively compare bacterial communities between "smelly" and "non-smelly" washing machines and assess whether specific taxa or bacterial loads are associated with perceived odor.

**Materials & Methods:** Swab samples were collected from three machine sites. DNA was extracted, quantified, and analyzed by full-length 16S rRNA gene amplicon sequencing (Oxford Nanopore Technologies) and qPCR targeting the 16S rRNA gene. Reads were processed with Epi2ME v5.2.4 and classified to species level. Alpha and beta diversity metrics were calculated in R and QIIME2. Statistical differences were evaluated using the Wilcoxon–Mann–Whitney U test, PERMANOVA, and ANOSIM.

**Results:** The bacterial community was dominated by Pseudomonadota, Actinomycetota, Bacillota, and Bacteroidota, consistent with previous studies [3,4]. Dominant bacteria varied mainly by sampling site, with minor differences between smell groups. Among all locations, the detergent drawer was the only site that exhibited a significant difference in both alpha diversity (Shannon index) and beta diversity, as determined by weighted and unweighted UniFrac PCoA analyses. *Moraxella osloensis*, a species linked to laundry malodor [5], dominated door seal samples in both groups. qPCR revealed no significant difference in bacterial DNA concentration between "smelly" and "non-smelly" machines, but a trend towards more bacterial DNA in "smelly" machines.

**Summary:** Washing machine microbiomes differed mainly by site, while odor status showed no clear effect. Standardized odor evaluation, metatranscriptomic analyses and larger sample sizes are recommended for future studies.

[1] Gattlen et al. (2010) Biofouling 26:873–882. [2] Munk et al. (2001) J Surfact Deterg 4:385–394. [3] Jacksch et al. (2019) Microorganisms 8:30. [4] Nix et al. (2015) Tenside Surfactants Detergents 52:432–440. [5] van Herreweghen et al. (2020) Microorganisms 8:1170.

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#### SL-MC-10

##### **Resource allocation controls the composition of synthetic microbial communities**

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Biophysical constraints in microbes have fundamental consequences for their physiology. The observed constant intracellular density across growth rates and culture conditions in bacteria leads to a trade-off between investing limited resources into anabolism or catabolism. Here, we investigate the role resource allocation plays in controlling the composition of minimal bacterial communities that are obligately coupled through amino acid auxotrophies.

We constructed 12 amino acid knockout strains of *Vibrio natriegens*, and co-cultured each combination in sparse minimal medium. Remarkably, we found that for each community the final composition (after multiple rounds of passaging) does not depend on the initial seed composition – each community seems to be attracted to its own stable composition equilibrium.

Since each member in a co-culture is coupled through auxotrophy, the community growth rate is constrained by the efficiency at which the members share resources to overcome their biosynthetic auxotrophies. We hypothesized that intracellular resource allocation constraints in each member is the primary determinant of their ability to engage in mutualism and leads to the observed equilibrium composition. For example, some pathways are costlier in energy or protein resources, and the equilibrium abundance of each member is a function of these differences. To test this hypothesis, we separately overexpressed an ATP and protein waster enzyme (ppsA and lacZ, respectively) in each member. Since each community member has a genomically integrated fluorophore, we could use cytometry to measure the shift in the equilibrium composition. We coupled this with quantitative proteomics to develop a simple model that links the equilibrium composition to the resource cost each member invests in the community. Under certain conditions, we show that the equilibrium abundances result from a near optimal resource allocation strategy across the community.

#### SL-MC-11

##### Tracking Tri-cultures: Resolving microbial interaction dynamics through microfluidic cultivation and live-cell imaging

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Microfluidic single-cell cultivation enables the study of microbial populations and individual cells by providing precisely controlled and dynamic environments at the micrometre scale; a unique platform for automated live-cell imaging. Conventional methods at laboratory scale and shake flask cultures, average and obscure spatio-temporal dynamics and cellular interdependencies within cell populations, making it challenging to study microbial interactions in complex biological systems such as co-cultures or more complicated microbial networks.

By using different microfluidic devices, we cultivated defined microbial model consortia at dynamic and static environmental conditions. We designed a synthetic tri-culture of *E. coli* and *C. glutamicum*, in which two *C. glutamicum* strains are dependent on each other, while the effect of *E. coli* on *C. glutamicum* depends on the microbial composition of the consortia. This tri-culture is based on co-cultures analysed at the projects Simbal 2.0 (DFG Priority Program 2170 "InterZell") and project "B01 Race for iron" (SFB 1535 MibiNet "Microbial networking – from organelles to cross-kingdom communities"). We also investigated a synthetic tri-culture of *E. coli*, *C. glutamicum* and *P. putida*, in which scarce iron resources drive competitive and exploitative interactions.

Novel DI-based single-cell segmentation and tracking over cell generations enables to derive precise information about the spatiotemporal performance and development of individual microbes inside the growing microbial colonies. We introduce new data analysis methods and visualizations

to transform microbial interaction dynamics into quantitative data sets. By combining these data with advanced visualisation techniques, we aim to extract general principles of microbial interaction and extend this framework to multi-species systems with four or more partners.

#### SL-MC-12

##### Personalized gut microbiome collections via automated culturomics unravel community functions and ecology

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Current gut microbiome models rely on either defined bottom-up communities or undefined stool-derived communities. Defined models offer tractability but neglect the native diversity and coevolution of natural communities, whereas stool communities preserve complexity but lack compositional control. To bridge the gap, we establish expandable personalized culture collections from healthy donor stool and assemble personalized microbiome communities (pComs) to study native interactions and ecological relationships *in vitro* and *in vivo*. We first disassemble native microbiomes into subcommunities through culturing-based enrichment, perturbing the community to achieve different compositions and allowing diverse and less abundant species to proliferate. Results show that subcommunity conditions modulate bacterial abundance profiles in native stool communities and lead to distinct enrichment patterns, enabling targeted sampling to increase the isolation of unique species. Conditions enriched in specific species are selected for subsequent isolation, employing an automated strain isolation platform, that will be coupled to a machine learning-guided picking algorithm that identifies species based on colony morphology. As proof-of-concept, we demonstrate ML-based identification of 22 gut bacterial species, achieving 70% species-level annotation accuracy by a ten-nearest neighbor classifier. We establish personalized microbiome culture collections representing the majority of bacterial species reproducibly detectable by culture-independent community sequencing. To validate how well pComs recapitulate the structure and function of their native stool communities, we benchmark a 35-member pCom, assessing stabilization and compositional dynamics under varying growth conditions as well as robustness against invading pathogens and commensals. Key to understanding microbial community dynamics is disentangling intrinsic microbiome effects from host-mediated influences in the gut. *In vitro* communities allow the study of host-independent bacterial functions, and once characterized in a controlled context, complex pComs will be introduced into gnotobiotic mouse models to systematically dissect host responses. Altogether, this work establishes a framework for generating personalized microbial communities to study microbiome function under laboratory conditions, guide our understanding of disease risk, drug administrations and help inform individualized therapeutic strategies.

## SL-MC-13

### Deciphering the metabolic languages of microbial pathogens at the interface of cross-feeding within the lung microbiota under Cystic Fibrosis condition

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**Introduction:** Even with the success of triplet CFTR modulators in improving immune function, patients with cystic fibrosis (CF) still battle recurring infections. Beneath this persistence lies a microbial dialogue called cross-feeding where commensal bacteria in low-oxygen CF lungs, ferment nutrients into neutral metabolites like 2,3-butanediol & acetoin. These compounds are then hijacked by opportunistic pathogens such as *Pseudomonas aeruginosa* (*Pa*), triggering virulence, phenazine production, and biofilm formation. Yet, the link between bacterial fermentation and signaling pathways remains unexplored. Understanding these silent metabolic exchanges shape early biofilm development, could reveal new dimensions of cross feeding in CF pathophysiology. This research explores how *Pa* (wild-type PAO1 & clinical strains) use fermented metabolites under varying oxygen conditions offering on how metabolic cooperation drives persistent infection despite advanced therapies

**Methodology:** Lung anaerobes and *Pa* strains were isolated from CF sputum samples through standard microbiological procedures. Initially, biofilm assays were performed with *Pa* in SCFM with 30mM of synthetic 2,3-butanediol in microaerophilic (6% O<sub>2</sub>) and anaerobic conditions over different time windows. RNA isolation was done and qPCR was performed for measuring the expression of Phenazine and Biofilm gene markers.

**Results:** In the presence of 2,3-butanediol, phenazine gene expression especially PhzS linked to pyocyanin increased twofold under aerobic conditions at 24 hours and showed delayed peaks under low oxygen and anaerobic conditions, highlighting its oxygen dependence. In synthetic cystic fibrosis sputum medium (SCFM), *Pseudomonas aeruginosa* formed biofilm-like aggregates with variable expression of biofilm genes (*LasR*, *LecA*, *PeIA*, *PeIE*, *PslB*, *AlgD*) across oxygen levels. Among clinical strains, the hyper mucoid type showed reduced pyocyanin but higher alginate gene expression, while the non-mucoid strain displayed stronger biofilm gene activation with 2,3-butanediol suggesting a key link between metabolite signaling, oxygen, and virulence regulation.

**Conclusion:** These findings reveal that metabolites like 2,3-butanediol modulate *P. aeruginosa* virulence pathways. In hyper-mucoid strains, alginate provides protection, perhaps inhibiting other gene expression. Future work will integrate co-cultures, metabolomics, and transcriptomics to understand the metabolite-driven gene regulation.

## SL-MC-14

### Coculture of *Collinsella aerofaciens* and *Bacteroides thetaiotaomicron* under bile acid stress reveals vitamin B6 exchange

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**Background:** Although the bile acid-mediated microbiome-host interaction is known to shape both the composition and functionality of the gut microbiome, the mechanisms by which bile acid stress influences specific microbial metabolic interactions remain poorly understood. To address this gap, we examine the metabolic interplay between two key gut microbes. *Bacteroides thetaiotaomicron*, one of the most abundant species, possesses a broad enzymatic repertoire for polysaccharide degradation, while *Collinsella aerofaciens* is associated with liver-related diseases and plays a role in modifying primary bile acids.

**Results:** In anaerobic coculture, *C. aerofaciens* mitigated the inhibitory effects of deoxycholic acid (DCA) on *B. thetaiotaomicron* by absorbing DCA from the medium and partially converting it into glycine-conjugated derivatives. Proteomic analysis showed that DCA broadly disrupted amino acid and vitamin metabolism pathways, particularly in *B. thetaiotaomicron*. In contrast, coculture led to a general upregulation of these pathways in *C. aerofaciens*, with a marked activation of vitamin B6 metabolism. Additionally, *C. aerofaciens* exhibited increased production of citrulline and ornithine in coculture.

**Conclusions:** *C. aerofaciens* alleviates DCA toxicity on *B. thetaiotaomicron* through absorption, while promoting amino acid and vitamin metabolism, including the vitamin B6 synthesis pathway, during coculture. These results suggest that microbial interactions can enhance resistance to bile acid stress and may influence gut microbiome resilience, with potential relevance for liver- and bile acid-related disorders.

**Keywords:** gut-liver axis, *Collinsella aerofaciens*, *Bacteroides thetaiotaomicron*, bile acids, deoxycholic acid, vitamin B6, pyridoxal 5"-phosphate, arginine metabolism, branched-chain amino acid, cross-feeding.

## SL-MC-15

### Molecular communication in the perpetual interaction of *Azolla* and cyanobacterial symbiont

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Filamentous cyanobacteria engage in diverse nitrogen fixing symbioses found across the tree of life. In land plants, only in the case of the interaction with the water fern *Azolla* this cyanobacterial symbiosis has been taken to the next level: both partners interact in perpetuity for more than 60 million years. Neither host nor cyanobacteria are able to survive without the other in their natural habitat. This is only possible through unfailingly coordinated inheritance of the



cyanobacterial symbiont throughout the life cycle of *Azolla*. And can only be assured, if a tight communication between the partners occurs. However, our knowledge of the signals that underpin this interaction are limited. Using RNASeq and untargeted metabolomics, my team and I have pinpointed a set of phenolic compounds that act as modulators of cyanobacterial physiology and behaviour. We have profiled the responses triggered by these compounds and found that they specifically induce cyanobacterial motility and symbiont abundance, and possibly alterations in nitrogen-fixing behaviour. At the same time these phenolic compounds are induced during abiotic and biotic stress cues, linking stress responses with symbiotic communication and co-ordinated inheritance. With these molecules at hand, we can specifically scrutinize the molecular interaction throughout the life cycle, shedding light on the processes that underpin symbiotic perpetuity. For this, we currently profile a fine-grained developmental gradient capturing all major developmental transitions in cyanobacterial inheritance during vegetative and sexual reproduction. Additionally, we investigate the interaction of the cyanobacterial symbiont with its microbial community in *Azolla*'s symbiotic structure, the leaf cavity.

#### SL-MC-16

##### Various mechanisms influence how bacterial interactions alter the impacts of fungal-derived antimicrobial proteins

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Plants actively recruit beneficial microbes into their root microbiota in response to pathogen invasion, in order to try and halt disease development. Most soil-borne pathogens infect plants through their roots, encountering the root microbiota as a first layer of plant defense. The soil-borne plant pathogenic fungus *Verticillium dahliae* secretes antimicrobial proteins (AMPs) to manipulate host microbiota, facilitating host plant invasion. These AMPs show selective, yet distinct, activity spectra. However, the fungus not only expresses AMP genes during host-colonization, but also during soil-dwelling life stages. Considering that early fungal evolution predates the emergence of land plants, and the likely occurrence of intense competition with soil microbes for nutrient sources in ancestral environments, AMPs are likely to have originally evolved as tools for microbial competition rather than for host-colonization. However, the impact of AMPs on soil microbial communities in the absence of a plant host has hardly been explored. Here, we investigate the effect of the AMPs Av2 and CP1, expressed by *V. dahliae* during soil-dwelling stages, on a collection of 22 soil-derived microbes. First, the sensitivity of each of the bacteria to the AMPs was assessed, revealing differential sensitivity among the bacteria for each of the AMPs. Next, we treated the coculture of all 22 soil-derived bacteria with each of the AMPs and analysed strain abundances using amplicon sequencing. Intriguingly, the impact of the AMP on the bacterial community did not reflect individual sensitivities, as some strains that are sensitive to the AMP when treated individually were not depleted in coculture, while others that were depleted showed no sensitivity in the individual culture. To investigate these unexpected bacterial sensitivity changes in the bacterial community, we analyzed all possible pairwise combinations among the 22 bacterial strains in presence of each of the AMPs. We identified three bacterial pairs which exhibit sensitivity changes to CP1. In two cases, a strain which is insensitive to CP1 becomes sensitive in

presence of an *Aeromonas* strain. In another case a *Microbacterium* strain which is sensitive to CP1 becomes insensitive in presence of a *Pseudomonas* strain. We will use these three pairs to study the molecular mechanisms responsible for the observed sensitivity changes.

#### SL-MC-17

##### Serial enrichment drives deterministic module selection and functional convergence in anaerobic digestion consortia

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Microbial consortia mediate anaerobic digestion (AD) by partitioning metabolism across interacting bacterial and archaeal guilds. Although AD communities often contain hundreds to thousands of taxa with overlapping metabolic capacities, it remains unclear whether environmental selection stabilizes AD function by acting on specific organisms, on functionally cohesive guilds, or on core metabolic modules that can be carried by phylogenetically diverse lineages.

Here, we combined serial enrichments with metagenomic analysis to disentangle deterministic environmental filtering from stochastic community reassembly. Microbial communities originating from four distinct inocula were cultivated for seven weekly transfers under factorial combinations of pH, substrate type, and nutrient supplementation. 16S rRNA profiling showed a consistent decline in  $\alpha$ -diversity and a convergence of  $\beta$ -diversity by condition, indicating streamlining toward environment-specific consortia. Despite pronounced taxonomic turnover, end-point metabolite profiles (VFAs, CH<sub>4</sub>) clustered by pH and substrate rather than inoculum, demonstrating functional convergence.

Genome-resolved metagenomics revealed that all consortia retained a conserved set of metabolic modules - central fermentation and Wood-Ljungdahl pathway- while pH deterministically partitioned peripheral routes such as methanogenesis and chain elongation. Thus, distinct taxa assembled into equivalent metabolic guilds that sustained reproducible biochemical fluxes.

Together, our results show that AD microbiomes self-organize into functional attractors, where environmental filters determine which metabolic modules are active, while stochastic reassembly determines which taxa perform them. This framework explains how reproducible ecosystem function emerges from divergent community compositions and offers principles for engineering microbial bioprocesses for desired function.

#### SL-MC-18

##### Engineering a photoautotrophic microbial coculture toward enhanced biohydrogen production

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#### Introduction

Phototautotrophic microbes play an important role in the natural carbon and nitrogen cycles, as they provide biological fixation of CO<sub>2</sub> and N<sub>2</sub> through light-driven processes. These traits lay the basis for a range of food webs around the globe. In Biotechnology, the application of synthetic phototrophic microbial consortia holds the promise of enabling sustainable bioenergy production, by solely using sunlight, water, CO<sub>2</sub>, and N<sub>2</sub> as main resources. Nevertheless, strategies for efficient construction, application, and regulation of such consortia remain challenging.

## Goals & Methods

Applying a combination of genetic engineering and light-regulation, this study aimed at constructing a synthetic community of phototrophs composed of a purple non-sulfur bacterium and a cyanobacterium to generate a productive co-culture thriving on CO<sub>2</sub> and N<sub>2</sub> (1).

## Results and Conclusion

Using *Rhodospseudomonas palustris* (*R. palustris*) and a strain of *Synechocystis* sp PCC 6803 engineered for acetate production (*Synechocystis\_acs*), it was possible to achieve a consortium growing on CO<sub>2</sub> and N<sub>2</sub>. Both organisms were in a trophic dependency, with *Synechocystis* fixing carbon and *R. palustris* fixing N<sub>2</sub>. We developed a circadian light regime that allowed shifting the community between hydrogen production and fatty acid storage metabolism. The strategy of circadian illumination effectively limited oxygen levels in the system, ensuring the activity of the nitrogenase in *R. palustris*, despite oxygenic photosynthesis happening in *Synechocystis*. When infrared light was introduced into the circadian illumination, the production of H<sub>2</sub> and fatty acids (especially C16 and C18) was significantly enhanced. Proteomic analysis indicated acetate exchange and light-dependent regulation of metabolic activities. Infrared illumination significantly stimulated the expression of proteins coding for nitrogen fixation, carbohydrate metabolism, and transporters in *R. palustris*, while constant white light led to the most upregulation of photosynthesis-related proteins in *Synechocystis\_acs*.

This study demonstrated the successful construction and light-regulation of a phototrophic community enabling H<sub>2</sub> and fatty acid production through carbon and nitrogen fixation.

(1) M Pan, RA Colpo, S Roussou, C Ding, P Lindblad, JO Krömer (2024) Engineering a photoautotrophic microbial coculture toward enhanced biohydrogen production. *Environmental Science & Technology* 59 (1), 337-348

## Prokaryotic physiology and metabolism

### SL-PPM-01

#### From processed food to microbial fitness: resolving the underground metabolism of the advanced glycation end product N<sub>ε</sub>-carboxymethyllysine in *Escherichia coli*

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Food processing drives the Maillard reaction, thus generating advanced glycation end products (AGEs) – modified amino acids that accumulate in our regular diet. These AGEs are only partially absorbed in the small intestine and therefore reach the colon, placing them in direct contact with gut microbes. Decoding their microbial fate is biologically important because it establishes mechanistic links between diet, microbial physiology, and host biology; yet even for the prevalent AGE N<sub>ε</sub>-carboxymethyllysine (CML), the pathway had remained unresolved.

We delineate the underground metabolism of CML in *Escherichia coli*. An ornithine decarboxylase (SpeC) first converts CML to N<sub>ε</sub>-carboxymethylcadaverine (CM-Cad)—a proton-consuming step. Second, broad-specificity aminotransferases partition flux with defined substrate scopes: IlvE (branched-chain amino-acid aminotransferase), GabT (GABA aminotransferase) and PatA (putrescine aminotransferase) accept either CML, CM-Cad or both as off-substrates, respectively. The corresponding transamination reactions yield two stable end products: N<sub>ε</sub>-carboxymethyl-5-aminopentanoate (CM-APA) and the CML-derived α-keto acid (CM-amino-2-oxohexanoic acid). On the CM-Cad branch, transamination produces a transient CM-aminopentanal that is not directly detected but undergoes spontaneous cyclization to a piperidinium ion and non-enzymatic oxidation to CM-APA.

Functionally, we show that this pathway channels a common dietary AGE into usable nitrogen (via aminotransferase chemistry) and contributes to pH homeostasis (via proton-consuming decarboxylation), revealing concrete mechanisms by which enterobacteria translate processed-food chemistry into fitness advantages in the colon.

### SL-PPM-02

#### Illuminating the role of a cell wall glycopolymer ligase in *Streptomyces*' development and stress response

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### Introduction:

Given the essential roles of cell wall glycopolymers in maintaining cellular integrity, determining cell shape, supporting bacterial vitality, and mediating other physiological processes, their synthesis, transfer, ligation, and function have been extensively studied across diverse bacterial species.<sup>1</sup> However, the composition and function of the cell wall of filamentous, multicellular actinobacteria remain obscure, despite their immense significance in biotechnology and medicine as antibiotic producers.

### Goals:

The major goal of this study was to understand the molecular link between cell wall architecture and signalling mediated by the second messenger c-di-AMP in *Streptomyces*, with a special focus on functional characterization of the LytR-CpsA-Psr (LCP)-LytR\_C domain protein CglA (Vnz\_13690).

### Methodology & Results:

Through whole genome sequencing of spontaneous suppressor mutants, we identified that a *cglA* loss-of-function mutation restores the growth of *S. venezuelae* with reduced intracellular c-di-AMP level under osmotic stress conditions. The *cglA* mutant exhibited decreased glycopolymer content in the cell wall, resulting in enlarged vegetative hyphae, misplaced FtsZ rings, aberrant cell compartments, misshaped spores, and diminished cell vitality.<sup>2</sup> Further analysis revealed that CglA localizes specifically to zones of cell wall biosynthesis in *S. venezuelae*, suggesting potential interactions with other cell wall biosynthetic proteins. Our initial interaction analysis revealed that CglA interacts with the membrane-bound putative glycosyltransferase Vnz\_13700 *in vivo*. Identification of additional interaction partners of CglA using Co-immunoprecipitation analysis is currently ongoing.

#### Summary:

Overall, this project characterizes the cell wall glycopolymer ligase CglA in *S. venezuelae*, which is required for hyphal vitality as a part of cell wall biosynthesis and modification machinery in filamentous, multicellular bacteria.

1 – D'Elia, M. A., Millar, K. E., Beveridge, T. J. & Brown, E. D. Wall teichoic acid polymers are dispensable for cell viability in *Bacillus subtilis*. *J Bacteriol* **188**, 8313–8316; 10.1128/JB.01336-06 (2006).

2 – Bhowmick, S. *et al.* Cell shape and division septa positioning in filamentous *Streptomyces* require a functional cell wall glycopolymer ligase CglA. *mBio* **15**, e0149224; 10.1128/mbio.01492-24 (2024).

#### SL-PPM-03

##### Near-atomic resolution structure of the sHdr enzyme complex indicates a new mechanism for sulfur oxidation

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Dissimilatory sulfur-oxidizing bacteria and archaea are crucial to planetary sulfur cycling. They conserve energy by coupling the oxidation of inorganic sulfur compounds, such as thiosulfate, to photosynthesis or respiration. Our research focuses on understanding the distribution, structure and reaction mechanisms of cytoplasmic sulfane sulfur-oxidizing systems, including the widespread sHdr system [1,2]. Here, we describe the atomic resolution structure of the sulfur-oxidizing sHdr complex from the chemolithoautotrophic Gammaproteobacterium *Thioalkalivibrio thiocyanoxydans*. In this and other members of the Pseudomonatodota, the sHdr core proteins are encoded in a highly conserved *shdrC1B1AHC2B2* gene cluster and resemble the HdrABC subunits of heterodisulfide reductase found in methanogenic archaea. The sHdrAB1B2C1 and -C2 proteins are co-purified after two steps of anion exchange chromatography. Cryo-electron microscopy reveals an asymmetric sHdr(AA'B1B2C1C2) complex. One sHdrA monomer is associated with sHdrC1B1 and the other binds sHdrC2B2. Each sHdrA subunit contains one [4Fe-4S] cluster and a covalently bound FAD. sHdrC1/C2 each bind two [4Fe-4S] clusters, and function as redox relays between sHdrA and

sHdrB1/B2. Two non-cubane FeS clusters, ligated by ten cysteines, reside in the sHdrB2 active site, likely enabling the catalysis of a dithiol-disulfide switch. The sHdrB1 active site also contains two non-cubane iron-sulfur clusters. While one cysteinyl ligand of the first cluster is replaced by aspartate, the second cluster features unprecedented ligation by two serine and three cysteine residues. These unusual properties highlight sHdrB1 as the prime candidate for catalyzing the oxidation of sulfane sulfur to sulfite. The proposed roles of the non-cubane iron-sulfur clusters in sHdrB1 and sHdrB2 are investigated using electron paramagnetic resonance spectroscopy. DsrE3B, which is encoded in immediate vicinity of the *shdr* genes, could present the sulfur substrate to sHdr, because a substantial portion of the complex co-purifies with this established sulfur-binding protein. Additionally, sHdrG, a cofactor-less protein encoded downstream of the *shdr* genes in the reverse transcription direction, is tightly bound to the sHdrC2B2 side of the heterocomplex and may fulfill a stabilizing function.

[1] Koch & Dahl 2018 ISME J 10, 2479

[2] Ernst et al. 2021 FEBS J 288, 1664

#### SL-PPM-04

##### Breaking bonds without breaking ATP – A novel superfamily of ATP-dependent, nonhydrolyzing C-C lyases

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To proliferate, pathogens must metabolize nutrients provided by the host. The genomes of 15% of sequenced enterobacteria, mostly pathogens, contain a gene cluster for the fermentation of glutamate (Kronen & Berg 2015), an abundant amino acid in the mammalian gut (Reeds et al., 2000). Here, we show that this cluster encodes a citramalate lyase dependent on an ATP cofactor that is not hydrolyzed but participates indirectly in catalyzing retro-aldolase reactions. This enzyme belongs to a novel superfamily of C-C lyases found in 12% of all bacterial species, across 82 phyla. Utilization of ATP as a cofactor ensures functionality following horizontal gene transfer and facilitates adaptation to novel activities, as evidenced by the involvement of several closely related enzymes in diverse, oftentimes uncharacterized, metabolic pathways. We have characterized the functions of four additional enzymes of this family, all of which both share conserved amino acid positioning required for ATP binding and use ATP as a cofactor to catalyze C-C-lyase reactions. A variety of substrates, characterized by a carboxylate in  $\alpha$ -position to the electropositive carbon, invites methodological perspectives to exploit these C-C-cleaving and -forming enzymes for organic syntheses.

Kronen, M. & Berg, I. A. Meseconase/fumarase FumD in *Escherichia coli* O157:H7 and promiscuity of *Escherichia coli* class I fumarases FumA and FumB. *PLoS One* **10**, e0145098 (2015). doi:10.1371/journal.pone.0145098

## SL-PPM-05

### When a chaotropic agent turns into a nutrient – Deciphering guanidine assimilation and riboswitch-based gene regulation in cyanobacteria for synthetic biology applications

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Guanidine is well-known as a denaturing agent. However, recent studies have demonstrated both the widespread synthesis of guanidine, e.g. in plants and mammals, as well as the widespread occurrence of guanidine metabolism in bacteria, suggesting a broader biological role. Here, we provide insights into guanidine assimilation via previously identified guanidine hydrolases (GdmH, [1]) in cyanobacteria. The corresponding *gdmH* gene is widespread among cyanobacteria and enables growth on guanidine as sole nitrogen source [2]. Consistent with this, *gdmH* gene expression increased under nitrogen limitation, regulated by the transcription factor NtcA. However, guanidine is toxic above 5 mM, necessitating GdmH activity and adaptive mutations activating the multidrug efflux system PrqA. The *gdmH* gene is frequently co-localized with ABC transporter genes (named *gimABC*), which are driven by an additional NtcA-regulated promoter. The corresponding substrate-binding protein GimA showed high affinity to guanidine. Consistent with a high affinity import system, disruption of genes *gimA* or *gimB* impaired guanidine-dependent growth of *Synechocystis* sp. PCC 6803 at low concentrations. However, in presence of >1 mM guanidine, these mutants grew like wildtype, suggesting the existence of additional uptake mechanisms for guanidine. We also demonstrate the high-affinity binding of guanidine to a previously described, conserved RNA motif located within the *gdmH* 5' UTR, validating it as a guanidine-I riboswitch. By combining it with various promoters, we achieved precise, titratable control of heterologous gene expression in cyanobacteria *in vivo*. Compared to widely used chemical inducers, guanidine is inexpensive and effective at low concentrations, making large-scale applications in cyanobacterial cell factories cost-efficient. Altogether, our findings establish guanidine assimilation as an integral element of cyanobacterial nitrogen metabolism and highlight guanidine riboswitches as valuable tools for synthetic biology [2].

[1] Funck, D., [...], Hartig, J.S., 2022. Discovery of a Ni<sup>2+</sup>-dependent guanidine hydrolase in bacteria. *Nature* **603**, 515–521.

[2] Itzenhäuser, M.A., [...], Klähn, S., 2025. Deciphering guanidine assimilation and riboswitch-based gene regulation in cyanobacteria for synthetic biology applications. *PNAS*, *in press* (a preprint is available at bioRxiv, doi: <https://doi.org/10.1101/2025.03.14.643060>)

## SL-PPM-06

### Regulation of glycogen phosphorylase isoforms in cyanobacteria

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Glycogen is a widespread storage polymer that supports survival under environmental stress in diverse organisms. In the cyanobacterium *Synechocystis* sp. PCC 6803, glycogen is essential for enduring periods of darkness and nutrient deprivation. Given its central role, glycogen metabolism is tightly regulated to balance synthesis and degradation according to cellular needs. Glycogen phosphorylase (GlgP) catalyzes the first step of glycogen breakdown, releasing glucose-1-phosphate from the polymer. *Synechocystis* possesses two GlgP isoforms, GlgP1 and GlgP2, which exhibit similar catalytic efficiencies but differ in regulatory mechanisms and physiological roles. Their regulation is mediated by a C-terminal domain whose distinct amino acid composition enables specific activation under different environmental conditions. Moreover, the subcellular localization of GlgPs appears linked to their activation state: the enzymes form aggregates when inactive, which dissolve upon activation. These mechanisms allow rapid responses to ensure adaptation to environmental changes.

## SL-PPM-07

### Bisphosphosugars and Beyond: Regulating *Synechocystis* Phosphoglucomutase

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*Synechocystis* sp. PCC 6803 (*Synechocystis*) relies on glycogen as a carbon and energy source during dark periods and transient nutrient deprivation. In *Synechocystis* glycogen metabolism, the two phosphoglucomutase (PGM) isoenzymes SII0726 (PGM1) and Slr1334 (PGM2) play distinct key roles. PGM1 drives glycogen accumulation and mobilization by catalyzing the interconversion of glucose-1-phosphate and glucose-6-phosphate [1]. PGM2 regulates PGM1 by converting fructose-1,6-bisphosphate (fructose-1,6-BP), an inhibitor of the PGM1 reaction, into glucose-1,6-bisphosphate (glucose-1,6-BP), an activator of PGM1. While the activator functions by providing the essential initial phosphorylation of the active-site serine, the mechanism of fructose-1,6-BP inhibition remains poorly understood [2]. PGM1 activity is additionally modulated by phosphorylation of serine 47 (S47), which inhibits PGM1 activity during nitrogen starvation, preventing premature glycogen degradation to ensure survival [1]. This study explores the inhibition mechanism of fructose-1,6-BP and its regulatory interplay with the phosphosite S47. We show that fructose-1,6-BP phosphorylates PGM1 and PGM1 S47A, a phosphosite mutant mimicking a permanently dephosphorylated active PGM1. However, fructose-1,6-BP showed no inhibitory effect on the PGM1 S47A variant, indicating that S47 is involved in the fructose-1,6-BP-mediated inhibition. Additionally, kinetic analyses and kinetic modeling revealed that fructose-1,6-BP and glucose-1,6-BP competitively regulate PGM1 at an allosteric site, with

glucose-1,6-BP mitigating the effect of fructose-1,6-BP. This suggests an elaborate regulatory interplay between the two bisphosphosugars. These findings further illuminate the complex mechanisms controlling PGM1 activity and underscore its central role in *Synechocystis* glycogen metabolism.

[1] Doello, S., N. Neumann, and K. Forchhammer, 2022. The FEBS Journal, **289** (19): p. 6005-6020.

[2] Neumann, N., S. Friz, and K. Forchhammer, 2022. mBio, **13** (4): p. e01469-01422.

## SL-PPM-08

### The small protein SbtC is a functional component of the CO<sub>2</sub> concentrating mechanism of cyanobacteria

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Oxygenic phototrophs fix CO<sub>2</sub> via the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) that is considered the rate-limiting step of photosynthesis under many conditions. To circumvent low and fluctuating CO<sub>2</sub> concentrations in aquatic systems, cyanobacteria and algae have evolved sophisticated inorganic carbon (Ci) concentrating mechanisms (CCMs). Bicarbonate transporters such as SbtA play a crucial role in the cyanobacterial CCM and hence are tightly regulated at multiple layers. The control of sbtA gene expression and corresponding transporter activity involves the PII-like protein SbtB, whose gene is frequently co-transcribed with sbtA. Here we report on the discovery of a so far non-annotated gene in the model *Synechocystis* sp. PCC 6803 that encodes the small protein SbtC composed of 80 amino acids. Presence of SbtC was confirmed by immunoblotting after fusing the sbtC-coding sequence to a Flag-tag. Similar to sbtAB, transcription of the sbtC locus is induced by low CO<sub>2</sub> availability but controlled independently. Mutation of the sbtC locus in a wild-type background showed only a mild phenotype even under low CO<sub>2</sub>, but the diurnal growth was impaired as found before in the mutant  $\Delta$ sbtB. Nevertheless, biochemical analysis provided evidence for a trimeric SbtABC complex in the membrane. Recombinant *Synechocystis* strains harboring only SbtA as single Ci uptake system with either deleted sbtB or sbtC genes showed that bicarbonate leakage from the cell is strongly elevated in both mutants. We confirm that SbtC contributes to the formation of the SbtAB complex, thereby, regulating bicarbonate exchange at the cytoplasm membrane. Well-conserved SbtC-like proteins encoded in the neighborhood of sbtAB exist in many cyanobacterial genomes pointing towards an important role in the cyanobacterial CCM.

## SL-PPM-09

### Monitoring light-induced H<sub>2</sub>O<sub>2</sub> dynamics in *Chlamydomonas reinhardtii* mutants using a fluorescent H<sub>2</sub>O<sub>2</sub> biosensor

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Fluorescent biosensors such as HyPer7 and roGFP2-Tsa2 $\Delta$ CR have revolutionized real-time investigation of redox dynamics in living cells. While HyPer7 offers

ultrasensitive H<sub>2</sub>O<sub>2</sub> detection in *Arabidopsis thaliana*, roGFP2-Tsa2 $\Delta$ CR is the benchmark for monitoring H<sub>2</sub>O<sub>2</sub> in *Chlamydomonas reinhardtii*. In this project, we aim to express HyPer7 in *Chlamydomonas* and directly compare its accuracy to that of roGFP2-Tsa2 $\Delta$ CR under physiological and stress conditions. Expression of both sensors will be validated through quantitative fluorescence assays and confirmed by Western blotting and fluorescence gel analysis. To identify novel components involved in oxidative stress and chloroplast-to-nucleus retrograde signaling, we will perform a forward genetic screen in a *Chlamydomonas* strain expressing roGFP2-Tsa2 $\Delta$ CR. This involves generating thousands of random insertional mutants, screening for high light and H<sub>2</sub>O<sub>2</sub> sensitivity, and characterizing redox dynamics in an Internal Light Unit (ILU). Candidates showing altered H<sub>2</sub>O<sub>2</sub> signatures will undergo adapter-linked PCR and Sanger sequencing to localize genomic disruption sites and pinpoint genes putatively involved in retrograde signaling. Additionally, we seek to elucidate the reductant specificity of HyPer7 and roGFP2-Tsa2 $\Delta$ CR. Using *Chlamydomonas* strains deficient in glutathione reductase (GR) and NADPH-dependent thioredoxin reductase C (NTRC), we will assess biosensor responses to validate the links between the glutathione and thioredoxin pools and sensor reduction. This integrated approach combining biosensing, high-throughput genetics, and targeted mutant analysis promises new insights into H<sub>2</sub>O<sub>2</sub> signaling and the molecular basis of chloroplast retrograde pathways in photosynthetic eukaryotes.

## SL-PPM-10

### Glycan Uptake in Marine Bacteria: A Reductionist Approach

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Approximately 80% of photosynthetically fixed marine carbon is stored as glycans. The turnover of these glycans is an important process in marine ecosystems, influencing the flow of organic matter. Heterotrophic bacteria contribute substantially to this turnover by degrading glycans through two active foraging mechanisms: selfish uptake and external hydrolysis. Selfish uptake involves the binding and partial hydrolysis of glycans on the cell surface, followed by the uptake of oligosaccharides into the periplasm where further hydrolysis occurs. This mode minimises substrate loss and thus presents a unique advantage for organisms to maximise their energetic yield. Selfish uptake occurs in diverse microbiomes, including the human gut, limnic and marine. However, elucidating the factors driving this uptake mechanism in complex environmental samples is challenging. To address this knowledge gap, we employed a reductionist, laboratory-based approach to investigate the conditions that favour selfish activity. We examined how variations in carbon and glycan concentrations drive selfish activity in distinct bacterial strains, building on environmental findings that selfish uptake increases when samples are primed with the target glycan. Furthermore, we explored the role of extrinsic factors, including the effects of vitamins in the growth medium and biotic competition in co-cultures.

To do this we phenotypically characterized 38 new bacterial isolates from the North Sea and assessed their ability to

grow on two key glycans, laminarin and glycogen. We assessed selfish uptake using fluorescently labelled polysaccharides and microscopy. Cultivation experiments revealed distinct growth patterns across bacterial groups and confirmed that members of the *Flavobacteriia* and *Gammaproteobacteria* are capable of selfish uptake. We identified a subset of strains that responded to the presence of heterologous species and were influenced by environmental factors such as carbon concentration and vitamin availability.

Co-culture experiments revealed contrasting dynamics between *Flavobacteriia* members, with cooperative coexistence observed in certain combinations, but not in others, particularly when paired with *Gammaproteobacteria*. Despite considerable functional redundancy, differences in foraging strategies emerged in response to varying conditions, underscoring the importance of phenotypic characterization for understanding complex ecological interactions.

## SL-PPM-11

### Understanding triacylglycerol synthesis and functions in *Streptomyces venezuelae*

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In bacteria, the primary lipid-based storage compound is polyhydroxyalkanoic acid. However, some, including *Streptomyces* accumulate triacylglycerol (TAG). Recently, it was shown that TAG has roles beyond storage, such as to protect *Mycobacterium* from oxidative stress[1] and *Rhodococcus* from UV and desiccation[2,3]. In comparison, roles of TAG in *Streptomyces* remain poorly understood. Here, we characterise the bi-functional wax synthase/diacylglycerol acyltransferase (WSD) in *S. venezuelae*, focusing on its regulation and effects of TAG on *Streptomyces* physiology under different conditions.

Our bioinformatic analysis of 117 *Streptomyces* genomes revealed that WSD orthologs form two distinct clades. Each species contains at least one conserved WSD, while some possess additional, more divergent copies. Strikingly, we observed that promoters of the conserved clade frequently contain a putative BldD-binding motif. BldD is a c-di-GMP-dependent master regulator in *Streptomyces* development[4]. We chose *S. venezuelae* as model system for further study, which carries two WSD. Our study identifies Vnz\_33585 as a core enzyme for TAG biosynthesis, which is under BldD-(c-di-GMP) control proven by our electrophoretic mobility shift assays. Furthermore, we observe the differing TAG levels in wild-type and in a *bldD* mutant using total lipid extractions separated on thin-layer chromatography (TLC). This demonstrates, that TAG production is likely integrated into the developmental regulation in *Streptomyces*.

In our TLC we see that a deletion of *vnz\_33585* leads to drastic decrease of TAG, whereas overexpression leads to an increase. To understand whether TAG accumulation affects cellular fitness, relevant strains were exposed to various stress conditions (SDS, DNA damage, desiccation), and survivability was assessed. Lower TAG and higher TAG levels correlate to the varying degree of tolerance to SDS stress. This supports the hypothesis that TAG metabolism and membrane lipid biosynthesis need to be carefully balanced for optimal adaptation to environmental challenges.

[1] Mallick et al., (2021). *FEMS Microbiology Reviews*, 45(6)

[2] Alvarez et al., (2004). *FEMS Microbial Ecology*, 50(2)

[3] Bequer et al., (2013). *Extremophiles* 17(2)

[4] Tschowri et al., (2014). *Cell*, 158(5)

## SL-PPM-12

### A novel layer in metabolic regulation in bacteria: *B. subtilis* TCA enzymes adapt metabolon formation in response to substrate flux

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It has been proposed that metabolic enzymes can transiently assemble into higher-order complexes known as metabolons, enabling substrate channeling and coordinated metabolic regulation [1,2]. However, evidence for their dynamic formation in living cells is scarce. We have investigated how dynamics of tricarboxylic acid (TCA) cycle enzymes in *Bacillus subtilis* respond to changes in carbon source availability.

Using single-molecule tracking [3], we quantified the mobilities of five key TCA enzymes, citrate synthase (CitZ), aconitase (CitB), isocitrate dehydrogenase (Icd), malate dehydrogenase (Mdh) and phosphoenolpyruvate carboxykinase (PckA), in cells grown on different carbon sources. Squared displacement analysis revealed two diffusive states: a fast-diffusing population and a slow-diffusing population with >10-fold reduced diffusion rate. The slow population showed pronounced substrate-dependent differences and may reflect partial complex formation. Diffusion similarity analysis indicated that some enzymes co-diffuse, consistent with complex assembly. During growth on malate, associated with high TCA flux, slow-diffusing populations were substantially increased, whereas on glucose, all enzymes predominantly diffused freely. Pairwise Förster resonance energy transfer (FRET) confirmed physical interactions: specific enzyme pairs exhibited considerably higher FRET on malate than on glucose, supporting substrate-dependent assembly into metabolons.

These results provide quantitative *in vivo* evidence that TCA cycle enzymes dynamically reorganize in response to metabolic flux. Such transient assemblies may optimize flux distribution, enhance substrate channeling, and represent a versatile regulatory mechanism in bacterial metabolism.

[1] Srere, P.A. (1972). Is there an organization of Krebs cycle enzymes in the mitochondrial matrix? *Energy Metabolism and the Regulation of Metabolic Processes in Mitochondria*. Academic Press

[2] Meyer, F.M., Gerwig, J., Hammer, E., Herzberg, C., Commichau, F.M., Völker, U., & Stülke, J. (2010). Physical interactions between tricarboxylic acid cycle enzymes in *Bacillus subtilis*: Evidence for a metabolon. *Metabolic Engineering*.

[3] Rösch TC, Oviedo Bocanegra LM, Fritz G, Graumann PL (2018). SMTracker: a tool for quantitative analysis, exploration and visualization of single molecule tracking data reveals highly dynamic binding of *B. subtilis* global repressor

### SL-PPM-13

#### Cold cases – what we can learn from old inhibitors and antimetabolites

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The shikimate pathway, found in prokaryotes, fungi and plants produces chorismate, which serves as a building block for the synthesis of phenylalanine (Phe), tryptophan (Trp), and tyrosine (Tyr), as well as quinones and folate. Since the pathway does not exist in animals, it is an excellent target for inhibitors. For example, glyphosate-dependent inhibition of the pathway leads to a chorismate deficiency and thus cell death. We are interested in the biosynthesis of aromatic amino acids and in their uptake systems in the Gram-positive model bacterium *Bacillus subtilis*. Although unknown, the uptake systems for Phe, Trp and Tyr must exist in this organism. Firstly, the glyphosate-dependent inhibition of *B. subtilis* can be partially relieved by supplying the amino acids to the cells [1]. Secondly, *B. subtilis* mutants that are unable to synthesize Phe, Trp and Tyr import the amino acids from the environment. Toxic analogues of the aromatic amino acids are suitable for the identification of transport systems. The Phe analogue  $\beta$ -2-Thienylalanine ( $\beta$ TA) was identified many years ago and inhibits the prephenate dehydratase (PheA), which catalyzes the penultimate step in PheA biosynthesis [2].  $\beta$ TA resistant *B. subtilis* mutants have been described but the resistance-mediating mutations were never identified [3]. Genome-sequencing of  $\beta$ TA-resistant *B. subtilis* mutants revealed that the alteration of a regulatory domain of PheA, which is essential for Phe-dependent feedback inhibition, leads to high-level  $\beta$ TA resistance. We also identified a highly conserved residue whose modification eliminates feedback inhibition in plants. Serendipitously, we discovered that a widespread *B. subtilis* laboratory strain synthesizes a regulation reversal PheA variant that is even activated by  $\beta$ TA. Moreover, a single  $\beta$ TA resistant mutant carries a mutation in a putative PheA transporter gene. The current status of the project will be presented.

[1] Wicke et al. (2019) Environ Microbiol 21: 1287-1305.

[2] du Vigenagaud et al. (1945) J Biol Chem 159: 385-394.

[3] Coats & Nester (1967) J Biol Chem 240: 3034-38.

### SL-PPM-14

#### An acetophenone carboxylase homolog from *Rhodococcus aetherivorans* initiates acetone rather than acetophenone catabolism

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Acetone and acetophenone are common intermediates of natural secondary metabolism. As these substrates are chemically inert, they must be activated prior to microbial degradation. Ketone activation proceeds either by

oxygenation or by ATP-dependent carboxylation [1,2]. Acetone carboxylase (Acx) and acetophenone carboxylase (Apc) are homologous enzymes, but have different subunit compositions. While Acx forms a hexameric complex out of three different subunits ( $[\alpha\beta\gamma]_2$ ), the octameric Apc core complex comprises four distinct subunits ( $[\alpha\alpha'\beta\gamma]_2$ ). Furthermore, Apc activity requires a fifth subunit (Apc $\epsilon$ ), which is not present in Acx [2,3].

Here, we show that the Apc homolog from *Rhodococcus aetherivorans* unexpectedly catalyzes acetone carboxylation. Thereby, the unique subunit  $\epsilon$  is indispensable, as confirmed by growth experiments with deletion strains. In accordance with previous studies on the partially purified Acx from an unsequenced *R. rhodochrous* strain [4], our enzyme activity measurements revealed an exceptionally broad substrate spectrum (e.g., pentanones) and nucleotide usage (e.g., ITP, GTP). We propose that the Apc-like subunit composition of *Rhodococcus* Acx accounts for its unique biochemistry.

To our surprise, expression of the *apc* operon was not induced by acetophenone. Instead, we observed upregulation of several oxygenase homologs during *R. aetherivorans* growth on acetophenone. We elucidate the acetophenone degradation pathway of *R. aetherivorans* and characterize the involved enzymes.

[1] Heider et al., J Mol Microbiol Biotechnol 26:152-64 (2016).

[2] Weidenweber et al., Sci Rep 7:39674 (2017).

[3] Mus et al., Sci Rep 7:7234 (2017).

[4] Clark and Ensign, J Bacteriol 181(9):2752-58 (1999).

### SL-PPM-15

#### Biochemical and Physiological Analysis of a Recently Evolved Bacterial Degradation Pathway for the Synthetic Buffer Compound TRIS

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The widely used synthetic buffer compound TRIS (2-amino-2-hydroxymethyl-propane-1,3-diol) was long considered biologically inert. We recently isolated *Pseudomonas* and *Shinella* bacteria from different wastewater sources that can grow with TRIS as only carbon and nitrogen source under aerobic conditions [1]. Transcriptome analyses, genome comparisons, and heterologous gene expression revealed that a choline dehydrogenase (CDH)-like protein (TaoA), an aldehyde dehydrogenase (TaoB), a serine hydroxymethyltransferase, and a serine dehydratase catalyze the initial degradation reactions of TRIS. The corresponding genes are encoded in two adjacent gene clusters on a transposable megaplasmid, suggesting that the TRIS degradation pathway evolved by a recombination of genes involved in the metabolism of polyamines, choline, and serine.

In this study we characterized the initial TRIS degradation reactions, using a Clark-type oxygen electrode to measure enzyme activity of cell extracts of a TRIS-degrading *Pseudomonas* strain. Results indicate that the TRIS alcohol dehydrogenase TaoA can use phenazine methosulfate (PMS) as an artificial quinone-like electron acceptor and is associated with the membrane. We identified TRIS aldehyde as a novel reaction intermediate in the oxidation of TRIS to 2-hydroxymethylserine (2-HMS) and determined a  $K_m$  value of 22 mM TRIS for TaoA in cell free extract. A significant increase in 2-HMS production upon the addition of  $NAD^+$  or  $NADP^+$  supports the notion that the  $NAD^+/NADP^+$ -dependent TRIS aldehyde dehydrogenase TaoB catalyses the subsequent oxidation of TRIS aldehyde to 2-HMS. We further isolated a *Stutzerimonas degradans* strain that degrades TRIS under denitrifying conditions. Anaerobic Tris degradation was also confirmed for other TRIS-degrading *Pseudomonas* and *Shinella* strains and genome comparisons suggested that the same set of proteins catalyzes TRIS degradation under anaerobic and aerobic conditions, indicating that TaoA does not require oxygen as a co-substrate. In anaerobic cultures, formate was identified as a Tris degradation product, confirming the involvement of the serine hydroxymethyltransferase in the degradation.

These results advance the physiological and biochemical understanding of the recently evolved TRIS degradation pathway and provide further insight into the origin and recombination of the involved enzymes.

[1] Holert et al., 2024, The ISME journal, <https://doi.org/10.1093/ismejo/wrad023>

#### SL-PPM-16 Proton gradient generation by periplasmic sugar oxidation and cytoplasmic ketosugar reduction

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In this contribution, I present current evidence for a vectorial redox chemistry on sugars to generate a proton gradient.

Quinone redox chemistry in the respiratory chain is characterized by a reduction on the cytoplasmic surface of the membrane consuming two protons and two electrons. The oxidation on the periplasmic surface yields quinone, two electrons to cytochrome c and two protons. Overall, the vectorial redox chemistry contributes with two protons per cycle to the proton gradient. A second example for vectorial redox chemistry are *Escherichia coli* and other anaerobic bacteria that use cytoplasmic hydrogen production and periplasmic hydrogen oxidation for the generation of a proton gradient.

The presence of cytochrome c in microorganisms lacking a quinone-cytochrome c oxidoreductase (complex III of the respiratory chain) caught my interest. Periplasmic sugar dehydrogenases using cytochrome c as electron acceptor and cytoplasmic NAD(P)H-oxidizing keto-sugar reductases have been characterized in many microorganisms. Genetic operons with transporter genes located between the genes for the oxidoreductases argue for the existence of specific keto-sugar transporters. At this moment, these transporters have not been experimentally characterized. This redox modification of the sugar transport is thermodynamically feasible with NAD(P)H as electron donor and cytochrome c as electron acceptor and yields two translocated protons per

imported sugar. The strategy may also be feasible with other organic compounds. The process may explain the presence of periplasmic cytochrome c in microorganisms lacking a quinone-cytochrome c oxidoreductase, i.e. *Flavobacteriia*.

#### SL-PPM-17

#### Innovations in bacterial solute transport: Discovery of novel types of binding-protein dependent secondary transporters

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#### Introduction and goals.

For many years it was assumed that only the ABC (ATP-Binding Cassette) transporter family employ extracytoplasmic solute binding-proteins (SBPs) for initial substrate recognition and capture. However, the discovery of the TRAP (Tripartite ATP-independent Periplasmic) and TT (Tripartite Tricarboxylate) transporters challenged this dogma; SBPs can also be associated with unrelated *secondary* transporters powered by ion-gradients. To date however, these remain the only types of characterised systems that are known to employ SBPs for high affinity uptake. Our goal was to determine if other, novel types of SBP dependent transporters exist.

#### Methods and Results.

We used the INTERPRO database to interrogate the domain architecture of ABC, TRAP and TT SBPs, combined with a systematic examination of the architectures of all of the classes of secondary importers listed in the Transporter Classification Database (TCDB). We found many novel examples of SBPs (most commonly from the ABC superfamily) fused or associated with a variety of unrelated membrane transport proteins. TRAP-type SBPs of the TAXI subfamily were specifically fused with the Na<sup>+</sup>:glutamate transporter GltS in the family Lachnospiraceae while in other bacteria we found operons encoding contiguous genes for the TAXI SBP and GltS. We characterised one such TAXI-GltS system in detail, from the pathogen *Bordetella pertussis* (1). Here, the TAXI SBP is an unusual helix-swapped dimer with a C-terminal solute-binding domain which ligand-induced fluorescence changes showed L-glutamate binding with nanomolar affinity. Thus, at least in this system, the SBP functions to increase the affinity of transport, rather than changing the substrate specificity of GltS itself.

#### Summary

Diverse transporters employ SBPs beyond the well-known ABC and TRAP systems. Use of an SBP offers a higher substrate affinity compared with the membrane transporter operating alone, allowing better scavenging in low nutrient environments.

1. Jaques, L. M., Davies, J. F. S., Sheldon-Towler, J. J., Kelly, D. J., Leone, V., & Mulligan, C. (2025). A new class of binding-protein dependent solute transporter exemplified by the TAXI-GltS system from *Bordetella pertussis*. *Communications biology*, 8(1), 1201.



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## SL-PPM-18

### Elucidating the Structure of the Purinosome

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Purines are essential components of nucleotides and play a critical role in DNA/RNA synthesis and cellular energy metabolism. The *de novo* purine biosynthesis (DNPB) pathway, which synthesizes purines from simple precursors, is highly conserved and involves six enzymes catalyzing ten steps in humans. Due to the instability of key intermediates, it has been hypothesized that DNPB enzymes form a dynamic multi-enzyme complex known as the purinosome, enabling efficient substrate channeling<sup>1</sup>. While purinosome formation and localization have been studied in mammalian cells, its structural organization remains unresolved. Emerging evidence suggests an organized architecture, but a comprehensive structural model is still lacking. We propose a novel strategy to elucidate purinosome architecture by leveraging the model organism *Bacillus subtilis*, which offers genetic tractability and evolutionary conservation of the DNPB pathway. Preliminary pulldown experiments with tagged PurE in *B. subtilis* demonstrate successful enrichment of the DNPB pathway, validating the system for structural investigation. We aim to determine interaction interfaces using cross-linking mass spectrometry, providing distance constraints to inform complex topology. The goal is to translate these findings to generate a structural model of the human purinosome, validated by cutting-edge structural biology techniques.

<sup>1</sup>An et al. Reversible Compartmentalization of de Novo Purine Biosynthetic Complexes in Living Cells. *Science* **2008**, 320 (5872), 103–106. <https://doi.org/10.1126/science.1152241>.

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## Systems and synthetic microbiology

### SL-SSM-01

#### Re-routing photosynthetic electron fluxes towards hydrogen production in cyanobacteria using an engineered small regulatory protein

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Photoautotrophic microorganisms like cyanobacteria hold great potential for sustainable production of chemicals and fuels from light, CO<sub>2</sub>, and water. However, their applicability is limited, in part due to low product yields achieved to date. Recent insights into their metabolism, energy and metabolite fluxes, and metabolic regulation provide new opportunities for interventions to enhance product yields. For conversions that only require electrons – such as redox catalysis or the formation of hydrogen by hydrogenases - the partitioning of electrons from the photosynthetic electron transport chain (PETC) is a determinant for the yield. In these processes the Calvin-Benson-Bassham (CBB) cycle is the main competitor for electrons. Here, engineered small regulatory proteins based on CP12 were used to reduce electron flux into the CBB cycle and redirect them into hydrogen in the model cyanobacterium *Synechocystis* sp. PCC 6803. In most

phototrophic organisms, CP12 is used to control the CBB cycle in dark-night cycles by binding and inhibiting the enzymes glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and phosphofructokinase (PRK). The native CP12 of *Synechocystis* itself is redox-regulated, inhibiting the CBB cycle only in the dark and becoming deactivated by a reduced cell status when light is present and the PETC is running<sup>1,2</sup>. We therefore employed variants of CP12 either derived from cyanophages - which use them during infection in light as auxiliary metabolic genes yielding a more reduced status of their hosts<sup>3</sup> - or rationally designed to be unaffected by the redox-status. By inducible expression of these CP12 variants, we were able to modulate the CBB cycle activity, photoautotrophic growth, and shift the intracellular redox potential to a more reduced state. This altered metabolic state increased the electron flux from photosynthesis into hydrogen production and, in combination with disruption of cyclic electron flow and increasing hydrogenase capacity, enabled higher hydrogen production. By utilizing CP12 variants to redirect electrons from the CBB cycle toward hydrogen production in cyanobacteria, we highlight the potential of small regulatory proteins for metabolic engineering to optimize resource allocation towards desired products.

1 McFarlane, et al. 2019, PNAS 116(42): 20984-20990.

2 Lucius et al. 2022, Front. Plant Sci. 13: 1028794.

3 Thompson et al. 2011, PNAS 108: E757-E764

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### SL-SSM-02

#### Improving Rubisco Through Allosteric Modulation

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Rubisco's intrinsic oxygenase activity imposes a fundamental limitation on photosynthetic efficiency by driving energetically costly photorespiration. While direct engineering of the active site to enhance CO<sub>2</sub> specificity has been hindered by the evolutionary trade-off between catalytic efficiency and substrate discrimination, we propose a paradigm-shifting strategy: allosteric regulation of the catalytic large subunit (LSU) through engineered interactions at its interface with a synthetic small subunit (SSU). To overcome the classic compromise between increased specificity and diminished turnover rate, we employ *de novo* designed protein binders that access vast regions of sequence space beyond the constraints of natural evolution. These synthetic binders are engineered as minimalist, high-affinity modules specifically tailored to the LSU-SSU interface of the ancestral Rubisco variant *AncL+7 e170N*. This variant serves as an ideal platform due to its reconstructed subunit interaction landscape, post-entrenchment and structurally simplified enabling precise, targeted engineering without the confounding legacy of co-evolved interfaces. Crucially, our binder design does not incorporate explicit functional selection for turnover or specificity. Instead, our core hypothesis rests on a fundamental principle of protein allostery: high-affinity binding at the LSU-SSU interface functionally mimicking the ancestral SSU will induce long-range conformational changes that propagate to the active site, thereby modulating catalytic function. By exploiting the intrinsic physical connectivity between the subunit interface

and the catalytic center, this approach bypasses the need for pre-evolved allosteric networks and opens a new frontier in enzyme engineering. This strategy represents a shift from *active-site-centric* redesign to *interface-driven allostery*, offering a powerful, generalizable framework for enhancing the performance of complex, multi-subunit enzymes like Rubisco, without compromising their inherent catalytic machinery.

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### SL-SSM-03

#### A system for large-scale and precise inter-species genomic DNA transfer

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With the advent of CRISPR technology, genome editing was lifted to a new level. With this technology it is possible to marker-free select for classical insertion and deletion. Moreover, more complex constructs can be designed that use this technology amongst others. Here, we present a genome editing system that is capable to transfer large fragments of genomic DNA from one organism to another without technical size limitations and with basepair accuracy. The system utilizes CRISPR nucleases to excise genomic DNA transfer it onto a shuttle vector and insert it into the desired host at any location. We demonstrate its capabilities by multi-editing and transfer of more than 100kb fragments between different species. The system is currently available for gram-negative bacteria and is further developed for the application in gram-positive bacteria that harbour a huge diversity of secondary metabolite pathways.

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### SL-SSM-04

#### Lighting Up the Efflux: Biosensor-Based Insights into Solvent Tolerance Mechanisms in *Pseudomonas taiwanensis* VLB120

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As a contribution to the transition to a sustainable bioeconomy, we focus on the bio-based production of hydrophobic aromatics from renewable resources applying *Pseudomonas taiwanensis* as a solvent-tolerant whole-cell biocatalyst. For developing production strains with optimized tolerance phenotypes, a detailed understanding of tolerance mechanisms and stress responses is required. The presented work focuses on the quantification of intracellular solvent concentrations, which are directly linked to the extrusion mediated by the RND efflux pump TtgGHI. In the bacterium *Pseudomonas taiwanensis*, TtgGHI is the primary factor responsible for a distinct solvent tolerance phenotype.

Consequently, we developed fluorescent biosensors to quantify intracellular solvent concentrations *in vivo* and gain a deeper mechanistic insight into the molecular basis of solvent tolerance. These biosensors are based on transcriptional regulators of solvent efflux pump genes, which were shown to respond to a broad range of hydrophobic aromatics with a high dynamic range in an inducer screening.

A second constitutively expressed fluorescent protein was incorporated into the biosensor construct to normalize biosensor readouts to cell fitness under solvent stress, enabling ratiometric analysis. For the establishment of this

construct, we demonstrated that increased expression of the *ttgGHI* efflux pump led to lower biosensor signals, indicating reduced intracellular solvent concentrations. Strains with lower internal solvent levels exhibited enhanced tolerance to externally added solvents, confirming that active extrusion is a key tolerance mechanism. Through the biosensor, we gained quantitative insights into the relationship between efflux pump expression and intracellular stressor levels over a wide range of concentrations. We are currently engaged in the identification of constitutive synthetic promoters for *ttgGHI* expression that result in superior tolerance phenotypes for solvent-specific applications. The primary challenge lies in achieving a balance between TtgGHI's energy demand for operation and expression while ensuring sufficient solvent extrusion.

Since the biosensors are easy to construct and designed for stable *attTn7* integration, they are a versatile "plug-and-play" tool for rapid characterization of *Pseudomonas* strains under solvent stress. Consequently, they possess considerable utility for the scientific community.

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### SL-SSM-05

#### PyrAux: Engineering a pyruvate auxotrophic *E. coli* strain to use growth-coupling for metabolite sensing, enzyme engineering and bioproduction

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Synthetic microbiology aims to establish sustainable bioprocesses as a replacement for traditional, often fossil-based processes. Metabolic engineering targets the establishment of tools for synthetic microbiology or efficient biocatalysts for bioproduction. An emerging strategy is growth-coupling, introducing an obligatory metabolic link between the activity of a desired pathway and an essential cellular function. This coupling results in a sensor strain where growth serves as the readout for the activity of the desired pathway. Such strains provide a versatile application platform, including semi-quantitative monitoring of secreted metabolites in a co-culture, functional screening of enzymatic activity and *in vivo* enzyme engineering, as well as growth-coupled bioproduction.

Pyruvate is a key metabolite in central metabolism. Here, we engineered a pyruvate-auxotrophic *E. coli* strain (PyrAux) by deleting the native pyruvate-releasing reactions while maintaining growth on pyruvate as the sole carbon source. The growth of PyrAux can be rescued by either external pyruvate supplementation or by a synthetic pyruvate-releasing pathway.

To demonstrate the application potential, we first showcased PyrAux as a metabolite sensor for lactate. Reintroducing the D-lactate dehydrogenase restored growth on lactate, and in co-culture the fluorescently labelled sensor strain enabled time-resolved, easy, and low-cost semiquantitative determination of lactate produced by a second strain. Next, we demonstrated the potential of PyrAux for selecting enzyme activity by introducing the DAHMS pathway, which converts xylose into pyruvate and glyceraldehyde. PyrAux could only grow on xylose as a sole carbon source after introducing the DAHMS pathway, confirming pathway activity. This concept enables *in vivo* enzyme engineering, allowing screening of enzyme libraries and adaptive laboratory evolution to select improved pathway enzymes.

Furthermore, we demonstrated growth-coupled ethylene glycol production from xylose via DAHMS pathway, highlighting growth-coupling as a strategy for stable, continuous bioproduction while ensuring a high minimal product yield.

Taken together, PyrAux adds to the repertoire of growth-coupled platform strains. Leveraging such strains provides a powerful tool to develop efficient enzymes and pathways for the use of sustainable carbon sources and sustainable bioproduction.

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## SL-SSM-06

### **Towards a multiplexed synthetic layered biosystem governing dynamic responses**

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Gene regulatory networks can act at different levels of gene expression and exhibit dynamic, time-varying responses to multiple dynamic inputs. However, studies to date have focussed primarily on single inputs, and many questions remain about how these networks decode and process multiple stimuli. Here, we present a novel approach for a synthetic biological control system by mimicking multiple signals with dynamic optogenetic multiplexing, i.e. time- and amplitude-varying light inputs, and by combining layered optogenetic regulation at transcriptional and translational levels within one bacterial host. Through mathematical modelling, we found sufficiently distinct activation and deactivation kinetics of the optogenetic systems used, enabling sequential activation of the systems by dynamic light inputs. Using the bioreactor platform "Chi.Bio" and varying light intensities and patterns, we experimentally characterised the two systems individually as well as together on one host. This enabled us to successfully identify the dynamic light-input constraints within which the two combined regulatory systems remain controllable. Furthermore, we demonstrated that under appropriate conditions, the systems can be precisely regulated by modulating the dynamics of the light input while maintaining a constant total light exposure level. Building upon these fundamentals, we next aim to further optimise the syn-bio system and establish step-by-step approaches to independently control it at different gene expression levels and at the single cell level using optogenetics and microfluidics. In addition, the newly acquired data will be integrated into the mathematical model to enhance its performance in predicting the biosystem's behaviour. Our efforts will advance the development of a robust, highly controllable multi-gene expression system to understand the complexity of layered gene regulatory networks and how cells process multiplexed information.

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## SL-SSM-07

### **Tuning expression in cyanobacteria: Towards programmable photoautotrophic cell factories**

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Cyanobacteria are attractive platform organisms for sustainable biotechnology, because of their ability to rapidly convert CO<sub>2</sub> and sunlight into biomass. In recent years,

research has expanded across multiple areas, from metabolic engineering of high-value compounds to constructing microbial consortia and understanding the fundamental biology of photoautotrophs. The development of genetic tools, including CRISPRi, Golden Gate assembly toolboxes, and heterologous expression platforms has greatly accelerated precise strain engineering. When combined with modern cultivation technologies, these advances have the potential to bring cyanobacterial hosts closer to the versatility and productivity of established heterotrophic systems.

In this study, we established a system for the comparative screening of both previously reported and newly developed genetic tools in the model cyanobacterium *Synechocystis*, with a particular focus on chemically inducible promoters. Using their individual components in a modular fashion, we constructed logic gates capable of responding to multiple inducer combinations, allowing us to study their individual behaviors in a controlled manner.

By leveraging high-density cultivation approaches, we evaluated these tools in a production-relevant context, examining intrinsic and extrinsic properties including dynamic range, leakiness, orthogonality and temporal response. This framework allows us to systematically assess tool performance in cyanobacteria, improve our understanding of their underlying behaviors, and provide a foundation for building more complex, predictable genetic circuits. Our work aims to not only advance the toolkit for cyanobacterial engineering, but also to support the rational design of synthetic systems capable of robust and programmable function in photoautotrophic hosts.

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## SL-SSM-08

### **RGB & UV Programmable Optogenetic Toolbox in *Pseudomonas putida*: A Platform for Light-Driven Synthetic Biology**

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Optogenetics uses light to provide precise, reversible, and non-invasive control over gene expression in bacteria with unique spatiotemporal resolution. Although optogenetic systems have been widely explored and developed for *E. coli*, very few light-responsive tools are currently available for other prokaryotes. In this study, we developed a modular optogenetic toolbox for *Pseudomonas putida*, a Gram-negative bacterium that is characterized by its metabolic flexibility and relevance for biotechnological and environmental applications. The light responsive switches enable dynamic and fine-tuned regulation of gene expression across the full spectral range of light (UV-A, red, green, and blue light) and include both photoreceptor-based two-component systems and photocaged inducers, which allow tight and gradual control via defined illumination conditions. Remarkably, the systems exhibited strong expression of up to ~250-fold upon induction through illumination, demonstrating high regulatory strength and reliable light-dependent activation.

To validate this platform, we implemented light-controlled regulation of pyoverdine (PVD) biosynthesis in *P. putida* as a proof of concept. PVD is a fluorescent siderophore, produced

by many *Pseudomonads*, which plays a central role in iron acquisition and can act as a public good. By replacing the intrinsic iron-dependent signal transduction with light-dependent regulatory circuits, we achieved gradual control over PVD biosynthesis, ranging from no production to overproduction of the siderophore. For the first time, this enables the direct investigation of siderophore-mediated social interactions under defined and dynamically adjustable conditions.

Overall, the optogenetic toolbox presented here broadens the genetic engineering capabilities for *P. putida* and provides a versatile platform to program cellular behaviour and study microbial interactions with high spatiotemporal resolution. Furthermore, implementing user-defined, light-regulated gene expression creates new opportunities in synthetic biology, bioprocess engineering, and microbiome studies.

## SL-SSM-09

### Transcriptomic adaptability of *Neobacillus vireti* under different conditions, including nitrate ammonification and nitrous oxide respiration

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The facultatively anaerobic, Gram-positive bacterium *Neobacillus vireti* reduces nitrate (NO<sub>3</sub><sup>-</sup>) preferentially to ammonium (dissimilatory nitrate reduction to ammonium, DNRA) or, at high nitrate concentrations, to a mixture of ammonium and dinitrogen (N<sub>2</sub>). The latter process transiently produces nitrite (NO<sub>2</sub><sup>-</sup>), nitrogen monoxide (NO) and laughing gas (N<sub>2</sub>O). In addition, *N. vireti* cells can grow by N<sub>2</sub>O respiration. This study aimed to explore the transcriptomic response to the presence of nitrate, nitrite, NO or N<sub>2</sub>O as well as anaerobiosis in the absence of added nitrogen compounds.

Cultures of *N. vireti* were grown aerobically or anaerobically, either with or without nitrate (5 mM, which favours DNRA, or 20 mM). In addition, anaerobic cultures in the early exponential phase were exposed to nitrite (5 mM), NO (1 µM), or a mixture of 5 mM nitrate and 10 % N<sub>2</sub>O. RNA was isolated at appropriate time points and sequenced. Transcriptome alignment, quantification and statistical evaluation were performed using customised R workflows.

Transcriptome analysis revealed differential expression profiles of various genes, including those encoding key enzymes involved in aerobic respiration, mixed acid fermentation, DNRA and N<sub>2</sub>O respiration. Nitrite stress elicited a rapid response involving the upregulation of *hmp* (encoding flavohaemoglobin) and *hcp* (encoding a hybrid cluster protein), whereas the reaction to NO was milder and slower.

The results highlight the transcriptomic flexibility of *N. vireti* and corroborate the occurrence of fermentative DNRA. Analysis of transcriptome dynamics indicates that shifts between metabolic pathways and stress responses occur rapidly.

## Phage biology

### SL-PB-01

#### Diversity of large macromolecular complexes in phage-infected cells

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Jumbo phages encode hundreds of uncharacterized factors that orchestrate host takeover and stress responses. Yet, our understanding of which of these proteins engage in stable interactions with the host gene expression machinery remains limited. Many of these factors lack sequence similarity and have poorly predictable structures, hindering computational annotation of functions and prediction of interaction partners. At the same time these factors represent a playground for discoveries.

Our goal is to map the complexes formed between phage factors and host RNA polymerases and ribosomes to uncover novel mechanisms of host takeover at the level of gene expression and cellular stress response. Using gradient fractionation coupled to proteomics, we previously identified a ΦKZ phage factor, ΦKZ014, that targets the ribosome in *Pseudomonas* cells to boost phage replication (Gerovac et al. 2024, Wannasrichan et al. 2025). We now extend this approach to additional jumbo phages that form a phage nucleus, including e.g. *Escherichia* phage Goslarvirus and others and compare the insights on the phage and host sides.

Subsequently, we functionally characterise these factors and explore the complex replication cycle of the *Chimalliviridae* family by integrating transcriptomics and structure-based interaction predictions, as well as knockdowns (Gerovac et al., 2025). Furthermore, we characterise their contribution to phage replication under stress conditions. Together, our findings reveal previously unknown interfaces and regulatory pathways exploited by jumbo phages, offering potential strategies to combat antimicrobial resistance.

## References

Gerovac et al. 2025 *Nature*

Wannasrichan et al. 2025 *PLoS Pathogens*

Gerovac et al. 2024 *Nature Microbiology*

### SL-PB-02

#### Functional dissection of the *Bacillus subtilis* SPβ prophage lysogeny-lysis system

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SPβ is a temperate bacteriophage infecting the Gram-positive model bacterium *Bacillus subtilis*. SPβ-like phages possess a unique lysogeny control system [1]. Our recent work identified the master repressor of SPβ and an essential activator of the lytic cycle [2]; however, the regulatory network governing the lysis-lysogeny decision requires

further elucidation [1]. Furthermore, fundamental mechanisms such as virion assembly or the adsorption of SP $\beta$  to its bacterial hosts remain poorly understood [3].

To address these knowledge gaps, we have started a series of investigations. The isolation and whole-genome sequencing of non-infectious *B. subtilis* strains confirmed the role of the teichoic acid biosynthesis gene *ggaA* in phage resistance [4]. Moreover, we are screening mutants of potential receptor-binding proteins to identify a secondary phage receptor analogous to that of phage SPP1. Furthermore, we are examining the function of 'Yop proteins' in preserving lysogeny, along with their capacity to assemble into a sensor complex, using cross-linking experiments.

In addition, we are conducting mass spectrometry profiling of purified SP $\beta$  particles to identify the most abundant proteins, which are presumed to be structural components of the virion. We are also investigating their roles in infection and prophage induction. Furthermore, we are using time-resolved quantitative proteomics to better understand regulatory dynamics of SP $\beta$ . To this end, we are analysing three *B. subtilis* strains: (a) prophage-free strain, (b) an inducible lysogen, and (c) a non-inducible lysogen, at multiple time points before and after heat shock induction.

[1] **Kohm K**, v. Clanner A, Hertel R, Commichau FM (2025) Closely related and yet special - how SP $\beta$  phages control lysis-lysogeny decisions. *Trends Microbiol.* 33: 387-396.

[2] **Kohm K**, Jalomo-Kharyova E, Krüger A, Basu S, Steinchen W, Bange G, Frunzke J, Hertel R, Commichau FM, Czech L (2023b) Structural and functional characterization of MrpR, the master repressor of the *Bacillus subtilis* prophage SP $\beta$ . *Nucleic Acids Res.* 51: 9452-9474.

[3] **Kohm K** Hertel R (2021) The life cycle of SP $\beta$  and related phages. *Arch Virol* 166: 2119-2130.

[4] Estrela AI, De Lencastre H, Archer LJ (1986) Resistance of a *Bacillus subtilis* mutant to a group of temperate bacteriophages. *Microbiology* 132:411-415.

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### SL-PB-03

#### Multicellular strategies of *Streptomyces* to counteract phage predation

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Viral predation exerted by bacteriophages has driven the continuous evolution of diverse antiphage defense mechanisms. Most known defense systems operate at the cellular level, targeting the process of phage infection at various levels<sup>1</sup>. Bacteria of the genus of *Streptomyces* are characterized by a complex developmental lifestyle, which is intricately linked to the production of specialized metabolites and proteins, some of which have already been shown to confer protection against viral infection<sup>2, 3</sup>. Considering the prolific production of specialized metabolites by *Streptomyces*, we hypothesize that these multicellular bacteria also evolved strategies aiming at the inactivation of free phages after bursts of infections.

To investigate this phenomenon, we examined the interplay between *Streptomyces* mycelial development and phage infection. We observed that a burst of phage infection in a *Streptomyces* culture was frequently followed by regrowth of phage-resistant mycelium coinciding with a significant drop in the titer of infectious phage particles - a distinctive feature of *Streptomyces* phage interactions. This regrowth was accompanied by a decrease in the pH of the culture supernatant and elevated levels of organic acids, such as citrate. Therefore, we hypothesize that regrowth of phage-resistant mycelium together with a possible reduction of phage adsorption and acidification of the extracellular environment would ultimately protect the younger, more susceptible regions of the mycelium from phage attacks, thereby conferring protection on a multicellular level. Further, a screening of different *Streptomyces* spent medium revealed secretion of compounds that inactivate and degrade extracellular phage particles.

Notably, the antiphage potential of this repertoire has only been recently recognized; with phage infection also identified as an elicitor of specialized metabolism<sup>4, 5</sup>. Taken together, these findings highlight the multifaceted defense strategies employed by *Streptomyces* to protect the community and shape its ecological interactions with phages.

### References:

<sup>1</sup>Georjon, H., & Bernheim, A. (2023). doi:10.1038/s41579-023-00934-x

<sup>2</sup>Hardy, A., *et al.*, (2023). doi:10.1016/j.tim.2022.08.001

<sup>3</sup>Kever, L., *et al.*, (2022). doi: 10.1128/mbio.00783-22

<sup>4</sup>Luthe, T., *et al.*, (2023). doi:10.1093/femsml/uqad002

<sup>5</sup>Hardy, A., *et al.*, (2020). doi: 10.3390/v12101065

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### SL-PB-04

#### Unveiling the translational landscape of double-stranded RNA phages using ribosome profiling

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Bacteriophages employ sophisticated regulatory networks to optimize gene expression to efficiently infect and replicate within their bacterial hosts. While phages with DNA genomes primarily regulate their protein output at the transcriptional level, phages with RNA genomes do not undergo a DNA stage during replication and therefore rely exclusively on post-transcriptional regulation. However, the mechanisms of post-transcriptional regulation in RNA phages remain largely unknown.

Here, we globally study translation during infection of *Pseudomonas syringae* by the double-stranded RNA (dsRNA) phage  $\phi 6$  using ribosome profiling. We show the temporal regulation of phage protein expression and how  $\phi 6$  achieves optimal protein stoichiometries for virion production. Additionally, we investigate our ribosome profiling data to identify ribosome pausing and frameshifting events, which are post-transcriptional regulatory mechanisms frequently

employed by RNA viruses. We further examine putative new open reading frames in both the phage and the host to provide insights into genomic regions currently annotated as noncoding. Our work sheds light on how dsRNA phages use post-transcriptional regulation to optimize gene expression during host takeover and replication, highlighting the importance of these processes in this understudied group of phages.

## SL-PB-05

### Studying bacteriophage-bacteria interactions: Analysis of the Zorya anti-phage defence system at the single-cell resolution

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Bacteria deploy diverse molecular systems to protect themselves from viral infection, yet how these defences operate dynamically at the single-cell level remains poorly understood. The Zorya-I (ZorI) system represents a recently discovered, multi-component bacterial anti-phage defence that couples membrane sensing to phage DNA degradation. Here, we combine high-resolution time-lapse microscopy, fluorescence labeling of phage particles and DNA, and microfluidic mother-machine assays to dissect ZorI activation dynamics in *Escherichia coli*. Quantitative imaging reveals that ZorI mediates phage resistance by rapidly degrading injected phage DNA, preventing productive infection. We show that ZorI activation follows distinct input–output modes depending on the infecting phage: graded recruitment of the effector ZorD upon Bas24 infection contrasts with an all-or-none, switch-like response to phage T4. Developing and leveraging on a multi-inlet microfluidic design, we achieve precise temporal control over phage exposure and media exchange, enabling lineage-resolved analysis of infection outcomes. Our findings establish a single-cell framework for dissecting bacterial immune responses, uncovering how Zorya integrates phage recognition and effector activation to achieve robust protection.

## Biology of yeast and fungi

### SL-BYF-01

#### Pmal-dependent pH gradients initiate inter- and intraspecies germling fusion in filamentous fungi

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Somatic cell fusion among spore germlings is a complex process in filamentous fungi that enables the formation of a syncytial unit and facilitates the exchange of genetic material apart from sexual reproduction. The fusion process can be divided into three main parts: (I) the formation of conidial anastomosis tubes (CATs), (II) bidirectional chemotropic growth of CATs towards each other (CAT homing), and (III) cell fusion at the CAT contact side. In the last decades, substantial knowledge about genes and proteins involved in the fusion process was gathered, however, the signals provoking the bidirectional growth of the fusion partners towards each other remain unknown.

In this study, we investigated the molecular mechanisms underlying germling fusion in the maize pathogen *Colletotrichum graminicola*, the grey mold *Botrytis cinerea* and the saprophytic model organism *Neurospora crassa*. Based on secretomes harvested during the germling fusion process of fusion-competent oval and fusion-deficient falcate conidia of *C. graminicola*, we identified pH as a communication signal in all three fungi. We showed further that the plasma membrane localized ATPase Pmal is a probable source for pH gradient formation, linking germination and germling fusion. In *C. graminicola*, we identified the  $\alpha$ -pheromone receptor CgSte3 and the scaffold protein CgSo of the Cell Wall Integrity (CWI) Mitogen Activated Protein Kinase (MAPK) pathway as responsible for the perception of acidic pH and subsequent downstream signalling. Since all three fungal species show bidirectional growth in intraspecies fusion assays, a conserved role of pH in germling interactions is anticipated.

From our data, we propose a new model for germling interactions, in which pH serves as an interaction initiation signal, resulting in the activation of the cell-dialogue. Our findings have implications for our understanding of fungal communication and pathogenicity, and highlight the importance of pH as a signal molecule in germling fusion.

### SL-BYF-02

#### A novel sesquiterpene from *D. discoideum* attracts the predatory nematode *C. elegans*

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The social amoeba *Dictyostelium discoideum* potentially serves as a powerful model for the investigation of bioactive natural products, owing to its intricate life cycle and established role in biological research and natural product discovery. This organism harbors a diverse set of biosynthetic genes, including 11 terpene synthase (TPS) genes that are primarily responsible for the production of sesquiterpenes and diterpenes. These genes are predominantly expressed in later developmental stages, where they hypothetically mediate intra- and interspecific chemical communication. TPS3 in particular exhibits high and tightly regulated expression during the slug and fruiting body formation, suggesting that it's as yet unidentified main metabolic products have distinct biological functions.

To elucidate the role of terpene biosynthesis in social amoebae, we first established constitutive overexpression of *tps5* as proof-of-principle, confirming in vivo production of the diterpene  $\beta$ -pinacene. We then generated a *tps3* overexpression strain to characterize its elusive metabolites. GC/MS analysis revealed several TPS3-derived volatile compounds, mainly sesquiterpenes, including three previously uncharacterized substances. Comprehensive volatile profiling throughout fruiting body development, indicates that TPS3 yields its final products also in vegetative cells without further modification during the developmental cycle. Scaling up the *tps3* overexpression strain in a 25 L bioreactor enabled the isolation and NMR-based structural elucidation of the major product, resulting in the identification of a novel sesquiterpene. Exposure assays with purified TPS3 products and the predatory nematode *Caenorhabditis*

*elegans* revealed a strong attractant effect, suggesting a potential role for these metabolites in interspecies communication within the soil ecosystems.

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### SL-BYF-03

#### Need for Sweet: Exploring the How and Why of Erythritol Biosynthesis in Fungi

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Fungi of the Ustilaginaceae family naturally produce a variety of value-added chemicals, including organic acids, glycolipids, and polyols. While primarily known for their plant pathogenicity, these fungi are gaining biotechnological interest due to their diverse product spectra [1]. *Ustilago maydis* serves as a model organism with available genetic tools transferable to other family members. Erythritol, a four-carbon polyol primarily used as a sugar substitute, is 60–80% as sweet as sucrose and is noncaloric, noncariogenic, and generally recognized as safe [2]. Its growing demand reflects rising awareness of the negative health impacts of excessive sugar consumption. In this study, 123 wild-type strains from the genera *Pseudozyma*, *Ustilago*, *Macalpinomyces*, *Sporisorium*, and *Anthracoecystis* were screened for natural erythritol production. Selected strains were subsequently characterized and genetically engineered to gain deeper insights into the conditions promoting erythritol accumulation, as well as the underlying biosynthetic pathways and enzymes involved.

Screening of the strain library identified *Ustilago tridiae* as an outstanding natural erythritol producer, reaching titers of up to 175 g L<sup>-1</sup> and yields of approximately 0.5 g g<sup>-1</sup> glucose in laboratory-scale batch fermentations. Switching to a fed-batch process increased the titer to 207.2 ± 2.5 g L<sup>-1</sup>. Additionally, deletion of the erythritol dehydrogenase gene, preventing re-assimilation of the produced erythritol, led to a further increase in titer to 236.8 ± 3.5 g L<sup>-1</sup>. Furthermore, the erythritol biosynthetic pathway in Ustilaginaceae was investigated through comparative transcriptomic and proteomic analyses, complemented by insights from other yeasts, particularly *Yarrowia lipolytica*. This approach enabled the identification of candidate enzymes for the final steps of erythritol biosynthesis — erythrose-4-phosphate phosphatase and erythrose reductase. Current efforts focus on elucidating the regulation of erythritol production under osmotic stress, mediated by activation of the HOG (High Osmolarity Glycerol) signaling pathway. A comprehensive understanding of the erythritol biosynthetic pathway and its regulation is essential for rational strain engineering and process optimization, as fungal erythritol production remains incompletely understood despite its industrial relevance.

[1] Wierckx et al. (2021) *Essays in Biochemistry*, 65:365–379.

[2] Boesten et al. (2015) *Nutrafoods*, 14:3–9.

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### SL-BYF-04

#### Spatiotemporal control of fungal pathogenesis: The role of Khd4 at the endoplasmic reticulum in *Ustilago maydis*

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Successful infection by fungal pathogens depends on precise gene expression programs. A crucial component of this regulation is mediated by RNA-binding proteins (RBPs). However, the spatiotemporal mechanisms driving this RNA control remain largely unexplored. In the corn smut fungus *Ustilago maydis*, the RBP Khd4 is known to undergo phase separation into cytoplasmic stress granules during heat stress. We hypothesize that Khd4 contributes to the stress response network specifically in proximity to the endoplasmic reticulum (ER), thereby supporting pathogenic development in maize. Preliminary data indicate that upon heat stress, Khd4-positive condensates accumulate at the cell periphery, particularly at the growth poles of yeast and hyphae. This localization mirrors that of the cortical ER. Since the ER is pivotal for membrane trafficking required for polarized growth, we propose Khd4 regulates local translation at the ER surface. To test this, we will confirm Khd4/ER co-localization and employ spatial proteomics and transcriptomics to characterize the Khd4-dependent regulon at the ER. This study will uncover novel spatial post-transcriptional control mechanisms essential for fungal virulence.

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### SL-BYF-05

#### Getting a "taste" for the global carbon cycle: Identification of the first cellulose receptor in filamentous fungi

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### Introduction and Goal

With their ability to mineralize (ligno-)cellulose, fungi are contributing massively to the global carbon turnover. Cellulose is the most abundant carbon source on this planet, yet the identity of a dedicated cellulose receptor that allows fungi to perceive the presence of this polymer has remained elusive so far. This is in contrast to the transcriptional regulators responding to cellulose, which have been well-described in many fungi. We therefore aimed to identify the first cellulose receptor in filamentous fungi and elucidate its molecular mechanism of action.

### Methods

Using *Neurospora crassa*, being a well-known reference system for biochemical genetics, we combined genetic approaches (targeted deletions, growth assays), biochemical analyses (e.g. isothermal titration calorimetry), structural studies (e.g. hydrogen-deuterium exchange mass spectrometry, docking), and yeast two-hybrid interaction assays. Phylogenetic analyses assessed conservation across fungi.

### Results

The repressor CLR-3 had already been speculated to be involved in cellulose sensing. We now identified it as the physical cellulose receptor in fungi and elucidated its mechanism. In absence of cellulose, CLR-3 binds to and thereby inhibits the cellulolytic transcription factor CLR-1.

The primary cellulose breakdown product – cellobiose – then binds to CLR-3 via a "domain of unknown function (DUF)." This leads to dissociation of CLR-3 from CLR-1, which can activate the cellulolytic regulon when released. Structural analyses identified key residues mediating sugar binding, and mutational studies confirmed their functional relevance. Experiments with native gels of purified proteins allowed to monitor the CLR-3:CLR-1 complex dynamics. Moreover, phylogenetic analysis revealed CLR-3 conservation across major fungal lineages, indicating an ancient role in carbohydrate sensing.

## Conclusion

This work resolves a long-standing question by identifying CLR-3 as the first cellulose receptor in the Eumycota and elucidating its mechanism. The discovery of a DUF as a sugar-binding domain opens new avenues for understanding carbohydrate regulation and for engineering fungi with enhanced cellulase production for biotechnological applications.

### SL-BYF-06

#### From data to model, linking *Knufia petricola* traits to emergent biofilm properties using individual-based experiments and modelling

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Black fungi, a diverse group of ascomycetes, well known for their ability to thrive in extreme environments, are found to colonize and grow on subaerial surfaces where few other microbes survive. This includes bare rock as well as marble monuments and solar panels. Black fungi can deteriorate hard substrates which is of particular concern for the preservation of cultural heritage, while their ability to colonize solar panels can cause a reduction in green energy yield. We have been developing computational modelling tools to gain predictive insight into their growth and proliferation.

In this work we focus on the black fungus *Knufia petricola*, a fungus that possesses many of the characteristic features of black fungi generally and for which genetic engineering tools have been developed. Detailed observations and systematic characterization of the organism are fundamental to developing a predictive model.

We combined the lessons from individual-based and biofilm characterization studies of *K. petricola* by Dehkoneh et al. (Manuscripts in preparation) with the predictive potential of Individual-based Modelling (IbM). In these systematic characterization studies *K. petricola* was grown and characterized on agar and on a glass substrate. *K. petricola* was grown under a variety of conditions; different carbon and nitrogen sources, different degrees of nutrient limitation, and C:N ratios. We've thus gained insight on how these conditions affect the growth characteristics and morphological development of *K. petricola*, including how nutrient limitation can limit branch development and shift generation times, and how the C:N ratio affects substrate penetration. We employed this knowledge to develop a new *K. petricola* IbM.

We used the IbM framework iDynoMiCS 2.0 (Cockx et al. 2024) to formulate our model. The model captures both morphological and kinetic aspects of *K. petricola*. By formulating characteristics of individual cells and the interactions with their neighbors and environment mathematically we can simulate emergent properties, such as the architecture and expansion of *K. petricola* micro-colonies, which gives us the unique ability to link microbial traits with biofilm characteristics. The model gives us insight on how environmental parameters can be manipulated to steer the development of *K. petricola* biofilms, it can thus help forecast *K. petricola* biofilm proliferation and can be used to inform new bio-colonization mitigation strategies.

### SL-BYF-07

#### Chromosome-scale metagenome assemblies reveal transposable element dynamics in *Peltigera* lichens

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Many fungi engage in diverse symbiotic relationships, ranging from pathogenic and commensal to mutualistic interactions. Lichens represent one of the most stable and ecologically successful mutualistic systems, typically formed between a fungal partner (mycobiont) and one or more photosynthetic partners (photobionts), such as green algae or cyanobacteria. In addition to this core partnership, lichens harbor diverse fungal and bacterial communities, forming a complex microbiome.

The genus *Peltigera* primarily forms symbioses with the cyanobacterial photobiont *Nostoc*, characterizing it as a representative cyanolichen. However, a few *Peltigera* species form tripartite associations, in which the primary photobiont is a green alga of the genus *Coccomyxa* and the secondary symbiont a cyanobacterium (*Nostoc*). Despite more than two centuries of research, many genomic aspects of lichen symbiosis remain poorly understood. Recent advances in combining long-read sequencing methods enable the resolution of complex lichen metagenomes.

Here, we present 38 high-quality metagenome assemblies from eight *Peltigera* species primarily associated with the cyanobacterial photobiont *Nostoc*, collected across five European countries. In addition to this core cyanolichen dataset, we have assembled three additional samples from two *Peltigera* species forming tripartite associations involving *Coccomyxa* and *Nostoc*, allowing us to investigate why some *Peltigera* species engage in these additional algal partnerships while others remain exclusively cyanobacterial. In our dataset 29 of the 38 mycobiont assemblies reached chromosome-scale resolution, consistently showcasing a total of 13 chromosomes across the genus. Transposable element (TE) annotations showed variable abundance across species, which correlated with genome size variation, suggesting that species-specific genome expansions were



likely driven by TE proliferation. In addition, we will present analyses of photobiont genomes and associated microbiomes to assess species-level variation and identify core members shared across samples.

This dataset represents the largest genomic resource currently available for a single lichen genus and provides a robust foundation for investigating the molecular basis of lichen symbioses. By jointly analyzing the mycobiont, photobiont, and associated microbiome, our study advances the genomic understanding of *Peltigera* and contributes broadly to microbial symbiosis research.

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## SL-BYF-08

### Investigating the role of psychrotolerant microorganisms and extremophilic yeasts to icy moon-relevant conditions

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Extremophilic microorganisms play a key role in the identification of the habitability of celestial bodies. Excluding Mars, two other locations in the Solar System could support life, the icy moons of Jupiter and Saturn, Europa and Enceladus respectively. Space missions starting in the 1970s have already elucidated the environmental characteristics of these moons, however, with the launch of the Jupiter icy moons explorer (JUICE) and Europa clipper missions, our understanding of the environmental characteristics of these locations will be improved. On Earth, the analysis of the adaptation strategies of psychrotolerant microorganisms, isolated from analogue environments, such as Antarctic and high arctic ones, will elucidate how these celestial bodies could support life.

To identify microbial organisms capable of tolerating icy moon conditions, we selected one psychrotolerant microorganism from each domain of life. Growth was performed under oligotrophic media conditions. To simulate space conditions, we exposed the organisms to the following: desiccation at different temperatures, UV-C (254 nm) and polychromatic UV (200-400 nm) radiation, X-ray radiation and freezing and thawing cycles without additional cryoprotectants to determine their survival. Our results revealed that the yeasts were more tolerant than the investigated bacteria and that the yeast *Rhodotorula frigidicola* was the most tolerant under all exposure conditions. Therefore, in order to reflect more accurately the extreme conditions of the icy moons, we exposed *R. frigidicola* to combined conditions. Remarkably the yeast can tolerate 7 days of desiccation combined with 7500 J/m<sup>2</sup> of polychromatic UV and 750 Gy of X-ray radiation at 200 kV and 15 mA. We then isolated the RNA of the yeast prior to, during, and after exposure we were able to identify a high number of differentially expressed genes.

Undoubtedly the damage the yeast cells received during exposure to the icy moon conditions requires repair for survival. The transcriptomic analyses we performed revealed the capability of the yeast to perform metabolic functions under desiccated state. Furthermore, the analysis shows the yeast halts cell cycle progression to upregulate DNA repair genes and cell structure components. Our study reveals how important multifactorial studies are for astrobiology and the role fungal candidates play as extremophiles and for the forward planetary protection of the icy moons.

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## SL-BYF-09

### Identification of Carbohydrate-Degrading Enzymes Secreted by *Fomes fomentarius* on (Non)-Lignocellulosic Substrates

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*Fomes fomentarius*, an up-and-coming biomaterials cell factory, lives as a saprophytic endophyte. As such, it accesses complex carbon polymers by secreting Carbohydrate-Active enZymes (CAZymes) that break down, for instance, lignin and cellulose. Upon their degradation, the resulting products may be internalised and metabolised. *F. fomentarius* grows on and utilises a wide range of lignocellulosic and non-lignocellulosic substrates, which renders it a suitable candidate for the valorisation of industrial by-products. Since it is also a promising industrial biomaterials cell factory candidate, learning how to best incorporate by-products as substrates for fungal biomass generation throughout the biomaterial production process will contribute to advancing the field of fungal biomaterials. However, it is not yet fully understood which CAZymes are responsible for the utilisation of which substrates. Therefore, we analysed CAZymes that end up in droplet-forming water exudates, droplets, on the growing fungal mycelium. These droplets contain a range of secreted and passively released compounds collectively referred to as the dropletome. By dissected the dropletome of *F. fomentarius* cultivated on 11 different industrial lignocellulosic and non-lignocellulosic by-products, 185 CAZymes were found in total. In a previous study, we reported that around 45% (226 from 504) of the predicted CAZyme-encoding genes are actively transcribed (Cairns *et al.*, 2025). By analysing the dropletome, we have been able to show that almost all of the corresponding gene products appear to end up in droplets, shining new light onto the function of fungal guttation. The number of CAZymes from each CAZy class in the lignocellulose-derived droplets was two to sixfold higher than in the non-lignocellulose-derived droplets, with the highest increase observed for carbohydrate esterases. We have previously reported transcriptional fingerprints for CAZymes required for lignin degradation (AA class), the genes of which have most likely undergone gene family expansion. From 103 actively transcribed AA CAZymes, 7 were detected in lignocellulose-derived droplets. This points to there being (post)translational layers of divergence within these gene families. Gauging the CAZyme suite *F. fomentarius* deploys to degrade and utilise substrates will advance our understanding of this fungus's potential for upgrading industrial by-products and how to best exploit its natural carbon catabolic capabilities.

# Anaerobic metabolism

## P-AM-001

### Reconstructing *Dehalococcoides mccartyi*'s organohalide respiratory complex in the fast-growing host *Priestia megaterium*

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The organohalide respiratory (OHR) complex of *Dehalococcoides mccartyi* strain CBDB1 is a membrane-bound protein supercomplex responsible for the reductive dehalogenation of toxic halogenated organic compounds. This complex consists of seven subunits organized into three functional modules: (i) the hydrogen uptake module, comprising the [Ni-Fe]-hydrogenase subunits HupL and HupS, catalyzing hydrogen oxidation; (ii) the putative proton-translocating core module, including HupX, OmeA, and OmeB subunits; and (iii) the dehalogenation module composed of the reductive dehalogenase RdhA anchored to the membrane by RdhB. Except for the integral membrane proteins OmeB and RdhB, each subunit contains multiple metal cofactors, including ten [4Fe-4S] clusters, one [Ni-Fe] cofactor, a putative *bis*-MGD cofactor, and a corrinoid cofactor in RdhA.

The OHR complex has great potential for biotechnological applications in bioremediation. However, its use is limited by the extremely slow growth and low biomass yield of *D. mccartyi* strains. To overcome this limitation, we aim to heterologously reconstruct the OHR complex in a fast-growing production host.

The Gram-positive bacterium *Priestia megaterium* was chosen as a production host because it encodes a complete corrinoid biosynthesis pathway, and its monoderm cell architecture facilitates the outward-facing (periplasmic) orientation of the OHR complex, making it accessible for potential bioelectrochemical applications. Since *P. megaterium* lacks the [Ni-Fe] cofactor biosynthesis machinery, the respective maturation genes from *D. mccartyi* strain CBDB1 were integrated into its genome.

All seven OHR subunits were cloned into the expression vector p3STOP1623hp and produced in *P. megaterium* WH320. To enhance production yields and metal cofactor maturation, cultivation conditions were tested. Protein mass spectrometry confirmed the localization of all seven OHR subunits in the membrane fraction of *P. megaterium*. Further analyses, including dehalogenase and hydrogenase activity assays, native polyacrylamide gel electrophoresis, hydrogenase in-gel activity staining, and ICP-MS-based metal quantification, were performed to assess the integrity and functionality of the recombinant OHR complex.

## P-AM-002

### Examination of anaerobic degradation of bioplastics: Microbial communities and potential pathways for PHBV

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Plastic waste in the environment has developed into a major environmental and human health concern due to its accumulation and disintegration into micro- and nanoplastics. Therefore, "bioplastics" are being developed that are not only

derived from sustainable resources, but are also biodegradable, at least under oxic conditions. However, a large proportion of plastic waste ends up in environments where oxygen is limited or absent. Therefore, we tested anaerobic degradation of the following bioplastics under fermentative conditions: two chemically recyclable, and under oxic conditions biodegradable bioplastics, i.e., the aliphatic long-chain polyesters PE-18,18 and PE-12,12, comprised of ester-linked long-chain alkyl dialcohols and dicarboxylic acid monomers with chain lengths of C18 and C12, respectively [1]. Furthermore, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), poly(L-lactic acid) (PLLA), and low-density polyethylene (LDPE), in comparison to polyethylene terephthalate (PET) [2, 3].

The plastics were supplied in form of foils to anoxic enrichment cultures inoculated with sewage sludge. Only the PHBV foils were completely degraded within weeks, whereas the other plastics remained macroscopically intact during an incubation time of up to two years. The PHBV cultures were subcultivated and further examined for biogas production, fermentation products, community composition, and potential degradation pathways. Metaproteomics indicated that the PHBV-degrading bacteria utilized PHB depolymerases and that PHBV-derived carbon was likely stored intracellularly as polyhydroxyalkanoate granules or in the cellular fatty acid pools via fatty acid synthesis, in addition to energy conservation through substrate-level phosphorylation.

Our findings provide a deeper understanding of anaerobic bacterial polymer degradation, important for the development of new bio-based and bio-degradable plastic materials.

#### Acknowledgments:

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#### References:

- [1] Eck M, *et al.* Biodegradable High-Density Polyethylene-like Material. *Angew Chem Int Ed Engl.* 2023;62(6):e202213438.
- [2] Kim MS, *et al.* A Review of Biodegradable Plastics: Chemistry, Applications, Properties, and Future Research Needs. *Chemical Reviews.* 2023;123(16):9915-39
- [3] Bátori V, *et al.* Anaerobic degradation of bioplastics: A review. *Waste Management.* 2018;80:406-13.

## P-AM-003

### Physiological characterization and biotechnological application of anaerobic gut fungi

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Anaerobic fungi, with a unique diversity of carbohydrate-active enzymes and the ability to produce hydrogen, have great potential in the utilisation of lignocellulosic materials. Despite the first classification as anaerobic fungi nearly 50 years ago and the re-categorisation into the phylum

*Neocallimastigomycota* in 2007, growth conditions and metabolism remain largely unknown. While many genetic analyses have been performed, physiological data is scarce.

The work presented here describes the metabolic response of the organisms *Aestipascuomyces dupliciliberans*, *Caecomyces churrovis*, *Khyollomyces ramosus*, *Orpinomyces joyonii*, *Pecoramyces ruminantium* and *Neocallimastix cameroonii* at different growth temperatures, straw particle sizes, different alternative C-Sources, different soluble carbon sources and their concentrations. In addition, a comparison of batch and fed-batch cultivation was carried out. As an example, for biotechnological application caproate production from the lignocellulosic biomass *Enset* fiber in a one-pot two-step fermentation using the anaerobic fungi *Neocallimastix cameroonii* and an anaerobic chain-elongating *Clostridium kluyveri* is shown.

This study delivers on its premise on providing physiological growth data on anaerobic fungi while analysing metabolite production in different conditions. While bioprocess engineering will lead to maximum metabolite production, the observed behaviours with different straw particle sizes show huge potential in reducing costs for biomass pretreatment in big scale applications. The analysis of the effect of temperature improves the understanding of handling anaerobic fungi.

#### P-AM-004

##### **Pyruvate oxidoreductase - An enzyme-decorated molecular nanowire from *Methanococcus maripaludis***

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#### **Introduction:**

Hydrogenotrophic methanogens harness the small energy gain of converting H<sub>2</sub> and CO<sub>2</sub> into methane for their survival. Under lithotrophic conditions, their entire biomass is generated through the Wood-Ljungdahl pathway (WLP). Acetyl-CoA, the WLP's end-product, is further converted into pyruvate. The pyruvate oxidoreductase complex (POR/PFOR) from *Methanococcus maripaludis* (Mmar\_POR) catalyzes the reversible conversion of acetyl-CoA to pyruvate using low-potential electrons from an unknown source. Compared to related enzymes from the OFOR superfamily, Mmar\_POR stands out with its two additional ferredoxin-like subunits, PorE and PorF. While previous studies highlighted their importance for efficient POR function, their role in the POR complex is still unknown.

#### **Goals:**

We aim to elucidate the structure of the POR complex, with particular emphasis on the interactions with PorE and PorF. In combination with further biochemical and mutation experiments, the structure will aid in understanding the function of PorE and PorF in their physiological context.

#### **Methods:**

We transformed *Methanococcus maripaludis* with a pLW40 plasmid carrying the *porA* gene, fused to an N-terminal Twin-Strep tag. We purified the full POR complex via affinity

purification with a simultaneous pulldown of the untagged POR subunits. The purified POR complex was subjected to cryoEM for structure determination and is used for ongoing biochemical and bioinformatic experiments.

#### **Results:**

We could confirm that POR forms long molecular nanowires similar to the HDCR supercomplex from *Thermoanaerobacter kivui* and determine the structure of the complex. The minimal repeating unit of Mmar\_POR consists of three PFOR units binding the ferredoxin filament formed by PorE and PorF at roughly a 120° angle. We further identified the presence of the *porE* and *porF* genes in more than 20 species, all characterized as class I methanogens.

#### **Summary:**

The efficient conversion of acetyl-CoA to pyruvate by PFORs is essential for hydrogenotrophic methanogens growing under lithotrophic conditions. We characterize the structure of a novel PFOR subfamily from class I methanogens that forms long molecular nanowires using ferredoxin-like subunits.

#### **P-AM-005**

##### **Enzymes involved in anaerobic naphthalene degradation including a novel aldolase family**

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Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic, mutagenic, or toxic to reproduction and, at the same time, persistent and bio-accumulative. Soils and groundwater are often highly contaminated leading to oxygen depletion and anaerobic degradation of PAHs.

We are studying the anaerobic degradation of naphthalene by the sulfate-reducing enrichment cultures N47, which proceeds in three main steps: (i) activation by carboxylation and CoA-ligation, (ii) reduction of the aromatic ring system, and (iii) ring-cleavage by  $\beta$ -oxidation-like reactions finally generating acetyl-CoA. The enzymes for the  $\beta$ -oxidation-like steps appear to be encoded in a single gene cluster named *thn*. Using heterologous expression of genes from the *thn* cluster and enzyme assays analysed by LC-MS, we identified the enzymes mediating the second-ring cleavage, including a novel family of aldolases that cleave a C–C bond at a tertiary hydroxyl group.

Starting with from naphthalene degradation intermediate 2-carboxymethyl-cyclohexane-1-carboxyl-CoA, the CoA-transferase ThnP transfers the CoA moiety to the carboxymethyl group, producing 2-carboxy-cyclohexylacetyl-CoA. The acyl-dehydrogenase ThnO oxidises the C-C bond of the acetyl-CoA side chain to a double bond, which is hydrated by ThnL. The resulting tertiary alcohol cannot be further processed by beta-oxidation. Instead, we identified the tetrameric aldolase ThnQS which presents a novel lyase cleaving the C1-C2 bond of the cyclohexane moiety of 2-carboxy-cyclohexylacetyl-CoA in a retro-aldol reaction. The open chain product is immediately cleaved by the thiolase ThnV, yielding acetyl-CoA and pimeloyl-CoA, which can be fuelled into the central metabolism. ThnQS is similar to the

enzyme AtuA with an  $\alpha\beta\beta\alpha$  architecture and adopts a previously unknown fold indicating a new class of aldolases.

These findings close the gap between the upper naphthalene degradation pathway and the central metabolism and reveal a new class of aldolases capable of processing tertiary alcohols—an activity that may have valuable biotechnological applications.

#### P-AM-006

##### Characterization of the cobalamin-dependent Radical SAM methyltransferase QCMT involved in maturation of methyl-coenzyme M reductase

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The enzyme methyl-coenzyme M reductase (MCR) plays a crucial role in the global carbon cycle owing to its ability to catalyze both the formation and consumption of methane in methanogenic archaea and anaerobic methane-oxidizing archaea, respectively [1]. MCR exhibits several unusual post-translational amino acid modifications, including 2-(S)-methylglutamine [2]. The Radical SAM methyltransferase QCMT is responsible for the chemically demanding methylation of the unreactive  $sp^3$  carbon atom (C2) of glutamine [3]. In addition to the Radical SAM domain, QCMT contains an N-terminal cobalamin-binding domain, and the enzyme employs methylcobalamin as an additional cofactor [3]. A detailed understanding of QCMT function provides valuable insights into the biochemical intricacies of the complex reactions catalyzed by Radical SAM enzymes.

QCMT has been shown to catalyze the transfer of a methyl group from methylcobalamin to the glutamine residue of a peptide substrate via a radical-based mechanism. During catalysis, two molecules of S-adenosyl-L-methionine (SAM) are consumed, yielding 5'-deoxyadenosine (5'-dA) and S-adenosyl-L-homocysteine (SAH) as byproducts in an approximately 1:1 ratio. QCMT accepts peptides of varying lengths as substrates, and methylation of these peptides was verified by MALDI-TOF MS analysis. To obtain deeper insights into substrate recognition and peptide binding, several QCMT variants were generated, in which conserved amino acid residues were specifically substituted guided by an initial crystal structure. The specific enzymatic activities of these variants were determined for comparative evaluation. In addition, cobalamin binding to QCMT was investigated in detail. Both methylcobalamin and hydroxycobalamin were found to support enzymatic activity, as the cob(II)alamin form of the cofactor is re-methylated during each catalytic cycle. KD values for methyl- and hydroxycobalamin binding were determined, and the binding kinetics were characterized.

#### References:

- [1] Kallistova, A. Y. et al. *Microbiology* **86**, 671–691 (2017)
- [2] Kahnt, J. et al. *The FEBS journal* **274**, 4913–4921 (2007)
- [3] Gagsteiger, J. et al. *Angew. Chem. Int. Ed.* **61**, e202204198 (2022)

#### P-AM-007

##### Engineering *Clostridium ljungdahlii* for sustainable acetone production

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For a sustainable future, the reliance on fossil fuels must be further reduced. While significant progress has been made in replacing fossil fuels in electricity production, most industrial production of commodity chemicals still relies on fossil raw materials. Decarbonizing the chemical industry, therefore, requires implementing sustainable production routes. Acetone is a key chemical as a solvent and an essential resource in the production of various specialty chemicals, but it is currently primarily sourced from fossil raw materials.

*Clostridia* spp. have a long history in industrial bioprocesses. The solventogenic bacterium *Clostridium acetobutylicum* was already used in the early 20th century to produce acetone, butanol, and ethanol from starch. While this process is sustainable, its reliance on carbohydrates as substrate puts it in direct competition with food production. *Clostridium ljungdahlii*, on the other hand, can grow with hydrogen and carbon dioxide as sole electron and carbon sources, which makes it attractive for the sustainable bioproduction of chemical commodities. However, wild-type *C. ljungdahlii* only produces ethanol, acetate, and small amounts of 2,3-butanediol as metabolic end products.

In the project BETA (bioethanol to acetone), we aim to provide an efficient process for the sustainable production of the base chemical acetone from bioethanol. Here, we present the progress on the development of a microbial biocatalyst within the overall process. We illustrate ways to produce acetone with *C. ljungdahlii* and aim to optimize the production through pathway engineering and metabolic modeling.

#### P-AM-008

##### The enzymology of fermentative nitrate reduction to ammonium and nitrous oxide respiration in *Neobacillus vireti*

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#### Introduction

*Neobacillus vireti* is a facultatively anaerobic bacterium isolated from grassland soil. Depending on the environmental conditions, it exhibits a versatile metabolism involving respiratory and fermentative pathways. When incubated in tryptic soy broth (TSB) medium in the absence of nitrate and oxygen, the cells produce acetate, formate and lactate as fermentation products. In the presence of 5 mM nitrate, *N. vireti* performs dissimilatory nitrate reduction to ammonium (DNRA), with fermentation shifting towards acetate production. At elevated nitrate concentrations (20 mM), alongside ammonium, the cells produce nitric oxide (NO), nitrous oxide (N<sub>2</sub>O) and, ultimately, dinitrogen (N<sub>2</sub>). The *N. vireti* genome encodes putative membrane-bound respiratory nitrate and nitrite reductases (NarGHI, NrfHA), as well as cytoplasmic nitrate (NasC) and nitrite (NirBD) reductases. *N. vireti* also reduces externally provided nitrous

oxide (N<sub>2</sub>O) and grows by N<sub>2</sub>O respiration using a clade II N<sub>2</sub>O reductase (NosZ).

## Goals

This work aimed to elucidate the enzymology of nitrate, nitrite and N<sub>2</sub>O reduction by constructing and characterising markerless gene deletion mutants of *N. vireti* DSM 15602. The pathway of N<sub>2</sub>O production from nitrite or NO was also investigated.

## Methods

Deletion mutants lacking intact gene copies of either *narG*, *nrfHA*, *nasC*, *nirBD*, *aor* (encoding a putative NO-producing nitrite reductase), *cbaBA* (encoding N<sub>2</sub>O-producing NO reductase) or *nosZ* were constructed. The kinetics of nitrate, nitrite, ammonium, NO, N<sub>2</sub>O and N<sub>2</sub> turnover were determined by using a robotised incubation system.

## Results and Conclusion

Initially, it was thought that *N. vireti* cells could grow by nitrate respiration. However, nitrate reduction was found to be unaffected in the *narG* mutant, whereas it was completely abolished in the *nasC* mutant. These results suggest that nitrate reduction serves to oxidise cytoplasmic NADH in order to regenerate the NAD<sup>+</sup> needed for fermentation, and this would explain the observed increase in acetate production in the presence of nitrate. Similarly, mutant phenotypes indicate that nitrite is predominantly reduced by the cytoplasmic NADH-dependent NirBD, rather than by menaquinol-dependent NrfHA. We will present a model of the energy metabolism of *N. vireti* that addresses 'fermentative nitrate ammonification (FNRA)', the function of the Nos system in N<sub>2</sub>O respiration, and the roles of Aor and Cba in NO turnover and nitrosative stress defence.

### P-AM-009

#### Unraveling the conversion of methoxylated aromatic compounds in *Tepidanaerobacter acetatoxydans*

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Methoxylated aromatic compounds (MACs) are components of lignin and are therefore very abundant on Earth. However, their impact on the global carbon cycle is not well understood. To convert these compounds microorganisms use O-demethylase systems that comprise four different enzymes. So far, the O-demethylase systems of several bacteria and one archaeon [1] have been characterized. The anaerobic bacterium *Tepidanaerobacter acetatoxydans* is known to use different organic compounds as carbon and energy source such as organic acids, alcohols, sugars and amino acids [2]. Although the conversion of MACs has not been previously observed, the organism harbors a genomic region with several genes encoding components of O-demethylase systems in its genome. Methylo-trophic growth and acetogenesis have not yet been described for *T. acetatoxydans*, which encodes most key enzymes for acetogenesis, but lacks formate dehydrogenase genes [3]. The question rises if the O-demethylase systems of *T. acetatoxydans* are functional and if the organism can produce acetate from MACs. To answer this question, we analyzed the growth of *T. acetatoxydans* on MACs and

studied several O-demethylase systems from *T. acetatoxydans* in regard to their biochemical properties and phylogeny. We were able to show that *T. acetatoxydans* grows on various MACs. By analyzing the utilization of 2-methoxybenzoate we found that acetate is produced despite the absence of a formate dehydrogenase. Additionally, we observed that methylo-trophic growth of *T. acetatoxydans* might be dependent on the presence of ammonia. The characterization of different O-demethylase systems shows that each of them catalyzes the demethylation of a variety of MACs as well as chloromethane with different substrate specificities. Furthermore, we observed that one O-demethylase system from *T. acetatoxydans* converts 2-MB and TMB, which differs from all previously characterized bacterial O-demethylase systems but is similar to the archaeal system [1], a similarity also reflected in its close phylogenetic relationship to the archaeal system. This study provides novel insights into bacterial O-demethylase systems and suggests that methylo-trophic growth on MACs is more widespread than previously thought, even occurring in acetogens lacking a formate dehydrogenase.

[1] Kurth JM et al. (2021). *ISME J.* 15:3549–65.

[2] Westerholm M, Roos S, Schnürer A. (2011). *Syst Appl Microbiol.* 34:260–6.

[3] Müller B et al. (2015). *PLoS ONE.* 10:e0121237.

### P-AM-010

#### A methylene-THF reducing supercomplex consisting of HdrCBA, MvhD, MetVF from *Sporomusa ovata*

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**Introduction:** Acetogenic bacteria are a group of strictly anaerobic bacteria that fix CO<sub>2</sub> and produce acetate as the main product using the Wood-Ljungdahl pathway (WLP)<sup>[1]</sup> but the energetics of the WLP is at the thermodynamic limit of life. Reduction of methylene-THF to methyl-THF (E<sub>0'</sub> = -200 mV) using NADH as reductant is the only reaction of the WLP exergonic enough to be coupled to energy conservation (ΔG<sub>0'</sub> = -23 kJ/mol)<sup>[2]</sup>. The genetic organization and subunit composition of methylene-THF reductases (MTHFR) differ among acetogenic bacteria. The core subunits of the MTHFR consists of MetF and MetV that are present in all different MTHFRs and catalyze methylene-THF reduction<sup>[3]</sup>.

**Goals:** We aimed to elucidate the subunit composition and electron carriers involved in the reduction of methylene-THF to methyl-THF in *S. ovata*.

**Materials & Methods:** Protein purification, biochemical characterization, enzymatic assays and bioinformatic analysis.

**Results:** The MTHFR of *S. ovata* is a type IV MTHFR and encoded by *metVF*, *mvhD* and *hdrBCA*<sup>[2]</sup>. The presence of the subunits HdrBCA, which are known from electron-bifurcating enzymes, indicates that the MTHFR of *S. ovata* may also be electron-bifurcating. Unfortunately, the native purification of the MTHFR only yielded the core subunits MetVF. The MTHFR was homologously produced using a plasmid-based production system in *S. ovata*<sup>[4]</sup> that allows to overexpress and purify a His-tagged version of the MTHFR for the first time. The purified protein consisted of the

expected six subunits (HdrBCA, MvhD, MetV and MetF) which were verified by Western Blot analysis and catalyzed MV<sub>red</sub>:methylene-THF oxidoreductase activity. NADH-dependent methylene-THF reduction or electron bifurcation was not observed.

**Summary:** The MTHFR consisting of HdrBCAMvhDMetVF can be produced and purified via affinity chromatography.

[1] Li, L.F., Ljungdahl, L., Wood, H.G. (1966) J. Bacteriol. 92:405-412

[2] Schuchmann, K., Müller V. (2014) Nat. Rev. Microbiol. 12:809-821

[3] Öppinger, C., Kremp, F., Müller V. (2022) Int. Microbiol. 25:75-88

[4] Tremblay, P.L., Zhang T. (2024) Appl. Environ. Microbiol. 90:e0175723

### P-AM-011

#### Structural and functional characterization of the heme *d<sub>I</sub>* biosynthesis enzyme NirJ

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Heme *d<sub>I</sub>* is a dioxo-isobacteriochlorin used as a prosthetic group by bacterial cytochrome *cd<sub>I</sub>* nitrite reductase NirS in the process of denitrification. Heme *d<sub>I</sub>* biosynthesis proceeds from the common tetrapyrrole precursor molecule uroporphyrinogen-III via multiple enzymatic steps forming siroheme, which is then decarboxylated by siroheme decarboxylase NirDLGH to form 12, 18-didecarboxy-siroheme (DDSH), the substrate of the radical S-adenosyl-methionine (SAM) enzyme NirJ. Recent investigations of NirJ revealed its auxiliary Fe-S cluster to be a [4Fe-4S] cluster and demonstrated that it eliminates the C3 and C8 propionate side chains of DDSH as acrylate, leaving methylene groups at C3 and C8.<sup>(1)</sup> The precise mechanism of this reaction as well as the function of the auxiliary Fe-S cluster remain unknown. The aim of this study is to elucidate the reaction mechanism of NirJ using a combination of X-ray crystallography, site-directed mutagenesis and *in vitro* enzyme activity assays followed by HPLC analysis of reaction products to investigate the effect of exchanging amino acid residues suspected to be either directly involved in catalysis, critical to substrate coordination or serving as auxiliary Fe-S cluster ligands. Serine substitution of individual cysteine residues in the auxiliary cluster binding motif resulted in production of insoluble protein. Co-production of the NirJ variant C343S with its substrate had a stabilizing effect, allowing the purification of soluble, monomeric NirJ-C343S with bound DDSH. NirJ-C343S was inactive in enzyme activity assays, forming neither the tetrapyrrole product nor the SAM cleavage product 5"-deoxyadenosine. *In vitro* reconstitution of Fe-S clusters did not restore enzyme activity, implying that even conservative modification of the auxiliary cluster binding motif affects cluster coordination and severely impairs enzyme activity. Additional mutagenesis experiments are to be performed to investigate substrate binding and radical quenching mechanisms in the active site.

1. Meyer et al., FEBS J. **2025** Oct;292(19):5151-5167. doi: 10.1111/febs.70105.

### P-AM-012

#### Deciphering anaerobic *myo*-inositol metabolism in intestinal microbiomes using proteomic stable isotope probing

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**Introduction:** *Myo*-inositol (MI) is a polyol with essential metabolic and regulatory functions in mammals and poultry. Despite its physiological relevance, MI utilization by the intestinal microbiome remains poorly characterized. Previous studies showed a possible microbial utilization of MI as a carbon source and the presence of related catabolic genes involved in MI turnover in mammals, but experimental evidence of MI metabolism in poultry is lacking till date.

**Goals:** This study aimed to identify intestinal microbes from laying hens involved in MI metabolism. We characterized their temporal and segment-specific metabolic activity including the detection of the respective functional pathways, using isotopically labelled MI (<sup>13</sup>C), metaproteomic-based stable isotope probing (SIP) and metabolomics.

**Materials & Methods:** Ileal and caecal digesta from commercial *Gallus gallus domesticus* (laying hens) were incubated anaerobically in a custom minimal growth media containing <sup>13</sup>C-MI as the principal carbon source. Caecal samples were directly inoculated and incubated for 0.5-24 hours, whereas ileal samples underwent a 24-hour pre-incubation to stabilize microbial activity and were subsequently inoculated and incubated for 0.5-36 hours. Samples collected at multiple time points were subjected to protein extraction and LC-MS/MS analysis, and metabolomics of labelled products (NMR). The SIPROS 4 algorithm was used to detect <sup>13</sup>C-labelled proteins and assign their microbial origins.

**Results:** The results showed higher abundance of labelled proteins associated with energy production and conversion, carbohydrate transport and metabolism, and translation, ribosomal structure and biogenesis. Temporal dynamics were observed with more rapid and pronounced MI metabolism in caeca and delayed metabolism in the ileum. Taxonomic profiling identified *Megamonas* as the key MI-utilizing player in the caeca, whereas the ileal community exhibited activity across multiple taxa, suggesting distinct metabolic niches along the intestinal tract.

**Summary:** This study provides the first detailed insight on anaerobic MI metabolism by intestinal bacterial community in poultry. The findings also reveal section-specific dynamics in microbial MI utilization and highlight *Megamonas* as a key caecal anaerobic metabolizer.

### P-AM-013

#### Structural aspects on the coenzyme F430 biosynthetic reductase CfbC/D

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Methanogenic archaea preserve energy anaerobically by the reduction of alternative electron acceptors, such as CO<sub>2</sub>, acetate and methanol or methylamines to methane, respectively [1]. This process, which is known as methanogenesis, is coupled to a chemiosmotic mechanism that drives ATP synthesis [2]. The methyl-coenzyme M reductase (MCR) catalyzes the terminal step of methanogenesis, which leads to the formation of methane. The MCR active site utilizes a unique Ni-containing porphyrinoid cofactor, called coenzyme F430 [3]. Starting from the precursor sirohydrochlorin, the *cfb* gene products are responsible for the formation of coenzyme F430. The energetically challenging six electron reduction of the tetrapyrrole macrocycle is thereby performed by the CfbC/D complex in an ATP-dependent reaction [4]. Phylogenetic studies have identified the CfbC/D complex as an ancient relative of nitrogenase, the only enzyme that is capable to reduce molecular nitrogen to bioavailable ammonia [5]. While a few nitrogenase-like complexes, including dark-operative protochlorophyllide oxidoreductase (DPOR), have been structurally characterized in the past years, the experimental structure of the CfbC/D complex remains a missing piece in the mechanistic understanding of nitrogenase-like enzymes and further, the relation between nitrogen fixation and methanogenesis in terms of enzyme catalysis. Our work presents the production and purification of the CfbC/D complex from *Methanocaldococcus jannaschii*. Using single-particle cryogenic electron microscopy, we were able to obtain the first structure of the complex at a nominal resolution of 3.47 Å.

[1] Thauer RK et al. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nat Rev Microbiol*. 2008 Aug;6(8):579-91.

[2] Dybas M & Konisky J. Energy transduction in the methanogen *Methanococcus voltae* is based on a sodium current. *J Bacteriol*. 1992 Sep;174(17):5575-83.

[3] Ermler U et al. Crystal structure of methyl-coenzyme M reductase: the key enzyme of biological methane formation. *Science*. 1997 Nov 21;278(5342):1457-62.

[4] Moore SJ et al. Elucidation of the biosynthesis of the methane catalyst coenzyme F430. *Nature*. 2017 Mar 2;543(7643):78-82.

[5] Jasper J et al. Chimeric Interaction of Nitrogenase-Like Reductases with the MoFe Protein of Nitrogenase. *Chembiochem*. 2020 Jun 15;21(12):1733-1741.

#### P-AM-014

##### Electron acceptor availability determines the use of methyltransferase systems in *Methanomassiliicoccus luminyensis*, as shown by comparative proteomics

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The hydrogen (H<sub>2</sub>)-dependent methylophilic methanogenesis has emerged as a novel type of

methanogenesis. Although organisms capable of performing this metabolism have been detected in metagenomic datasets for several years, their physiology remained unexplored in the absence of cultivated representatives. This changed with the discovery of the *Methanomassiliicoccales* and the isolation of *Methanomassiliicoccus luminyensis*<sup>1</sup>. H<sub>2</sub>-dependent methylophilic methanogens utilize methylated electron acceptors like methanol and trimethylamines (TMA) and require H<sub>2</sub> for their complete reduction to methane. The reduction of these electron acceptors is mediated by distinct sets of methyltransferases. However, a comprehensive comparison under different growth conditions has not yet been conducted. In this study, we employ comparative proteomics to systematically investigate differences in protein utilization during growth on various carbon sources.

For this purpose, the type strain *M. luminyensis* B10 was cultivated with methanol alone, methanol and acetate, methanol and TMA, and TMA as sole carbon source and a proteomic analysis was conducted. Additional growth characteristics were determined physiologically.

Across all growth conditions, the most abundant proteins were methyl-coenzyme M reductase (Mcr; 28.5%) and methanol:coenzyme M methyltransferase (Mta; 10.6%). Other methyltransferases were abundant only when *M. luminyensis* was grown with TMA, where methyltransferases specific for TMA, dimethylamine, and monomethylamine were identified. The pyrrolysine biosynthetic machinery was detected exclusively under TMA growth. Methyltransferases acting on methylated sulfides appeared in low abundance under methanol conditions but were absent with TMA. The acetate assimilation machinery was detected under all tested conditions. High levels of the energy-conserving hydrogenase Ech1, as well as moderate levels of the headless Fpo complex and HdrD, were observed. This study demonstrates how comparative proteomics can illuminate the metabolism of understudied organisms and emerging metabolic pathways like the H<sub>2</sub>-dependent methylophilic methanogenesis and might pave the way for *M. luminyensis* as model organism.

1. Dridi, B., Fardeau, M. L., Ollivier, B., Raoult, D. & Drancourt, M. *Methanomassiliicoccus luminyensis* gen. nov., sp. nov., a methanogenic archaeon isolated from human faeces. *Int J Syst Evol Microbiol* **62**, 1902–1907 (2012).

#### P-AM-015

##### Radical mediated fumarate addition initiates anaerobic butane degradation

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As a major part of natural gas and crude oil, fully saturated alkanes represent a significant ecological threat when released into the environment. Although energy-rich, their degradation poses a major challenge due to the high stability of C-H bonds. Under anaerobic conditions the difficult initial activation step is catalysed by methylalkylsuccinate synthases (MAS), a class of glycyl radical enzymes (GRE). MAS activate the alkane by fumarate addition at the secondary carbon, producing methylalkylsuccinate as the product. GRE harbour an essential stable glycyl radical, which is initially generated by an GRE activating radical SAM enzyme (GRE-AE)<sup>1</sup>.

Genes for MAS enzymes are commonly found within microbial communities surrounding deep sea hydrocarbon seeps, indicating fumarate addition as the prevalent method for anaerobic alkane activation<sup>2</sup>. However, *Desulfosarcina aeriohaga* BuS5, a propane- and butane-oxidizing Deltaproteobacteria, remains the only bacterium that can be cultivated from gaseous alkanes<sup>3</sup>. To date, no MAS has been fully characterized.

In this study, we could successfully purify MAS from butane grown *D. aeriohaga* BuS5 cells and obtained first structural and spectroscopic data, using cryo-electron microscopy and electron paramagnetic resonance spectroscopy (EPR).

The substrate-bound structure, which could be solved to a resolution of 2 Å, formed a Mas(BCDEX)<sub>2</sub> hetero-decamer. Butane, an active site cysteine residue and the co-substrate fumarate were aligned in an ideal position within the active site to achieve (i) hydrogen atom abstraction from the substrate by a cysteinyl radical intermediate. And (ii) addition of the substrate radical to fumarate giving a product radical. The presence of a glycyl radical within MAS was confirmed by EPR and first-time evidence of a complex between MAS and its activating enzyme was observed.

1. Grundmann, O. *et al. Environ Microbiol* **10**, 376–385 (2008).
2. Warkhade, Y. *et al.* **13**, 222 (2025).
3. Chen, S. C. *et al. Environ Microbiol* **24**, 1964–1976 (2022).

#### P-AM-016

##### 1-Testosterone Dehydrogenase and a Ring A Cleaving Beta-Diketone Hydrolase as Key Enzymes of Steroid Degradation in Anaerobic Bacteria

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Steroids, including steroid hormones, are widespread and persistent organic compounds which bacterial degradation plays a crucial role in their environmental removal. While aerobic pathways of steroid catabolism are well characterized, the mechanisms underlying oxygen-independent degradation of the sterane skeleton remain unclear. To elucidate key steps involved in the cleavage of ring A, we identified and characterized two enzymes catalysing consecutive reactions in anaerobic steroid degradation. A molybdenum-dependent 1-testosterone dehydrogenase (1-TDH) belonging to the xanthine oxidase family catalyses the oxidation of androsta-1-en-3,17-dione (1-AD) to androsta-1,3,17-trione (1,3,17-ATO). Subsequently, a metal-dependent ring A hydrolase mediates the hydrolytic cleavage of the cyclic 1,3-diketone moiety of 1,3,17-ATO, yielding 1,17-dioxo-2,3-seco-androstan-3-oate (DSAO), a hallmark intermediate of anaerobic steroid degradation. Structural analyses of both enzymes revealed the respective cofactors within their active sites and provided molecular insight into substrate recognition and catalysis. Comparative genomic analyses showed that both 1-TDH and the ring A hydrolase are highly conserved among known and newly identified steroid-degrading proteobacteria. These findings identify two key enzymes in anaerobic steroid catabolism and establish them as potential functional biomarkers for monitoring steroid contaminant degradation in anoxic environments.

Jacoby C, Peller L *et al.* "Ring A Cleaving Beta-Diketone Hydrolase Is a Key Enzyme of Steroid Degradation in Anaerobic Bacteria." *Environmental microbiology* vol. 27,1 (2025): e70034. doi:10.1111/1462-2920.70034

#### P-AM-017

##### Effects of bioaugmentation on pectin degradation in anaerobic digester microbiomes

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Climate change has led to a cross-generational effort to move away from fossil fuel-based energy supplies. In Germany, renewable energy sources account for more than 60% of total electricity generation. The most common renewables are wind and solar, with installed capacities of 76.1 GW and 103.7 GW, respectively. Since their productivity depends on the season and weather conditions, the grid must balance mismatches between electricity supply and demand. In addition to battery storage and pumped storage plants, biogas produced by anaerobic digestion (AD) of biomass residues is a renewable energy source that can help close the gap between electricity generation and demand. However, this requires flexible power generation from biogas, which can be achieved by expanding gas storage capacities or increasing biogas production on demand. Sugar beet pulp is an abundant feedstock that can be used in agricultural AD plants for short-term boosts in biogas production. However, the high pectin content of sugar beet can cause foaming and acidification problems in non-adapted AD processes. We hypothesized that pectin hydrolysis is the bottleneck that could be overcome by bioaugmentation with pre-adapted pectinolytic microbes.

Our goal was to test the effectiveness of bioaugmentation on biogas production with enriched pectinolytic microbial consortia in a standardized biogas production experiment fed with pectin.

The Automatic Methane Potential Test System provides a standardized test method for methane yield. Using starting material from a full-scale biogas reactor fed with corn silage and cow manure and different consortia enriched on pectin, we systematically compared methane yields from pectin in bioaugmented batch cultures and non-bioaugmented controls.

The bioaugmented cultures inoculated with enriched pectinolytic consortia degraded pectin faster and consistently showed higher initial methane production than the non-bioaugmented controls. Our experiment demonstrated an advantage in using bioaugmentation specifically for pectin digestion.

Bioaugmentation is a promising approach in flexible biogas production, allowing better process stability in feed flexibilization. Further work will evaluate the effective dosage of bioaugmentation culture in pectin degradation, identify the composition of the most effective pectinolytic consortia, and test if this effect is reproducible with sugar beet pulp as feedstock.



## P-AM-018

### Genome and proteome analysis of triethanolamine-degrading mesophilic acetogen *Acetobacterium* sp. LuTria 3

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*Acetobacterium* sp. LuTria 3 (DSM 8909) is an anaerobic, mesophilic acetogen, originally isolated for the ability to utilize triethanolamine as sole source of energy and carbon. This organism breaks down the relatively stable alkyl-nitrogen linkage anaerobically, forming acetate and ammonia. Although the underlying reactions were described previously, the responsible enzymes remain poorly characterized. In this study, we performed genome and proteome analysis of *Acetobacterium* sp. LuTria 3 to investigate the metabolic pathways involved in triethanolamine and ethanolamine utilization.

Genome analysis revealed that the total genome length of strain LuTria 3 is 3.82 Mb, with a GC content of 44.32 mol%. The 16S rRNA gene sequence shows 99% similarity to the type strain *Acetobacterium wieringae* DSM 1911; therefore, strain LuTria 3 is likely a substrain of *A. wieringae*. Genes encoding bacterial microcompartment (BMC) and potential ethanolamine-degrading enzymes were identified. Comparative proteome analyses were conducted under three growth conditions—ethanolamine, triethanolamine, and lactate—to investigate substrate-dependent protein expression. Proteomics data showed that BMC-associated genes were highly upregulated during growth with ethanolamine. Notably, a protein whose gene is annotated as "hydroxylamine reductase" was one of the most abundant proteins in the triethanolamine metabolism and, therefore, may play a role in triethanolamine degradation. In addition, we present other potential protein candidates that may be involved in ethanolamine- and triethanolamine metabolism of strain LuTria 3.

## P-AM-019

### Characterize novel (Mcr-independent) energy metabolism in *Methanosarcina acetivorans*

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Methanogenic archaea play a pivotal role in the global carbon cycle, as methanogenesis (the biological formation of methane) represents the terminal step in the degradation of organic matter under anaerobic conditions lacking alternative respiratory electron acceptors such as sulfate or nitrate. Among these microorganisms, *Methanosarcina acetivorans* stands out for its remarkable metabolic flexibility.

Alongside methylated compounds, *M. acetivorans* has been shown to grow using alternative substrates, such as carbon monoxide during acetogenesis and pyruvate for methanogenesis, revealing unexpected metabolic capabilities. However, little is known about the full range of compounds it can utilize.

Our project aims to further explore the catabolic versatility of *M. acetivorans* by assessing its growth and metabolic performance under different conditions that promote methanogenesis, methyl reduction, and acetogenesis. A variety of known and novel compounds are being tested as

potential electron donors, electron acceptors, and carbon sources, respectively. Particular attention will be paid to the organism's ability to adapt to and metabolize new substrates, which often requires physiological adaptation and/or genetic mutation. Understanding the metabolic potential and flexibility of *M. acetivorans* will contribute to a deeper knowledge of archaeal bioenergetics and evolution and may also offer the prospect for expanding its biotechnological applicability.

## P-AM-020

### Gut Microbial Enzymes as Predictors of Steroid Hormone Responses

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Steroid hormones are central regulators of mammalian physiology and are metabolized by both host and gut bacteria, with major implications for inflammation and drug efficacy. Natural and synthetic steroids are widely used to treat autoimmune diseases, inflammatory disorders, hormone deficiencies, and cancer, yet their microbial transformation remains poorly understood.

We identified two distinct gut microbial pathways that reduce diverse steroid hormones, including synthetic glucocorticoids, to either 5 $\beta$ - or 5 $\alpha$ -reduced metabolites. The 5 $\beta$ -reductive pathway, encoded by the *osrABC* operon in *Clostridium steroidoreducens*,<sup>1</sup> sequentially reduces the  $\Delta^1$  (*osrA*) and  $\Delta^4$  (*osrB*) double bonds, producing biologically inactive 5 $\beta$ -metabolites. In *C. steroidoreducens*-monocolonized mice, oral prednisolone administration caused ~80 % lower colonic and ~50 % lower systemic drug levels, demonstrating that microbial steroid reduction can directly compromise therapeutic efficacy *in vivo*. Moreover, *osrB* homologs are enriched in pro-inflammatory gut microbes such as *Clostridium\_AQ\_innocuum* and *Ruminococcus\_B\_gnavus*, both linked to Crohn's disease.

In contrast, *Holdemania filiformis* and related taxa encode a single 5 $\alpha$ -steroid hormone reductase that converts natural and synthetic glucocorticoids to 5 $\alpha$ -reduced metabolites that retain glucocorticoid-receptor (GR) affinity. Together with other microbial enzymes, such as steroid side-chain cleaving desmolases, this activity could generate androgen-like metabolites resembling dihydrotestosterone (DHT). Such transformations may contribute to treatment resistance, for example during chemical castration therapy with abiraterone and prednisone.

These findings reveal previously unrecognized microbial steroid-reducing mechanisms that can inactivate or reactivate host and drug-derived steroids, linking gut microbial metabolism to inflammatory disease and variable responses to glucocorticoid therapy.

1. Jacoby, C. *et al.* Gut bacteria metabolize natural and synthetic steroid hormones via the reductive OsrABC pathway. *Cell Host Microbe* S1931312825003804 (2025) doi:10.1016/j.chom.2025.09.014.

## P-AM-021

### Chemiosmotic energy conservation during nitrate reduction in the acetogenic bacterium *Sporomusa ovata*

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**Introduction:** The acetogenic bacterium *Sporomusa ovata* is known for its ability to use a wide range of electron acceptors. While acetogenesis *via* the Wood-Ljungdahl pathway with CO<sub>2</sub> as electron acceptor and its process of energy conservation are well known, the mechanism of energy conservation during the use of alternative electron acceptors remains unknown. Nitrate is known as an alternative electron acceptor in *S. ovata*, but the bioenergetic principles of nitrate reduction and its potential coupling to ATP synthesis remain to be elucidated.

**Goals:** To determine whether *S. ovata* conserves energy during nitrate respiration.

**Materials & Methods:** Resting cells experiments, metabolite analyses, preparation of membrane fractions, determination of intracellular ATP content, studies with protonophores, sodium ionophores and ATPase inhibitors.

**Results:** Resting cells of *Sporomusa ovata* grown on fructose + nitrate catalyzed nitrate reduction to ammonia with electrons derived from molecular hydrogen. Nitrate reduction was strictly hydrogen-dependent and coupled to the synthesis of ATP. ATP synthesis driven by hydrogen-dependent nitrate reduction was abolished by protonophores, indicating the involvement of a transmembrane electrochemical proton gradient in ATP synthesis. Furthermore, ATP synthesis and at the same time nitrate reduction were inhibited in the presence of the ATPase inhibitor *N,N'*-dicyclohexylcarbodiimide (DCCD). The inhibitory effect of DCCD on nitrate reduction was reversed almost completely by the addition of the uncoupler 2-(3,5-di-*tert*-butyl-4-hydroxy-benzylidene)-malononitrile (SF6847).

**Summary:** *S. ovata* conserves energy during nitrate respiration by a chemiosmotic mechanism.

## P-AM-022

### Phosphoketolase with CO<sub>2</sub>-bicarbonate balance directs methanol conversion to butyrate in *Eubacterium callanderi*

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Acetogenic *Eubacterium callanderi* converts one-carbon feedstocks to short-chain fatty acids through the Wood-Ljungdahl pathway, a carbon-fixing route. We examined whether installing a phosphoketolase-driven reductive acetyl-CoA bi-cycle would improve carbon use and steer methanol conversion toward butyrate.

To do so, a phosphoketolase gene from *Clostridium drakei* SL1 was cloned into a constitutive plasmid and introduced into *E. callanderi*, yielding a recombinant strain alongside the reference wild-type strain. Cultures were grown anaerobically on glucose, fructose, methanol, and on gases (H<sub>2</sub> + CO<sub>2</sub>), and products were quantified by gas and liquid chromatography.

On fructose, the recombinant increased carbon recovery by ~12% relative to the wild type and shifted products from ethanol/acetate toward butyrate; the butyrate production rate rose by ~20% while acetate decreased slightly under the same conditions. During methylotrophic growth in batch, varying the initial methanol:bicarbonate concentration ratio shifted product distributions. At 3:1, both strains favored acetate and butyrate remained minor, although the recombinant showed a higher butyrate fraction than the wild type. At 5:1, the wild type reached 74 mM butyrate and 58 mM acetate, while the recombinant reached 76 mM butyrate and 64 mM acetate. Because bicarbonate availability depends on pH, adding 3-(*N*-morpholino)propanesulfonic acid (MOPS) stabilized pH and maintained bicarbonate in solution; with 250 mM methanol plus 21 g L<sup>-1</sup> MOPS, the wild type formed 47 mM butyrate and 52 mM acetate after 168 h. Under H<sub>2</sub> + CO<sub>2</sub>, adding 60 mM bicarbonate was essential for robust growth: the recombinant strain did not grow without bicarbonate, whereas with bicarbonate both strains reached OD<sub>600</sub> ≈ 1 and produced ~140 mM acetate with only trace butyrate. In mixotrophic tests (methanol with H<sub>2</sub> + CO<sub>2</sub>) using the wild type, gas uptake accelerated and methanol consumption rose from ~98 to ~128 mM when bicarbonate was added; acetate increased to ~132 mM, while butyrate remained ~13 mM.

Altogether, integrating phosphoketolase pathway engineering with tuning of the CO<sub>2</sub>-bicarbonate balance and pH buffering offers a practical route to direct methanol to butyrate in *E. callanderi*, with the recombinant strain showing a clearer shift toward butyrate and higher carbon recovery than the wild type under fructose and methanol conditions.

## P-AM-023

### Heterologous expression of the *Dehalococcoides mccartyi* organohalide respiratory complex in *Escherichia coli*

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Organohalides are prevalent and pervasive environmental contaminants, introduced into soil and groundwater as chemical intermediates, biocides, and industrial solvents, often with toxic effects on human and ecosystem health. *In situ* biological remediation is an established and effective method for the treatment of organohalide pollution, relying on anaerobic mixed cultures enriched in organohalide-respiring bacteria (OHRB). The OHRB *Dehalococcoides* is of particular scientific interest due to its unique quinone-independent respiratory machinery. This group encodes a membrane-bound protein complex consisting of three modules: (i) the HupLS module oxidizes hydrogen and shuttles the resulting electrons to (ii) OmeAB-HupX module, the putative proton pump and electron shunt, which transfers the electrons to (iii) the RdhAB module, reducing the corrinoid cofactor in RdhA, which can then dehalogenate

organohalides. This complex has great potential as a bioengineering target, but research has been limited by the slow growth and fastidious nature of the native organisms. Heterologous expression would allow for greater yields and higher purity, as well as expanding the molecular techniques available for research. The objective of this study is to express the complete OHR complex from *Dehalococcoides mccartyi* strain CBDB1 in *Escherichia coli*.

To achieve this, all seven subunits of the OHR complex were cloned into the expression vector pACYC:Duet and produced in different *E. coli* strains containing a supplementary cobalamin uptake pathway encoded on the pBAD42:*btuCEDFB* vector. Here we evaluate different production conditions for protein yields and cofactor incorporation using protein mass spectrometry (nLC-MS/MS) and inductively coupled plasma-mass spectrometry. Moreover, association of complex members was investigated by native polyacrylamide gel electrophoresis and nLC-MS/MS. To test the functionality of the recombinant reductive dehalogenase activity assays using different organohalides were conducted.

This study not only advances our understanding of a unique, quinone-independent respiratory mechanism but also lays the foundation for engineering bioelectrochemical systems and synthetic organisms capable of pollutant degradation and sustainable redox biocatalysis.

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#### P-AM-024

##### Energy conservation in the gut acetogen *Blautia schinkii*

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**Introduction:** The gut microbiome is a highly complex community, not only involved in the digestion of nutrients but also playing a central role in the development of diseases as well as contributing to human health. Gut acetogens of the genus *Blautia* are often connected to the human well-being<sup>[1]</sup>. However, only little is known about their physiology and bioenergetics. Here, we characterized the respiratory chain of the human gut acetogen *Blautia schinkii*.

**Goals:** To unravel the physiology and bioenergetics of the human gut acetogen *B. schinkii*.

**Materials & methods:** Growth experiments, fermentation and metabolite analysis, genome analysis and enzyme assays.

**Results:** *B. schinkii* ferments glucose to mainly acetate and ethanol as well as to trace amounts of lactate, formate and H<sub>2</sub>. In the absence of NaCl, the growth rates and fermentation rates decreased, indicating a function of Na<sup>+</sup> in the metabolism of *B. schinkii*. The addition of 20 mM NaCl led to a threefold increase of Rnf activity at the cytoplasmic membrane. Additionally, ATP hydrolysis catalyzed by the cytoplasmic membrane was stimulated by addition of NaCl. In the absence of NaCl resting cells of *B. schinkii* produced 9 mM acetate from H<sub>2</sub> + CO<sub>2</sub>, which was increased by addition of 20 mM NaCl to 24 mM. Furthermore, acetate production was completely inhibited by the Na<sup>+</sup>-ionophore ETH2120, whereas the protonophore TCS had no effect. Bioinformatic analysis of the genome of *B. schinkii* revealed only one gene cluster encoding an ATP-synthase; the enzyme is of the A<sub>1</sub>A<sub>0</sub>-type and has a Na<sup>+</sup>-binding site in subunit c.

Interestingly, this c-subunit has four transmembrane helices but only one Na<sup>+</sup>-binding site.

**Summary:** The human gut acetogen *B. schinkii* has a Na<sup>+</sup>-dependent respiratory chain, consisting of a Rnf-complex and an A<sub>1</sub>A<sub>0</sub> ATP-synthase.

[1] Liu, X., Mao, B., Gu, J., Cui, S., Wang, G., Zhao, J., Zhang, H., Chen, W. (2021) Gut Microbes 13: e1875796

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#### P-AM-025

##### Comparative Kinetic Analysis of Trimethylamine Formation and Trimethylamine N-oxide Reduction by the Human and Rat Gut Microbiota

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The human gut microbiota is a complex and adaptive community which is under constant influence of environmental and nutritional changes. Dietary compounds derived from animal products include (phosphatidyl)-choline, carnitine and betaine. The gut microbiota is able to convert these compounds to trimethylamine (TMA), a tertiary amine associated with several diseases and syndromes (Day-Walsh et al. 2021). While conditions like the trimethylaminuria are unpleasant side effects of a high TMA blood level, the conversion of TMA to trimethylamine-N-oxide (TMAO) by human hepatic flavin monooxygenases are of higher concern as TMAO may contribute to severe health conditions like the promotion of cardio vascular diseases (Wang et al. 2011)

The main goals of our experiments were to kinetically characterize the formation of TMA from three dietary compounds as well as the conversion capability of TMAO to TMA by the human and rat microbiota.

Human or rat colon bacteria were isolated from fecal samples using a density gradient centrifugation and adjusted to OD<sub>600</sub> values of 10 and 5, respectively. The bacterial preparations were incubated with 1 g L<sup>-1</sup> of pectin (human) or carboxymethyl-cellulose (rat), xylan and mucin under anaerobic conditions. The substrates choline, L-carnitine, betaine and TMAO were adjusted to a final concentration of 5 mM. Samples were taken at different time point and analyzed by HPLC.

Both human- and rat microbiota converted choline, L-carnitine and TMAO to TMA over a 24 h period. In contrast, betaine was only minimally converted to TMA by cells of the human gut microbiota and not by the rat gut microbiota within the same timeframe. The conversion of L-carnitine to TMA proceeded via a two-step pathway, involving the intermediate formation of γ-butyrobetaine. Choline and TMAO were the most rapidly converted substrates in both systems.

Day-Walsh, P. et al. (2021). *European Journal of Nutrition*, 60(7), 3987–3999

Wang, Z. et al. (2011).. *Nature*, 472(7341), 57–63

# Poster

## Archaea

### P-AR-001

#### Ultrastructure of archaeal virocells from the continental subsurface

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"*Candidatus* Altiarchaeum hamiconexum" dominates and contributes to carbon fixation in continental subsurface ecosystems through a Factor420-free reductive acetyl-CoA pathway, whereas viral infections of these organisms likely initiate the heterotrophic carbon cycling in these otherwise oligotrophic systems. Despite these findings, the diversity of virulent viruses infecting *Ca. Altiarchaeum* and the cellular changes during infection remain underexplored. Here, we detected interactions between *Ca. Altiarchaeum* and its viruses using Hi-C metagenomics, while directly imaging extra- and intracellular virus-host interactions using cryo-electron tomography (cryo-ET). To this end, we generated metagenomic and Hi-C data from the *Ca. Altiarchaeum*-dominated groundwater biofilms collected at the Mühlbacher Schwefelquelle in Isling, Regensburg (Germany). Hi-C data was mapped to both a draft genome of *Ca. Altiarchaeum* and predicted high-quality viral contigs to retrieve linkage information on physically associated virus-host pairs. Taxonomic classification of the dominant infected virus, detected via quantification of Hi-C contact frequency and relative abundance, was inferred through homology searches and clustering along with computing a phylogenetic tree of the major capsid protein. Microdiversity within this dominant infected virus was examined by identifying single nucleotide polymorphisms (SNPs) across viral genomes recovered from samples collected in 2012, 2018, 2022, and 2024, revealing high infection rates and accumulation of SNPs. To directly visualize the virus-host interactions, cryo-ET data of *Ca. Altiarchaeum* cells undergoing infection was analyzed using the IMOD software, resulting in 12 models from 19 tomograms. Structural segmentation revealed rod-shaped and spherical virus-like particles (VLP) closely associated with both inner and outer membranes of the host. ISO-based segmentation enhanced feature resolution and demonstrated direct virus-host envelope contacts. This work combines Hi-C-detected virus-host interactions with cryo-ET-based structural visualization to provide a comprehensive, multi-scale understanding of the infection processes of an uncultivated, yet key carbon-fixing archaeon in subsurface ecosystems.

### P-AR-002

#### Biochemical characterization of the archaeal heme biosynthesis enzyme AhbC

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While the 'canonical' protoporphyrin IX-dependent pathway for heme biosynthesis occurs in many bacteria and eukaryotes, archaea utilize a distinct siroheme-dependent route. In this pathway, siroheme is decarboxylated to 12,18-didecarboxysiroheme (DDSH), which is subsequently

converted to Fe-coproporphyrin III (Fe-CPIII). During AhbC catalysis, two acetate chains of DDSH are oxidatively removed [1,3,4]. Despite its central role for archaeal heme biosynthesis, detailed biochemical data on purified AhbC and the function of its auxiliary [4Fe-4S] cluster have remained limited [2,4].

We heterologously produced archaeal AhbC in *Escherichia coli* BL21(DE3), optimized anaerobic purification, and established an in vitro enzymatic activity assay to monitor conversion of DDSH to Fe-CPIII. Product formation was analyzed and quantified by HPLC, supported by UV-visible spectroscopy, providing direct evidence for AhbC-catalyzed conversion of DDSH. Complementary in vivo assays in engineered *E. coli* cells producing the substrate DDSH verified Fe-CPIII formation in a cellular context.

The recombinant AhbC contained two [4Fe-4S] clusters after chemical reconstitution. Exchange of one auxiliary cluster ligand (Cys → Ala) resulted in the production of a soluble but strongly activity-reduced AhbC variant, implicating an important role for the auxiliary cluster in catalysis or structural stabilization.

This study provides the first quantitative in vitro assay for Fe-CPIII formation by archaeal AhbC. Future studies will aim to elucidate the AhbC reaction mechanism, Fe-S cluster functions and structural properties.

[1] Layer G. (2021) Heme biosynthesis in prokaryotes. *Biochim. Biophys. Acta Mol. Cell Res.*, 1868, 118861.

[2] Layer G. (2022) Radical SAM enzymes involved in tetrapyrrole biosynthesis. *Compr. Nat. Prod. III Chem. Biol.*, Volume 5, 349-363.

[3] Kühner M., *et al.* (2014). The alternative route to heme in the methanogenic archaeon *Methanosarcina barkeri*. *Archaea* 2014, 327637.

[4] Bali S., *et al.* (2011) Molecular hijacking of siroheme for the synthesis of heme and d<sub>1</sub> heme. *Proc. Natl. Acad. Sci. U.S.A.*, 108, 18260–18265.

### P-AR-003

#### The Patchwork of Nucleoid-Associated Proteins in Archaea

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Genomic DNA requires structural organization to maintain its integrity and accessibility. In Bacteria, this is achieved primarily by nucleoid-associated proteins (NAPs). In Eukaryotes, histones are responsible for chromatin packaging. Archaea combine both strategies, using histones and diverse NAPs. Among these, the histone-like protein HU is almost ubiquitous in Bacteria but occurs only sporadically in Archaea (Hochoer et al., 2022). Bacterial HUs form dimers that bind DNA without sequence specificity, bending it to maintain supercoiling and nucleoid compaction. They also contribute to replication, recombination, transcription, and repair (Dillon & Dorman, 2010). However, the functions of archaeal HU homologs remain poorly understood, except for the HU of *Thermoplasma acidophilum*, which functions as a

histone analog, as Thermoplasmata lack histones (Hoher et al., 2019; Laursen & Luger, 2025).

Using phylogenomics, biochemistry, and structural biology, we investigate the evolutionary origin and DNA-binding properties of archaeal HUs. Our analysis identified 327 archaeal HU homologs across 16 phyla. Most were acquired from Bacteria through recent horizontal gene transfers of single HU genes, while Thermoplasmata, Halobacteria, and some DPANN lineages show evidence of more ancient transfers followed by vertical inheritance and intra-lineage transfers. Remarkably, Halobacteria encode a unique monomeric HU consisting of two fused HU domains and an N-terminal Twin-Arginine-Translocation signal peptide.

We recombinantly overexpressed the *Halorubrum aquaticum* HU gene in *E. coli* and purified the protein to homogeneity. Electrophoretic mobility shift assays showed the highest affinity to be toward double-stranded DNA, followed by mismatch, 3' or 5' overhang substrates, indicating a preference for intact duplex DNA. Co-crystallization of HU-dsDNA complexes yielded fibrous and needle-like crystals; and conditions are being optimized for diffraction.

Our results reveal that archaeal HU proteins are evolutionary mosaics of bacterial origin, possibly adapted to meet lineage-specific demands for DNA organization. For the halobacterial HU, key questions remain: does it bend or coat DNA, and does it protect the genome under extreme conditions? Future micrococcal nuclease assays, using plasmid DNA or heterologous expression in *E. coli*, will help determine how this archaeal HU organizes DNA and whether it forms distinctive protective nucleoprotein filaments.

#### P-AR-004

##### **Heterotrophic Bacteria Facilitate Methanogen Survival under Oxygen Stress through Metabolic Cooperation**

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#### **Aims**

Methanogens are key contributors to global carbon cycling but are generally considered highly sensitive to oxygen. However, in natural microoxic environments, methanogens often coexist with aerobic or facultatively aerobic microorganisms. This study aims to explore how heterotrophic bacteria mitigate O<sub>2</sub> stress for methanogens and to identify bacterial partners that enhance methanogen tolerance and activity under microaerobic conditions.

#### **Methods**

The model strain *Methanococcus maripaludis* MM901 was co-cultured in batch systems with aerobic microorganisms obtained through enrichment from specific environmental samples using *M. maripaludis* medium. Enriched aerobic partners were isolated by flow cytometry. Single strains were then tested in different combinations with *M. maripaludis* MM901 to evaluate cooperative performance under O<sub>2</sub> exposure. The co-cultures were periodically supplied with a gas mixture of 4:1 H<sub>2</sub>/CO<sub>2</sub> containing defined concentrations of O<sub>2</sub>. We monitored OD<sub>600</sub>, methane production, and oxygen consumption during incubation.

## **Results**

An isolated *Vibrio* species from coastal sediments exhibited effective cooperation with *M. maripaludis* MM901. The co-culture maintained stable methane production when exposed to gas mixtures containing less than 1.1 kPa O<sub>2</sub> (1 %). At O<sub>2</sub> levels below 2.3 kPa, methane production temporarily ceased but recovered within several hours. After long-term adaptation, the culture was able to resume methane production within a few days even after exposure to 4.7 kPa O<sub>2</sub> (4 %). The co-culture sustained with no addition of organic carbon or reduced sulfur in the medium, we hypothesize that *Vibrio* digests *M. maripaludis* biomass for O<sub>2</sub> consumption and growth, and *Vibrio* reduces sulfate to forms accessible to *M. maripaludis* MM901 as sulfur source.

## **Conclusions**

These results reveal that heterotrophic bacteria can protect methanogens from O<sub>2</sub> toxicity through metabolic cooperation. Such interactions present a valuable example of sustaining methanogenesis in fluctuating redox environments with help of heterotrophs, providing new ecological insight into methane production under microaerobic conditions.

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#### **P-AR-005**

##### **Guided tour through the small protein landscape of *Methanosarcina mazei***

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Small open reading frame-encoded proteins (≤100 amino acids) fulfill important roles in many cellular processes. In the methanoarchaeon *Methanosarcina mazei*, numerous small proteins have previously been identified under different nitrogen-availabilities, with only few being subject to functional characterization. Consequently, a detailed expression analysis of small proteins translated under other stress conditions, in conjunction with conservation, homology and sequence-based analyses, may reveal interesting candidates for future downstream analyses. Here, we investigated the small proteome of *M. mazei* under five growth conditions. By enriching the low molecular weight proteome and combining top-down and bottom-up proteomic analysis, a total of 234 small proteins were validated at the protein level; 130 of these were identified by top-down proteomics, giving rise to 408 distinct proteoforms. Beyond a large cohort of hypothetical proteins, we detected small ferredoxin-like proteins, toxin-antitoxin system components, and subunits of well-known macromolecular complexes. Especially small ribosomal proteins were well represented within the mass spectrometry data. *M. mazei* possesses at least 15 small ribosomal proteins, all identified across every experimental condition, underscoring their functional importance in ribosomes. The extensive repertoire of ribosomal-protein proteoforms also raises the possibility that post-translational modifications of these small subunits modulate translation. Overall, this study provides a systematic, condition-dependent profiling of the *M. mazei* small proteome using complementary proteomic strategies, coupled with homology and sequence-based analyses. The dataset constitutes a valuable resource for downstream functional investigations of archaeal small proteins.

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## P-AR-006

### Biochemical characterization of a methyltransferase system involved in the demethylation of methylated selenium compounds

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Selenium is an essential trace element for organisms of all three domains of life, as it is incorporated into selenoproteins as selenocysteine [1]. *Methanococcus voltae* is a methanogenic archaeon that encodes selenoproteins involved in its metabolism, and selenium limitation was shown to lead to reduced growth [2]. In the absence of selenium, genes were found to be induced that encode a corrinoid protein and two methyltransferases [3]. This methyltransferase system is most probably responsible for the demethylation of dimethyl selenide to provide selenium for biosynthesis of selenoproteins. While knockout studies demonstrated that the corrinoid protein and one of the methyltransferases was involved in selenium scavenge, for the second methyltransferase this could not be verified [3]. However, biochemical characterization and substrate analysis of the methyltransferase system has not yet been performed. Our aim is to biochemically characterize this corrinoid-dependent methyltransferase system and to understand which methylated selenium compounds can compensate for selenium depletion. To achieve this, we heterologously produced and purified the corresponding proteins in *E. coli* and started performing UV-Vis spectroscopy as well as activity assays to determine the substrate range and reaction kinetics of the two substrate methyltransferase paralogs. Furthermore, we will analyse the growth behaviour of *M. voltae* cultivated in medium lacking selenium as a trace element but supplemented with distinct methylated selenium compounds. This study will lead to a better understanding of microbial selenium scavenge using a corrinoid-dependent methyltransferase system for demethylation of methylated selenium compounds.

[1] Stock T, et al. (2009). *Biochim Biophys Acta*. 1790, 1520-1532.

[2] Whitman W. B., et al. (1982). *J Bacteriol*. 149, 852-63.

[3] Niess U. M., et al. (2004). *J Bacteriol*. 186, 3640-3648.

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## P-AR-007

### Investigating the conversion of methoxylated aromatic compounds by archaea

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Lignin, one of the most abundant organic polymers on Earth, serves as a major natural source of methoxylated aromatic compounds (MACs). While bacteria-mediated MAC conversion is well-documented, archaeal degradation of MACs has only recently been discovered. The methanogenic archaeon *Methermicoccus shengliensis* was the first archaeon found to be capable to convert MACs and use them for methane production [1]. *M. shengliensis* uses an O-demethylase system (Mto) composed of four distinct enzymes that, despite being archaeal, shows notable similarity to bacterial methyltransferase systems for MAC

conversion [2]. Recent findings suggest that such methyltransferase systems may be more widespread among archaea than previously assumed. The hydrogenotrophic methanogen *Methanolacinia petrolearia* that primarily uses H<sub>2</sub> and CO<sub>2</sub> for methane production encodes four proteins that are homologous to the Mto proteins. Despite lacking canonical MAC transporters in close proximity, growth experiments with *M. petrolearia* revealed tolerance to multiple MACs and to toxic methyl halides (e.g., chloromethane), suggesting these compounds might be converted/detoxified by the Mto system. Activity assays with heterologously produced Mto proteins from *M. petrolearia* verified demethylation of a variety of MACs as well as chloromethane. To our knowledge, we have identified the first methyltransferase system of a hydrogenotrophic methanogen that converts MACs and chloromethane. To better understand the MAC and chloromethane conversion in *M. petrolearia*, RT-qPCR analyses have been initiated to investigate the expression of the *mto* genes under various growth conditions. Furthermore, comprehensive growth experiments including substrate and product analyses will be performed. These studies are expected to shed light on methylated compound conversion in hydrogenotrophic methanogens such as *M. petrolearia*.

[1] Mayumi et al. (2016) *Science* 354: 222. [2] Kurth et al. (2021) *ISME J* 15: 3549.

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## P-AR-008

### Investigating the Role of OrfY in the unidirectional trehalose glycosyltransferring synthase TreT pathway

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Trehalose, a non-reducing disaccharide of two glucose units, is increasingly recognized for its multifunctional role in microorganisms, where it contributes to stress resistance, protein and membrane stabilization, and virulence. These properties make trehalose biosynthesis a promising target for therapeutic and diagnostic development. Due to its unique 1,1- $\alpha$ , $\alpha$ -glycosidic linkage, chemical synthesis of trehalose remains challenging, and enzymatic production, particularly via trehalose glycosyltransferring synthase (TreT), has gained industrial relevance (1).

Among TreT enzymes, the enzyme from *Thermoproteus tenax* (TxTreT) catalyzes the unidirectional formation of trehalose and hence gained special interest for biotechnological applications. Gene neighbourhood analysis revealed that the *treT* gene forms an operon with an open reading frame of unknown function, designated, *orfY*, present in (hyper)thermophilic Archaea (2). Based on analysis of the crystal structure, a putative role as a transcriptional regulator was proposed (3).

In this study, we investigated the function of *orfY* in trehalose metabolism. The *orfY*-*treT* operon as well as the single gene from *T. tenax* was recombinantly expressed in the crenarchaeal model organism *Sulfolobus acidocaldarius*. The proteins were purified via affinity chromatography and analysed for complex formation via size-exclusion chromatography. Notably, both genes form a stable enzyme complex and enzymatic assays demonstrated that OrfY enhances TreT activity and increases TreT expression by up to two-fold.

These findings support a modulatory role of OrfY in trehalose biosynthesis, possibly through protein stabilization or regulatory interaction. This work expands our understanding of trehalose metabolism in Archaea and identifies OrfY-like proteins as potential regulators in this pathway.

## References

1. O'Neill et al 2017. *Pure Appl Chem* 89(9): 1223–1249 DOI: 10.1515/pac-2016-1025
2. Kouril et al 2008. *Archives of Microbiology* 190 (3): 355–69. DOI: 10.1007/s00203-008-0377-3
3. Kryshchuk et al 2014. *Proteins* 82 Suppl 2(0 2): 26–42. DOI: 10.1002/prot.24489

## P-AR-009

### Unraveling the distribution and diversity of methoxydotrophic archaea

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Methoxylated aromatic compounds (MACs) are a diverse group of aromatic ethers with significant relevance in biological systems. Lignin represents the primary source of MACs, comprising a substantial portion of organic matter in global ecosystems<sup>[1]</sup>. Conversion of MACs by bacteria and fungi has already been observed since over 40 years<sup>[2]</sup>. Yet only recently, it has been discovered that also archaea have this ability: Methoxydotrophic archaea use designated methyltransferase systems, namely the Mto and Mtg system, for MAC conversion<sup>[3,4]</sup>. Given the abundance of MACs on Earth, the presence of methyltransferase genes in a variety of archaeal taxa<sup>[3]</sup> and the lacking knowledge about their ecology, methoxydotrophic archaea might exert an underestimated yet important influence on global climate due to CH<sub>4</sub> and CO<sub>2</sub> formation from MACs.

Using a collection of primer pairs targeting genes of substrate methyltransferases, a variety of different environments was screened, and amplicon sequencing was conducted. We could show that methoxydotrophic archaea appear to be widespread in different habitats ranging from hot springs, hydrothermal vents and marine sediments to peatlands, oilfields as well as termite guts. Particularly, Bathyarchaea appear to be the most widespread taxon, encoding both Mto and Mtg systems, while Halobacteriota and other Thermoproteota appear to be more niche-specific. Furthermore, a screening of publicly available metagenomes aims to verify and deepen the knowledge about the biogeography and diversity of methoxydotrophic archaea.

In summary, we provided first insight into the biogeography of methoxydotrophic archaea in distinct habitats.

## Bibliography

- [1] D. J. Burdige, *Global Biogeochem. Cycles* **2005**, *19*.
- [2] R. Bache, N. Pfennig, *Arch. Microbiol.* **1981**, *130*, 255–261.
- [3] C. U. Welte, R. de Graaf, P. Dalcin Martins, R. S. Jansen, M. S. M. Jetten, J. M. Kurth, *Environ. Microbiol.* **2021**, *23*, 4017–4033.
- [4] J. M. Kurth, M. K. Nobu, H. Tamaki, N. de Jonge, S. Berger, M. S. M. Jetten, K. Yamamoto, D. Mayumi, S. Sakata, L. Bai, L. Cheng, J. L. Nielsen, Y. Kamagata, T. Wagner, C. U. Welte, *ISME J.* **2021**, *15*, 3549–3565.

## P-AR-010

### Characterisation of lactate oxidoreductase LutBC from *Haloferax volcanii*

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Lactate is a key intermediate in microbial metabolic networks, occurring in almost all ecological niches and serving an easily accessible nutrient for many microorganisms. Whereas NAD-dependent lactate dehydrogenases serve for lactate production during fermentations, the utilization of lactate requires usage of different than NAD electron acceptors, or electron bifurcating enzymes. The *lutABC* gene cluster was first identified as essential for lactate oxidation in *Bacillus subtilis* and *Shewanella oneidensis* (Chai *et al.*, 2009; Pinchuk *et al.*, 2009) and encodes a set of highly conserved proteins that enable lactate utilization. Bioinformatic analyses have revealed that these proteins share a conserved Lactate Utilizing Domain (LUD) (Hwang *et al.*, 2013), being widely distributed among both bacteria and archaea. The halophilic model archaeon *Haloferax volcanii* is among the archaea that possess a homologue of these proteins. It can grow using lactate as its sole source of energy and carbon. In this organism, the proteins of the *lut* operon are represented by LutB, an iron-sulfur oxidoreductase (a fusion of the *B. subtilis* LutA and LutB proteins), and LutC, the associated subunit. We produced the *H. volcanii* LutBC homologously in an active form and characterized it biochemically. We demonstrated that the protein is specific to its substrate, L-lactate, exhibiting a specific activity of 73 U/mg protein. We discuss the functions of Lut(A)BC proteins and their distribution in prokaryotes.

Chai, Y., *et al.* (2009). *Journal of Bacteriology*, **191**(8), 2423–2430.

Hwang, W. C., *et al.* (2013). *BMC Bioinformatics*, **14**, 341.

Pinchuk, G. E., *et al.* (2009). *Proceedings of the National Academy of Sciences of the United States of America*, **106**(8), 2874–2879.

Reinhardt, A., Johnsen, U., & Schönheit, P. (2019). *Molecular Microbiology*, **111**(4), 1093–1108.

## P-AR-011

### A new set of autofluorescence-free *Haloferax volcanii* strains for robust imaging of *in vivo* cell biology

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Fluorescence microscopy has become a key technique to study protein dynamics, mechanisms, and subcellular localization in Archaea [1]. We previously established robust fluorescence imaging in the halophilic model archaeon *H. volcanii* using the autofluorescence-free strain WR806 and the pUE001 plasmid system, which facilitated controlled expression of different proteins of interest and is particularly suited for expression-level-sensitive studies like single-molecule localization microscopy (SMLM) of low abundant proteins [2, 3]. However, current SMLM approaches in *H. volcanii* are limited to WR806, a strain with an extensively modified genetic background. Genetic deletion of auxotrophic selection markers in this pleomorphic archaeon, followed by either plasmid-based complementation or exogenous supplementation of metabolic compounds, consistently produces cells with aberrant morphology and altered physiology, thereby compromising the integrity of cell biological phenotypes [4, 5, 6].

In this work we aim to broaden the availability of imaging-compatible strains, while maintaining comparability to the most commonly used genetic combinations and approximating native-like systems. Our novel strains enable selection of optimal imaging strategies based on protein level requirements and selection markers, facilitating native-like single-molecule studies across various biological processes.

[1] Van Wolferen et al., Nat Microbiol. (2022) doi: 10.1038/s41564-022-01215-8

[2] Turkowyd et al., Front Microbiol. (2020) doi: 10.3389/fmicb.2020.583010

[3] Schrage et al., Preprint Biorxiv (2025) doi: 10.1101/2025.02.24.639823

[4] Patro et al., Front Microbiol. (2023) doi: 10.3389/fmicb.2023.1270665

[5] Abdul Halim et al., J Bacteriol. (2016) doi: 10.1128/JB.00849-15

[6] De Silva et al., Microbiol. (2021) doi: 10.1099/mic.0.001012

## P-AR-012

### Investigating chromatin conformation in Archaea

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Unlike eukaryotes, most Archaea encode only one or a few small histones. These histones form dimers, and each dimer wraps ~30 bp of DNA, generating "nucleosome-like" particles of variable length (Henneman 2018). Recent cryo-EM data revealed that magnesium strongly modulates these assemblies: the Asgard histone HHoB can form a continuum of architectures, from short to extended hypernucleosomes,

where MgCl<sub>2</sub> concentration drives increased "stacking" and thus longer hypernucleosome formation (Ranawat 2025).

We now aim to systematically probe how other archaeal histones stack into extended fibers. To screen this in a high-throughput manner we want to establish a FRET-based compaction assay. In this assay, DNA labeled with 5'-Cy3 and 5'-Cy5 is incubated with archaeal histones. When histone-induced stacking brings the dyes into close proximity, energy transfer occurs and a FRET signal is detected. This provides an initial readout of both stacking propensity and/or—potentially—binding orientation preferences.

Using HHoB as a benchmark we observe FRET upon addition of MgCl<sub>2</sub>. We will now apply the assay to a panel of archaeal histones to systematically map which proteins have a stacking capacity at what MgCl<sub>2</sub> concentration.

Finally, candidates that show strong FRET-based stacking will be examined using cryo-EM to directly visualize fiber architecture and confirm the presence or absence of stacked nucleosomes.

In summary, we are developing a FRET-first screening pipeline to survey archaeal histone stacking, followed by cryo-EM validation for selected hits.

**References** Henneman B et al., PLOS genetics, 2018  
Ranawat H M et al., Mol Cell 2025

## P-AR-013

### Equipositioning of Chromosomes in the Polyploid Archaeon *Haloferax volcanii* by HpaAB

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Proper genome segregation is essential for all life forms, but in archaea, this process is less understood. Archaea show considerable variability in chromosome and plasmid copy number, and organization. Stable maintenance of chromosomes and low-copy plasmids in organisms requires an active segregation system. *Haloferax volcanii* DS2 is a polyploid archaeon that contains a fragmented genome composed of a main chromosome, three secondary chromosomes, pHV1, pHV3 and pHV4, as well as a plasmid, pHV2. Due to the high copy number of all replicons, genomic segregation in *H. volcanii* is typically considered random. However, we identified several two-gene operons encoding a ParA-like homologue and an uncharacterized protein. Here we characterized one of these systems, termed here hpaA (parA homologue) and hpaB that are encoded on the secondary chromosome pHV3. HpaA, showing conserved ATPase walker motifs, is likely a ParA protein with a bacterial origin acquired via horizontal gene transfer. Mutation of key residues in HpaA alters its localization, and HpaA is necessary for proper spatial distribution of HpaB. HpaB, an archaeal protein capable of forming foci in the cytosol, likely functions as a centromere-binding protein, thereby acting as a functional ParB homologue. qPCR data confirm that HpaB is crucial for maintaining the plasmid-to-chromosome ratio, as deletion of *hpaB* reduces pHV3 copy number. Single-particle tracking suggests that HpaB influences HpaA dynamics. Deletion of the genes *hpaA* and *hpaB* results in severe growth defects when cells are grown on xylose as a



sole carbon source, in line with the location of the genes for the xylose degradation pathway on chromosome pHV3. We propose that *H. volcanii* has an active chromosome segregation system, essential for the two-dimensional segregation of its replicons. This is likely important for replicon maintenance in different cell morphologies observed in *H. volcanii*, where cells transition between rod-shaped or disc-shaped at different growth stages.

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#### P-AR-014

##### Unraveling the mechanism of selenocysteine translation in Archaea

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*Methanococcus maripaludis* is a strictly anaerobic archaeon which conserves energy via hydrogenotrophic methanogenesis. In this metabolic process, proteins containing selenocysteine (selenoproteins) are directly and indirectly involved. Depending on the selenium availability in the cell either the selenoproteins are utilized or a set of cysteine-containing isoforms which replace the selenoproteins. Regulation of the isoforms is governed by the LysR-type transcriptional regulator HrsM. In order to disrupt selenoprotein synthesis and express the Cys isoform in the presence of selenium, HrsM needs to be eliminated.

Biosynthesis of selenocysteine (Sec) proceeds bound to Sec-specific tRNA<sup>Sec</sup>. Sec is co-translationally inserted into the growing polypeptide chain by recoding the stop codon UGA. The recoding event requires the Selenocysteine Insertion Sequence (SECIS) element located in the untranslated region of the mRNA, but the exact mechanism is unknown, particularly a SECIS-binding protein has not been found in Archaea. Identifying this unknown binding factor is the aim of the research presented. For this purpose, an established random mutagenesis approach is being adapted to *M. maripaludis* and the scientific question at hand. The results of our efforts will be presented and discussed.

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## Biology of yeast and fungi

#### P-BYF-002

##### Deciphering the (T)SEN complex localization in lower and higher eukaryotes

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Transfer RNAs (tRNAs) are synthesized by RNAPolIII in the nucleus and modified by multiple enzymes before participating in protein translation. In yeast, end-processed, partially modified, intron-containing tRNAs are exported to the cytoplasm and should reach the tRNA splicing endonuclease ((T)SEN) complex for intron removal.

The (T)SEN complex comprises four essential subunits: Sen2, Sen34, Sen15, and Sen54. The first two subunits are catalytically active (cleaves pre-tRNA in 5' and 3', respectively), forming a composed site, while the last two are structural. Sen15 does not show interactions with tRNA, but Sen54, the largest subunit of the complex, acts like a ruler to help position pre-tRNAs and recruits the tRNA maturation regulator Clp1 RNA kinase. Even though the (T)SEN

complex is conserved through the eukaryotes, its location and tRNA splicing process are highly diverged. It is postulated that 5'-leader and 3'-trailer removal occurs before the end of maturation in vertebrates but after nuclear export in lower eukaryotes. Moreover, in the yeast, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, the (T)SEN complex is peripherally associated with the cytosolic surface of the outer membrane of mitochondria, while in humans and *Xenopus laevis* oocytes, its localization is nuclear. *Ustilago maydis* has emerged as a model fungus for studying RNA biology, due to the high percentage of identity of SEN complex subunits compared to the human ones (Sen2, 29%; Sen34, 28%; Sen15, 50%; Sen54, 29%) and the fact that nearly almost all their pre-tRNAs contain introns, this organism is the perfect subject to understand the process in basidiomycetes, a group closely related to vertebrates. Here, we aim to unravel the localization of the (T)SEN complex in *Ustilago maydis* and the implications of its placement near the mitochondria or nucleus surface.

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#### P-BYF-003

##### An inducible *Ac/Ds* transposon system for *in vivo* mutagenesis of the black fungus *Knufia petricola*

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Microcolonial black fungi, commonly found on sun-exposed natural and man-made surfaces worldwide, belong to different classes within the Ascomycota but convergently evolved similar morpho-physiological adaptations to colonize extreme low-competitive environments. Genetic studies of these organisms have long been hampered by slow growth, lack of sexual cycles and difficulties in transformation. To overcome these limitations, CRISPR/Cas9-based genome editing was implemented in the rock-inhabiting fungus *Knufia petricola* (Eurotiomycetes, Chaetothyriales). This enables efficient generation of deletion mutants and overexpression strains for functional analyses, and thus hypothesis-driven targeted mutagenesis. However, the unique ability of black fungi to colonize oligotrophic extreme environments remains poorly understood, and genes involved are unknown. To address this, a hypothesis-generating tool for functional assessment of new species- and/or trait-specific genes was implemented in *K. petricola*. Specifically, we adapted the two-component *Activator/Dissociation (Ac/Ds)* transposon system from maize for generating insertional mutants by *in vivo* mutagenesis. For controlling the transposition of a *Ds* transposon carrying a resistance cassette, the inducible and metabolism-independent Tet-on promoter system was combined with the *Ac* transposase (*AcTPase*) coding sequence fused to a functional nuclear localization signal. In total, six auxotrophic *Ac/Ds* starter strains were generated, each harboring the *Ds* transposon at different positions within *ade2*, *ura3* or *ppt1*. Induction of TET::AcTPase with doxycycline followed by selection on ADE/URA/LYS-lacking media resulted in prototrophic revertants for most *Ac/Ds* strains. Sequencing of excision sites revealed characteristic footprints. Mapping of *Ds* re-insertion sites demonstrated transpositions both within the same chromosome and across different chromosomes, identifying 30 genes as non-essential. Current efforts include scaling up mutant generation, as the generation and sequencing of saturated mutant libraries combined with mutation mapping will enable the systematic identification of essential genes under diverse culture conditions.

## P-BYF-004

### Dark stipe mutants in fruiting body development of *Coprinopsis cinerea*

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The saprotrophic basidiomycete *Coprinopsis cinerea* is a well-established model organism for studying mushroom development. Fruiting body development is strictly controlled by environmental conditions including light, temperature, nutrients, aeration, and humidity. It follows a specific developmental scheme of seven days (D0 to D7) to completion under standard fruiting conditions of alternating 12 h light and 12 h dark periods at 25 °C, good aeration, and high humidity. The differentiation process begins with the formation of primary hyphal knots (Pks) in the dark, which, when exposed to light, transform into compact secondary hyphal knots (Sks). Further light and dark signals control primordium development (P1 to P5) which takes five days to culminate on day 6 of the process in light-induced karyogamy within the basidia followed by meiosis and basidiospore production. Failure in light signaling or in aeration leads to formation of so-called "dark stipes", under unusual proliferation of stipe tissues and a block in further cap development. Defects in formerly described genes *dst1* (*wc1*) and *dst2* caused P1-induced "dark-stipe" phenotypes by defects in blue-light receptors and a FAD/FMN-binding GlcD-type dehydrogenase. Two other mutants (*dst3*, *dst4*) with defects in dark stipe formation at the later stages P3 and P4 are addressed in this study.

Cultivation under various growth conditions, such as different illumination schemes, ventilating and blocking aeration as well as CO<sub>2</sub> scavenger experiments with KOH, revealed that the defects link to CO<sub>2</sub> metabolism. DNA sequencing of the *dst3* (7K17) and *dst4* (B1918) mutants revealed multiple SNPs in the fungal genomes (64 and 56 SNPs, respectively). Genes with SNP-defects (missense and early stop codon mutations) were identified. Defective genes for enzymes associated with glycolysis and citrate cycle as well as for the Csn5 subunit of the Cop9 signalosome were predicted as likely candidates for the altered phenotypes. Wildtypes of these mutated genes have been cloned for complementation experiments by fungal transformation into the *dst3* and *dst4* mutants.

## P-BYF-005

### Novel, Fast and Cost-effective Real-Time PCR-based Method for the Differential Diagnosis of Dermatophytes in Veterinary Medicine

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#### Introduction:

Diagnosing dermatophyte infections in animals remains challenging due to the limitations of traditional diagnostic tools, which often lack sensitivity and specificity. This study aimed to develop a robust, real-time PCR (qPCR)-based diagnostic assay tailored for veterinary use to detect zoophilic dermatophytes rapidly and accurately.

#### Materials and Methods:

Species-specific primers and probes were designed for key dermatophyte pathogens, including *Microsporum canis* and members of the *Trichophyton* and *Nannizzia* genera. The project followed a modular strategy: sample collection, rapid DNA extraction (developed by Xpedite Diagnostics), primer design, assay development (with Anchor Diagnostics optimizing the multiplex PCR system), and final validation. Initial validations were performed in singleplex qPCR format. Statistical analyses included sensitivity and specificity assessments using standard diagnostic metrics.

#### Results:

The first module—comprising DNA extraction, primer/probe design, and singleplex qPCR validation—was successfully completed. Preliminary data demonstrated high specificity, with no cross-reactivity observed with non-target species, and high sensitivity, capable of detecting the target pathogens at levels as low as approximately 10 copies. Current efforts are directed toward multiplexing these primers to enhance diagnostic throughput and efficiency.

#### Conclusion:

This qPCR-based method offers a rapid, sensitive, and cost-effective diagnostic alternative for veterinary dermatophytosis. Its modular structure allows for further expansion and adaptation, representing a promising advancement in molecular diagnostics for veterinary dermatology.

## P-BYF-006

### Why does yeast smell like yeast?

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Smell, mediated by volatile organic compounds (VOCs), plays key roles across all life domains, from facilitating plant communication<sup>1</sup> to triggering odor-linked memories in humans<sup>2</sup>. The total VOC output of an organism, known as its volatilome, reflects both metabolic activity and environmental interactions. The baker's yeast *Saccharomyces cerevisiae* volatilome is particularly interesting because it shapes the aroma of our daily products such as bread, wine, and beer. Current VOC analyses typically rely on solid phase microextraction (SPME) coupled with gas chromatography (GC), methods that are invasive and limited by fiber-specific affinities. In contrast, secondary electrospray ionization (SESI) combined with high-resolution Orbitrap mass spectrometry (MS) offers a non-invasive, online approach capable of detecting a broader spectrum of VOCs in real time. It can serve as the key technology to discover the yeast volatilome and address the question: "Why does yeast smell like yeast?"

The goal of this study is to optimize SESI Orbitrap MS methods to maximize the number of detectable VOCs from the yeast volatilome while minimizing ionization suppression, therefore enabling more comprehensive yeast VOC profiling.

Volatiles emitted from *S. cerevisiae* are comparatively analyzed using three SESI Orbitrap MS acquisition modes<sup>3</sup>: Full scan with data-dependent acquisition (DDA), globally optimized targeted (GOT) method, and the database-assisted globally optimized targeted (dGOT) method. These approaches are first developed, tested, and refined using

yeast fermentation condensate (Mauvais). Subsequently, the optimized method is applied to *S. cerevisiae* strains, including targeted gene knockout variants affecting specific VOC biosynthetic pathways, to evaluate its suitability for strain-level differentiation.

The results of this study provide both a deeper insight into the scent of one of the most prominent microorganisms used in food production, as well as the analytical method development of microbially emitted VOC studies, consequently defined by the composition of the researched organism's volatilome.

This study aims to optimize *S. cerevisiae* volatilome studies using the SESI Orbitrap MS to gain a deeper understanding of the emitted VOC spectrum of baker's yeast, advancing our understanding of the chemical basis of yeast aroma and its genetic determinants.

## References

<sup>1</sup> DOI: 10.1016/j.cub.2023.04.025

<sup>2</sup> DOI: 10.3758/BF03193837

<sup>3</sup> DOI: 10.1039/d3an01487h

## P-BYF-007

### Heterologous expression of cryptic biosynthetic gene clusters from *A. cruciatus* 763 using *Aspergillus* as biotechnological production platform

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Marine-derived fungi are a prominent source for secondary metabolites including steroids, peptides, terpenes, alkaloids, and polyketides with unique structural features and broad biological activities. These compounds not only expand the overall spectrum of known biomolecules but also offer promising applications in medicine and pharmaceutical industries. Within this group the filamentous fungus *Asteromyces cruciatus* 763 (*Pleiosporaceae*, *Ascomycota*), collected from the coast of La Jolla (San Diego, USA), has been identified as the natural producer of the cyclic non-ribosomal pentapeptide lajollamide A, whose structure was elucidated in 2012 by HPLC-MS and NMR [1]. Subsequent genome mining of *A. cruciatus* 763 revealed in total 29 putative biosynthetic gene clusters (BGCs) covering also the lajollamide A BGC, which was previously characterized [2]. However the majority of the remaining BGCs appear cryptic or silent under laboratory conditions, suggesting that *A. cruciatus* 763 harbours yet underexplored biosynthetic potential. To exploit this hidden metabolic capacity we aimed to establish a heterologous production platform for the expression of cryptic biosynthetic gene clusters from *A. cruciatus* 763 in *Aspergillus*. Therefore six BGCs harbouring different type I PKS core genes from *A. cruciatus* 763 were selected and cloned individually under a strong constitutive *eno* promoter. The constructed vectors were then introduced into the auxotrophic *Aspergillus oryzae* NSAR1 strain via PEG-mediated protoplast transformation. This procedure is expected to yield prototrophic transformants generated by multiple random ectopic integration of the introduced plasmid DNA. Subsequent small-scale chemical screening of several transformants will be required including detailed HPLC-MS and MS/MS analysis to confirm the presence of putative novel compounds produced by the prototrophic strains. In summary, this strategy aims to unlock the cryptic biosynthetic potential of *A. cruciatus* 763, thereby facilitating

the discovery of structurally unique marine-derived natural products and expanding the chemical repertoire for future drug development and biotechnological applications. [1]Gulder, T., H. Hong, J. Correa, E. Egereva, J. Wiese, J. Imhoff and H. Gross (2012). Isolation, Structure Elucidation and Total Synthesis of lajollamide A from the Marine Fungus *Asteromyces cruciatus*. *Marine Drugs* 10: 2912-2935. [2] Maier, H. (2025). Expanding the molecular tool box for the exploration of natural products from non-model ascomycetes. Doctoral thesis. Eberhard Karls Universität Tübingen

## P-BYF-008

### Biological degradation of Ethylenediaminetetra(methylenephosphonsäure) (EDTMP) by three fungi species

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The persistence of phosphonates, primarily due to the presence of multiple carbon-phosphorus (C–P) bonds in their molecules, poses an increasing challenge for wastewater treatment plant operators, as these compounds can interfere with phosphorus removal processes.

This study aimed to evaluate the potential of a complex fungi–substrate system (wood chips or pellets) as a biofiltration material for the removal of ethylenediaminetetra(methylenephosphonic acid) (EDTMP) from water. Four white-rot fungi—*Pleurotus ostreatus* MG 1005, *Trametes versicolor* MG 1700, *Phanerochaete chrysosporium* DSM 1556, and *Pleurotus ostreatus* DSM 1020—were selected due to their documented degradation abilities. The NL-D0.4-G15s (NL.Med) minimal medium was used, as it supported optimal fungal growth among several tested media. Since phosphorus is essential for all organisms, EDTMP served as the sole phosphorus source in the degradation experiments.

Two degradation experiments were conducted: (1) in liquid NL.Med cultures under phosphorus-limited conditions, and (2) in water containing EDTMP and the complex fungi–substrate system (fungal mycelium pre-grown on wood substrate). The second experiment aimed to assess the degradation potential of the fungi–substrate system as a potential biofiltration material.

The results of these experiments are intended to provide a foundation for the use of fungi and wood-based substrates in biofiltration systems for phosphonate removal, representing a significant step toward mitigating the challenges posed by phosphonates in wastewater treatment.

## P-BYF-009

### Unveiling Basidiomycete Biosynthetic Potential for Sustainable Chemical Production

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Our heavy dependence on fossil-derived chemicals as the basis of countless products and processes essential to modern life urges the exploration of more sustainable replacements. The specialized metabolism of fungi holds promise for advancing sustainable bio-based chemical production. Cataloguing the fungal chemical and biosynthetic repertoire is crucial to leverage their full potential. Basidiomycetes, a diverse fungal division encompassing both culinary mushrooms and toxic species, remain largely unexplored in terms of their specialized metabolism. A major challenge for genome-based specialized metabolite discovery from basidiomycete is the lack of both genomic data and bioinformatic tools. Here, we present the genome-guided investigation of basidiomycete specialized metabolism. We developed a bioinformatic tool for facilitated and enhanced gene-calling, ultimately improving prediction of biosynthetic genes encoded in basidiomycete genomes. This tool supported the comprehensive investigation of biosynthetic potential across a wide range of basidiomycetes. We then sequenced the genomes of select species from metabolically gifted genera and employed a semi-automated workflow to extract and analyze their metabolomes. Through integrative omics, this work offers insight into the chemical repertoire of basidiomycetes and guides our heterologous expression-based discovery of previously unknown chemical architectures and enzymatic functions. We identified an NRPS of unusual domain composition, which is conserved across the genomes of a broad range of basidiomycetes and heterologously produced the corresponding metabolite of previously unknown structure. Ultimately, this work will broaden biocatalytic toolkit for microbial chemistry and support the development of sustainable methods for chemical production.

#### P-BYF-010

##### Iron acquisition in rock-inhabiting black fungi: genomic insights and mitigation strategies

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Iron is an essential micronutrient for all organisms, driving intense competition for its acquisition. This competition is particularly evident in parasitic interactions, where hosts actively restrict iron availability to inhibit the growth of fungal invaders (nutritional immunity). Saprobic fungi, on the other hand, acquire iron by digesting dead organic material and may protect their nutrient sources from competitors through efficient iron uptake, rapid growth, and the production of toxic secondary metabolites. Rock-inhabiting black fungi colonize – alone or within microbial communities (subaerial biofilms) – oligotrophic, exposed surfaces such as rocks, stone monuments, and photovoltaic panels, where competition from fast-growing saprobes is minimal. To understand how these fungi acquire iron, we combined comparative genomics with targeted gene deletions in the model species *Knufia petricola*. Genome analysis revealed a reduced repertoire of iron acquisition-related genes compared to those of pathogenic relatives: a single reductive iron assimilation (RIA) complex, one non-ribosomal peptide synthetase (NRPS) for siderophore biosynthesis, and absence of vacuolar iron transporters. Functional assays demonstrated that *K. petricola* primarily relies on RIA, mediated by FTR1–FET1, and secondarily on siderophore-mediated iron acquisition (SIA) via NPS1. Mutants lacking both systems were nearly non-viable under iron limitation, confirming that RIA and SIA are the only relevant uptake routes under laboratory conditions, that simulate the free-

living lifestyle. Cross-feeding and chemotropism assays indicated secretion of an extracellular siderophore enabling growth toward the iron-containing mineral olivine. This siderophore, however, failed to mobilize iron from strong chelators such as BPS and EDTA, and its chemical nature remains unknown. Although melanin adsorbs and reduces iron, results on deletion mutants showed that melanin does not contribute to iron uptake or chelator resistance, functioning instead as a passive iron sink. Sensitivity assays revealed unusually low minimal inhibitory concentrations (MICs) for these chelators in *K. petricola* and other rock-inhabiting fungi, suggesting limited iron acquisition and storage capacity. These findings highlight the vulnerability of rock-inhabiting fungi to iron depletion and suggest that strong iron chelators could serve as an effective strategy to inhibit fungal colonization of human-made surfaces.

#### P-BYF-011

##### Biodiversity on materials: black fungi as emerging colonizers of solar panels

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Sun-exposed surfaces are extreme environments characterized by intense UV radiation, temperature fluctuations, desiccation, and nutrient scarcity. These habitats are colonized by extremotolerant microorganisms that often form multispecies biofilms. Among them, black fungi (Ascomycota) stand out for their unique morphophysiological traits: slow growth, small compact colonies, reproduction by budding or meristematic growth, and continuous deposition of 1,8-dihydroxynaphthalene (DHN) melanin on thickened cell walls. This melanin is thought to provide protection against abiotic stress, for both the fungi and their microbial partners. Originally associated with natural substrates such as rocks in hot, cold, or saline deserts, black fungi have recently been detected on human-made surfaces, including solar panels, where they may reduce system efficiency. Despite their simple morphology, black fungi represent a vast and underestimated genetic diversity with representatives in Eurotiomycetes, Dothideomycetes, and Arthoniomycetes. They exhibit diverse lifestyles, including lichen symbioses, opportunistic pathogenicity, and saprobic growth. However, only a few genomes of black fungi have been sequenced to date. Initial analyses indicate haploid or diploid states, occasional genome duplications, and gene loss, reflecting adaptation to extreme conditions. For even fewer species, genetic engineering tools are available. To address this gap, we develop genetic tools for functional studies and compare genome sequences. The STRES Community Science Program, funded by the Joint Genome Institute (JGI), aims to sequence up to 600 black fungal genomes from diverse extreme environments (<https://stresblackfungi.org/>). Our contribution includes black fungi isolated from solar panels in Germany and the United States – among some several new species. Annotated genomes are now becoming available, enabling comparative analyses to discover novel genes for enzymes and secondary metabolites. These efforts also support strategies to mitigate undesired biofilm formation on man-made surfaces such as facades and solar panels.

### P-BYF-013

#### Cell wall modification of *Fomes fomentarius* to develop biobased filter materials for antibiotic removal from wastewater

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*Fomes fomentarius* efficiently converts lignocellulosic biomass into valuable composite materials that can be applied in packaging and insulation. These fungal-based composites provide a scalable, low-cost and biodegradable alternative to conventional petrol-based products. However, there has been limited progress in the targeted engineering of such materials using synthetic biology.

This study therefore aims to functionalize the cell wall of *F. fomentarius* by introducing tailored surface characteristics that extend its functionality. As a proof of concept,  $\beta$ -lactamase enzymes were fused to a glucan binding domain, expressed in *E. coli* and demonstrated immobilization on the cell wall of *F. fomentarius* on the outer  $\beta$  1,3-glucan layer.

Specific and stable cell wall binding of the fusion proteins was confirmed by confocal fluorescence microscopy, SDS-PAGE, and spectroscopic fluorescence quantification. Furthermore, the hydrolysis of Penicillin G by coated *F. fomentarius* cells was verified by HPLC analysis. This study demonstrates how the surface properties of fungal-based biomaterials can be engineered to introduce new functionalities, expanding their potential use to filter materials or catalytic surfaces for wastewater treatment and beyond.

### P-BYF-014

#### Structure-function analysis of Ubp3, Bre5 and the Ubp3/Bre5 complex

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Ubiquitination is a post-translational protein modification in eukaryotes with broad signaling functions. In this process, the small protein ubiquitin is covalently attached to target proteins through a complex, multi-step enzyme cascade, which can also result in the formation of polyubiquitin chains of various architectures. Ubiquitin-mediated signaling is involved in a multitude of cellular functions and quality control mechanisms, requiring precise regulation. These quality control pathways include different types of selective autophagy such as ribophagy and mitophagy. To maintain cellular homeostasis and counteract ubiquitination, deubiquitinating enzymes can remove or modify ubiquitin signals by cleaving ubiquitin or polyubiquitin chains. In *Saccharomyces cerevisiae*, one such deubiquitinating enzyme is Ubp3, which can utilize Bre5 for specific activity. The Ubp3-Bre5 complex plays a vital role in various cellular processes, particularly in stress response. Interestingly, it exhibits a dual role in cellular functions. For instance, it protects mitochondria from mitophagy while simultaneously promoting other forms of general autophagy. This investigation aims to elucidate how these distinct functions are coordinated and to explore the nature of the interaction between Ubp3 and Bre5. We demonstrate the heterologous expression, purification, and *in vitro* activity of Ubp3, which does not require Bre5. The proteins were expressed in *E. coli* and purified using a series of different chromatography

steps to obtain protein in high purity, suitable for structural studies. *In vitro* activity of Ubp3 against different substrates such as Ub-AMC and Ub-GFP could be observed and does not require Bre5. With this approach, it is possible to elucidate the molecular mechanisms that coordinate the interaction between Ubp3 and Bre5 which will allow a better understanding of their natural behavior *in vivo*. This study should therefore help to decipher the complex regulatory network in which Ubp3 is involved and advance our understanding of deubiquitinases.

### P-BYF-015

#### Plasticity of *Ustilago maydis* metabolism for sustainable biosurfactants production

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The environmental impact of paper waste recycling has become an important topic and raised concerns. Paper mills consume a significant amount of energy through the use of electricity and gas<sup>1</sup>. One contributor to the energy consumption is deinking. This process utilizes detergents to separate ink from the pulp via floatation. However, the used detergents splits the cellulose fibers, thereby diminishing the quantity of recycled paper produced<sup>2</sup>.

To increase overall carbon efficiency of paper recycling, we propose to use these split fibers, which can be found in the so called rejects fraction, as carbon and energy source for microbes producing tailored biosurfactants. The cellulose fibers are hydrolyzed to glucose by enzymes, followed by the consumption of the glucose by microorganisms.

Here, we explore the production of the biosurfactants mannosylerythritol lipids (MELs) by *Ustilago maydis*. MELs are mild surfactants commonly used in the cosmetics industry, with a considerable range of different congeners that can be tailored to the deinking process, helping to preserve cellulose fibers<sup>3</sup>. *U. maydis* cultivations were performed with various carbon sources (glucose, oil, glucose and oil mixtures, and waste hydrolysate), using different nitrogen concentrations to examine MEL production. Thin-layer chromatography (TLC) was used for MEL identification, and HPLC-CAD for quantification.

Notably, *U. maydis* produces MELs on various carbon sources. The process highlighting metabolic plasticity. Waste paper hydrolysate could be shown to be a viable substrate for MELs production.

The results will be discussed in the context of waste valorization in the paper recycling industry. The aim to valorize a side stream to produce a product required in paper recycling, might one day contribute to the envisaged circular bioeconomy.

#### Literature

1. Marilena Hantke, Marie-Charlotte Lück, Katrin Brabender, Alexander von Reibnitz, DIE PAPIERINDUSTRIE - Leistungsbericht PAPIER 2025.
2. Redlinger-Pohn JD, Grabner M, Zauner P and Radl S, *Separation and Purification Technology* **169**:304–313 (2016).

3. Valkenburg AD, Ncube MZ, Teke GM, van Rensburg E and Pott RWM, *Biotechnol Bioeng* 121(3):853–876 (2024).

#### P-BYF-016

##### A comparative genomic analysis of extracellular peptidases in *Aspergillus* fungi

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*Aspergillus* fungi are ubiquitous and inhabit various ecological niches. Some of them are saprophytic, some may facultatively cause plant or animal infections, and extracellular hydrolytic enzymes play a key role in adaptations to these diverse lifestyles. Among those, extracellular peptidases have the widest range of substrates to cleave.

We decided to describe repertoires of extracellular peptidases for *Aspergillus* fungi. This may shed light on the evolution of fungal proteolytic systems, reveal relationships between lifestyle and set of produced peptidases and their inhibitors and uncover dependencies between phylogeny of *Aspergillus* and these enzymes.

98 sequenced and assembled *Aspergillus* genomes were downloaded from MycoCosm and GenBank, some of them were manually annotated using AUGUSTUS. To predict peptidases and their inhibitors, a python-based tool previously created in our group, called Proty and available on GitHub was used. Extracellular enzymes were left after SignalP preprocessing and manual validation of the results.

It was found that there are representatives of 14 peptidase families, namely A01, G01, M14, M19, M20, M28, M35, M36, M43, S01, S08, S10, S28, S53. Among them there are endo-, oligo- and exopeptidases, and the majority of the peptidases encoded in the genome are exopeptidases, which suggests that they probably mainly serve for nutrition. However, some rare and unique examples were also found, and their detailed functions going beyond nutrition are to be discovered. Interestingly, black *Aspergilli* have the highest (up to 10) number of aspergillopepsins (family A01 and G01). Interestingly, the number of encoded inhibitors varied from 0 to 7 for different species. However, their precise mechanism of action and role in physiology needs to be studied in more detail.

Thus, the set of fungal extracellular peptidases reflects fungal lifestyle and its features may be taken into account when studying fungal ecology. Such complex bioinformatic investigations may help in identification of peptidases with high potential for biomedicine and biotechnology when working with genomes of novel species.

#### P-BYF-017

##### Development of a PEG-based transformation protocol for CRISPR-mediated genome editing in *Fomes fomentarius*

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The fungal species *Fomes fomentarius* is emerging as a promising basidiomycete model for sustainable biomaterial development. To enable its genetic accessibility, we established a transformation workflow that is both robust and reproducible. The heterokaryotic strain PaPF11 — established as production strain for composite materials in our group — served as the basis for systematic optimization of cultivation and PEG-mediated transformation conditions.

Refinement of the protoplast isolation procedure led to an 84-fold increase in yield and a strong reduction in mycelial debris, allowing up to  $6 \times 10^7$  viable protoplasts to be used per transformation. Although the regeneration frequency remained low (0.15%), this improvement enabled the recovery of multiple transformants carrying ectopic integrations of a *ku70* deletion cassette, as well as putative transformants for *ura3*. Among 53 colonies subcultured for two months, 49 contained the deletion cassette, yet all retained the unmodified *ku70* locus. Over 70 primary transformants targeted with a *ura3* deletion cassette showed no modifications in the locus as well. Follow-up experiments with split markers resulted in a dramatically reduced yield of putative transformants ( $n=4$ ) and no successful targeting.

Transformation efficiency was not influenced reproducibly by the length of homologous flanks for recombination or the use of CRISPR/Cas. Given that all putative transformants so far have ectopic integrations of the cassette, this observation is likely conclusive.

The resulting protocol provides a stable basis for subsequent targeted and non-targeted genetic studies in *F. fomentarius*. Identifying the parameters most critical for efficiency will also streamline adaptation of this method to the monokaryotic strain Pdm1 and accelerate functional studies, such as promoter analyses and gene-specific manipulations.

#### P-BYF-018

##### Towards improved fungal soil remediation: Assessing drivers of mycelial growth of white-rot fungus *Pleurotus ostreatus*

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*Pleurotus ostreatus* (oyster mushroom), is one of the most common globally produced and consumed edible mushrooms. After mushroom production, the remaining growth substrate, i.e. the spent mushroom substrate (SMS), is considered agricultural waste, although the white-rot fungus remains metabolically highly active enabling it to transform a high variety of recalcitrant contaminants e.g. by its ligninolytic enzymes. In the frame of the EUREKA program-funded PLEUOREM project to upcycle SMS into a mycoadditive that can facilitate bioremediation processes in polluted soil systems, we here studied environmental drivers

influencing growth of *P. ostreatus*. Such knowledge is needed as soil habitats differ from typical natural habitats of lignin-degrading *P. ostreatus*. To do so we here quantified the effects of varying pH (5 – 9), substrate C/N ratios (1/1 – 100/1) and temperatures (5 – 20 °C) on mycelial growth in synthetic laboratory ecosystems using optical microscopy and using OpenCV machine vision. Preliminary data show that *P. ostreatus* is able to efficiently grow at C/N ratios, T, and pH as typically found in topsoil (i.e. C/N ratio of 12/1, average T between 10 – 20 °C, wide range of pH up to 8). However, the best growth was observed at pH 6 and C/N ratios of 10/1 and 100/1. Our results point towards the importance of assessing the effects of combined environmental factors in hyphal growth beyond the known optima, as it may help find the conditions that aid the colonization of "non-ideal" habitats, thus increasing the likelihood of successful remediation attempts.

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## P-BYF-019

### Large Scale cultivation of the fungivorous micropredator *Protostelium aurantium*

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Chemical synthetic plant protection agents are an integral part of modern agriculture. The use of fungicides alone in Germany in the 21st century has exceeded 10,000 tons p.a. With global warming, biotrophic fungi and fungal plant pathogens find increasingly favorable conditions further aggravating the need for synthetic chemical plant protection agents. The ecological risks of these agents have led the European commission to attempt cutting their use and risk by 50% by 2030. One tool to reach that goal are Biological Control Agents (BCA). Several products based on Fungi, Bacteria or nematodes are already utilized. The goal of this project is to monitor the antifungal activity of the leaf-borne fungivorous amoeba *Protostelium aurantium* and to develop a reliable large scale cultivation process for this eukaryotic micropredator.

*P. aurantium* covers a wide range of fungal prey organisms, some of which are known plant pathogens. However, few laboratory and cultivation techniques have been developed, hindering its use as BCA. Here, we show that large scale cultivation of *P. aurantium* can be achieved in a multistep process with the living yeast *Rhodotorula mucilaginosa* as substrate. Suspending the required amount of *R. mucilaginosa* in a buffering solution for the final fermentation volume increased the reproducibility of the *P. aurantium* fermentation, but required an entire separate fermentation of *R. mucilaginosa*. With data acquired from previous fermentations and lower scale trial runs the beginning of *R. mucilaginosa*'s stationary phase was determined and utilized as the optimal inoculation point for *P. aurantium*. This strategy of parallel fermentation led to the final fermentation protocol requiring less inoculation volume, better predictability and finally yielding enough biomass for efficacy testing *in planta* and downstream processing trials.

## P-BYF-020

### Laccase expression in the dung fungus *Coprinopsis cinerea* with 17 natural laccase genes

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Laccases are phenoloxidases capable of oxidizing phenolic and aromatic compounds and are found in bacteria, fungi, insects, and plants. Among fungi, wood- and litter-rotting basidiomycetes are the primary natural producers of laccases. These fungi secrete various forms of the enzyme under different environmental conditions, including isoforms encoded by the same gene, isoenzymes from different laccase genes, or allozymes from different alleles. The aim of this study is to explore and harness the diversity and expression patterns of recombinant laccase genes in *Coprinopsis cinerea* for biotechnological applications, with the target of achieving high yields of properly glycosylated enzymes performed in natural manner in the original biological organism. *C. cinerea* contains in its genome 17 different laccase genes divided into two subfamilies, differentially expressed during growth on distinct media, at varying temperatures, during fungal differentiation, and as a response to microbial competition. Monokaryotic strains may carry inactivated laccase genes, most frequently *lcc15*, while *lcc1* and *lcc5* are the predominant laccases, especially at 25–28 °C under stress conditions. *lcc9* is expressed at trace levels but is fully induced in the presence of competitors. Laccase genes were cloned behind native and other strong promoters, transformed into suitable monokaryotic strains, and cultivated under optimized conditions. Laccase activity in culture supernatants reached up to 30 U/ml, depending on the cloned gene and cultivation conditions. Crossing of transformants further enhanced yields, producing dikaryons expressing either a single laccase or mixtures of laccases when transformants of different genes were mated. The regulated expression of multiple laccase genes in *C. cinerea* can be exploited for industrial enzyme production. Genetic engineering combined with strategic strain crossing enables high yields of functional, glycosylated laccases, highlighting the fungus as a valuable platform for biotechnological applications.

## P-BYF-021

### Sexual and asexual reproductive spores of the basidiomycete mushroom *Coprinopsis cinerea*

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*Coprinopsis cinerea* produces two distinct types of reproductive spores: sexual basidiospores and asexual oidia. Basidiospores develop on specialized basidia located on gills in fruiting body caps and are the result of a complex sexual cycle involving plasmogamy by hyphal fusion and later karyogamy and meiosis in basidia with four resulting haploid meiotic nuclei migrating into individual spores. Thick-walled brown basidiospores can forcibly be discharged into the air, enabling long-distance dispersal and genetic recombination within populations. Others are released through cap autolysis and fall in droplets to the ground. Germination gives rise to sterile primary homokaryotic mycelia (monokaryons). Through fusion with a compatible mating type, a fertile

secondary mycelium (dikaryon) is created that can reproduce sexually. In contrast, thin-walled, uninucleate, haploid oidia, are formed mitotically in sticky liquid droplets by fragmentation of short oidial hyphae at the tips of oidiophores that differentiated on aerial mycelial hyphae. Oidia are produced constitutively in large numbers on monokaryons and light-induced in small numbers on dikaryons. They attach to flies attracted by the substrate dung and spread as clonal propagules by the flies to fresh dung heaps, enabling rapid colony expansion onto new substrate without genetic recombination. Here, we compare growth and developmental properties of monokaryons, mated dikaryons and a co-isogenic self-compatible mutant AmutBmut mimicking a dikaryon. Growth rates of monokaryons were ca 4-6 mm/d in dark and slightly higher in light, those of AmutBmut and dikaryons >7 mm/d in dark and ca 6 mm/d in light. Hyphal diameters varied by type of mycelium. Leading hyphae ranged from 2.2 µm in sterile to 2.9 µm in fertile strains, side branches progressively decreased in size across successive branch generations. Morphologies and sizes of oidia and basidiospores were similar in strains. It contrasts with the dormant, thick-walled, nutrient-rich mitotic chlamydospores formed endogenously in hyphae within substrate to withstand stress of adverse environmental conditions until improvement. In monokaryons, chlamydospores are half as wide and long as in AmutBmut and dikaryons. The results show that the coexistence of sexual and asexual reproductive strategies reflects an evolutionary adaptation to fast fluctuating environments, balancing genetic diversity with rapid colonization of the short-lived scattered habitat of dung.

## Biotechnology

### P-BT-001

#### Discovery and Engineering of a Novel Dienelactone Hydrolase from *Nannochloropsis salina* and its associated microbiome: Antibiofilm Activity and Prospects for Aquaculture Applications

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Antimicrobial resistance and biofilm formation present major challenges in aquaculture and environmental health. Recent meta-omics mining of microalgae-associated bacterial communities has led to the identification and functional characterization of novel dienelactone hydrolases (DlHs). Phylogenetic comparison revealed that this dienelactone hydrolase belongs to the alpha/beta hydrolase family and classified as Type I dienelactone hydrolase which is, classically involved in the breakdown of aromatic compounds and effective on trans-dienelactone substrate. In this study, recombinant DlH displayed robust antibiofilm activity, with significant inhibition rates observed for fish pathogens such as *Aeromonas salmonicida*, *Edwardsiella anguillarum*, and *Pseudomonas aeruginosa*.

Given its promising biofilm disrupting effects, prospective protein engineering approaches could further enhance this DlH catalytic efficiency, substrate range, and stability. Such advances would optimize DlH based biofilm management strategies, offering sustainable solutions for disease control and environmental stewardship in the blue bioeconomy. This presentation will highlight the protein mining, functional assays, phylogenetic analysis, and the outlook for protein engineering, positioning these enzymes as next generation tools for aquatic health and microbial management.

### References:

1. **Bergmann, L. et al.** Marine microalgae and bacteria interactions: unraveling microbial networks in algal cultures. *Sci. Rep.* **13**, Article 50734 (2023). <https://doi.org/10.1038/s41598-023-50734-9>
2. **Astafyeva, Y. et al.** Insights into algal-bacterial consortia and their ecological roles in aquatic environments. *Front. Microbiol.* **13**, 1006609 (2022). <https://doi.org/10.3389/fmicb.2022.1006609>
3. **Krohn, I. et al.** Characterization of novel microbial enzymes with potential for biotechnological applications. *Microb. Biotechnol.* **16**, e14082 (2023). <https://doi.org/10.1111/1751-7915.14082>

### P-BT-002

#### Harnessing the Potential of the Fungi *Poriella subacida* and *Phoma* sp. for Polyester-Polyurethane-Degradation

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The increasing usage of plastic and the resulting accumulation of plastic waste pose a severe danger for the environment and living beings, especially as microplastic. Consequently, the demand for sustainable plastic recycling is greater than ever. We investigated the so far unexplored fungus *Poriella subacida* for the potential to biologically degrade polyester-polyurethanes. Additionally, we isolated the polyester-polyurethane-degrading fungus *Phoma* sp. from mouldy silicone and present novel insights into its degradation processes. Both fungi were cultivated in medium containing either the model compound Impranil® DLN-SD or the polyester and polyurethane fabric Alcantara® which is commonly used in the automotive industry. GC-MS analysis was used to search for potential degradation products in the culture filtrate. FT-IR spectroscopy was conducted with Alcantara® before and after incubation to analyse changes in the molecular structure of the plastic. These studies are complemented by genome sequencing and, in future work, by transcriptome and secretome analysis. During long-term fermentation, it was found that *P. subacida* caused increase in hydroxy and carboxyl groups in Alcantara®. This indicates oxidation of the plastic polymer and leads to the hypothesis that degradative processes occur on the plastic surface. The genomic sequence of *P. subacida* was compared to the database "PlasticDB", which contains genes previously implicated in plastic degradation. A gene encoding a putative promiscuous esterase was identified. This gene has homologs in several bacterial species which were reported to be involved in the degradation of various plastics, for example polyurethane and polyethylene terephthalate. Current studies aim to heterologously overexpress this gene in the model fungus *Magnaporthe oryzae* for further characterisation and potential application in plastic degradation. For cultivation of *Phoma* sp., GC-MS analysis revealed typical products of Impranil® DLN-SD degradation, such as neopentyl glycol. This represents the first report of plastic degradation products in *Phoma* sp. and provides a promising basis for further optimising degradation conditions. In summary, degradative activity of *P. subacida* and *Phoma* sp. on the polyester-polyurethanes Alcantara® and Impranil® DLN-SD, respectively, was observed which sets the basis for enhancing the efficiency of microbial plastic degradation using these fungi.



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### P-BT-003

#### Enhancing the resilience and productivity of cucumber in salty soils: A sustainable approach employing biogenic silicon nanoparticles in the United Arab Emirates

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Salinity is a critical environmental stressor that impairs cucumber growth and yield, particularly in arid and semi-arid regions such as the United Arab Emirates (UAE). It disrupts essential physiological processes, including photosynthesis, nutrient uptake, and metabolic pathways. This study employed selenium nanoparticles (SeNPs) biosynthesized by *Streptomyces* sp. (UAE3) on salt-stressed cucumber plants via foliar application and soil soaking. The effects on oxidative stability, photosynthetic efficiency, physicochemical properties, and yield were assessed. Spherical silicon nanoparticles (SeNPs) with an average size of 50 nm and a negative charge of -24.7 mV exhibited 97% efficacy in free radical scavenging. Biogenic SeNPs (80 mg/L), predominantly through drenching, significantly enhanced chlorophyll content, net photosynthesis, stomatal conductance, and water use efficiency by 47%, 75%, 31%, and 91%, respectively. Proline increased by 22% and gibberellic acid by 39%, whereas abscisic acid and malondialdehyde decreased by 41% and 75.36%, respectively, relative to stressed controls. The activity of antioxidant enzymes returned to near-normal levels, demonstrating reductions of 28-38%. Physicochemical modifications resulted in a 49% increase in weight and a 79.3% boost in overall yield. The carbohydrate and protein content increased by 16% and 37%, respectively. The application of biogenic SeNPs is a sustainable approach to improve cucumber yield under saline conditions, thereby promoting resilient agricultural practices in salt-affected soils in the UAE and abroad. This study is the inaugural research illustrating the capacity of biogenic SeNPs derived from actinobacteria to alleviate salt stress in cucumbers.

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### P-BT-004

#### Concurrent overproduction of essential feed amino acids (CoFeed) by metabolic engineering of *Corynebacterium glutamicum*

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Introduction: Despite environmental drawbacks, global meat consumption continues to rise, with poultry showing the greatest growth. Feed conversion is limited by a mismatch between feed composition and animal dietary requirements, causing environmental impacts such as increased land use for crop production and groundwater pollution from nitrogen-rich manure. To improve both economic and environmental aspects of meat production, limiting essential amino acids are added to animal feed. This is a major application for amino acids, and the demand is rising with global meat production. Most amino acids are produced biotechnologically using metabolically engineered microorganisms, mainly *Corynebacterium glutamicum* and *Escherichia coli*, optimized for production of a single amino acid. The individually produced amino acids are then

blended and added to feed to meet nutritional demands. A notable advantage of using *C. glutamicum* is the GRAS status of its products. Due to lower purity requirements for feed-grade amino acids, the entire fermentation broth, including inactivated *C. glutamicum* biomass, can be used as a feed additive after downstream processing, if the producer strain is classified as non-GMO.

Objective: The aim of this project is to construct a non-GMO *C. glutamicum* strain, based on ATCC 13032, capable of co-overproducing four essential feed amino acids—L-lysine, L-threonine, L-valine, and L-isoleucine—in a defined ratio, consistent with the rank order of limiting amino acids in poultry feed. Metabolic engineering efforts targeted glucose uptake, release of feedback inhibitions, deletion of competing pathways, and gene expression changes.

Materials and Methods: Genomic mutations were introduced in *C. glutamicum* ATCC13032 via homologous recombination. Production and growth were tested in shake flask or BioLector® experiments with CGXII media. Amino acid and glucose concentrations were detected via HPLC.

Results and Conclusion: At present, 3 of 4 amino acid were co-overproduced, with a total yield of 5.7 g/L from 40 g/L glucose in shake flask experiments with the most highly engineered strain. Future efforts will focus on increasing and rebalancing amino acid production to achieve the desired ratio, consistent with the rank order of limiting amino acids in poultry feed. The final strain will serve as the basis for developing a laboratory-scale production process, integrated with a downstream processing strategy suitable for potential scale-up.

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### P-BT-005

#### Mycelium-based composite materials

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Mycelium-based composites (MBCs) have gained attention as eco-friendly substitutes for traditional packaging materials due to the growing need for sustainable, biodegradable materials. Basidiomycetes that proliferate well on resistant lignocellulosic materials are particularly suitable for MBC production. Prescreening of over 70 fungal species identified six different species with excellent growth properties for the development of MBCs. They were evaluated under varying environmental conditions, including temperature, light and dark exposure, different culture media and lignocellulosic substrates, for growth speed and density. Mycelial morphology was assessed in microscopic analyses. *Ganoderma resinaceum* had the most favorable growth performance and structural characteristics, and was chosen for optimizing substrate compositions. The fastest and most uniform mycelial colonization was achieved by combining hemp fibers or wheat straw with spelt husks, adding wheat flour as a nutrient source, and using a 1:1.5 substrate:water ratio. Alternative nutrient sources, addition of nitrogen, vitamins, micronutrients, minerals and inert filling materials are currently being researched as possible growth enhancers and wheat flour substitutes to increase the process's sustainability and cost-effectiveness. A variety of molding

materials and designs were examined, like aluminum BBQ trays (with plain bottom or with folds, with and without aeration holes), different hard-plastic, silicone kitchen and custom-made molds from PLA (polylactic acid). In silicone and PLA molds, MBCs showed consistent mycelial development throughout the composite matrix. Due to the lack of surface adhesion, the finished MBCs could easily and undamaged be removed from these molds. To preserve the structural integrity of the composites and stop fungal regrowth, a variety of drying and deactivation methods (heat press, oven drying, microwave drying) were assessed. Heat pressing was best in production of a smooth and compact surface texture by simultaneously drying, sterilizing, and densifying the material. Physical-mechanical tests (density, tensile and compression strength, water absorption, hydrophobicity, moisture absorption, biodegradability) are ongoing. In order to promote the development of high-performance, sustainable mycelium-based packaging materials, this study offers a fundamental workflow for optimizing fungal species, substrate composition, molding, and post-processing conditions.

#### P-BT-006

##### **Sustainable, Structurally Coloured Materials Derived from Flavobacteria**

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**INTRODUCTION:** Structural colour (SC) results from the reflection of light from organised, nano- to micro-scale, materials. Many organisms have the capacity to create SC. This requires a periodicity of refractive indexes on the scale of to the wavelength of incident light. The result is to create vivid, often iridescent (angle-dependent) colours. SC is a distinct optical mechanism to pigmentation. SC is based on interference of light scattered from the periodic structure, conveniently reveals the capacity of many living systems to create order. Examples of SC in higher organisms include insects, cephalopods, chameleons, terrestrial plants, and macroalgae. SC implies a formidable capacity for life to create highly precise nanostructures. The structures leading to SC may have many uses, including light management and communication, but also roles which appear unconnected with light. SC in eukaryotes is often well characterised in terms of optics and biological function, but knowledge of the underlying genetics is limited. Gains in researching SC are likely to further our knowledge of how life organises and builds complex, coloured materials.

Colonies of some bacteria, notably flavobacteria, can organise to create vivid SC. This allows us to study the genomics and optics of SC in an accessible organism. Further, it is possible to fine tune the colours created by the cultivation method and genetic modification, and, importantly, to preserve the colour after the death of the bacteria. This opens the way to creating pigment-replacing, sustainable colourants from bacteria...!

**GOALS:** We will demonstrate how bacterially-derived SC is made, can be used as a biomaterial and show strategies to scale production.

**METHODS:** A combination of molecular microbiology, manipulation of cultivation conditions and preservation were used to create SC biomaterials from *Cellulophaga lyica* PLA2. Genetic modification was used to create

additional colours, notably blue-shifted. Optical analysis, including goniometry, as well as cryo-electron microscopy and modelling were used to characterise the resulting materials.

**RESULTS:** Novel, stable, vivid optical materials (SC flakes, films, particles) were created with SC across the visible spectrum. Samples will be brought to the meeting.

**REFERENCE:** Zomer, Aldert, et al. "Structural color in the bacterial domain: The ecogenomics of a 2-dimensional optical phenotype." *Proceedings of the National Academy of Sciences* 121.29 (2024): e2309757121.

#### P-BT-007

##### **Engineering of *Pseudomonas putida* to access polyamide hydrolysates as a feedstock for microbial biotechnology**

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Aliphatic polyamides (PA) are extensively utilised in the textile and automotive industries due to their high durability and tensile strength. However, the current recycling rate of PA is below 5%. Bio-upcycling of plastics is an emerging alternative approach for valorising diverse polymer waste streams, which are low in purity and challenge conventional recycling technologies. *Pseudomonas* sp. possess a highly adaptable metabolism, which allows for the engineering of entry points for artificial key components of numerous plastics, such as styrene, aliphatic diols, and aliphatic, branched, and aromatic dicarboxylic acids. In this study, we aimed to establish the metabolism of  $\omega$ -amino acids, diamines, and their oligomers in *Pseudomonas putida*.

Building on this versatile metabolism, adaptive laboratory evolution (ALE) emerged as a powerful tool to realize plastic monomer metabolism and oligomer import. Transcriptomics and whole-genome sequencing of the ALE-derived strains facilitated targeted reverse engineering of *P. putida* for the metabolism of polyamide compounds. Gaps in native metabolic pathways were addressed by integrating synthetic expression modules for nylon oligomer hydrolases.

Guided by ALE, we engineered *P. putida* to metabolise 6-aminoheptanoic acid,  $\epsilon$ -caprolactam, and 1,6-hexamethylenediamine via a previously introduced heterologous  $\beta$ -oxidation pathway for dicarboxylic acids<sup>1</sup>. To enable the utilization of mixtures of monomers and oligomers, these genetic adaptations for strengthened polyamine metabolism and improved uptake were combined with oligomer hydrolases. Here, we expanded the so far limited toolkit of polyamide oligomer hydrolases by accessing new enzymes with different specificity for implementation in *Pseudomonas*. We could demonstrate the conversion of acidic hydrolysate of Polyamide6 as the sole carbon and nitrogen source into natural products, like polyhydroxybutyrate as a bio-based polyester material, via the central metabolism of the engineered *P. putida*<sup>2</sup>.

In summary, the engineered strains access different polyamides for biological upcycling, including Polyamide6,

Polyamide66, and polyesteramides. These developments have the potential to establish these materials as feedstock for biological catalysts and may, hence, contribute to novel recycling approaches for mixed plastic waste streams to strengthen the development of a circular plastic economy.

<sup>1</sup>10.1186/s12934-024-02310-7

<sup>2</sup>10.1038/s41564-025-01929-5

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#### P-BT-008

##### Coordinated enzyme programs enable novel aromatic production in *Phlebia radiata* during lignin valorization

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White-rot fungi are nature's master chemists of lignocellulose breakdown, yet the interplay between gene expression, enzyme secretion, and bioactive metabolite formation remains poorly understood. We used a time-resolved multi-omics approach to dissect how the fungus *Phlebia radiata* (IBWF-87241) orchestrates lignin valorization.

Cultures of IBWF-87241 were grown in flasks containing half-soy medium, with and without sterile wood powder supplementation. Enzymatic oxidation was monitored by laccase activity assays using ABTS substrates. RNA was sampled at 1–6 hours after wood addition and sequenced to profile transcriptomic changes. Secreted proteins were analyzed by LC-MS/MS, and lignocellulose structural changes characterized by ATR-FTIR. Mandelic and phenyllactic acid levels were quantified using GC-MS against standards. Wood supplementation triggers a laccase-dominated oxidative surge, potentiated by redox mediators, driving accelerated lignin remodeling as shown by ATR-FTIR spectral loss of aromatic and phenolic C–O bonds. Integrative RNA-seq data revealed upregulation of oxidoreductase and transporter genes, while secretome profiling uncovered a cascade of multicopper oxidases, cellulases, and hemicellulases. Strikingly, mandelic acid and 3-phenyllactic acid, two high-value  $\alpha$ -hydroxy aromatics, are found exclusively in cultures supplemented with wood, highlighting the metabolic induction. Gene ontology enrichments suggest coordinated regulation of phenylalanine catabolism and redox cycling, supporting a hypothesis where lignin-derived aromatic intermediates and amino acid metabolism come together. This multi-omics framework links enzyme programs, transcriptional regulation, surface chemistry, and product profiles, while identifying key levers, like medium composition, mediator presence, and time, for tuning yield and selectivity. This study gives critical mechanistic insight into fungal lignin valorization, revealing how transcriptional programs and extracellular enzyme cascades orchestrate production of  $\alpha$ -hydroxy aromatics. The selective biosynthesis restricted to wood-plus-soy cultures underscores the complexity of signaling in lignocellulose conversion. These insights advance *P. radiata* as a versatile fungal chassis for sustainable biomanufacturing and circular bioeconomy, with implications for integrating fungal bioprocesses with electrified upgrading and green chemistry.

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#### P-BT-009

#### Hydroxylation of Alcohols by P450 Monooxygenases

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One of the primary motivations for application-driven research is the urgent need to lower greenhouse gas emissions and develop sustainable alternatives for chemicals derived from fossil fuels. Within this framework, the enzymatic upcycling of simple compounds from renewable resources to produce high-quality compounds is a desired goal. As an example, diols can be produced as important precursors for multiple applications, such as polymers and cosmetics. We aim to optimize cytochrome P450 monooxygenases (CYPs) as biocatalysts for the selective hydroxylation of alcohols. These enzymes catalyze the incorporation of an oxygen atom into chemically inert C-H bonds supported by an electron transfer partner. By developing fusion constructs between CYPs and their redox partners, improved catalytic efficiency, stability, and selectivity may be achieved. The focus is on understanding and optimizing the influence of the fusion constructs' linkers and the modification of the enzyme itself by modifying the substrate binding site. A multidisciplinary approach, combining computational modelling and experimental protein engineering, is employed. Structural predictions using AlphaFold guide the rational design and modification of CYP-fusion constructs, which are supported by detailed active-site and interface analyses using Caver and YASARA. The resulting variants are expressed and analyzed using *in vivo* whole-cell biocatalysis in 96-well plate formats, which enables efficient screening. Non-native alcohol substrates obtained from industrial partners are used to evaluate the activity and selectivity of the enzymes under relevant conditions. Preliminary results underscore the crucial role of the linker type in determining the catalytic efficiency of CYP fusion constructs<sup>1</sup>. Initial experimental data suggest that certain fusion variants exhibit increased hydroxylation rates and improved coupling efficiency. These findings provide valuable insights into the relationships between the structure and function of P450 enzymes and underscore the potential of rational fusion design for optimizing biocatalytic processes. Ultimately, this project contributes to the development of sustainable biotransformations that replace energy-intensive chemical syntheses with gentle, selective, and environmentally friendly enzymatic processes. <sup>1</sup>Schultes FPJ, Welter L, Schmidtke M, Tischler D, Mügge C. A tailored cytochrome P450 monooxygenase from *Gordonia rubripertincta* CWB2 for selective aliphatic monooxygenation. Biol Chem. 2024 Sep 30.

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#### P-BT-010

##### Artificially engineered P450 monooxygenase fusion constructs with esterification module in oxyfunctionalization reactions

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Cytochrome P450s (CYPs) are heme-containing enzymes, present in a wide range of organisms, with manifold functions such as hydrocarbon degradation, fatty acid metabolism, hormone biosynthesis and detoxification. The CYP153A family has a large substrate scope with particularly high catalytic efficiency converting long carbon chain groups into alcohols.<sup>1</sup> Challenges associated with purified CYPs due to reliance on electron transfer partners (e.g., Ferredoxin Reductase and Ferredoxin), nicotinamide cofactors (NAD(P)H) and lowered activity outside of their native environment make it difficult for these enzymes to be scaled up for industrial use.

This work addresses the limitations of cytochrome P450 monooxygenases and proposes whole-cell biotransformation as a viable solution for scaling up their use, making CYPs a cost-effective and environmentally friendly option for the production and extraction of valuable chemicals. To simplify extraction and downstream processing, an esterification module was integrated into the host cell. The experiments involved construction of fusion constructs of various class I bacterial cytochrome P450 enzymes (CYPs) artificially linked to different electron transfer partners using distinct linkers. Whole-cell biotransformation has the potential to address both the stability and cofactor dependency of P450 monooxygenase enzymes, while the fusion construct enhances the efficiency of the electron transport chain.

Reaction optimization experiments were conducted to increase the yield of the desired product, reducing side product formation as well as controlling for toxicity of chemicals. Resting cell assays were performed in phosphate buffer with various carbon sources to maintain cell viability without promoting growth. The focus of this research was on compounds where current literature reports low yields with existing biocatalytic methods. Specifically, improving the efficiency of hydroxylation reactions to produce industrially important chemicals has been challenging due to stability issues and the limited catalytic efficiency of known P450s.

1. Mirhadiyev M, Mügge C. (2025). Cytochrome P450 monooxygenases in whole-cell format: Application notes from a biotechnological perspective. *Methods Enzymol.* 714(97-113). doi: 10.1016/bs.mie.2025.01.014

## P-BT-011

### Plastic monomer degradation with engineered *Pseudomonas putida* KT2440

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## Introduction

The traditional chemical industry is a linear economy, relying on fossil-based feedstocks such as petroleum, converted through unsustainable processes into numerous products. To date, no established sustainable recycling processes for the rising amounts of end-of-life plastic waste exists. Consequently, this waste is commonly thermally recycled, landfilled or released into natural ecosystems, aggravating environmental challenges.

## Goals

The project Catalaix aims to convert this linear economy into a multidimensional circular economy by developing novel chemical and biological recycling technologies, with catalysis as key technology. The objective is to close and interlink existing material flows via an open-loop principle while incorporating renewable carbon and energy sources, enabling the recovery of high-quality chemical building blocks from diverse plastic wastes. Thereby, the iAMB focuses on the expansion of the "bow tie" of microbial metabolism in *Pseudomonas putida* to biocatalytically convert toxic monomers derived from hydrogenolyzed plastic polymers. The resulting biomass will be valorized into high-value products.

## Methods

Metabolic engineering of *P. putida* to enable degradation of amine monomers and metabolic flux optimization. Continuous fermentation simulates a realistic waste stream. Isolation and characterization of environmental microorganisms utilizing plastic-derived monomers.

## Results and Conclusion

A *P. putida* strain capable of degrading PET and PU monomers simultaneously within 24 hours served as a starting point. A continuous fermentation with different plastic monomer mixtures whereby a real waste stream was simulated reached steady state, revealing mutations that are requiring further investigations. By metabolic engineering, the substrate spectrum of the strain was expanded and enabled the degradation of a monomer precursor of the target monomer. In the following, the integration of additional genes will enable the degradation of one of the desired monomers. Furthermore, the isolation and cultivation of microorganisms from diverse environmental samples led to the identification of several strains capable of utilizing different in part toxic, plastic-derived monomers for growth.

In conclusion, this study establishes a foundation for the development of *P. putida* as a microbial platform capable of degrading toxic amine monomers and contributes to a multidimensional circular economy.

## P-BT-012

### Microbial Cross-feeding on Brown Juice for Short-chain Carboxylic Acid Production

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To meet the growing demand for protein from sustainable sources, green biorefineries are used to extract protein from green biomass such as grass, clover, and alfalfa. The process by-product, brown juice, contains water-soluble carbohydrates, minerals, and residual protein. With the overall goal to establish the suitability of brown juice as substrate for microbial fermentation systems, this study investigates the production of short-chain carboxylic acids (SCCAs) by synthetic consortia. SCCAs confer antimicrobial activity and prevent biofilm formation in food and other biotechnological systems (Ng, 2024). For the production of SCCAs, we designed microbial cross-feeding systems composed of lactate producers and utilizers that form SCCAs. We isolated lactate producers indigenous to the brown juice and quantified monosaccharides and SCCAs via UHPLC-RI and UHPLC-DAD. The brown juices had an initial

pH around pH 5.5 and contained glucose, fructose and arabinose in various amounts dependent on the batch. Spontaneous fermentation of brown juice led to lactate accumulate and the enrichment of *Bacillus licheniformis*, *Niallia circulans*, *Heyndrickxia coagulans*, *Cytobacillus kochii*, which are spore-forming bacilli that persisted heat-treatment and freezing during the biorefinery process and storage of the brown juice. Added starter strains of *Liquorilactobacillus*, *Latilactobacillus*, and *Lactococcus* utilized the present sugars and produced mainly lactate. As a lactate utilizer, we will use *Latilactobacillus fuchuensis*, which produced up to 146 mM lactate in buffered brown juice. *Megasphaera elsdenii* and *Caproicibacter lactatifermentans* were tested for formation of SCCAs with a carbon backbone  $n > 4$ , while *Levilactobacillus lettrarii* was tested for propionate production. The process will be scaled up in a 200 mL bioreactor and fermentates will be tested for microbial safety and optimised product yield.

This study contributes to the development of sustainable bioprocesses for agricultural side-stream valorisation, aligning with circular bioeconomy principles. The results indicate that brown juice is a promising substrate for SCCA production using bacteria cross-feeding.

Ng, K. S. (2024). Environmental pH and compound structure affect the activity of short-chain carboxylic acids against planktonic growth, biofilm formation, and eradication of the food pathogen *Salmonella enterica*. *Microbiology Spectrum*, 12(11), e01658-24.

#### P-BT-013

##### Methods for Establishing *Fomes fomentarius* as a Basidiomycete Model Organism

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The basidiomycete *Fomes fomentarius* has recently gained much attention for its abilities to form strong and durable composite materials. While a composite production pipeline has been established, methods that ensure reproducible results at the wet-lab scale regarding its cultivation, characterisation, and biomass quantification were still missing. To establish *F. fomentarius* not only as a cell factory for fungal biomaterials but also as a basidiomycete model organism, we have developed standard operating procedures (SOPs) for i) small- to medium-scale liquid cultivations with minimised culture heterogeneity, ii) reliable biomass quantification from a range of different liquid media, and iii) an ergosterol assay for biomass quantification from fungal composite materials. To optimise liquid cultivation and establish protocols that minimise pelleting and morphological heterogeneity as well as to prevent wall growth, a range of standard laboratory media and lignocellulosic growth media were tested and process parameters were optimised. Biomass determination using cell dry weight is limited to liquid cultures in which the medium does not contain solid particles that become degraded and metabolised over time as this would render any mass-based quantification impossible. Further, cell dry weight determination is affected by a delay in data availability due to the time spent drying the sampled fungal biomass. Instead, we have established a calcofluor white-based staining method as a faster, less laborious alternative. This method can be used in chitin- and cellulose-free growth medium. Moreover, we have

established a qPCR-based method useful for lignocellulosic media containing insoluble particles. Lastly, we will present our protocol for determining the ergosterol content of the fungal cell wall for the purpose of measuring fungal biomass in composite materials. Our protocol can be performed without physical separation of fungal hyphae from the substrate particles that make up the composite, which is not feasible anyway. Yet, analysing the distribution of fungal biomass inside larger volumes of material is essential for quality control and crucial for preventing nutrient and oxygen gradients inside the material. Further, our medium throughput method only requires small sample amounts. Taken together, our SOPs provide essential methods for handling *F. fomentarius* in the wet-laboratory and will, thus, contribute to its establishment as a basidiomycete model.

#### P-BT-014

##### Purification and characterization of cyanophycin synthetase and cyanophycinase from *Clostridium ljungdahlii*

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Cyanophycin is a biopolymer consisting of a poly aspartate chain with arginine bound to the side groups of the aspartate chain. The biosynthesis of cyanophycin requires only a single enzyme: cyanophycin synthetase (encoded by *cphA*). It was first discovered in cyanobacteria, and later it was found that many other bacteria, including *Clostridia*, possess the genetic capability to produce cyanophycin. The degradation of the polymer happens via two enzymes: cyanophycinase (encoded by *cphB*), which cleaves cyanophycin into dipeptides, and promiscuous isoaspartyl dipeptidases, which cleave dipeptides into single amino acids. Due to its favorable properties compared to classic polymers (not toxic, biodegradable), the potential for widespread applications of cyanophycin are currently explored.

The acetogenic bacterium *Clostridium ljungdahlii* is an often-used model microbe for syngas fermentation and of interest to the industry for recycling waste gas to valuable compounds, e.g. biocompatible polymers like cyanophycin. So far, not much is known about the physiological role of cyanophycin in *Clostridia*. Only one transcriptomic study exists that hints toward a role in spore formation and germination [1]. Also, currently there is a lack of biochemical characterization of CphA and CphB outside of cyanobacteria. Preliminary *in vivo* data suggest that CphA from *C. ljungdahlii* might have different properties than cyanobacterial variants. Biochemical investigations of CphA and CphB from *C. ljungdahlii* will be presented and compared to the properties of previously characterized CphA and CphB from cyanobacteria.

[1] Liu H, Ray WK, Helm RF, Popham DL, Melville SB. 2016. Analysis of the spore membrane proteome in *Clostridium perfringens* implicates cyanophycin in spore assembly. *J Bacteriol* 198:1773–1782. doi:10.1128/JB.00212-16.

## P-BT-015

### Using functional metaproteomics for enzyme discovery: identifying gut microbiota enzymes linked to neuroimmune diseases and blood group antigen conversion

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**Introduction:** Microbial communities represent an immense source of enzymatic diversity with high potential for medical and biotechnological applications. Functional metaproteomics enables cultivation-independent enzyme discovery by combining gel-based activity assays with mass spectrometric protein identification using metagenomic data as reference. Building on this concept, we developed an in-gel zymography workflow focusing on two applications:

Investigating gut microbiota-derived enzymes that release propionic acid (PA) in the context of neuroimmune diseases such as multiple sclerosis (MS) and chronic inflammatory demyelinating polyneuropathy (CIDP).

Identifying glycosidases capable of converting A and B blood group antigens into H antigens, including those acting on fucosylated structures relevant to the Bombay phenotype.

#### Goals:

1. Evaluate whether compositional or activity-based differences in PA-releasing enzymes can be detected between patient and control samples.
2. Establish an in-gel screening workflow to identify microbial glycosidases that target A, B, or fucosylated blood group determinants.

**Materials & Methods:** Protein extracts from stool samples of healthy controls and patients (before and after 30 days of PA or placebo treatment) were analyzed using a zymography-based approach. PA ester-hydrolyzing activities were visualized using 4-methylumbelliferyl (4-MU) propionate. In parallel, initial screenings for blood group antigen-cleaving activities were conducted using 4-MU-based glycosidic substrates.

**Results:** PA-releasing enzyme activities were detected in all sample groups, showing comparable overall activity and enzyme diversity independent of disease status or treatment. Glycosidase activity screenings revealed distinct fluorescence signals and substrate-specific band patterns on 1D zymograms of stool protein extracts from donors of different blood groups. Future experiments aim to extend the analysis to 2D gels for subsequent mass spectrometric identification.

**Summary:** Our in-gel zymography assay enables detection of both PA ester-hydrolyzing enzymes relevant to neuroimmune diseases and glycosidases capable of cleaving terminal sugar moieties in blood group antigens from gut microbiota protein extracts. These findings establish a foundation for targeted enzyme discovery through

functional metaproteomics, linking microbial enzyme activity with medical and biotechnological applications.

## P-BT-016

### Cascade utilization of algal biomass for the production of valuable products

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**Introduction:** In recent years, extremophilic red microalgae have gained significant attention as a sustainable feedstock for industrial biotechnology. *Galdieria javensis* and *Cyanidioschyzon merolae* are of particular interest due to their ability to grow in harsh environments and to switch between autotrophic, mixotrophic, and heterotrophic modes. Furthermore, microalgae produce valuable metabolites such as phycocyanin, chlorophyll, and carotenoids. Following pigment extraction, the residual biomass, which is typically rich in carbohydrates and lipids, can be used as a substrate for microbial fermentation, thereby supporting a circular bioeconomy.

**Goals:** This study compared the red microalgae *Galdieria javensis* and *Cyanidioschyzon merolae* in terms of biomass production, pigment and fatty acid accumulation, and elemental composition in different photobioreactors. Additionally, the residual algal biomass after pigment extraction was evaluated as 3rd generation feedstock for the production of value-added compounds using heterotrophic platform organisms.

**Results:** Small-scale cultivations revealed faster growth of *Cyanidioschyzon merolae*, but slightly higher final biomass concentrations for *Galdieria javensis*. Due to its lack of a rigid cell wall, *Cyanidioschyzon merolae* was selected for further analysis. The highest biomass ( $11.66 \pm 0.37$  g/L) and phycocyanin ( $0.76 \pm 0.004$  g/L) concentrations were achieved using internally illuminated photobioreactors. Complete *Cyanidioschyzon merolae* biomass supported both growth and itaconic acid production in *Ustilago maydis*, whereas the pigment-extracted fraction could not be metabolized. In search of enzymes enabling efficient algal valorization, several *Bacillus* strains were evaluated and identified as additional promising candidates for future biomass valorization.

**Conclusions:** This work outlines optimized cultivation and extraction strategies for *Cyanidioschyzon merolae*, demonstrating its potential for utilizing and converting CO<sub>2</sub> into valuable compounds essential for the pharmaceutical and polymer industries.

## P-BT-017

### Introducing an optogenetic switch in *Pseudomonas taiwanensis* VLB120

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## 1. Introduction

The possibility to control cellular behavior by light-dependent regulation via an optogenetic switch opens a whole new toolbox for the regulation and induction of microbial systems. Different optogenetic proteins have been designed and applied already for *Escherichia coli* [1]. Here however, we want to transfer the use of optogenetic systems to the biocatalytic workhorse *Pseudomonas taiwanensis* VLB120 [2]. As the optogenetic element, we will use the blue light-dependent DNA-binding protein EL222 from *Erythrobacter litoralis* [3].

## 2. Goals

This study investigates the application spectrum of the optogenetic element EL222 for *P. taiwanensis* VLB120. The aim is to determine the overall functionality of the optogenetic system and to further test the sensitivity and performance of EL222 inside the biocatalytic workhorse.

## 3. Material & Method

To test the functionality of the EL222-based optogenetic system in *P. taiwanensis* VLB120 we will delete a key enzyme involved in the biosynthesis of the essential amino acid tryptophan and complement it with a plasmid encoding the enzyme under the control of EL222. With this we want to achieve light-dependent growth of *P. taiwanensis* VLB120.

To further characterize the performance of the optogenetic system in *P. taiwanensis* VLB120, we will place the red fluorescent protein (RFP) gene under the control of the light-inducible promoter and monitor RFP expression under varying illumination intensities and light-pulse frequencies.

## 4. Results

First results point towards the general functionality of the optogenetic system in *P. taiwanensis* VLB120. Also monitoring the RFP expression showed a correlation between fluorescence levels and light exposure, indicating a high sensitivity and a reliable regulation of RFP expression by EL222.

## 5. Summary

This optogenetic system offers a promising approach for spatial and temporal control of growth and activity in *P. taiwanensis* VLB120. With this light-dependent regulation we encounter new possibilities of reliable and tunable inducers for various microbial applications.

[1] Baumschläger et al., Adv Biol (Weinh). **2021**, 5, 2000256

[2] Blombach et al., Trends Biotechnol. **2022**, 40, 385

[3] Jayaraman et al., Nucleic Acids Res. **2016**, 44, 6994

The biotechnological production of high-value proteins such as enzymes and biopharmaceuticals is gaining increasing importance across various industrial sectors. To enable production of the requested heterologous proteins, there is a strong demand for suitable production chassis. One promising candidate is the fungal model *Ustilago maydis*. We recently uncovered a cytokinesis-dependent unconventional secretion pathway which we harness for production of heterologous proteins. This pathway utilizes a lock-type secretion mechanism, in which the chitinase Cts1 is released from the so-called fragmentation zone, a small compartment that is formed between mother and daughter cell during cell division. Using Cts1 as carrier protein the pathway can be exploited to produce heterologous high value proteins like biopharmaceuticals. Advantages include the bypassing of posttranslational modifications and the putative secretion of relatively large proteins. However, a complete understanding of the mechanistic details of the pathway is lacking and the secretory capacity is not competitive.

Our recent findings suggest that one central limitation of the system is defined by the expression strength of the heterologous genes. Cts1 fusions with fluorescent reporters showed that, contrary to expectations, high expression levels can lead to reduced secretion into the supernatant due to impaired transition to the fragmentation zone. Interestingly, when testing a range of expression strengths, a promising intermediate-range promoter was identified, yielding improved localization and comparatively high accumulation of functional secreted protein. In essence, the unconventional secretion pathway is likely overwhelmed in response to high gene expression levels. In the future, we will build on these insights and use a genetic screen to identify cellular factors required for enhanced transition of target proteins to the fragmentation zone and hence, secretion. In addition, the size limits for unconventional secretion will be addressed by multimerization approaches.

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### P-BT-019

#### Retiring *E. coli*: Towards Stable Plasmid Maintenance in *Vibrio natriegens*

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Plasmid DNA has a broad spectrum of applications, ranging from daily laboratory workflows to mRNA manufacturing and gene therapy. However, pDNA production faces several challenges such as instability of repeat sequences and residual endotoxins. While *Escherichia coli* strains have been tailored to the needs of individual downstream applications, these specifications can compromise plasmid yield, highlighting the importance of further optimization.

*Vibrio natriegens* is a Gram-negative, non-pathogenic marine bacterium with a doubling time nearly twice as fast as *E. coli*. In addition to its rapid growth, it produces fewer endotoxins, showing potential as a novel production host. Applying *V. natriegens* in pDNA production could increase plasmid yield while cutting production costs.

Although it is known that commonly used plasmids replicate in *V. natriegens*, little is known about plasmid maintenance or copy number - a research gap that this work is aiming to fill. First experiments focus especially on maintenance of plasmids without selective pressure over several days.

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### P-BT-018

#### Less is sometimes more - Finetuning unconventional secretion in *Ustilago maydis* at the transcriptional level

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Using an endonuclease-deficient *V. natriegens* strain harbouring ColE1-based plasmids, we analysed segregational stability in a culture without selective pressure. Plasmids containing a pUC19 origin showed rapid loss, whereas those with a pET origin remained stable for several days. Plasmid loss correlated with the plasmid size, with smaller constructs being more stable. As a complementary approach, we are quantifying the copy numbers of ColE1-based plasmids using digital PCR.

We set up and tested a lab evolution approach to develop strains with increased plasmid stability and to gain deeper insight into plasmid maintenance.

This work lays the foundation for employing *V. natriegens* as fast-growing future host for pDNA production.

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#### P-BT-020

##### Optimization of microbial transglutaminase from *Streptomyces mobaraense* for biotechnological applications

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Microbial transglutaminase (MTG, EC: 2.3.2.13) from *Streptomyces mobaraense* catalyzes the formation of isopeptide bonds between proteinaceous glutamine and lysine residues. In addition, MTG catalyzes the conjugation of various amines to glutamine residues. These characteristics make it a valuable tool for usage in different industrial applications from medicine to food production. However, several challenges have to be overcome, for MTG to unleash its full potential as a biotechnological tool. These challenges include the simplification of the production process. Although investigated for decades, the current procedure includes a step in which the inactive zymogen is catalytically activated resulting in the mature enzyme. In order to address this issue, we have introduced several destabilizing amino acid exchanges into the MTG pro-peptide by systematic screening of the pro-peptide sequence using *in silico* site-saturation mutagenesis and comparison of Rosetta  $\Delta\Delta G$  predictions. This strategy should allow a temperature-dependent activation as alternative to enzymatic cleavage. Another challenge for applications is the low thermostability of the wild-type MTG. By combination of the identified destabilizing pro-peptide mutations with the already highly thermostable MTG-variant TG2A, we obtained new MTG-variants that can be activated on-demand simply by temperature change. In addition, the half-life at a typical application temperature of 60 °C is improved by several orders of magnitude. This allowed us to create Pro-MTG variants, that can be purified in a streamlined two-step process and are active towards both small peptidic as well as large proteinogenic substrates.

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#### P-BT-021

##### Biofilm formation of *Methanosarcina acetivorans*

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In light of the climate crisis and an accelerating transition towards a circular economy, there is a growing need to explore the potential of microorganisms to produce valuable

chemicals from conventional waste streams. One such model organism is *Methanosarcina acetivorans*.

*M. acetivorans* is a well-studied, strictly anaerobic methane-producing archaeon. It is capable of utilising methanol, CO<sub>2</sub> and CO, amongst other substrates, for its growth.

Methanogens already play an important role in the circularization of the economy, as they are crucial for the production of biogas. However, a recent study has shown that *M. acetivorans* can be genetically engineered to produce complex terpenes from CO. Terpenes represent a highly diverse class of chemicals, that are typically seen as plant secondary metabolites. Numerous terpenes are of industrial and pharmaceutical relevance.

A challenge to fully unlock the biotechnological potential of *M. acetivorans* is that large-scale liquid culture of strict anaerobes have proven difficult in the past. An alternative method for the large-scale fermentation of microorganisms is a biofilm reactor. However, to date research into the biofilm formation of *M. acetivorans* is limited.

We initiated research into *M. acetivorans* biofilm formation via an initial screening. To this end, a diverse array of materials was examined for their capacity to support *M. acetivorans* biofilm formation. The potential biofilms are analysed via scanning electron microscopy (SEM) and growth is monitored via quantification of biofilm biomass after extraction from the substratum. The results and implications of our initial screening will be discussed.

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#### P-BT-022

##### Modular cobalamin-dependent methylation systems enabling sustainable methylations

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The chemical industry must adopt more sustainable and efficient processes to meet the increasing demands of a growing global population while protecting the environment. Methylation reactions play a crucial role in both the bioenergy and pharmaceutical sectors by modifying the physical and biological properties of compounds. Methyltransferases (MTases) offer a biological and environmentally friendly alternative, enabling selective methylations under mild conditions. In particular, cobalamin (B<sub>12</sub>)-dependent MTase systems present several advantages over the well-known S-adenosylmethionine-dependent enzymes, including greater stability of the methyl group carrier and the potential for ATP-independent cofactor regeneration. However, research on B<sub>12</sub>-dependent MTase systems for biotechnological applications remains limited.

Our previous studies on B<sub>12</sub>-dependent MTases suggest that the enzymes MTase I, corrinoid protein (CoP), and MTase II can function in a modular manner [1,2], raising the question of whether these modules are universally exchangeable. The main goal of this work is to produce B<sub>12</sub>-dependent MTase systems from different microorganisms and demonstrate that they can transfer a methyl group from inexpensive small organic compounds to various industrially relevant substrates. To this end, we heterologously expressed the three components of the B<sub>12</sub>-dependent methylation system (MTase I, CoP, and MTase II) in different *Escherichia coli* strains. The proteins were purified by affinity



chromatography, their interactions were characterized, and their activity was assessed based on the transfer of a methyl group from a sustainable donor to a target acceptor molecule. Due to the broad distribution and high similarity of these MTases across microorganisms, we demonstrate that the three protein modules can interact functionally, even when derived from different microorganism, to methylate target molecules.

This research presents an innovative, sustainable, and versatile alternative for methylation reactions, contributing to the development of a more future-oriented and bio-based chemical industry.

#### References:

[1] Deobald D, Hanna R, Shahryari S, Layer G, Adrian L. Identification and characterization of a bacterial core methionine synthase. *Sci Rep* 10(1), 2100 (2020).

[2] Soder-Walz, J. M., Deobald, D., Vicent, T., Marco-Urrea, E., & Adrian, L. (2024). Applied and Environmental Microbiology, 90(10), e00978-24.

#### P-BT-023

##### Cytosolic biotransformation of D-glucose to 2-keto-D-glucose using the acetic acid bacterium *Gluconobacter oxydans*

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**Introduction and Goals:** The acetic acid bacterium and industrial workhorse *Gluconobacter oxydans* harbors the beneficial ability of regio- and stereoselective incomplete oxidation of sugars, sugar alcohols and other substrates in the periplasm by membrane-bound dehydrogenases and release of resulting products into the cultivation medium. Furthermore, due to its ability to also take up such compounds into the cytosol and excrete related products both in the non-phosphorylated form, *G. oxydans* is also very interesting for intracellular biotransformation applications. In this study we tested the applicability of *G. oxydans* for the cytosolic biotransformation of D-glucose to 2-keto-D-glucose (2KDG) using heterologous expression of cytosolic pyranose 2-oxidase (P2Ox) enzymes. P2Ox converts D-glucose with oxygen to 2KDG and hydrogen peroxide. 2KDG can serve as precursor for commercially interesting compounds, such as the low-calorie sweetener D-allulose and the antibiotic beta-prone cortalcerone.

**Methods:** Two fungal *p2ox* genes from *Trametes ochracea* and from *Phanerochaete chrysosporium*, and a bacterial one from *Kitasatospora aureofaciens* were used to construct expression plasmids for *G. oxydans*. The plasmids were transferred into the previously engineered *G. oxydans* strain IK003.1 [1], followed by biotransformation assays. Metabolite analysis was carried out by HPLC and GC-ToF-MS analysis.

**Results and Conclusions:** SDS-PAGE and mass spectrometric analysis confirmed that heterologous P2Ox enzymes can successfully be produced in *G. oxydans*. The FAD cofactor was found to be associated with or covalently bound to the flavoprotein based on fluorescence detection of

protein bands separated on an agarose gel. Whole-cell biotransformation assays with D-glucose led to 2KDG yields up to 66 % while D-glucose was fully used up within 48 h. The highest 2KDG yield was obtained with P2Ox from *P. chrysosporium*. GC-ToF-MS analysis of the cell-free supernatant revealed significant amounts of unwanted side products indicating that hydrogen peroxide decomposed metabolites. In conclusion, in *G. oxydans*, FAD-containing P2Ox enzymes can successfully be produced. For whole-cell biotransformation applications, where hydrogen peroxide is a co-product, the catalase activity needs to be increased in *G. oxydans* to abolish unwanted side products caused by hydrogen peroxide.

[1] Kiefler et al. (2017). Appl Microbiol Biotechnol 101(13):5453-5467

#### P-BT-024

##### Efficient Electron Transfer Systems for the Fatty Acid Decarboxylase OleT<sub>JE</sub>

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The cytochrome P450 OleT<sub>JE</sub>, a member of the CYP152 family from *Jeotgalicoccus* sp. 8456, catalyzes the oxidative decarboxylation of long-chain fatty acids to terminal alkenes. These alkenes represent valuable platform chemicals for the production of fuels, lubricants, and polymers. While OleT<sub>JE</sub> can utilize hydrogen peroxide as a direct oxidant, the enzyme can also be operated in "reductase mode" where canonical P450 electron transfer systems fuel the reaction.<sup>1</sup> The presented work aims to improve the electron transfer efficiency in OleT<sub>JE</sub>-catalyzed reactions by systematically comparing different redox partner systems. Electron transfer routes mediated by flavin adenine dinucleotide (FAD) and iron-sulfur clusters, as well as those involving flavin mononucleotide (FMN), were investigated. These systems were evaluated against a putative native electron transfer chain found in *Jeotgalicoccus* sp. 8456 to identify the most effective combination for productive electron delivery.

Biocatalytic reactions were carried out *in-vitro* and product formation was quantitatively analyzed by gas chromatography with flame ionization detection (GC-FID). In addition, kinetic parameters such as turnover frequency and catalytic efficiency were determined. The comparative study aims to elucidate the impact of different redox partner combinations on alkene yield and selectivity. The insights obtained are expected to contribute to the rational optimization of electron transport systems in P450-driven alkene biosynthesis and to advance the development of efficient biocatalytic routes for sustainable hydrocarbon production.

(1) Belcher, J. et al., *The Journal of biological chemistry* **2014**, 289 (10), 6535–6550.

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## P-BT-025

### Expanding the expression toolbox of *Gluconobacter oxydans* to enable light-controlled gene expression using caged inducers for bioprocess engineering studies

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**Introduction and Goals:** Recently, the first two regulatable expression systems functional in the acetic acid bacterium and industrial workhorse *Gluconobacter oxydans* were reported, namely the L-arabinose-dependent AraC-P<sub>araBAD</sub> and the L-rhamnose-dependent RhaS(R)-P<sub>rhaBAD</sub> systems both originating from *E. coli*. With these systems, the use of photocaged inducers - molecules whose biological activities are temporarily blocked by light-sensitive protecting groups and which are cleaved off by light irradiation of a certain wavelength - could now allow control of cellular functions in *Gluconobacter* in a targeted manner with high spatial and temporal precision, thereby providing new opportunities in bioprocess engineering studies. In this study we synthesized and tested the UV-A-activatable photocaged inducers NP-L-arabinose and NP-L-rhamnose for applications with *G. oxydans*.

**Methods:** UV-A sensitive photocaged inducers were synthesized in a synthetic approach described in the literature. Microfluidic 2D growth chambers were used to analyse on the single cell level the response in *G. oxydans* carrying respective expression plasmids for the mNeonGreen fluorescence reporter gene *mNG* under control of either AraC-P<sub>araBAD</sub> or RhaS-P<sub>rhaBAD</sub>.

**Results and Conclusion:** *G. oxydans* strains with reporter plasmid-based AraC-P<sub>araBAD</sub> or RhaS-P<sub>rhaBAD</sub> system were successfully cultivated in microfluidic setup and in-depth analysis of growth rates and cell morphology was established using computer-assisted analysis of image data. Expression studies with the conventional inducers L-arabinose and L-rhamnose showed that also in the microfluidic setup the expression performance of both systems was very similar to the results in BioLector setup published previously. Therefore, the microfluidic setup is well suited for further studies with *G. oxydans*. However, UV-A-sensitive caged NP-L-arabinose and NP-L-rhamnose did not result in reporter expression changes, most likely due to limited solubility in medium. An attempt to increase the sensitivity of the AraC-P<sub>araBAD</sub> system in *G. oxydans* toward lower external L-arabinose concentrations by plasmid-based co-expression of the L-arabinose transporter gene *araE* from *E. coli* was not successful yet. Initial translational *araE-mNG* reporter assays suggested that at least codon-harmonization could be required for functional expression of AraE in *G. oxydans*. Further studies are required to establish applicability of caged inducers for *G. oxydans*.

## P-BT-026

### Utilization of orange peel waste for sustainable amino acid production by *Corynebacterium glutamicum*

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Oranges are the most processed fruit in the world—it is therefore apparent that the industrial production of orange juice generates large quantities of orange peel as a by-product. Unfortunately, the management of the orange peel waste leads to economic and environmental problems. Meanwhile, the use of sustainable raw materials for the production of bulk chemicals, such as amino acids, is becoming increasingly attractive. To address both issues, this study focused on the use of orange peel waste as a raw material for media preparation for the production of amino acids by engineered *Corynebacterium glutamicum*. *C. glutamicum* grew on pure orange peel hydrolysate (OPH) and growth was enhanced by the addition of a nitrogen source and a pH buffer. Inhibitory effects by the combination of high concentrations of OPH, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and MOPS buffer in the wild-type strain (WT), were overcome in the tyrosine-producing engineered *C. glutamicum* strain AROM3. Genetic modifications that we identified to allow for improved growth rates under these conditions included the deletions of the vanillin dehydrogenase gene *vdh*, the L-lactate dehydrogenase gene *ldhA* and the 19 genes comprising cluster cg2663-cg2686. A growth inhibiting compound present in high concentrations in the OPH is 5-(hydroxymethyl)furfural (HMF). We identified *vdh* as being primarily responsible for the oxidation of HMF to its acid 5-hydroxymethyl-2-furancarboxylic acid (HMFA), as the formation of HMFA was reduced by 97% upon deletion of *vdh* in *C. glutamicum* WT. In addition, we showed that growth limitations could be overcome by adjusting the media preparation, using a combination of cheap ammonia water and KOH for pH neutralization after acidic hydrolysis. Overall, we developed a sustainable medium based on orange peel waste for the cultivation of *C. glutamicum* and demonstrated the successful production of the exemplary amino acids L-arginine, L-lysine, L-serine, L-valine and L-tyrosine.

## P-BT-027

### Engineering *Corynebacterium glutamicum* for 7"-deoxysedoheptulose (7dSh) production

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Modern agricultural production heavily relies on the use of artificial chemicals like fertilisers, herbicides, and fungicides, which can have detrimental effects on the environment and human health. Therefore, replacing such chemicals with more environmentally friendly bioactive compounds is one important strategy to minimise the environmental impact of the agricultural industry. The unusual sugar 7"-deoxysedoheptulose (7dSh) was first discovered in the soil bacterium *Streptomyces setonensis* [1] and has become a promising candidate for agricultural applications due to its herbicidal properties. Like the controversial broad-spectrum herbicide glyphosate, 7dSh acts as an inhibitor of the shikimate pathway, which is only present in photoautotrophic organisms such as plants, bacteria and fungi [2]. Recently, the 7dSh biosynthesis pathway was characterised in the cyanobacterium *Synechococcus elongatus* [3], opening the door for biotechnological production of 7dSh. However, 7dSh is not yet commercially available, as its chemoenzymatic synthesis is expensive and inefficient. Therefore, this work aims to enable the biotechnological production of 7dSh through metabolic engineering of *Corynebacterium glutamicum*. For this goal, a synthetic 7dSh production pathway is designed based on the cyanobacterial biosynthesis starting from 5"-deoxyadenosine (5dAdo) and implemented into *C. glutamicum*. Furthermore, the

replacement of the phosphorylase-phosphatase steps of the pathway with a nucleosidase is explored to avoid the phosphorylated intermediate 5"-deoxyribose-1-phosphate (5dR-1P) and thus minimise loss through native salvage pathways. The precursor availability is also improved through the overexpression of native radical S-adenosyl-L-methionine (SAM) enzymes and the inactivation of salvage pathways. Lastly, *C. glutamicum* production strains are ultimately optimised for the utilisation of biogenic waste for large-scale fermentation-based production of 7dSh, thereby reducing production costs and improving sustainability. Overall, for the optimisation of metabolic flux distributions and the maximisation of 7dSh production, tools from the fields of metabolic engineering, synthetic biology and systems biology are employed, supported by mass-spectrometry-based metabolome analyses.

[1] T. Ito et al., *Carbohydr. Res.* 17.2, pp. 375–82, (1971).

[2] K. Brilisauer et al., *Nat. Commun.* 10.1, p. 545, (2019).

[3] J. Rap et al., *J. Biol. Chem.* 296, p. 100621, (2021).

#### P-BT-028

##### **Bio-based aromatics: Engineering *Pseudomonas taiwanensis* for efficient 2-phenylethyl acetate and 4-hydroxyphenylacetic acid production**

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The global demand for aromatic compounds such as 2-phenylethyl acetate (PEA) and 4-hydroxyphenylacetic acid (HPAA) continues to rise due to their wide applications in cosmetics, fragrances, pharmaceuticals, and polymers. Conventional production relies on fossil resources, and the associated processes are energy-intensive and environmentally harmful. Biotechnological production offers a sustainable alternative, but the hydrophobic and often cytotoxic nature of aromatics can limit microbial productivity. *Pseudomonas taiwanensis* has emerged as a promising host owing to its high tolerance toward aromatic compounds, enabled by resistance mechanisms such as active extrusion and adaptive membrane alterations.

In this work, *P. taiwanensis* was established as a production platform for PEA building upon a previously engineered phenylalanine-overproducing strain. In this context, a biosynthetic pathway was established by heterologous expression of a phenylpyruvate decarboxylase from *Enterobacter* sp., a phenylacetaldehyde reductase from *Solanum lycopersicum*, and an alcohol O-acetyltransferase from *Saccharomyces cerevisiae*. Deletion of five aldehyde oxidases and dehydrogenases reduced the formation of phenylacetate as a byproduct and thereby led to enhanced provision of 2-phenylethanol. Co-expression of a phosphotransferase from *Clostridium kluyveri* enabled acetyl-CoA regeneration for the final conversion of PE to PEA. Additionally, the deletion of an esterase gene involved in PEA degradation significantly increased PEA accumulation enabling production of 1 mM PEA with a yield of 8,3% (Cmol/Cmol) in a minimal medium. The next bottleneck to be addressed is the competition for acetyl-CoA between the biosynthetic pathway and central carbon metabolism.

Furthermore, *P. taiwanensis* was engineered for the biosynthesis of HPAA, a building block for novel bio-based polymers. Several pathways were evaluated, and the combination of a tyrosine decarboxylase and a tyramine oxidase showed the best performance. Process upscaling to fed-batch bioreactor cultivations was successful, although product inhibition was limiting. To address this, different process conditions (e.g., pH) and additional strain engineering is investigated to overcome this limitation. Additionally, alternative carbon sources such as paper hydrolysates are integrated into the HPAA production process to transition from purified glucose towards industrial waste streams as feedstock for aromatics production.

#### P-BT-029

##### **Employing CRISPR/Cas12a and Modular Cloning (MoClo) for Standardized & Modular Genomic Integrations in *Corynebacterium glutamicum***

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Genome engineering in the industrial workhorse *Corynebacterium glutamicum* remains limited by the small number of experimentally validated genomic integration sites. Although systems based on CRISPR/Cas or double-homologous recombination (e.g. *mobsacB*) have been successfully applied, a consistent and modular framework for genomic integrations has not yet been established. In this study, we developed a standardized genome engineering platform that integrates Modular Cloning (MoClo) with an all-in-one CRISPR/Cas12a system to identify and validate new chromosomal loci for targeted DNA integration in *C. glutamicum*. Seven intergenic regions were experimentally confirmed to permit stable integration of a 1.1 kb mNeonGreen reporter construct, showing efficiencies from 3% to 45%. Expression analysis verified functionality at all tested loci. The workflow was further optimized to support modular insertion of synthetic transcription units assembled from MoClo parts. Applying this framework, up to three copies of a raspberry ketone biosynthetic cassette and five copies of a noreugenin cassette were genomically integrated in *C. glutamicum* M-CoA, resulting in fully plasmid-free production strains. Collectively, this work introduces a robust and modular platform for standardized genomic integration in *C. glutamicum*, facilitating efficient strain design for biotechnological applications.

#### P-BT-030

##### **Characterization and Application of a Thermostable Catalase**

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Catalases (EC 1.11.1.X) are widely distributed enzymes among prokaryotes as well as eukaryotes and are known for efficiently degrading H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub>. Since the first enzyme ever described was a catalase, not surprisingly their catalytic mechanism and their structure are well known. Catalases are an efficient tool for H<sub>2</sub>O<sub>2</sub>-detoxification in the medical field as well as in the food and textile industry these days [1]. However, the application of catalases for processes at an industrial scale which occur at high temperatures is limited since most established catalases have an optimal

temperature at a maximum of 50 °C [2, 3]. To address this issue and to extend the range of applications of catalases, this study provides insight into an application focused investigation of the recently described thermostable catalase *PtCat* from *Parageobacillus thermoglucosidasius* [4].

The production of *PtCat* proved to be sufficient in *E. coli* with a yield of 180 mg/L culture using *E. coli* NiCo21 (DE3). *PtCat* was examined in two states, as the free enzyme and in its immobilized form on carriers enabling the reuse of the enzyme as well as easy separation from reaction media which is of particular interest for application. We determined favorable kinetic parameters with a  $K_m$  lower than described in the literature and revealed that free *PtCat* remains active when stored at simple conditions. For the immobilized enzyme, the choice of carrier material was investigated, where immobilization on a Ni-NTA loaded carrier could be used to achieve 54.7 % of the activity of the free enzyme. In addition, recycling and temperature tolerance up to 90 °C and the influence of frequently used solvents was tested. *PtCat* was shown to have a temperature optimum of 70 °C and tolerates most solvents including the commonly used 2-propanol and methanol at concentrations of up to 40 % (v/v). In conclusion, *PtCat* shows a high potential for a broad range of industrial processes due to its affordable production, its high activity, when immobilized, its high tolerance for unphysiological conditions and efficient H<sub>2</sub>O<sub>2</sub> degradation.

1: DOI: 10.1016/j.bcab.2018.07.035

2: DOI: 10.1016/S0021-9258(18)84618-X

3: DOI: 10.1016/0300-9084(88)90035-1

4: DOI: 10.1155/2016/7535604

#### P-BT-031

##### A new route for microbial synthesis of *N,N*-dimethyltryptamine (DMT)

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**Introduction:** Tryptamine and its derivatives, such as *N,N*-dimethyltryptamine (DMT), are pharmacologically interesting compounds due to their psychotropic effects and potential therapeutic applications. In this study, we investigated the catalytic performance of the recombinant enzymes tryptophan decarboxylase from *Ruminococcus gnavus* (RgnTDC) and *N*-methyltransferase from *Rhinella marina* (RmNMT) when expressed in *E. coli* for the production of tryptamine and DMT. Furthermore, we explored establishing a *de novo* DMT biosynthetic route using sequential cultures of *Corynebacterium glutamicum* and *Escherichia coli*. **Goals:** To express RgnTDC and RmNMT in *E. coli*, optimize DMT production conditions, and establish a *de novo* biosynthetic route using tryptophan supplied by engineered *C. glutamicum*. **Materials & Methods:** *E. coli* strains harboring expression vectors for RgnTDC and/or RmNMT were cultivated in AMM minimal medium supplemented with 0.5 mM tryptophan under various temperature and pH conditions. For *de novo* production, *C. glutamicum* TP679<sup>[1]</sup> was used as a tryptophan-producing strain, followed by sequential cultivation with *E. coli* TN1 expressing both enzymes. DMT was quantified by HPLC-UV after 52 h of

cultivation in minimal and rich media. **Results:** RgnTDC exhibited the highest catalytic efficiency at 37°C and pH 8.0, while RmNMT showed optimal DMT formation at 25°C, pH 7.5, and 0.1 to 1 g/L methionine. When co-expressed in *E. coli* TN1, the overall optimal temperature was 25°C, with RmNMT identified as the rate-limiting step. Sequential co-culture of *C. glutamicum* TP679 with *E. coli* TN1 resulted in DMT concentrations of 87 µM (16.38 mg/L) in minimal medium and 402 µM (75.68 mg/L) in rich medium after 52 h. Without substrate addition, this represents the highest DMT titers reported to date for a recombinant organism. **Conclusion:** Engineered *E. coli* expressing RgnTDC and RmNMT efficiently produced DMT under optimized conditions (25°C, pH 7.5). Sequential cultivation with tryptophan-producing *C. glutamicum* TP679 enabled *de novo* DMT biosynthesis, achieving a maximum of 402 µM. This study highlights the potential of microbial consortia for the sustainable biosynthesis of tryptamine-derived alkaloids.

[1] Kerbs A, Burgardt A, Veldmann KH, Schäffer T, Lee J, Wendisch VF. Fermentative production of halogenated tryptophan derivatives with *Corynebacterium glutamicum* overexpressing tryptophanase or decarboxylase genes. *Chembiochem*. 2022.

#### P-BT-032

##### Bacto-Click – A living electro-biocatalyst for sensing and cleaning

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Electroactive cells hold great potential in biotechnology as both biosensors and biocatalysts. By combining innovative conductive materials with synthetic biology and engineered bacteria, our goal is to develop Engineered Living Materials (ELM) with adaptive functions capable of sensing and bioremediating pollutants.

Specifically, we focus on engineering bacteria to express a membrane-bound metalloprotein complex from *Dehalococcoides mccartyi*, which is important for organohalide respiration and is responsible for transmembrane electron transfer [1,2].

Additionally, the engineered bacteria are designed to incorporate a chemical anchor - a non-canonical amino acid (ncAA) introduced into the protein complex through Genetic Code Expansion (GCE). The ncAA enables a bio-orthogonal reaction (a chemical reaction that occurs in living cells without impeding any of its biochemical processes) with a derivatized material - an innovative highly conductive hydrogel [3]. The orthogonal reaction results in a covalent and conductive linkage between the bacteria and the hydrogel, representing a novel achievement not yet realized by any other technique.

By coupling the bacteria to the hydrogel, a regenerable, conductive biomaterial is obtained that can be electronically monitored and modularly adapted for diverse applications, such as the dehalogenation of pollutants in wastewater.

## References:

- [1] K. Seidel *et al.*, *Frontiers in Microbiology*, 2018, 9:1130.
- [2] N. Hellmold *et al.*, *Frontiers in Microbiology*, 2023, 14:1305108.
- [3] Z. Altintas *et al.*, *Biosensors and Bioelectronics*, 2018, 100, 541–548.

### P-BT-033

#### A novel aldehyde reductase from the black poplar mushroom *Cyclocybe aegerita*

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C8 oxylipins are volatiles produced by many species across the fungal kingdom and key compounds in the aroma of mushrooms (Combet *et al.* 2006). In previous research, a biosynthetic pathway was postulated and genes putatively involved in the C8 biosynthesis were identified by analysing transcriptomic and volatilomic data through different developmental stages of the model organism *Cyclocybe aegerita* (Orban *et al.* 2021, Karrer *et al.* 2021). One of these genes is putatively coding for an aldehyde reductase catalysing the reduction of oct-1-en-3-one and oct-2-enal to oct-1-en-3-ol and oct-2-en-1-ol, respectively.

This aldehyde reductase gene was recombinantly expressed in *E. coli* and purified using chromatographic methods. The characterization of the enzyme via gas chromatography and mass spectrometry revealed that the enzyme catalysed the predicted reduction of oct-2-enal but did not convert oct-1-en-3-one. Activity tests showed that the enzyme only accepts NADPH/NADP<sup>+</sup> as cofactors, since there was no activity found with NADH/NAD<sup>+</sup>. Michaelis-Menten parameters were deduced based on photometric analysis of the conversion of NADPH and showed that the reduction reaction is favoured over the oxidation reaction with  $K_m$  values of  $83 \pm 15 \mu\text{M}$  for oct-2-enal and  $305 \pm 76 \mu\text{M}$  for oct-2-en-1-ol at the respective pH optima. The enzyme also showed activity on several other substrates, e.g. citral, cinnamaldehyde derivatives and *p*-anisaldehyde. This wide substrate spectrum demonstrates the biotechnological potential in the production of aroma active compounds like oct-2-en-1-ol or geraniol.

Combet, Emilie; Eastwood, Daniel C.; Burton, Kerry S.; Henderson, Janey (2006): Eight-carbon volatiles in mushrooms and fungi: properties, analysis, and biosynthesis. In: *Mycoscience* 47 (6), p. 317–326.

Karrer, Dominik; Weigel, Vanessa; Hoberg, Nikolas; Atamasov, Alexander; Rühl, Martin (2021): Biotransformation of [<sup>13</sup>C]linoleic acid suggests two independent ketonic and aldehydic cycles within C8-oxylipin biosynthesis in *Cyclocybe aegerita* (V. Brig.) Vizzini. In: *Mycol Progress* 20 (8), p. 929–940.

Orban, Axel; Weber, Annsophie; Herzog, Robert; Hennicke, Florian; Rühl, Martin (2021): Transcriptome of different fruiting stages in the cultivated mushroom *Cyclocybe aegerita* suggests a complex regulation of fruiting and reveals enzymes putatively involved in fungal oxylipin biosynthesis. In: *BMC Genomics* 22 (1), p. 324.

### P-BT-034

#### Experimental Assessment of Suitable Extraction Techniques for High-Molecular-Weight PHB for Application in Bio-Based Anti-Adhesive Coatings

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**Introduction** Polyhydroxybutyrate (PHB) is a bio-based, biodegradable polymer considered a key material for the future of sustainable plastics, with the potential to substitute not only conventional plastics used in packaging and housings, but also to serve as a backbone component or functional additive in a broad spectrum of polymer-based applications. This project targets the development of a bio-based alternative to PTFE (Teflon), known for its anti-adhesive properties. For high-performance applications, it is crucial not only to biosynthesize PHB with a high molecular weight—typically achievable through specific microbial strains and optimized cultivation conditions—but also to preserve this molecular integrity during downstream processing and extraction.

**Goals** The main objectives are to identify extraction protocols that yield high-molecular-weight PHB suitable for anti-adhesive coatings and to prioritize the use of sustainable, environmentally friendly chemicals. The study also aims to evaluate new solvents and methods not previously applied to PHB extraction, with a focus on minimizing environmental and occupational hazards.

**Methods** Various extraction techniques were compared and optimized, including traditional protocols (trichloromethane) and innovative approaches using green solvents such as propylene carbonate, dibasic esters, ethyl lactate, diacetylglycerol and dimethyl carbonate (DMC). The study also assessed the impact of extraction methods that either dissolve PHB directly or lyse cells and hydrolyze cellular components, as well as the effect of these methods on the molecular weight and purity of the recovered PHB.

**Results and Conclusion** Some of the tested green solvents achieved high PHB recovery rates and purity while preserving molecular weight, making them suitable for advanced applications. Extraction methods that involve cell lysis and hydrolysis can compromise the molecular length of PHB, especially for high-molecular-weight fractions, highlighting the importance of method selection. Overall, the research demonstrates that sustainable extraction protocols using green solvents can produce high-quality PHB, supporting its use as a bio-based replacement for PTFE in anti-adhesive coatings. However, classical solvents like trichloromethane still show some of the best results in terms of extraction quality and polymer chain length, suggesting they may remain relevant for certain application fields.

### P-BT-035

#### Heterologous Expression and Functional Characterization of Two Alginate Lyases in *Corynebacterium glutamicum*

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Alginates are linear, anionic polysaccharides consisting of  $\beta$ -D-mannuronate (M) and its C-5 epimer  $\alpha$ -L-guluronate (G), organized in block structures of M, G, and alternating MG-

blocks (1). Alginates are abundant in brown seaweeds, and seaweed extracts have a large potential as sustainable feedstock for industrial biotechnology. Here, the *alyB* and *alyD* genes associated with alginate degradation in *Vibrio alginivorius* (2) are heterologously expressed and secreted by *Corynebacterium glutamicum*. The mode of action of AlyB and AlyD, individually or in combination, were characterized using NMR spectroscopy, HPAEC-PAD, and an absorbance-based assay. AlyB was found to be *endo*-active degrading all types of alginates (G blocks, M block, GM) bonds forming short oligomers. AlyB also displayed C-5 epimerization activity. AlyD on the other hand, is *exo*-active when using the products formed by AlyB depolymerization as substrates, thus AlyD degrades  $\Delta$ -containing oligomers into  $\Delta$  monosaccharides. However, AlyD is not active on saturated oligomers. Surprisingly, when acting together, AlyB and AlyD synergistically depolymerize alginates to  $\Delta$  monosaccharides. The heterologously expressed and secreted AlyB and AlyD in *C. glutamicum* enabled the breakdown of alginate to the uronate monomers  $\beta$ -D-mannuronate and  $\alpha$ -L-guluronate. The resulting substrate was here used as feedstock for growth of *Escherichia coli* K12 MG1655 (2). Moreover, by using a genetically engineered *E. coli* MG1655 strain expressing a synthetic riboflavin operon we demonstrated riboflavin production to a concentration of  $2.1 \pm 0.1 \mu\text{g/mL}$  using alginate depolymerized by AlyB and AlyD heterologously produced in *C. glutamicum* as sole carbon source.

## References

- 1) Enquist-Newman M, Faust AM, Bravo DD, Santos CN, Raisner RM, Hanel A, et al. Efficient 542 ethanol production from brown macroalgae sugars by a synthetic yeast platform. 543 2014;505(7482):239–43. <https://doi.org/10.1038/nature12771>
- 2) Doi H, Tokura Y, Mori Y, Mori K, Asakura Y, Usuda Y, et al. Identification of enzymes 557 responsible for extracellular alginate depolymerization and alginate metabolism in *Vibrio* 558 *alginivorius*. Appl Microbiol Biotechnol. 2017;101(4):1581–92. 559 <https://doi.org/10.1007/s00253-016-8021-7>

## P-BT-036

### Chitin-binding properties of AFP from *Aspergillus giganteus*

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Climate change is promoting the emergence of pathogenic fungi, while fungicide overuse leads to antifungal resistance, threatening human health and food security [1-5]. Antifungal peptides offer a promising sustainable alternative to established antifungal drugs, with broad antifungal activity and low toxicity [6].

This study examines the chitin-binding properties of AFP from *Aspergillus giganteus*, focusing on regions with similarities of a hevein-like domain and bacterial chitin-binding sequence [7].

Recombinant production of a set of derivative proteins of AFP, with induced point mutations in putative functional regions were produced and will be compared in bioactivity and chitin-affinity. A susceptibility assay against *Aspergillus niger* revealed a putative antifungal region of AFP, and initial

dissociation constant measurement, using a modified frontal affinity chromatography approach, revealed a low affinity against chitin, comparable to other plant lectins containing a hevein-like domain [8]. Furthermore, docking simulations of AFP to chitin presents a potential protein-ligand interaction at the targeted binding site.

This study gives a closer insight into the mode of action of AFP and the putative correlation of bioactivity and chitin-affinity. The modification of the frontal affinity chromatography approach offers a practical and high-throughput alternative to protein–ligand affinity measurement techniques that are limited by insoluble glycans, such as surface plasmon resonance.

[1] Seidel et al. 2024. [https://doi.org/10.1016/S2666-5247\(24\)00039-9](https://doi.org/10.1016/S2666-5247(24)00039-9).

[2] Williams et al. 2024. <https://doi.org/10.1371/journal.ppat.1012219>.

[3] Nnadi et al. 2021. <https://doi.org/10.1371/journal.ppat.1009503>.

[4] Gusa, Asiya. 2024. <https://doi.org/10.1016/j.jbc.2024.106541>.

[5] Singh et al. 2023. <https://doi.org/10.1038/s41579-023-00900-7>.

[6] Hassan et al. 2023. <https://doi.org/10.3390/pharmaceutics15010072>.

[7] Hagen et al. 2007. <https://doi.org/10.1128/AEM.02497-06>.

[8] Solanke et al. 2019. <https://doi.org/10.1038/s41598-019-53815-w>

## P-BT-037

### Metabolic engineering of *Corynebacterium glutamicum* strain MB001 to enhance native decaprenoxanthin production

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Carotenoids are yellow to red pigments, that belong to the large group of terpenoids. The C5 isoprenoid diphosphates are the universal precursors for all terpenoids. The majority of carotenoids, such as  $\beta$ -carotene or astaxanthin, are C40 conjugated isoprenoids. They can be heterologously produced by the biotechnologically important bacterium *Corynebacterium glutamicum*<sup>1</sup>. However, this organism naturally synthesizes a rare yellow C50 carotenoid – decaprenoxanthin - which is supposed to have very strong UV-protection<sup>2</sup> as well as high antioxidative properties, as we could show here. In addition, we engineered a genome-reduced strain MB001<sup>3</sup> to produce high yields of this value added carotenoid by deleting the carotenoid biosynthesis repressor gene *crtR*, replacing the native carotenoid biosynthesis gene cluster with a reduced operon expressing only the final pathway genes *crtBIYeb* necessary for decaprenoxanthin production under a strong *Ptac* promoter,

replacing the native *dxs* promoter with a strong constitutive *P<sub>tuf</sub>* promoter, and finally replacing the native *idi* by a synthetic operon harboring a second copy of *idsA* and *idi* expressed under the strong *P<sub>syn</sub>* promoter. This plasmid-free strain already produced 0.13 g L<sup>-1</sup> decaprenoxanthin in shake flasks. Reintroduction of *crtE* and a second copy of the genes *crtB* and *crtI* on a plasmid increased shake flask production by 75%. In a 2 L fed-batch fermentation this strain produced up to 1.45 g L<sup>-1</sup> decaprenoxanthin.

1 Schmitt I. *et al. Molecules*. 2023 20;28(4):1996. doi: 10.3390/molecules28041996.

2 Sandmann G. *Antioxidants*. 2019 11;8(7):219. doi: 10.3390/antiox8070219.

3 Baumgart M. *et al. Appl Environ Microbiol*. 2013 79(19):6006-15. doi: 10.1128/AEM.01634-13.

### P-BT-038

#### Synthetic Biology Approaches to Engineer *Yarrowia lipolytica* for High-Value Carotenoid Biosynthesis

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Carotenoids are high-value natural isoprenoids that exhibit diverse biological functions, including antioxidant activity and photoprotection. Therefore, they have wide applications in the food, pharmaceutical, nutraceutical, and cosmetic industries. *Yarrowia lipolytica*, a dimorphic and oleaginous yeast, is a promising platform owing to its broad substrate range and high stress tolerance, and its ability to form large lipid droplets provides efficient storage sites for hydrophobic compounds such as carotenoids. Furthermore, *Y. lipolytica* synthesizes high levels of acetyl-CoA, a precursor of the mevalonate pathway and carotenoid biosynthesis.

In this research, we develop metabolic engineering strategies for producing C<sub>40</sub> and C<sub>50</sub> carotenoids in *Y. lipolytica*. Due to the poor integration efficiency at the target locus observed in our laboratory, the *KU70* gene was deleted in the *Y. lipolytica* strain CLIB122 to limit the repair of double-strand breaks via the non-homologous end joining pathway. Afterwards, we overexpressed key genes and confirmed their integration at the target locus. In particular, the rate-limiting enzymes of the mevalonate pathway, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*HMG1*) and geranylgeranyl pyrophosphate synthase (*crtE*), were overexpressed to enhance the availability of building blocks for carotenoid biosynthesis. To produce β-carotene and astaxanthin, the genes for phytoene desaturase (*carB*) and bifunctional phytoene synthase/lycopene cyclase (*carRP*) from *Mucor circinelloides*, along with β-carotene ketolase (*crtW*) and hydroxylase (*crtZ*) from *Haematococcus pluvialis*, were expressed in CLIB122 (*Δku70*). Moreover, we discuss how to expand carotenoid diversity, advance CRISPR/Cas9-based metabolic engineering strategies, and investigate cultivation strategies utilizing alternative carbon sources, such as acetate and glycerol, to enhance production titers.

### References:

Kretzschmar, Anne, et al. "Increased homologous integration frequency in *Yarrowia lipolytica* strains defective in non-homologous end-joining." *Current genetics* 59.1 (2013): 63-72.

Ma, Yongshuo, et al. "Removal of lycopene substrate inhibition enables high carotenoid productivity in *Yarrowia lipolytica*." *Nature Communications* 13.1 (2022): 572.

Zhu, Hang-Zhi, et al. "Production of high levels of 3 S, 3' S-astaxanthin in *Yarrowia lipolytica* via iterative metabolic engineering." *Journal of Agricultural and Food Chemistry* 70.8 (2022): 2673-2683.

### P-BT-039

#### The filamentous cell factory *Aspergillus niger* can be engineered for recombinant hydroxylated collagen production

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Collagen is a major structural protein with broad biomedical and industrial uses in fields such as tissue engineering, wound healing and bioprinting. Traditionally sourced from animals, its current production raises concern about safety, consistency, and scalability. Recombinant production offers a cleaner, more sustainable alternative, but current systems struggle with proper posttranslational modifications, particularly proline hydroxylation present in natural collagens, and low secretion yields. *Aspergillus niger*, a robust industrial host known for efficient protein secretion and posttranslational capacity, had not previously been applied to recombinant collagen production until now.

This study aimed to establish a proof-of-concept system for producing hydroxylated collagen in *A. niger*, identify key bottlenecks such as degradation by endogenous proteases, and implement genetic and media-based strategies to improve yield and stability. A modular cloning toolkit was used to assemble expression cassettes encoding a truncated collagen III and various prolyl-4-hydroxylase (P4H) isoforms, introduced into *A. niger* via CRISPR/Cas12a genome editing. Collagen expression was monitored using luciferase and eGFP reporters, while secretion and hydroxylation were finally assessed using HiBiT tagging, SDS-PAGE, and LC-MS.

Initial strains secreted collagen but showed rapid extracellular degradation. Transcriptomic data identified *protA*, encoding a proline-specific endopeptidase, as a major contributor. Deletion of *protA* in a protease-deficient background significantly enhanced collagen stability. Further improvements were achieved by optimizing nitrogen sources in the medium to suppress protease expression. The final isolate, TM44.2, secreted partially hydroxylated collagen (~16% of expected prolines) at initial titers up to 5 mg L<sup>-1</sup> in shake flasks, with protein remaining stable in the supernatant for over 140 hours. Process parameter optimisation of the growth and media conditions has shown promising results for increased recombinant collagen titres and increased hydroxylation.

This study provides the first evidence that *Aspergillus niger* can serve as a heterologous system to secrete partially hydroxylated collagen. These engineered strains and methodological strategies constitute a promising foundation for further improvements in yield and modification efficiency toward scalable, virus-free recombinant collagen production.

#### P-BT-040

##### Discovery and heterologous production of non-ribosomal peptides using *Vibrio natriegens* as microbial chassis

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Different and well established microbial chassis are used for the heterologous production of proteins, vaccines and natural products (NP). **Non-Ribosomal Peptides (NRPs)** stand out as a special kind of NP, widespread in bacteria and fungi, since they can function as antibiotics, toxins, siderophores, etc. generated by **Non-Ribosomal Peptides Synthetases (NRPSs)**, multifunctional and modular enzymes. For NRP production, and the production of other NP, the most common organism used is *E. coli*. Moreover, even though other microbial chassis have their own characteristic that makes them suitable for producing a particular NP, a common bottleneck process is the production time. In most of the cases, the production time to obtain high yields are long, which makes the process expensive. Here we present *Vibrio natriegens* as a solution to speed up NP production. Using established synthetic biology and analytical tools, we were able to produce and quantify three different NRP classes in *V. natriegens*: Indigoidine, Xenortide and GameXpeptides. When comparing the production times against *E. coli*, *V. natriegens* shows a much shorter production time. In the future, we want to use *V. natriegens* to heterologously express different NRPSs and discover novel natural products in metagenomic data from Colombia and other places.

#### P-BT-041

##### Microbial and Enzymatic Depolymerization of Natural Rubber

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Natural rubber (NR) is an abundant biopolymer used in automobile tires, gloves, seals, and many other industrial and everyday products. Industrial vulcanization and the addition of fillers and additives make NR highly resistant to microbial degradation. Biological depolymerization of NR provides an environmentally friendly alternative to conventional recycling methods; however, only few microorganisms and enzymes responsible for this process have been characterized so far. This study aims to identify new rubber-degrading microorganisms and their enzymes by integrating bioinformatic, microbiological, and analytical approaches.

Environmental samples from different sites were screened using a latex overlay assay specifically developed for detecting NR-degrading activity. Several isolates showing distinct clearing zones were obtained and identified by 16S rRNA sequencing for bacteria and MALDI-TOF or ITS sequencing for fungi. Degradation was also confirmed in liquid cultures using FTIR spectroscopy and Schiff's reagent staining to detect aldehyde and ketone groups formed during polymer oxidation.

To uncover potential genetic determinants of this activity, genome-mining analyses were performed using NCBI, UniProt, and KEGG databases to identify candidate genes

encoding known rubber-degrading enzymes such as latex-clearing proteins (Lcp), rubber oxygenases (RoxA/RoxB), laccases, and manganese peroxidases.

The current progress demonstrates the feasibility of combining microbiological isolation with bioinformatic prediction to identify novel NR-degrading enzymes. Ongoing work focuses on heterologous expression and biochemical characterization of newly discovered candidate genes, aiming to advance the development of sustainable enzymatic strategies for rubber recycling.

#### P-BT-042

##### Metabolic engineering of *Corynebacterium glutamicum* for sugar and methanol co-consumption for zero CO<sub>2</sub> conversions

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The atmospheric concentration of the greenhouse gas CO<sub>2</sub> has been steadily increasing for decades. Although biotechnology offers sustainable alternatives using renewable feedstocks, their conversion in microbial processes still results in CO<sub>2</sub> formation. The formation of CO<sub>2</sub> is caused by an electron imbalance between the substrates and the resulting products, which leads to the oxidation of carbon to CO<sub>2</sub>.

This study aims to achieve CO<sub>2</sub>-neutral and carbon-conserving microbial conversions in *Corynebacterium glutamicum* through the co-utilization of xylose and methanol. Methanol, a highly reduced carbon source, is predicted to serve as an electron donor to counterbalance the oxidative nature of xylose metabolism in *C. glutamicum* [1]. To enable efficient co-utilization of methanol and xylose, several genomic targets were engineered in *C. glutamicum*.

This work represents a first approach toward zero-CO<sub>2</sub> and fully carbon-conserving bioconversions, using methanol as an electron donor to achieve redox-balanced microbial production processes.

[1] Carlos Arevalo Villa, Jan Marienhagen, Stephan Noack, Sebastian Aljoscha Wahl, Achieving net zero CO<sub>2</sub> emission in the biobased production of reduced platform chemicals using defined co-feeding of methanol, Current Opinion in Biotechnology, Volume 82, 2023, 102967, ISSN 0958-1669, <https://doi.org/10.1016/j.copbio.2023.102967>.

#### P-BT-043

##### Influence of a 1064 nm-laser on *Escherichia coli* as an example of a biotechnologically relevant microorganism

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Laser technology is becoming increasingly important in measurement and sensor technology, including in biotechnological applications such as fermentation monitoring. Despite these advances, the biological interactions between laser radiation and microorganisms remain largely unexplored.



It is known that laser radiation interacts with biological tissue. Short wavelengths can damage DNA, leading to mutations or cell death, while long wavelengths can cause the evaporation and denaturation of proteins, which can also be lethal. In addition to wavelength, laser intensity and specific properties also play a decisive role.

This project aims to investigate the influence of a 1064 nm-laser on the growth of *Escherichia coli* under different irradiation scenarios and to classify the results in the literature.

For this purpose, an *Escherichia coli* culture was irradiated with a power of 100 mW and partially returned or tested directly. The irradiation duration was varied depending on the experiment. Growth behavior was analyzed based on the number of living and total cells and compared with reference samples. In addition, the metabolic processes were examined using a commercial biochemical identification stripes. Initial experiments show no observable changes in growth behavior or metabolic processes after laser irradiation. In addition, a comprehensive literature search was conducted to determine the current state of research and to classify the known positive, negative, and neutral effects of laser irradiation on microorganisms.

The results obtained so far contribute to a better understanding of biological laser interactions and form a basis for further investigations. Since the effects depend strongly on wavelength, irradiation duration and power as well as on the microorganism, further experiments are necessary to understand the underlying mechanisms in more detail.

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#### **P-BT-044** **Harnessing *Aspergillus oryzae* for efficient expression of thermostable and alkaline amylases in laundry enzyme development**

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Amylases are among the most widely applied enzymes in industrial biotechnology, particularly in laundry detergents, where they catalyze the removal of starch-based stains under alkaline and high-temperature conditions. However, the demand for sustainable and cost-effective production systems of robust amylases remains a challenge. Here, we develop *Aspergillus oryzae* as an efficient heterologous expression chassis for thermostable and alkaline  $\alpha$ -amylases with potential detergent applications. We chose *A. oryzae* for its exceptional natural capacity to secrete large amounts of amylolytic enzymes, simplifying downstream processing and enabling direct recovery of active proteins from culture supernatants.

Amylase genes from bacterial, fungal, and insect origins were selected based on their reported stability at elevated pH and temperature—conditions characteristic of laundry processes. These genes were codon-optimized for expression in *A. oryzae* and cloned into a tailored expression vector. Using a CRISPR/Cas9-based genome editing strategy with PEG-mediated protoplast transformation, constructs were introduced into an  $\alpha$ -amylase-deficient strain<sup>1</sup> to eliminate background activity and improve detection sensitivity. Heterologously expressed amylases exhibited measurable activity under alkaline and elevated-

temperature conditions, validating *A. oryzae* as a versatile platform for secretory expression of enzymes derived from diverse phylogenetic origins. Furthermore, the incorporation of GFP fusion constructs enabled real-time monitoring of amylase expression throughout cultivation, providing a valuable tool for subsequent optimization studies.

The successful expression of thermostable and alkaline-tolerant amylases highlights the potential of *A. oryzae* as a sustainable and industrially relevant fungal chassis for enzyme production. Furthermore, this work supports the development of next-generation, eco-friendly laundry formulations and establishes a framework for scalable biosynthetic production of other industrially important enzymes.

#### **References**

1. Haeger, G., Grankin, A. & Wagner, M. Construction of an *Aspergillus oryzae* triple amylase deletion mutant as a chassis to evaluate industrially relevant amylases using multiplex CRISPR/Cas9 editing technology. *Applied Research* 2, (2023).

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#### **P-BT-045** **Heterologous Expression of Peptides with *A. oryzae* in Submerged Cultivation and Solid-State Fermentation**

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*Aspergillus oryzae* is a filamentous fungus, which is used in industrial biotechnology due to its strong secretory capacity and robust protein processing machinery for both homologous and heterologous protein expression. These characteristics make it an interesting host for heterologous peptide production. In this study, we employed a CRISPR/Cas9-based integration system to introduce target peptide sequences into the *A. oryzae* genome to produce peptides relevant for consumer goods applications. Expression was driven by the inducible amyB promoter, which regulates  $\alpha$ -amylase expression during starch metabolism and allows induction of peptide synthesis.

We aimed to exploit the secretion potential of the *A. oryzae* amylase machinery for heterologous peptide expression under Submerged Cultivation (SmC) and Solid-State Fermentation (SSF) conditions. A triple amylase knockout strain of *A. oryzae* was used to eliminate background amylase expression (Haeger et al., 2023). As a model system, an antimicrobial peptide with activity against *E. coli*, but not against the expression host itself, was selected to serve both as an active and representative peptide for further downstream processing and application tests.

For SmC, sterile DPY medium consisting of dextrin, peptone, yeast extract, and salts, was inoculated with spores and incubated for 5 days under shaking conditions. The dominant form of mycelia in this cultivation method were pellets. For further analysis, pellets were removed from the medium. For SSF, rice grains were hydrated and cooked by autoclavation. DPY medium was mixed in before inoculation with spore suspension, followed by incubation for 5 days without shaking. Mycelium grew on the solid surface of the rice grains as a dense mycelial mat. After incubation, rice grains were washed with buffer to extract the secreted peptides.

Peptide expression was analyzed via SDS-PAGE and Western blot.

The integration of heterologous peptide sequences under the control of the *amyB* promoter successfully enabled peptide expression in *A. oryzae*. Our results indicate expression of peptides both in SmC and SSF. These findings demonstrate the potential of *A. oryzae* as a versatile platform for sustainable heterologous peptide production. In future work, we are going to compare the expression levels and secretion efficacy of the peptides in *A. oryzae* under SmC and SSF conditions to identify optimal cultivation parameters for Scale Up and Downstream Processing.

Haeger, G., Grankin, A., & Wagner, M. (2023). Construction of an *Aspergillus oryzae* triple amylase deletion mutant as a chassis to evaluate industrially relevant amylases using multiplex CRISPR/Cas9 editing technology. *Applied Research*.

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#### P-BT-046

##### Development of a biotech platform for tailor-made glycosylated glycomacropeptide with high potential in pharmaceutical and nutritional applications

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Glycomacropeptide (GMP) is a 64 amino acid glycopeptide, which is derived from enzymatic cleavage of  $\kappa$ -Casein. GMP is a proteinogenic component of whey and is a by-product during the production of cheese. As the name reflects, GMP is highly glycosylated. The glycosylation pattern is very heterogeneous with 14 identified forms with glycans from mono- to tetrasaccharides. Additionally, there are differences in the glycosylation pattern and the occurring sugar units among different milk-producing species e.g., human, bovine or goat. While positive effects of glycosylated GMP on cells and model systems are well known and described in the literature. Nevertheless, the detailed knowledge about how the biological activity is dependent on a certain sugar modification is yet to be elucidated. Therefore, GMP is an interesting peptide for pharmaceutical and nutritional applications and a starting point for further research. In that context one focus is on the improvement of (cardio-)metabolic and neurodegenerative diseases, which are results of the demographic change and aging of our population. To address the detailed investigations of the glycan functionality with regard to GMP glycosylation the current objective of our project deals with the establishment of a production process of tailor-made glycosylated GMP-fractions. We therefore want to apply different recombinant glycosyltransferases and recombinantly produced non-glycosylated GMP in an *in vitro* approach. Our research should result in a biotechnological platform for a controllable glycosylation pattern, i.e., with uniformly glycosylated glycopeptide-fractions. The pool of derived glycopeptide-fractions will be tested for bioactivity and response in appropriate model systems. The promising candidates of the identified bioactive glycopeptides shall be part of new health-relevant supplements and/or food products based on milk peptides.

#### P-BT-047

##### Expanding the Substrate Spectrum of *Corynebacterium glutamicum* to Include Novel Utilization of Seaweed-Based Deoxy Sugars

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The growing global population demands more sustainable exploitation of carbon sources for microbial bioprocesses. Seaweed represents a promising renewable feedstock, as it does not require arable land or freshwater and exhibits high growth rates. In this study, we aimed to expand the substrate utilization range of *Corynebacterium glutamicum*, a robust microbial workhorse widely used in industrial biotechnology, towards seaweed-derived carbohydrates and to demonstrate growth and L-lysine production from seaweed hydrolysates containing the deoxy sugars fucose and rhamnose.

Hence, we established efficient fucose utilization via heterologous expression of the *fucIKUA* operon in combination with the inositol permease gene *iolT2* from *Escherichia coli* and *C. glutamicum*, respectively. Conversely, we achieved efficient rhamnose utilization via heterologously expressing the *rhaBAD* operon, together with the fucose/rhamnose permease gene *fucP* from *E. coli*. Additionally, utilization of lactaldehyde, an intermediate of fucose and rhamnose metabolism, by *C. glutamicum* was enhanced through overexpression of the native acetaldehyde dehydrogenase gene *ald*. Engineered *C. glutamicum* strains were tested for growth on brown and green seaweed hydrolysates, derived from the seaweed species *Saccharina latissima* and *Ulva lactuca*, respectively. Furthermore, the newly developed synthetic operons were introduced into the *C. glutamicum* L-lysine-producing strain GSL, establishing L-lysine production from seaweed hydrolysates containing fucose or rhamnose.

In summary, this work expands the substrate spectrum of *C. glutamicum* to include seaweed-derived carbohydrates and identifies novel native enzymatic functions contributing to deoxy sugar metabolism. These findings highlight the potential of *C. glutamicum* as a platform for seaweed-based biorefineries and contribute to the development of sustainable microbial production platforms.

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#### P-BT-048

##### Plasmid persistence in single-species and mixotrophic biofilms

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**Introduction** Biofilms offer a promising alternative to suspended microbial cultures in biotechnology due to their robustness and self-regeneration, enabling continuous processes [1]. A key challenge of applying these systems lies in maintaining catalytic activity and selection pressure for specific enzymatic functions. The delivery of selection compounds, such as antibiotics used for plasmid stability, can be hindered by mass transfer limitations, resulting in heterogeneous plasmid distributions. Moreover, continuous antibiotic use increases ecological impact and operational cost.

**Goals** This study investigates the long-term persistence of recombinant plasmids in *P. taiwanensis* VLB120 and *Synechocystis* sp. PCC 6803 biofilms compared to suspended cultures. The aim is to determine whether antibiotics are necessary for plasmid maintenance and, if so, to define the minimal effective dosage and application frequency required for stable expression.

**Materials & Methods** Spatial and temporal analyses of plasmid persistence are conducted using fluorescently tagged plasmids. To visualize the respective cells, either autofluorescence of Chlorophyll A in case of *Synechocystis*, or DsRed in case of *Pseudomonas* will be used. Cultures are maintained for 1–2 weeks under identical conditions in both planktonic and biofilm cultures. Flow cytometry will be used to quantify populations with and without plasmids in suspended cultures and dispersed biofilms. To determine the spatial plasmid distribution within biofilms confocal laser scanning microscopy (CLSM) and optical coherence tomography (OCT) will be applied.

**Results** First data point towards a faster plasmid loss in suspended cultures in the absence of antibiotics, probably due to higher growth rates as compared to biofilm growing cells. In biofilms, a decrease in plasmid-containing cells is indicated in the layers in contact with the media, as seen in Røder et al. (2021), attributed to a higher duplication rate than those with less access to the growth media.

**Summary** This work provides insight into the spatial and temporal dynamics of plasmid stability in biofilms, offering a basis for minimizing antibiotic use while maintaining catalytic activity in continuous bioprocesses. Understanding these mechanisms will contribute to more sustainable and robust biofilm-based production systems.

1 Philipp, L.-A. et al. (2024). *Nat Rev Microbiol* 22, 276–290

2 Røder, H. L. et al. (2021). *npj Biofilms Microbiomes* 7, 78

## P-BT-049

### Machine-Learning-based Design of Experiment for Fermentation with *Pleurotus ostreatus*

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Every year, more than ten million tons of food waste are generated in Germany alone.<sup>1</sup> When utilized at all, this energy-rich material is converted into biogas, which is subsequently combusted – a clear example of resource underutilization. The **INGRAIN** alliance<sup>2</sup> strives to establish circular resource flows. Within this framework, our project **BasicalT** (Basidiomycetes for a Circular economy in the Agro-, Food-, and Textile Industries) pursues this goal by using edible mushrooms to upcycle side streams of the food industry into valuable raw materials for the food, textile or paper sector.

To achieve this, efficient fermentation processes are essential. Our immediate goal was to optimize biomass production in our model organism, *Pleurotus ostreatus*. This

fast-growing, basidiomycete is particularly well suited for biomass generation.

To maximize the space-time yield of *P. ostreatus* biomass, we applied an **iterative design-of-experiments (DoE)** strategy guided by **machine learning** – specifically, Gaussian process modeling and **Bayesian** optimization – as described by Schmitz et al.<sup>3</sup> The approach was implemented for cultivation of *P. ostreatus* DSM1833 on malt extract medium in 2 L bioreactor vessels controlled by Eppendorf SciVario®-twin systems. To reduce experimental complexity, only **pH**, **media composition**, and **cultivation time** were varied systematically.

Using this approach, we achieved the maximized space-time-yield of *P. ostreatus* biomass with a relatively small number of experiments. In parallel with biomass optimization, we also gathered activity data for secreted enzymes of interest. This data could serve as the basis for subsequent optimization towards specific enzyme activity.

The presented DoE approach efficiently identified optimal cultivation conditions with a minimal experimental burden. Owing to its simplicity and flexibility, the method can readily be extended to a larger set of process variables. Furthermore, the accompanying datasets provide a valuable basis for fermentation optimization towards different target parameters.

[1] Statistisches Bundesamt; *Umweltbundesamt* 2024; Textel144/2024

[2] Innovationsbündnis Agrar-Textil-Lebensmittel; <https://ingrain.nrw>

[3] Schmitz, C. et al.: Chapter 5 In Computational and Data-Driven Chemistry Using Artificial Intelligence; *Elsevier*, 2022; pp. 161–192; <https://doi.org/10.1016/B978-0-12-822249-2.00001-3>.

## P-BT-050

### Genome streamlining and application of *Vibrio natriegens* synSC1.0 for biotechnology

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Using microbes for industrial applications is one core element of the global bioeconomy. The biodiversity of microbes is enormous, but only a limited number of organisms are applied in biotechnology. Nevertheless, with recent technological advances in particular efforts in synthetic biology, non-model organisms can be domesticated within rapid time frames. *Vibrio natriegens* is a promising platform for biotechnological applications, because it can be cultivated as easy as *E. coli* and within a shorter time frame, a large number of genetic tools became available (Thoma & Blombach, 2021),(Stukenberg et al., 2022). In addition, *V. natriegens* can be cultivated using sea water which reduces the use of fresh water, a resource becoming more and more restricted. *Vibrio* species generally have a bi-partite genome with a larger main chromosome and a smaller secondary chromosome. We recently fused the two chromosomes and characterized the resulting strain *V. natriegens* synSC1.0.

The strain retains its rapid growth, and no phenotypic changes have been observed.

The presented study applies two strategies to our single chromosome strain: (i) the removal of larger gene fragments that are not needed in the future within biotechnological applications and streamlining synSC1.0 as a chassis, and (ii) the utilization of a synthetic chromosome based on the native secondary chromosome origin of replication region as a platform for expression of heterologous pathways. These results represent the first steps towards establishing *V. natriegens* synSC1.0 as a sustainable microbial cell factory.

#### References:

Stukenberg, D., Hoff, J., Faber, A., & Becker, A. (2022). NT-CRISPR, combining natural transformation and CRISPR-Cas9 counterselection for markerless and scarless genome editing in *Vibrio natriegens*. *Communications Biology*, 5(1). <https://doi.org/10.1038/s42003-022-03150-0>

Thoma, F., & Blombach, B. (2021). Metabolic engineering of *Vibrio natriegens*. *Essays in Biochemistry*, 65(2), 381–392. <https://doi.org/10.1042/ebc20200135>

#### P-BT-051

##### **Purification and characterization of a new glutenase secreted by the micromycete *Aspergillus parasiticus***

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The problem of human gluten sensitivity remains urgent, as existing glutenases show broad specificity and do not completely utilize gluten. In this study, a new producer of gluten-destroying protease, *Aspergillus parasiticus*, was isolated from the leaf litter of a tropical forest in Vietnam, Nghe An province.

The aim of the work was to study the hydrolytic potential of a new producer of glutenase, as well as to isolate, purify and characterize glutenolytic protease. The hydrolytic potential of the strain was assessed on agar media with certain protein substrates as a source of nitrogen. Glutenolytic protease was obtained in different ways to compare the effectiveness of the resulting preparation: by submerged cultivation and solid state cultivation. Enzymes were isolated by salting out, dialysis, and purification by isoelectric focusing. Proteolytic activity was evaluated in reactions with gluten, gliadin, and other native and colored protein substrates to determine substrate specificity. The purity and molecular weight of the protease were evaluated and the presence of proteolytic activity was confirmed by electrophoretic methods.

The hydrolytic potential was the highest in relation to casein (EI=1.33), slightly lower in relation to gliadin and fibrinogen (for both substrates, EI=1.23), and in relation to gluten, EI=1.19. Secretion of gelatinases, collagenases or keratinases in media containing these substrates was not observed. The efficiency of protein substrate hydrolysis decreased in a following sequence of proteins: azocasein (62.6 E/ml), fibrinogen (19.7 E/ml), fibrin (18.7 E/ml), blue fibrin in pH 5 (18.3 E/ml), bovine serum albumin (13.7 E/ml), hemoglobin in pH 8.2 (10.5 E/ml), casein (10.3 E/ml), hemoglobin in pH 5 (8.1 E/ml), keratin (7.2 E/ml), gliadin (4.0 E/ml), gluten (3.3 E/ml), azocoll (1.3 E/ml), human serum albumin (0.4 E/ml), and the hydrolysis of fibrin blue in pH 8.2,

keratin-azur, and elastin-congo red did not occur. When comparing the glutenase preparations obtained by different cultivation methods, the preparation obtained by submerged cultivation proved to be the most promising in biotechnology.

Thus, a previously unstudied glutenase was isolated and purified from the culture fluid of *A. parasiticus*, and its specificity towards gluten and the difficult-to-degrade protein fibrinogen was demonstrated. The production of a purified protease specific for gluten hydrolysis holds promise for the gluten-free food industry.

#### P-BT-052

##### **Rapid, Reliable DNA Extraction at the Point of Care: SwiftX DNA**

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#### Introduction:

Extraction of nucleic acids is the first step of every molecular diagnostic workflow. Depending on the pathogen and the specimen, nucleic acid extraction can be challenging and crucial for successful diagnosis. Conventional extraction workflows often involve multiple steps, specialized equipment, and long processing times, which limit their use in decentralized or resource-limited environments. Therefore, the aim was to develop and validate a rapid, simplified DNA extraction method designed for point-of-care (POC), delivering high-quality DNA in minutes without complex instrumentation.

#### Material & Methods:

SwiftX DNA employs a proprietary cell capturing and reverse purification technology optimized for a wide range of sample types, including blood, swabs, saliva, and tissue. It comprises of the following key steps: (a) optional enrichment from liquid samples, (b) rapid cell lysis, and (c) one-step inhibitor removal by reverse purification rendering the extracted DNA applicable to various molecular methods. The workflow eliminates the need for centrifugation, using a single-tube, heat-assisted protocol compatible with downstream PCR and isothermal amplification assays. Performance was evaluated across multiple clinical and environmental sample matrices, benchmarking DNA yield, and amplification efficiency against leading commercial kits.

#### Results:

The finalized workflow significantly reduces hands-on time and enables DNA extraction in under 15 minutes. Results demonstrate that the protocol performs equivalently to conventional silica membrane-based kits, while being significantly faster and easier to use. The workflow showed consistent performance across sample types, with yields sufficient for sensitive molecular detection. Integration with rapid PCR and LAMP assays demonstrated robust amplification.

#### Conclusion:

The rapid extraction procedure provides a fast, user-friendly, and instrument-minimal solution for high-quality DNA extraction directly at sampling sites or in small laboratories. Its streamlined workflow enables reliable molecular testing in

clinical, field, and emergency settings, supporting rapid decision-making and expanding access to molecular diagnostics.

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#### P-BT-053

##### Unveiling the catalytic potential of methyltransferases for C1-derived methylation reactions in biotechnology

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Methylation reactions play an important role as diversification reactions in chemical and pharmaceutical synthesis. Conventional chemical methylation, however, typically requires strong bases, metal catalysts, and toxic methyl donors, posing both environmental and safety challenges. In contrast, enzymatic methylation proceeds under mild conditions with high chemo- and regioselectivity.

Methyltransferases (MTases) offer a biological alternative, utilizing small methyl donors such as methanol, acetyl-CoA, or cofactors including methyl-tetrahydrofolate, S-adenosylmethionine (SAM), and methyl cobalamin. Among these, B12-dependent methylation systems remain relatively underexplored. This system typically comprise: (i) an MTase I that demethylates the methyl group donor, (ii) an MTase II that transfers the methyl group to an acceptor molecule, and (iii) a B12-shuttle protein mediating reversible methyl transfer between MTase I and MTase II.

In this project, recombinant production and characterization of MTase I enzymes capable of utilizing methanol and other small compounds as methyl group donors, sustainably derived via electrochemical CO<sub>2</sub> reduction. To enable biotechnological applications of MTases I, different expression vectors for heterologous expression in *Escherichia coli* have been tested, and production conditions have been optimized. The recombinant MTase I enzymes were purified, and underwent subsequent characterization, including anaerobic activity assays for kinetic parameter characterization ( $V_{max}$ ,  $K_m$ , and  $k_{cat}$ ) and metal quantification using ICP-MS. Furthermore, interaction of MTase I enzymes with different B12-shuttle proteins as well as with MTases II, followed by structural analysis is targeted.

Overall, this research aims to develop a sustainable, circular biological methylation platform utilizing methanol or other small compounds as renewable methyl group donors, offering a CO<sub>2</sub>-neutral, enzymatic alternative to conventional chemical methylations to produce, e.g., pharmaceuticals and biofuels.

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#### P-BT-054

##### Insight into the Metabolic Bottlenecks in the Biosynthesis of the Methylated Plant Metabolite Ferulic Acid in *Escherichia coli*

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The biotechnological production of ferulic acid (FA)—a valuable antioxidant and precursor molecule—is an attractive alternative to chemical synthesis or plant extraction. We set up a biosynthesis pathway in *E. coli*, which starts from tyrosine, followed by a deamination, a hydroxylation, and a final methylation step. The amount of ferulic acid produced in

lab-scale bioreactors is severely limited by the final SAM-dependent methylation reaction step, a bottleneck traditionally attributed to a limited intracellular supply of S-adenosylmethionine (SAM).

To investigate the limitations of this methylation step, we performed a targeted metabolomic analysis of the Activated Methyl Cycle (AMC), the pathway responsible for recycling the methylation byproduct S-adenosylhomocysteine (SAH) back to SAM. We utilized a fed-batch strategy (12h-growth, 48h-production) in a parallel bioreactor setup with minimal media. We compared four conditions with different SAM consumption rates and co-supplementation of methionine, SAM's direct precursor. Intracellular metabolites (methionine, tyrosine, SAM, SAH, and S-ribosylhomocysteine (SRH)), traced along the production process, were quantified by LC-MS/MS. Concentrations were normalized to relative abundance per mg dry weight (DW), and changes were visualized using a heatmap.

Metabolomics data revealed two distinct bottlenecks: 1) without methionine supplementation, FA production (max. 6 mM) was limited by SAM availability; SAM and its precursor methionine were rapidly depleted, while the inhibitory byproducts SAH and SRH slightly accumulated. 2) With methionine supplementation, SAM supply was increased, enabling higher FA production (15 mM), but with strong and continuous accumulation of SAH. These results indicated that the flux through the SAH recycling pathway (via SRH) was overwhelmed, unable to keep pace with the high methylation demand of biotechnological FA production, which suggested this as the factor limiting further conversion.

While SAM availability is the initial limiting factor, our results demonstrate that in a high-production state, the primary bottleneck shifts from SAM supply to the recycling of its byproduct, SAH. This work identifies the SAH recycling pathway as a critical and previously underestimated target for metabolic engineering to unlock further increases in ferulic acid production and other valuable methylated compounds.

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#### P-BT-055

##### *Staphylococcus aureus* adhesins Eap, Emp and FnBPs differentially orchestrate bacterial interaction with host tissue and implant materials

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*Staphylococcus aureus* (*S. aureus*) is a major cause of implant-associated infections, where successful colonisation depends on coordinated adhesion to host tissues and foreign materials. This process is mediated by a repertoire of surface adhesins with partly overlapping and context-dependent functions. Here, we dissect the contribution of the extracellular adherence protein (Eap), the extracellular matrix-binding protein (Emp) and fibronectin-binding proteins A and B (FnBPs) to *S. aureus* pathogenicity in tissue and implant-associated infection.

Using isogenic deletion mutants, we combined *in vitro* cell culture infection assays with *in vivo* murine footpad infection and a newly established implant-associated *Galleria mellonella* model. FnBPs were both necessary and sufficient for adhesion to and invasion of host cells, whereas loss of

Eap or Emp did not measurably affect these cellular processes. In the murine model, however, Eap- and Emp-deficient strains showed moderately reduced bacterial burdens, indicating a subtler contribution to infection fitness *in vivo*. Strikingly, in adhesion assays with titanium, Emp promoted bacterial attachment to a similar extent as FnBPs, underscoring its specific relevance at the implant interface. In the *Galleria* model, mutants lacking Eap/Emp or FnBPs were cleared more efficiently than the wild-type, with the impact of Eap and Emp becoming apparent primarily in the presence of foreign material.

These data identify FnBPs as central determinants of host interaction and reveal a distinct, implant-context-dependent role of Eap and Emp. Targeting this differential adhesin repertoire may inform the rational design of anti-adhesive coatings and adjunctive strategies to prevent or control orthopaedic implant-associated *S. aureus* infections.

#### P-BT-056

##### **Production, vesicle encapsulation, and plant growth promoting effects of the microbial siderophore pyoverdine**

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Iron deficiency poses significant challenges in agriculture, affecting plant growth and crop yields. In this context, certain microbes have shown to improve the bioavailability of this essential micronutrient by producing siderophores – a class of secondary metabolites capable of efficiently chelating insoluble Fe<sup>3+</sup>. Therefore, siderophores have tremendous application potential in agriculture as ingredients in alternative fertilizers. However, their native biosynthesis in bacteria is controlled by complex regulatory networks.

To address this challenge, we aimed to engineer the microbial host *Pseudomonas putida* for the efficient production of the siderophore pyoverdine (PVD) to gain access to this class of valuable compounds. We successfully developed novel *P. putida* strains for the controlled overproduction of PVD independently of intrinsic iron homeostasis control mechanisms. The PVD-enriched supernatants from these cultures, were subsequently tested for their ability to promote plant growth. It could be demonstrated that PVD-containing supernatants strongly enhanced plant growth parameters (leaf chlorophyll and root iron content) when applied to roots of *Arabidopsis thaliana*.

In addition, we successfully engineered *P. putida* strains for increased formation of outer membrane vesicles (OMVs) containing PVD. Initial plant growth promoting studies showed that OMV-encapsulated PVD was less effective in promoting plant growth, indicating that encapsulating the siderophore might be a privatization strategy of *P. putida* under iron depletion. The data obtained in this study clearly indicate that the plant growth promoting siderophore PVD can be efficiently synthesized and secreted in engineered *P. putida* production strains. Thus, PVD has great potential as a microbiological alternative to fossil-fuel based iron chelators

#### P-BT-057

##### **Development of efficient tools for metabolic engineering of *Clostridium kluyveri* DSM555 for syngas fermenting consortia**

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Microbial fermentation of syngas (CO, CO<sub>2</sub>, H<sub>2</sub>), which is produced as a by-product from the steel industry or from gasification of municipal waste, offers great potential to sustainably produce valuable bulk chemicals. Combining syngas fermenting organisms with the carbon chain elongating *Clostridium kluyveri* in co-cultures would facilitate efficient conversion of syngas to higher value products. For example, the syngas gas-fermenting *Clostridium carboxidivorans* produces ethanol and acetate. These are the substrates for *C. kluyveri*, which is converting them into medium chain fatty acids. Those fatty acids are in turn reduced to the corresponding alcohols butanol and hexanol by *C. carboxidivorans*. Unfortunately, we found that CO concentrations most favorable for good ethanol and acetate production by *C. carboxidivorans*, reversibly inhibit growth of *C. kluyveri*, presumably by inhibition of the [FeFe]-hydrogenases.

We achieved adaptation of *C. kluyveri* to high CO partial pressures using an automated laboratory evolution (ALE) strategy. CO-adapted clones were characterized by whole genome sequencing, and a mutation potentially responsible for the CO-resistant phenotype was reconstructed by reverse engineering. To this end, we developed two markerless genome editing tools for *C. kluyveri* – a system using 5-fluorocytosine for counterselection and a highly efficient Cas9-based genome editing system. To achieve the high conjugation rates required for such systems without cumbersome *in vivo* methylation, we have constructed restriction-deficient *C. kluyveri* strains devoid of its type I, type II and type III restriction-modification (RM) systems. Thereby, the RM type II knockout revealed by far the strongest increase of conjugation efficiency of at least five orders of magnitude. This resulted in a very useful *C. kluyveri* platform strain and the methods to do efficient metabolic engineering of this organism, needed for future development of optimized syngas fermenting, chain elongating consortia.

#### P-BT-058

##### **Biotechnological production of laccases by *Pleurotus ostreatus* and *Flammulina velutipes* in submerged culture using agricultural side streams**

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Laccases (EC 1.10.3.2) are versatile multicopper oxidases, widely applied in bioremediation, fine chemical synthesis, and green catalysis (Brugnari et al. 2021). This study investigated regional agri-food residues from North Rhine-Westphalia (NRW) - carrot ends, potato, and onion by-products - as alternative substrates for submerged cultivation of *Pleurotus ostreatus* (DSM 1833) and *Flammulina velutipes* (DSM 1658). Media were prepared from thermally pretreated extracts of the side streams and compared to a malt-extract-

peptone standard medium (MEB). Biomass formation, laccase activity (ABTS assay), and sugar dynamics (DNS assay) were quantified over 14 and 21 days.

*F. velutipes* achieved the highest biomass in potato medium ( $5,29 \pm 1,58 \text{ g L}^{-1}$ ), while *P. ostreatus* produced greater overall biomass, reaching  $10,43 \pm 2,38 \text{ g L}^{-1}$  in MEB and  $7,26 \pm 1,20 \text{ g L}^{-1}$  on potato residues. Laccase synthesis showed distinct substrate dependencies. *F. velutipes* produced measurable activity only in late stages, peaking at  $\sim 120 \text{ mU mL}^{-1}$  in potato medium. *P. ostreatus* exhibited induction in both potato and carrot substrates, reaching  $62 \text{ mU mL}^{-1}$  and  $214 \text{ mU mL}^{-1}$ , respectively, after 13 days. Extending cultivation to 21 days confirmed *P. ostreatus* as the superior producer. In this phase, the mixed onion–carrot–potato (OCP) medium yielded the highest activity ( $> 230 \text{ mU mL}^{-1}$ ) despite moderate biomass formation ( $3,84 \pm 0,04 \text{ g L}^{-1}$ ). Pure onion extract inhibited fungal growth, likely due to antimicrobial thiosulfates (Sorlozano-Puerto et al. 2020). Sugar profiling revealed strong substrate-dependent patterns. Bound carbohydrates contributed significantly to total carbon availability, decreasing markedly during enzyme production.

These findings demonstrate that regional side streams can replace synthetic media while enhancing laccase induction. The OCP mixture provided synergistic nutritional and inductive effects, enabling a chemically untreated and regionally adaptable process for sustainable fungal enzyme production within a circular bioeconomy framework.

Reference list: DOI: 10.1186/s40643-021-00484-1; DOI: 10.3390/ph14010021

#### P-BT-059

##### Old vehicle for cutting edge biotech; Expanding tool box of metabolic engineering by use of Cosmids

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Metabolic engineering relies on the efficient transfer of genetic information to a production host. While in most cases DNA is transferred into microorganisms by the use of plasmids, new engineering tools can also be used to achieve genomic modifications with moderate effort. However, it is not uncommon for genomic insertions to require significantly more effort than the transfer of episomal DNA. Still the workhorse in biotechnology, plasmid's advantages of easy transformation decrease inversely with size. For a multigene cascade with individual regulatory elements, several thousands of kbp need to be coded. To overcome this problem, we investigate the use of a single 48 kbp phage derived plasmid (Cosmid) for transfer of genetic information of multi-step biosynthesis cascades to a bacterial production strain. Cosmid are self-replicating episomal elements which are controlled by typical origins of replication (pBR322 ori). In addition, Cosmids can be transduced by phage particles to lamB<sup>+</sup> strains of Enterobacteria. Phage particles are synthesized *in vitro* and packed with linear DNA substrate. We use Gibson assembly to synthesize the linear fragment with cos sequence at both ends. For this modern cloning process, DNA fragments up to the size of 25 kb are amplified by PCR. We observed varying efficiencies among different proof-reading polymerases especially while amplifying very long PCR fragments. Gibson assembly formulation is modified to improve assembly efficiency for extended DNA constructs. Furthermore, the preparation and titration of the

packaging extract and associated reaction conditions were systematically optimized to achieve higher overall transduction efficiency.

#### P-BT-060

##### Genetic modification of *Sorangium cellulosum* species for the expression of bioactive natural compounds

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Myxobacteria are known for their rich secondary metabolism that subsequently holds huge potential for new natural products discovery. To this point, the lack of implemented genetic manipulation tools hindered the investigations on antibacterial natural compounds derived by *Sorangium cellulosum* (*S. cellulosum*) species. New studies revealed an optimized electroporation method for the transfer of foreign DNA. In this study, the technique is tested on a selection of *S. cellulosum* strains. The proof of concept was investigated by randomised DNA integration via transposon transformation. Targeted genetic manipulation are performed e.g. with the native Chlorotoniil producer strain SoCe1525 for product optimization and furthermore by integration of previously uncharacterized BGCs in various *S. cellulosum* strains.

#### P-BT-061

##### Identification of kinetic constraints for the enzymatic detoxification of deoxynivalenol in feed

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The infection of crops by mycotoxin-producing fungi poses a considerable problem nowadays. A well investigated example of these mycotoxins is Deoxynivalenol (DON) from *Fusarium* species. It causes health issues in livestock due to its ability to bind eukaryotic ribosomes thus inhibiting protein translation. The enzymatic detoxification of DON in feed could be a green and mild solution. A potential enzyme in that regard is the Specialized Glyoxalase I (SPG, EC: 4.4.1.5) from *Gossypium hirsutum*. Classified as an isomerase, native SPG is part of the gossypol pathway and was found to transform DON into its less toxic isomeric form (Huang et al., 2020). The SPG variant Y62A was shown to have an increased catalytic activity towards DON as well as increased thermal stability (Hut et al., 2022). Even though DON conversion was proven in analytic scale available kinetic information for SPG and DON is limited. In our study, we aimed to investigate relevant kinetic parameter as a base for technical application approaches. After a successful recombinant production of SPG<sup>Y62A</sup> in *E. coli* BL21 (DE3) pLysS in 7-L bioreactor scale and subsequent downstream processing, the enzyme was analyzed for thermal stability as well as isomerase activity towards DON. Our results show, that the holoenzyme is thermostable with a  $T_i$  of 70 °C. However, the onset of unfolding occurs already at 45 °C. For the application of the enzyme in feed production, the ability to withstand high temperatures is needed and further engineering is necessary. In addition, the kinetic analysis showed that the substrate binding constant  $K_M$  of SPG<sup>Y62A</sup> is rather high ( $K_M \sim 6 \text{ mM}$ , pH 9.0 and  $K_M \sim 23 \text{ mM}$ , pH 6.0). This parameter is unfavourable for the treatment of feedstock

with DON concentrations in the  $\mu\text{M}$ -range. Based on our investigations we aim to engineer SPG<sup>Y62A</sup> to further increase the thermostability, decrease  $K_M$  towards DON to improve catalytic conversion. We therefore applied computational methods (Docking, funcLib) and identified variants that are characterized subsequently.

Huang, J. Q. *et al.* (2020). Aromatization of natural products by a specialized detoxification enzyme. *Nature Chemical Biology*, 16(3), 250-256.

Hu, Y. *et al.* (2022). Crystal structure and biochemical analysis of the specialized deoxynivalenol–detoxifying glyoxalase SPG from *Gossypium hirsutum*. *International Journal of Biological Macromolecules*, 200, 388-396.

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## P-BT-062

### Cobalamin-dependent methyltransferases for sustainable methylation chemistry

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Methylation reactions are important reactions in chemical industry as they can alter physicochemical properties of a substance like solubility, viscosity, or pKa. However, traditional methylation chemistry is often not chemio-, regio-, or enantio-selective and mostly dependent on toxic and expensive reagents. A biotechnological exploitation of methyltransferases (MTases) can encounter this problem as MTases do not depend on harsh reaction conditions or aggressive reactants. Moreover, for MTases a regeneratable methyl group donor could be used. My project aims to explore and extend the substrate range of particularly cobalamin-dependent methyltransferases through structural and biochemical analysis and rational enzyme design for application in sustainable chemical industry.

In this study, data from heterologous production and preliminary biochemical characterization of core-MetE methionine synthase and MecC MTase are presented, which both catalyze biotechnologically relevant reactions. For both enzymes, heterologous overexpression in *Escherichia coli* could be achieved through systematic optimization of cultivation conditions and the proteins were successfully purified using affinity chromatography.

The results indicate that an efficient production of cobalamin-dependent MTases is feasible. Together with an aspired analysis of reaction products and a rational enzyme design, methylation of new substrates with a high market value, e.g., fatty acids or nucleotides, by using the MTases can be achieved, reshaping methylation chemistry in line with bioeconomic principles.

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## P-BT-063

### *In vivo* protein immobilization using split FP-based biosensors and catalytically-active inclusion bodies

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*In vivo* protein immobilization, i.e., the attachment of a protein of interest (POI) to intracellular biogenic material during its production in bacteria, offers a promising strategy to enhance protein stability and reusability for industrial applications. Despite numerous approaches, achieving efficient *in vivo* immobilization remains challenging, as no method is suitable for all enzymes and real-time monitoring of the attachment process is not possible. Here, we present two strategies for improving *in vivo* protein immobilization. The first strategy involves a novel approach in *E. coli*. In this approach, the POI is coupled to polyhydroxybutyrate (PHB) granules as a carrier matrix, and a split fluorescent protein, such as split GFP, is used as a linker and biosensor. The splitGFP linker is generated by dividing superfolder GFP into two non-fluorescent protein fragments, GFP1-10 and GFP11. These fragments can specifically self-assemble resulting in a green fluorescent signal. We used a PHB synthase GFP11 fusion protein as a PHB anchor protein whereas the POI is linked to GFP1-10. Therefore, successful immobilization is indicated by a green fluorescence signal. To demonstrate the applicability of the split GFP-based immobilization system, the red fluorescent protein mCherry, the squalene synthase (SQS) from *M. capsulatus*, and the ester hydrolase LipD from *A. borkumensis* served as POIs. Successful immobilization was confirmed by fluorescence spectroscopy and enzymatic activity of immobilized enzymes was demonstrated via HPLC (SQS) and 4-nitrophenyl butyrate conversion (LipD). The second strategy focuses on generating catalytically-active inclusion bodies (CatIBs) by fusing the POI to aggregation-inducing elements. Unlike conventional inclusion bodies, CatIBs contain POIs in a correctly folded form. For CatIB formation of tryptophan-5-halogenase PyrH from *S. rugosporus*, various CatIB tags, fusion strategies and linkers were tested. Successful immobilization was verified by fractionation of cellular proteins and PyrH activity was quantified via HPLC.

In conclusion, the split GFP system enables *in vivo* immobilization of POIs on PHB granules and quantitative real-time detection of the formed immobilisates. Moreover, we successfully used the CatIB strategy for *in vivo* immobilization of PyrH, which may facilitate its future use in flow synthesis. Both approaches represent promising tools for advancing *in vivo* protein immobilization in industrial biotechnology.

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## P-BT-064

### Investigating the interplay of autoinducer 2 formation and biotechnological production of methylated compounds in *E. coli*

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Biotechnological approaches for the industrial production of high-grade plant metabolites like ferulic acid demonstrate a promising alternative to current chemical extractions and syntheses. Whole cell catalysis is performed in *E. coli* by a three-step reaction cascade starting from the deamidation of tyrosine, followed by hydroxylation and subsequent methylation to ferulic acid. Till now the methylation by the SAM-dependent O-methyltransferase (OMT) still causes a bottleneck in the production, due to limited supply of S-Adenosylmethionine (SAM) and insufficient recycling of S-Adenosylhomocysteine. Attempts to enhance recycling



through the activated methyl cycle (AMC) by overexpression of LuxS and MtnN only led to moderate success in improving ferulic acid production, as it also drastically impacted cell vitality measured by carbon dioxide transfer rates.

To further elucidate reasons for impaired cell vitality by enhanced SAM recycling we identified (S)-4,5-dihydroxy-2,3-pentanedione (DPD) as a potential toxic metabolite. The AMC byproduct DPD is formed during the cleavage reaction of ribosylhomocysteine by LuxS and serves as a precursor for quorum sensing molecules referred to as autoinducer 2 (AI-2). Considering the increased demand on the AMC during ferulic acid production coupled with the LuxS overexpression, extracellular AI-2 might accumulate beyond physiological tolerated concentrations for the cells (100-500  $\mu$ M) leading to possible cytotoxic effects. Accordingly, our cytotoxicity studies revealed increasing extracellular AI-2 concentrations lead to progressive reductions in cell growth of the strain with an IC<sub>50</sub> of 1,11 mM. Moreover, genome data of the host strain *E. coli* BL21 revealed a spontaneous deletion of the *lsr*-Operon, disabling the canonical AI-2 response and degradation system of the cell.

To circumvent accumulation of AI-2 and relieve cell stress, we performed metabolic engineering of the production strain by reimplementing the AI-2 degrading enzymes LsrK, LsrF and LsrG. Further, we designed a LsrK variant with deficient binding to the phosphocarrier protein HPr to remove potential influences on the phosphotransferase system. We then tested our engineered strain for ferulic acid production in parallel bioreactor setups. Using a HPLC-FLD detection method, we could confirm the specific removal of any extracellular DPD/AI-2 signal during production for the engineered strain leading to improved cell vitality compared to control strains.

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## P-BT-065

### Waste wood as a circular feedstock for mycelium-bound biomaterials

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## Introduction

The transition from a fossil-based to a bio-based economy is expected to increase the utilization of wood. As the most important renewable raw material for a bioeconomy, wood plays a central role in sustainable resource management, with circular use being essential for efficient utilization. Waste wood is increasingly recognized as a valuable feedstock for the development of new materials, offering opportunities for innovative and sustainable product design.

## Goals

This study aims to evaluate the suitability of different classes of waste wood, as defined by the German *Altholzverordnung*, as substrates for mycelium-bound composites. The objective is to determine how non-wooden contaminants, and inorganic pollutants affect fungal growth and mycelium development.

## Materials & Methods

Waste wood samples were collected in 2025 from five recycling companies in Bavaria, Germany. To increase the homogeneity of the samples, sampling was performed on ten different parts of large piles of waste wood chips at each recycling company. The elemental composition of the samples was analyzed using X-ray fluorescence (XRF) and confirmed via microwave digestion followed by Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES). Water-soluble fractions and lignin content were determined through Soxhlet extraction. Following chemical characterization, the substrates were tested for fungal colonization with selected biotechnologically relevant Basidiomycetes.

## Results

The analyses revealed distinct elemental profiles among the waste wood classes, reflecting varying degrees of contamination with metals and non-wood materials. These compositional differences were correlated with the growth performance of the tested fungal species. Although the presence of several non-wood pollutants in the substrates affected fungal colonization, the tests showed that the fungi were able to colonize even highly contaminated waste wood classes.

## Summary

This study provided a comprehensive assessment of waste wood as a substrate for mycelium-bound composites. By linking chemical characteristics with biological performance, it demonstrated that specific waste wood classes hold strong potential for use in sustainable biomaterial production. The findings highlight the opportunities and limitations of integrating waste wood into circular bioeconomic value chains through biotechnological upcycling approaches.

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# Computational microbiology

## P-CM-002

### Uncovering Chlorophyll degradation in Prokaryotes

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Chlorophyll is the most abundant pigment on Earth and has an annual turnover of  $\sim 1.15 \times 10^9$  t<sup>[1]</sup>. Although its degradation is well understood in plants, the role of prokaryotes in this process - despite their vast metabolic capabilities - remains unknown. Recent developments in the field of AI-predicted protein structures<sup>[2]</sup> have opened new avenues for investigating functional homologies between evolutionary-distant organisms previously inaccessible through traditional sequence- or profile-based methods.

Here, we present the first evidence of chlorophyll a degradation by prokaryotes, discovered through a novel bioinformatic workflow leveraging these AI-predicted structures. This workflow bridges the gap across the domains of life via structural alignments of functionally characterized plant proteins against the Evolutionary Scale Modeling Metagenomic Atlas<sup>[3]</sup> using Foldseek<sup>[4]</sup>, followed by novel structure similarity graph-based clustering. After selecting clusters with the highest similarity to a curated

Hidden Markov Model (HMM) from corresponding plant sequences, final HMMs for prokaryotic homologs were obtained.

We then assessed publicly available datasets containing genomes from isolates, metagenome-assembled genomes (MAGs), and single-cell genomes. Furthermore, we also assembled and binned metagenomic sequencing data to obtain additional genomes, yielding in over 70,000 medium- to high-quality genomes in total. This dataset was then mined for prokaryotic homologs of chlorophyll *a* degradation genes using the previously obtained HMMs. Our analysis revealed over 400 genomes from diverse taxonomic groups and habitats that possess a complete chlorophyll *a* degradation pathway, of which over 50% stem from isolates. Additionally, many other genomes harbor a partial pathway, suggesting that chlorophyll *a* degradation capabilities are globally widespread across diverse ecosystems.

We validated our *in silico* findings using the model organism *Shewanella acanthi* and confirmed its chlorophyll *a* degradation capability *via* growth experiments and fluorescence spectroscopy. Our findings demonstrate a previously unrecognized metabolic capability in prokaryotes, highlighting the power of structure-based remote homology detection for uncovering functional pathways and evolutionary relationships.

[1] George et. al. 1987, New Phytologist

[2] Jumper et al. 2021, Nature

[3] Lin et al. 2023, Science

[4] Kempen et al. 2023, Nature Biotechnology

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### P-CM-003

#### Comparative genomic analyses of antimicrobial proteins encoded by lichen-forming fungi

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Fungi have evolved diverse lifestyles over hundreds of millions of years. In plant pathogenic fungi, antimicrobial effector proteins (AMPs) play key roles in microbiome manipulation and niche establishment. We hypothesize that such AMPs are also important in mutualistic associations such as lichens, which are partnerships between a fungal mycobiont and a green algal or cyanobacterial photosynthetic partner called the photobiont. We analysed the AMP repertoires of 80 lichen-forming fungi by clustering all AMPs based on sequence and predicted structural similarity to explore patterns of distribution. Fungi associating with cyanobacteria appeared to encode fewer AMPs than those associating exclusively with green algae. Interestingly, certain groups of AMPs were specific to fungal species association strictly with cyanobacteria or algae. Within-species comparison further revealed both presence-absence and sequence variation of AMPs between geographic populations, highlighting potential adaptive flexibility in their antimicrobial catalogues.

### P-CM-004

#### Microbial Taxa-Function Dynamics and Their Impact on Human Lung Development Affected by Cystic Fibrosis

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The human respiratory microbiome plays a vital role in health and disease. Emerging ideas of functional redundancy and taxa-function decoupling suggest that taxonomic shifts may not fully capture microbial functional dynamics. Drawing from island biogeography theory, we hypothesise that microbial colonisation in Cystic Fibrosis (CF) lungs is influenced by migration across upper airway sites and by species arrival order. Specifically, this study explores the functional signatures and taxa-function decoupling events of CF. We aim to identify functional biomarkers of dysbiosis and characterise the microbial functions driving community dynamics during early lung development.

In collaboration with clinicians, we established a longitudinal cohort of infants and children with CF (ages 0-6 years), from whom paired nasal and throat swabs were collected (n = 96). To enable functional profiling of low-biomass respiratory samples, we developed an enhanced DNA/RNA co-extraction protocol and a comprehensive metagenomics pipeline integrating multiple tools. Validation of our analytical pipeline using pilot samples resulted in 43 high-quality metagenome-assembled genomes from ~138 Mbp of assemblies constructed from 311.4 Gbp shotgun metagenomic data.

First results from taxonomic profiling revealed niche-specific microbial signatures: nasal samples were dominated by *Staphylococcus*, *Corynebacterium*, and *Abiotrophia*, while throat samples showed enrichment of *Veillonella*, *Streptococcus*, *Prevotella*, and *Neisseria*. Age-related shifts were observed within niches, despite the persistence of *Staphylococcus* in nasal samples from 6 to 16 months. Comparison with healthy samples from a recent study revealed that core metabolic pathways were shared but encoded by distinct taxa, consistent with taxa-function decoupling.

Our longitudinal multi-niche analysis investigates taxa-function relationships in early childhood CF compared with healthy controls. Our functional enrichment analyses framework, integrated with clinical lung function data, tests whether conserved functional signatures arise despite taxonomic variability and whether priority effects appear at the functional level during early colonisation.

We find that shared metabolic functions are maintained by distinct taxa across CF and healthy airway niches, supporting the taxa-function decoupling hypothesis. This functional perspective opens new opportunities to identify dysbiosis biomarkers and understand lung development.

## P-CM-005

### A niche-based dormancy model for soil microbial community responses to disturbance and dispersal

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Microbial behaviors influence the stability of communities and their ecological responses, such as resistance to perturbations. For example in soil, a majority fraction of the microbial community is dormant.

Dormant microbes have low metabolic activity and are expected to resist unfavorable perturbations. It has been proposed that the entry into dormancy is a bet-hedging behavior, providing a "microbial seed bank" that can enhance community resilience. An alternative hypothesis proposes that the role of dormancy is to avoid competition for resources between genetically related cells. Indeed, both strategies may play a role in community resistance: resuscitation of dormant cells can contribute to community reassembly after perturbation, while dormancy-mediated competition reduction may help maintain community diversity by favoring coexistence between species that would otherwise compete for resources.

Dispersal of microbes into a community is another key factor that may contribute to microbiome recovery following perturbations, by restoring species that were impacted by the perturbation. However, the relative interplay between dormancy and dispersal in determining the resilience of a microbial community to perturbation remains an open question.

Mathematical modeling is an important tool to investigate the contributions of different factors to microbial community dynamics. Motivated by data from long-term soil mesocosms exposed to heat perturbation with and without dispersal, we established a mathematical model for the dynamical response to perturbation. The model is niche-based; it includes dormancy and dispersal and accounts for different levels of microbial sensitivity to the heat perturbation. In the model, microbes occupy a carrying-capacity-limited niche, can switch between active and dormant states, and are affected by disturbance and dispersal. The model qualitatively reproduced observed succession patterns and predicted how niche occupancy, environmental filtering, and dispersal shape post-disturbance outcomes.

Using this model, we aim to provide new insights into the ecological roles of dormancy and dispersal in microbial communities exposed to perturbations. Our model further highlights the utility of niche modeling to understand microbial resistance, succession, and the ecological roles of dormancy, perturbations and dispersal in complex microbial processes.

## P-CM-006

### Illuminating Microbial Interactions and Cooperation in a Long-Term Sulfate-Reducing, Benzene-Degrading Community

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Microbial communities perform essential functions and interactions that shape their metabolic niches. Defining these niches is key to understanding microbial community structure, function and ecological roles, as well as to harnessing communities for targeted applications. However, no standardized workflow currently exists for defining niches from metagenomic data. In this study, we developed a genome-centric workflow to define metabolic niches and tested it in a model sulfate-reducing, benzene-degrading microbial community maintained as an enrichment culture for several years.

Our workflow integrates genome annotation, clustering, and network-based niche identification. From two metagenomes recovered from this system, we reconstructed metagenome-assembled genomes (MAGs) using MuDoGeR. To capture functional diversity, we built six presence-absence datasets based on annotation from specific biological knowledge domains (e.g., genes coding for aromatics transformation reactions), KEGG modules and reactions, genome-scale metabolic reconstructions, and PROKKA annotations. MAGs with similar functional traits were identified using K-means and hierarchical clustering, followed by ANOVA and chi-square tests for statistical filtering ( $p < 0.05$ ) and Spearman correlation ( $\rho \geq 0.5$ ). Correlation coefficients were used as edge weights to reflect the strength of association between features. Undirected, weighted networks were visualized in Cytoscape, with nodes representing clusters or features and edges reflecting strong co-occurrence. Network hubs were detected with the Markov clustering algorithm, and hubs sharing similar metabolic features were grouped into metabolic niches informed by known pathways.

Metagenomic sequencing and assembly yielded 127 MAGs (120 Bacteria, 7 Archaea), dereplicated into 52 species-level operational taxonomic units. We identified 49 distinct functional network hubs organized into seven metabolic niches, including aromatic compound degradation and carbon fixation niches. Most MAGs were linked to multiple niches, suggesting functional redundancy, while a few performed bottleneck functions (e.g., acetogenesis). Sulfate reducers formed the community's metabolic, mediating energy flow via acetate, formate, and hydrogen exchange.

Our approach not only resolves taxonomic and functional diversity but also illuminates microbial interactions and cooperation, providing a robust framework to define ecological niches using metagenomics.

## P-CM-007

### Supervised learning from protein embeddings enables targeted function discoveries

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The ever-expanding catalogue of uncharacterized microbial proteins - often referred to as *functional dark matter* - poses a major challenge for biotechnological and biomedical exploitation. Functional assessment of most proteins is hindered both by the limitations of annotation transfer based on sequence homology and by the widespread propagation of erroneous annotations in public databases. The common denominator of both issues is that conventional approaches for functional annotation, such as local alignments or profile-based methods (e.g. Hidden Markov Models), rely on sequence similarity. These can become unreliable below certain thresholds and can diverge substantially even at sequence identities around 70%.

To approach this challenge from a different perspective, we implemented a strategy for targeted function discovery using so called protein embeddings - sequence representations generated by protein language models (pLMs). Data sets of proteins representing a target function as well as non-target functions were used to train a range of supervised learning models. These machine learning and deep learning algorithms learn discriminative boundaries between the datasets in embedding space. The resulting ensemble models yielded interpretable prediction scores, enabling targeted exploration of large protein databases without relying on multiple sequence alignments or structural information, which are anyway scarce in the realm of uncharacterized proteins.

In this study we tested our pipeline for the discovery of novel hydrogenases. Models for the identification of previously unknown [NiFe], [FeFe], and [Fe] hydrogenases were trained and successfully predicted numerous previously unrecognized homologues missed by conventional sequence- or structure-based searches, thereby expanding the known hydrogenase diversity and revealing new candidate enzymes with potential biotechnological value. By bypassing database misannotations and the general limitations of sequence similarity, our framework enables robust and interpretable function discovery in large-scale microbial datasets.

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#### P-CM-008

##### **A FAIR-by-Design and Standard-Compliant System for Automated Submission of Microbial Genomes to the European Nucleotide Archive**

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The rapid expansion of metagenome-assembled genomes (MAGs) in environmental microbiology demands infrastructure that ensures rigorous standardisation, reproducibility, and efficient public archiving. Despite progress in metadata guidelines, submitting genome-resolved datasets to the European Nucleotide Archive (ENA) remains a major bottleneck, particularly for large-scale projects with heterogeneous sampling conditions.

We present the NFDI ENA Submission Tool, a FAIR-by-design platform that enables the construction, semantic validation, and automated packaging of metadata compliant with MxS Terrestrial specifications. The system performs ontology-aware structural checks (ENVO, CHEBI and NCBI Taxonomy), spatiotemporal normalisation (e.g., ISO-8601 timestamps, WGS84 coordinates), and constraint-based enforcement of required fields, minimising curatorial errors.

Its flexible architecture supports both individual and batch submissions, providing scalability across diverse project sizes. Future integration with ENA's programmatic APIs will enable fully automated data deposition workflows with job tracking and provenance preservation.

The tool has been operationally validated within the CLUE-TERRA consortium (CoLaborative mUlti-domain Exploration of TERRestrial metagenomes), a network spanning 13 institutions and nine countries. Across this initiative, more than 50,000 high-quality MAGs, reconstructed using MuDoGeR, were curated and prepared for deposition into ENA with minimal manual intervention, demonstrating both technological readiness and community impact.

By enforcing metadata standards at source and enabling scalable FAIR data publishing, the NFDI ENA Submission Tool enhances interoperability, accessibility and compliance of microbial genomic resources, positioning itself as an enabling technology for high-throughput environmental microbiology.

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#### P-CM-009

##### **Circularising metagenome-assembled genomes with JORG reveals complete Patescibacterial genome architecture and improved functional annotation**

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As most metagenome-assembled genomes (MAGs) are highly fragmented, refined phylogenetic and functional inferences remain limited. This issue is especially critical for genomes like those of Patescibacteria, an abundant yet understudied bacterial phylum with small, streamlined genomes, reduced metabolic capacity, and often symbiotic lifestyles. Standard short-read pipelines classify these MAGs as contaminants or low-quality bins. At the same time, long-read and hybrid approaches remain resource-intensive and prone to errors.

In this study, we used JORG, an iterative short-read assembly and binning tool, to circularise Patescibacteria genomes. We implemented pre- and post-filtering to enhance JORG's performance, evaluated its computational requirements for large-scale applications, created a Bioconda channel for easy installation, and integrated it into the Galaxy web server. We benchmarked JORG to determine the optimal contig counts and genome coverage at which it performs best. Finally, we screened over 11000 MAGs from marine and terrestrial environments for Patescibacterial genomes within these thresholds to attempt circularisation.

Out of 4454 Patescibacteria MAGs, 128 met criteria for JORG analysis based on contig count ( $\leq 10$ ) and coverage (25x - 300x). We circularised 60 MAGs (46.9%) and identified their origins or replication. These genomes were compared to their MAGs before circularisation by evaluating completeness and contamination. Circularisation improved genome completeness, with 46 of 60 (76.6%) MAGs showing increased completeness (paired t-test with a  $p < 0.001$ ). Analysis of functional annotations using KEGG, COG, Pfam, CAZymes, and GO revealed differences in annotations after circularisation. We aligned raw vs circularised MAGs to identify structural changes. Insertions or deletions were

found in 52 of 60 genomes (~86%), all in circularised assemblies. The average was 8 per genome, mostly small (<50 kb), indicating circularisation of trimmed or fixed low-confidence regions.

Our study highlights JORG's potential and limits in recovering elongated and complete genomes from short-read metagenomes. JORG works well for genomes under 1 Mbp, with fewer than 20 contigs, and with coverage from 30-300x. Circularised MAGs are a valuable approach for improving the general understanding of microbial dark matter. Our data show that the refined use of genomics to understand biodiversity and even biotechnological applications may require circularised genomes.

#### P-CM-010

##### **Mother Machine Analysis Platform (MMA) - A semi-automated, high-throughput pipeline for analysing microscopy time-lapse data**

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Quantitative analysis of microbial growth and cellular behavior is essential for advancing our understanding of bacterial defense systems, including anti-phage mechanisms. A widely used approach for obtaining single-cell data is the *mother machine* device, which enables precise control of the cellular environment. When combined with fluorescence microscopy, it allows detailed investigation of dynamic cellular processes under diverse experimental conditions. However, this workflow produces large volumes of data, creating a bottleneck that demands computational tools capable of high-throughput and reproducible image analysis. Conventional tools such as Fiji/ImageJ and other general-purpose image processing software often rely heavily on manual input. Segmentation and analysis tools that require command-line interaction are not easily accessible to biologists and typically lack integrated data management. A fully implemented analysis pipeline would provide greater automation, minimize manual intervention, improve consistency, and give researchers a more comprehensive overview of their data.

The Mother Machine Analysis Platform (MMA) is a versatile, user-friendly software solution designed to facilitate the analysis of time-lapse microscopy data from mother machine experiments. MMA streamlines the analysis pipeline by integrating metadata management, image preprocessing, and machine learning-based segmentation using tools such as *ilastik*<sup>1</sup>, *Omnipose*<sup>2</sup>, and *DeLTA*<sup>23</sup>. It further supports automated kymograph generation, providing intuitive visualization of temporal cellular dynamics and lineage relationships.

With broad compatibility for common microscopy formats (e.g., *.nd2*, *.tif*), customizable analysis parameters, and batch-processing capabilities, MMA enables rapid and reliable data processing with minimal manual intervention. By combining automation with flexibility, it serves as a robust platform for extracting high-resolution quantitative insights from microfluidic imaging experiments.

#### References

- 1) Berg S, Kutra D, Kroeger T, Straehle CN, Kausler BX, Haubold C, et al. *ilastik*: interactive

machine learning for (bio)image analysis. *Nat Methods*. 2019;16:1226–32.

- 2) Cutler, K.J., Stringer, C., Lo, T.W. *et al.* Omnipose: a high-precision morphology-independent solution for bacterial cell segmentation. *Nat Methods* 19, 1438–1448 (2022)
- 3) O'Connor OM, Alnahhas RN, Lugagne JB, Dunlop MJ. DeLTA 2.0: A deep learning pipeline for quantifying single-cell spatial and temporal dynamics. *PLoS Comput Biol*. 2022 Jan 18

#### P-CM-011

##### **The de.NBI Resource Center for Microbial Bioinformatics**

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The German Network for Bioinformatics Infrastructure – de.NBI is a national, academic, and non-profit research infrastructure. Within de.NBI, the Bielefeld-Gießen Resource Center for Microbial Bioinformatics (BiGi) supports scientists with bioinformatics software tools, pipelines and resources for microbial research with a focus on the analysis of microbial genomes and related datasets.

In the last decade, various software tools, pipelines and platforms for annotation, characterization and comparative analysis of genomic sequencing data have been developed in our group. For the comprehensive annotation of microbial genomes, the fully-automated annotation software tool **Bakta** was developed. Bakta is available as command line tool as well as via a user-friendly web version. Bakta quickly emerged to be one of the most-actively used annotation tools worldwide.

A further important project is the large-scale bacterial genome repository **BakRep**, which provides access to a comprehensive assembly and annotation data, enriched with taxonomic information, for a total of ~2.4 million genomes.

For the comparative analysis of bacterial genomes, we provide the tool **EDGAR** which offers custom, password protected databases with more than 100,000 included genomes. Furthermore, a new public database based on the Genome Taxonomy Database (GTDB) will be presented which increases the number of public projects from ~750 to >8000 and the number of publicly available genomes in the DB from ~20,000 to > 200,000.

Furthermore, a broad range of more specialized software tools were developed and maintained, e.g., **sORFdb** as a dedicated database for short open reading frames and small proteins as well as their functions in bacteria of different taxa, **Platon** for the detection of plasmid-borne contigs within draft genomes, and **ReferenceSeeker** for the taxonomic classification of microbial genomes.

Besides the described software and data resources, BiGi also hosts a branch of the federated de.NBI cloud, which provides large-scale compute resources to the scientific community on demand. Furthermore, BiGi offers training programs, workshops and user support to help the scientific community to use the various bioinformatics solutions effectively.

In summary, BiGi offers to the microbial researcher community:

- A broad set of well-maintained and constantly improved software solutions
- An accessible powerful and growing cloud-computing infrastructure
- Regular workshops and training courses

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## P-CM-012

### What\_the\_Phage 2.0: A Platform for Pre-Clinical Phage Characterization

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#### Introduction

Phages are a promising alternative strategy for treating multidrug-resistant infections. Analyzing genomic safety criteria and genetic features must be routine procedures before the widespread use of phages as a treatment method is approved. We initially developed "What the Phage" (WtP 1.0) in 2020 as a user-friendly and parallel multitool approach specifically for phage identification from sequencing data. The upgraded WtP 2.0 workflow integrates and runs multiple state-of-the-art bioinformatic tools across five additional categories: phage genome annotation, taxonomic classification, host prediction, phage lifestyle prediction, and essential prophage detection. Our parallel, multi-tool strategy mitigates the inherent differences in algorithmic performance among individual programs, ensuring robust, consensus-driven results for the user.

#### Methods

WtP 2.0 is currently in active development and is expected to be fully released in February 2026. The Workflow is written in Nextflow and utilizes Docker and Singularity containers for a simplistic workflow execution in any Linux environment. WtP automatically utilizes these containers, which means that the Installation of specific tools is no longer needed. All dependencies or databases are automatically downloaded. WtP is freely available on our test branch at [https://github.com/replikation/What\\_the\\_Phage/tree/backbone\\_rework](https://github.com/replikation/What_the_Phage/tree/backbone_rework), which includes a simple installation routine for Nextflow/Docker.

#### Results

While WtP is already a well-established, scalable, and easy-to-use workflow for phage identification, WtP 2.0 builds upon this foundation by integrating four tools for phage annotation, three tools for taxonomic classification, four tools for prophage detection, three tools for host prediction, and two tools for phage lifestyle prediction. The workflow supports both end-to-end analysis and modular task execution, giving

users complete control over their analysis pipeline. Moreover, multiple samples are computed in parallel, and if less hardware is available, WtP automatically decreases the parallelization.

#### Conclusion

WtP 2.0 is a highly robust and stable pipeline for the complete genomic analysis of phages. After a WtP 2.0 run, the user is provided with sufficient processed data (such as tool performance comparisons, taxonomic assessments, annotation maps, and comprehensive tables) to work reliably with the analyzed sequences.

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## P-CM-013

### Towards a holistic epidemiology of *Streptococcus agalactiae* using the BakRep repository

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*Streptococcus agalactiae* (GBS) is a bacterium well-known for causing neonatal infections in humans and is also a major pathogen in the dairy industry, responsible bovine mastitis. While GBS research has largely focused on human and bovine infections, this pathogen has a broader host range, infecting various animal species. Although numerous studies have examined GBS, most are limited to specific regions or outbreak events, providing only partial insight into the species' global population structure.

In 2024, the BakRep repository was introduced, featuring extensive genome characterizations based on 661,405 uniformly processed assemblies derived from all short-read sequencing data available in the European Nucleotide Archive (ENA) up to November 2018. Since then, this dataset has expanded to 2,438,386 assemblies, all uniformly processed, characterized, and made publicly accessible. With its extensive genomic and metadata collection, BakRep offers an unparalleled resource for exploring publicly available bacterial sequence data and gaining insights beyond individual studies.

In this study, we performed a large-scale comparative analysis of 37,970 GBS genomes included in BakRep v2. The isolates were characterized by MLST, capsular serotype, antimicrobial resistance (AMR) genes, and putative lineage-specific genes, complemented by metadata on geographic origin, host species, and disease context. This comprehensive approach enables the identification of current research trends, highlights well-studied GBS lineages, and uncovers areas where public data remain limited.

In summary, the analysis revealed substantial gaps in metadata, as most genome entries lacked information on location, year, host, and disease status. Despite this, some geographic and temporal variation in serotype and clonal complex (CC) distribution was observed, alongside strong associations between specific serotypes and CCs, suggesting the global spread of particularly successful clonal lineages. Additionally, the increasing prevalence of resistance genes such as *erm(A)* and *erm(B)* and that over 40% of genomes carried multiple resistance genes underscores an important emerging trend in antimicrobial resistance among GBS populations.

## P-CM-014

### The EDGAR platform for large-scale comparative genomics - Expanding into the ultra-high throughput era using GTDB as a taxonomically robust data source

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#### Introduction

EDGAR 3.5 provides precomputed orthology databases for more than 80,000 microbial genomes in public as well as private projects. The platform allows for the rapid identification of the differential gene content of related genomes, i.e., the pan genome, the core genome, or singleton genes. EDGAR provides a wide range of analyses and visualization features required for phylogenomic inter- and intraspecies taxonomic surveys and has recently implemented several features for the interactive exploration of functional categorization information based on the KEGG, COG and GO databases. Thus, the software enables a quick survey of genomic relationships and simplifies the process of obtaining new biological insights.

#### Objectives

Over the last decade, the number of genomes sequenced per year has increased dramatically, as has the need for available computational analysis capacity. Overall, sequencing output has outpaced analytical throughput, creating vast repositories of underexplored genomic information. As a consequence, the goal of recent EDGAR developments was twofold: First, to create the necessary infrastructure and accompanying back-end processes to accommodate a large, robust data source like GTDB and make it interactively examinable to the research community. Second, the continued development of the EDGAR frontend, creating new ways for scientists to investigate their genomes and relations between them.

#### Materials & Methods

The back-end for the calculation of orthologs and genomic subsets has been rewritten in Rust and is now deployed on an auto-scaling Kubernetes-Cluster. For the alignment workflow, BLAST was replaced by the much faster Diamond tool. Features for the visualisation and enrichment of functional categories and subcategories, the imbedding of KEGG and GO information into wider contextual nets and the comparison of annotated genomic clusters are now available or in closed testing.

#### Results

In the recent EDGAR version, functional category data was added for all genomes along with their respective visualization features. The technical infrastructure was further optimized for scalability, and will soon incorporate GTDB as its data source, increasing the amount of publicly available genomes from about 20,000 to 200,000. These developments ensure that EDGAR remains a convenient platform for comprehensive microbial gene content analysis in the ultra-high throughput era.

Our Web Server: <http://edgar3.computational.bio>

## P-CM-015

### Dynamic Visualization of Organism-Specific Metabolic Pathways

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Metabolic pathways are central to understanding the biochemical processes that sustain life, yet their reconstruction and visualization remain challenging. Existing resources are mostly static and manually drawn, limiting scalability and integration of organism-specific data. There is a strong need for computational tools that enable researchers to generate, explore and customize pathways interactively while adhering to FAIR principles – findable, accessible, interoperable and reusable – thereby supporting open science and enabling uptake by the community.

To overcome these limitations, we developed a dynamic, intuitive software platform for visualizing metabolic pathways, enabling exploration, manipulation, and direct access to detailed biochemical information. Built upon the curated BRENDA enzyme knowledgebase, the system employs a graph database architecture that enables automated visualization of organism-specific pathways through a hybrid layout approach that adapts existing algorithms.

The prototype delivers interactive reconstructions for 195 pathways across organisms from all domains of life, with particularly strong coverage across microbial taxa, supporting real-time customization and exploration. Users can dynamically reshape pathway layouts and investigate biochemical relationships through integrated access to reaction equations, protein information, references, and external links, enabling intuitive analysis and hypothesis generation. The underlying graph database will be made publicly available to support community-driven tool development.

We present a scalable, user-friendly platform for organism-specific pathway visualization that adheres to FAIR principles and addresses a critical gap in computational infrastructure by integrating curated data with interactive, research-ready tools. Future development will focus on omics data overlay and enhanced genome-level annotations to further support microbial systems biology.

## P-CM-016

### CnecH16-GEM: An enhanced standard-GEM of *Cupriavidus necator* H16 to guide strain and process design for gas fermentation

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#### Introduction:

*Cupriavidus necator* H16 is a gram-negative  $\beta$ -proteobacterium of industrial relevance, known for its ability to naturally produce polyhydroxybutyrate (PHB) and to grow lithoautotrophically on CO<sub>2</sub> and H<sub>2</sub>. This ability has increasingly proven popular as it poses the chance to establish carbon-negative bioprocesses to produce valuable products. In this context, constraint-based metabolic modeling serves as a powerful tool to predict maximum growth rates, estimate theoretical yields, and design strategies for metabolic engineering and strain optimization.

## Goals:

Our aim is to establish *C. necator* H16 as a host for producing biopolymers and pharmaceutical precursors in gas fermentation processes using CO<sub>2</sub> and H<sub>2</sub> as carbon and energy source, respectively. To facilitate the design of a suitable production chassis and process, we aimed to adapt the previously published genome-scale metabolic model iCN1361 (Pearcy et al., 2022) to predict phenotypes, flux distributions and production profiles under lithoautotrophic conditions.

## Methods:

iCN1361 is revised regarding stoichiometric consistency, thermodynamic constraints, biomass composition, blocked reactions, and energy metabolism. Continuous enhancement of the base model is enabled through the establishment of a reusable, modular, standard-GEM-compliant curation pipeline enabling community-driven refinement. Analysis of the model involves basic characterization of the solution space as well as network-wide thermodynamic analysis. Gas fermentation data will be used to validate and refine the model under lithoautotrophic conditions.

## Results and Conclusions:

Through systematic curation, iCN1361 has matured into the enhanced CneCH16-GEM which better reflects current knowledge and exhibits improved simulation capabilities under lithoautotrophic conditions. The model will continue to evolve through community-driven curation enabled by the standard-GEM framework and a Python-based pipeline that allows flexible data acquisition and ensures reproducible data integration via templates and reusable scripts. Current analyses highlight the central role of energy metabolism. In this context, thermodynamic analysis enables condition-specific simulation of ETC-routes and suggests potential targets for efficient redox-cofactor utilization. Ultimately, extension of the model towards a protein allocation framework allows more realistic simulation of cellular resource distribution and metabolic trade-offs.

### P-CM-017

#### Accelerating Microbial Genome Analysis Through Hash-Based Gene Annotation: Introducing AI-DB

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The exponential growth of microbial genome sequences has posed unprecedented challenges for computational annotation pipelines. Conventional annotation methodologies involve repeatedly analysing identical or highly similar gene sequences across millions of genomes, resulting in significant computational redundancy.

In order to address this bottleneck, we present **AI-DB** (Already Identified Database), a hash-based annotation service that accelerates the analysis of microbial sequencing data while ensuring data privacy and sovereignty.

**AI-DB** implements a multi-tiered annotation strategy that leverages pre-computed annotations from extensively characterized protein sequences. Predicted coding sequences are converted into MD5 hashes and queried

against a comprehensive hash database derived from UniRef protein clusters. These hash matches enable the instant retrieval of annotations, including functional classifications, database cross-references, and metadata, without the need for computationally expensive sequence alignments. This approach ensures that sequence information never leaves the researcher's infrastructure, as cryptographic hashes cannot be reverse-engineered. For sequences without hash matches, **AI-DB** transitions to classical alignment-based annotation using Diamond searches against curated reference databases. Newly characterized sequences can optionally be incorporated into the hash database to create a continuously expanding knowledge base.

**AI-DB** is implemented as a web service with a Rust backend for efficient hash lookups and a user-friendly web interface. Building upon the widely-used Bakta annotation framework, the service functions as both a standalone tool and as an acceleration layer for large-scale comparative genomics projects.

The hash-based approach offers several key advantages:

- (1) reduced computational time for well-characterized sequences,
- (2) data privacy ensured through cryptographic hashing, and
- (3) decentralized data storage, meaning sequence information remains with originating researchers.

**AI-DB** is freely available as part of the de.NBI Bielefeld-Gießen Resource Center for Microbial Bioinformatics.

### P-CM-018

#### Predictive Biocatalyst Selection for Metabolite Synthesis

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In the synthesis of new building blocks for pharmaceutical substances, biocatalysis offers a viable alternative to the conventional chemocatalytic methods. The high abundance, diversity and intrinsic chirality of enzymes make them a powerful tool for catalysing a wide range of chemical transformations. Despite their potential, current biocatalytic strategies often rely on empirical "brute-force" screening of extensive enzyme libraries. These approaches are typically time- and resource-intensive and depend exclusively on experimental data. With the advent of next-generation sequencing, high-throughput screening techniques and growing protein database availability, coupled with machine learning (ML), a shift towards data-driven enzyme engineering is emerging as a promising solution.

One application of biocatalysts is in synthesizing active pharmaceutical ingredients (API), the core components of drugs. Evaluating their metabolic degradation products for toxicity and biological activity is a critical step in the pharmaceutical development process. In this context, ML approaches offer the potential to identify promising biocatalysts which can be used for the *in vitro* synthesis of the desired metabolites by detecting latent patterns in



existing datasets. These models can predict enzyme–substrate compatibility and suggest novel or optimized enzyme variants resulting in a reduced experimental workload and accelerating the discovery of environmentally friendly biocatalysts.

This projects aims to use ML methods to select biocatalysts for synthesizing metabolites of next-generation therapeutic building blocks in a direct and selective manner compared to classical chemical approaches. We focus on the cytochrome P450 monooxygenase family, which comprises bacterial enzymes (bac CYP450) that catalyse a diverse range of substrates and play a central role in the biosynthesis of many natural products. Our goal is to create a benchmark dataset of bac CYP450 enzymes and their substrates to identify compatible enzymes for a given substrate. We created a high-quality dataset of enzyme–substrate/non-substrate pairs by combining extensive literature search, domain expertise, and different methods for sampling non-substrates molecules. Next, we intend to use our benchmark dataset to develop a robust ML workflow which is capable to predict compatible enzymes for a given substrate and to compare our framework with available prediction services. With this framework we aim to accelerate the discovery of biocatalysts and to increase our understanding of bac CYP450 enzymes in the biosynthesis of natural products.

## Environmental microbiology and ecology

### P-EME-001

#### Detection of the year-round succession of auto- and heterotrophic taxa in an Baltic Sea estuary monitored in high temporal resolution

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Due to their relevance for ecology and health, the detection and analysis of phytoplankton blooms is a central topic for aquatic biology worldwide. Available methods for the detection of blooming behaviour in time series data have limitations that make them unsuitable for the use with the novel and high amount of eDNA sequencing based data. Here, we present a simple method for the identification of bloom-like phenologies in a Baltic Sea time series data. At its core, it identifies change points using a simple statistical method and defines a bloom as a positive change in abundance followed by a negative change point in the relative abundance of an ASV. This way, our method implicitly handles missing data, irregular time series and possesses one open variable that can be used to determine its behaviour. The results of the change point bloom detection method are validated statistically and by using CARD-FISH.

Applying the change point bloom method to a 16S and 18S rRNA gene amplicon time series with high spatio-temporal resolution, we identify a bloom-like phenology in around a third of all ASVs present in the dataset that together form a year-round pattern of succession. Furthermore, we show that the method can be tuned to distinct kinds of bloom phenology using the open parameter. Taken together, our findings indicate that suitable detection methods for bloom-

like phenologies, coupled with high-frequency sampling, offer new perspectives for ecological research and biomonitoring.

### P-EME-002

#### When Microbes Meet Metal: Microbiologically influenced Corrosion in Offshore Wind Monopiles

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Offshore wind structures (OWS) must remain reliable for decades to ensure stable renewable energy production. However, microbiologically influenced corrosion (MIC) poses a significant threat to the long-term integrity of monopiles, particularly at the sediment–water interface. This study investigates how naturally occurring microbial communities contribute to carbon steel corrosion under conditions representative of monopile environments.

An in-house column system was established and inoculated with sediment and seawater from the North Sea. Controlled flow regimes were applied to mimic seabed hydrodynamics. Corrosion progression and biofilm development were characterized using molecular microbiological analyses, metabolomics, and 3D surface profilometry.

Distinct microbial and corrosion responses emerged under different hydrodynamic conditions. High-flow treatments were dominated by sulfur-oxidizing bacteria (SOB), which oxidize hydrogen sulfide to sulfate, reducing the persistence of corrosive sulfur intermediates near the steel surface. In contrast, low-flow conditions favored sulfate-reducing bacteria (*Desulfocapsaceae*, *Desulfonaceae*), associated with localized anoxia and moderate sulfide accumulation. Under static conditions, *Desulfovibrionaceae* prevailed, reflecting strict anoxia and high H<sub>2</sub>S concentrations that promote aggressive corrosion.

Surface analyses revealed treatment-dependent corrosion patterns. High-flow conditions produced the greatest variability and deepest pits, suggesting enhanced localized corrosion driven by turbulence and nutrient exchange. All microorganism-treated samples exhibited higher pitting depths compared to sterile controls, confirming the strong influence of microbial processes on corrosion intensity.

Our findings demonstrate that hydrodynamic conditions shape microbial community structure and biofilm-associated corrosion patterns, providing mechanistic insights into MIC in offshore settings. These results contribute to improving monitoring strategies and informing mitigation approaches for protecting OWS infrastructure.

### P-EME-003

#### Effects of Cyanobacterial Bloom Decay on *Vibrio vulnificus* Population Dynamics in Baltic Sea Microbial Communities

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Cyanobacterial blooms, particularly those formed by *Nodularia spumigena*, regularly occur in the Baltic Sea and play a pivotal role in shaping microbial community structures. Understanding the ecological consequences of the decay phase of such blooms is essential, as it can influence the growth and dynamics of opportunistic pathogens like *Vibrio vulnificus*, with implications for environmental health. This study explores how decay of *N. spumigena* blooms, especially through cell lysis, impacts the growth dynamics of *V. vulnificus* in Baltic seawater. The focus was on the nutrient release associated with bloom decay and its influence on bacterial abundance. A 12-day controlled laboratory experiment simulated Baltic seawater microcosms with varying concentrations of healthy and ultrasonically lysed *N. spumigena* cells. Total bacterial and *V. vulnificus* abundance were tracked using DAPI (4',6-diamidino-2-phenylindole) staining and droplet digital PCR targeting the *vvhA* gene, respectively. Dissolved (DOC) and particulate organic carbon (POC) levels were quantified to assess nutrient release. Lysed *N. spumigena* conditions produced elevated DOC (12.601 mg C/L) and POC (1.483 mg C/L) compared to controls. Total heterotrophic bacterial proliferation was significantly higher ( $p < 0.05$ ) in both healthy and lysed bloom conditions, especially at *Nodularia* concentrations between  $1 \times 10^4$  and  $1 \times 10^5$   $\mu\text{m/mL}$  (total filament length per mL), mostly at day 6. However, specific *V. vulnificus* bacterial proliferation was notably enhanced only in the lysed bloom at  $1 \times 10^5$   $\mu\text{m/mL}$ , with peak growth occurring from day 3. Minimum *N. spumigena* concentrations (a threshold concentration) of roughly  $1 \times 10^5$   $\mu\text{m/mL}$  were required for substantial *V. vulnificus* increases, demonstrating a close link between nutrient availability, especially DOC, and pathogen expansion. *N. spumigena* bloom decay, primarily via cell lysis, releases substantial organic nutrients that stimulate the growth of *V. vulnificus* in Baltic seawater. The study emphasized the need for further research on microbial growth dynamics in response to eutrophication and climate warming, contributing to efforts to mitigate the risks of marine pathogen proliferation in nutrient-rich environments.

#### P-EME-004

##### Sustainable Bioaugmentation using Microbial Consortia to Enhance Pharmaceutical Degradation in the Hyporheic Zone

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The increasing consumption of pharmaceuticals for therapeutic and preventive purposes has led to their persistent presence in aquatic environments, posing significant risks to ecosystems and human health. Commonly detected pharmaceutical compounds are metformin, an anti-diabetes drug, and guanyurea which was previously considered a dead-end metabolite of metformin. Thus, we developed and evaluated metformin and guanyurea degrading microbial consortia for bioaugmentation to enhance the biodegradation of pharmaceuticals in the hyporheic zone sediments (HZ) under controlled laboratory and field conditions. Enrichment of pharmaceutical-degrading microorganisms from HZ sediment was conducted in membrane bioreactors under oxic and anoxic conditions with nitrate. 16S rRNA gene amplicon analyses revealed *Nitrospira*, *Paracoccus*, *Rhodanobacter*, *Sphingomonas*, and *Zoogloea* as dominating genera. Such consortia stimulated

target pharmaceutical degradation in microcosms with HZ sediment matrix and river water as suggested by high-performance liquid chromatography. The developed consortia were further validated in U-shaped *in situ* mesocosms placed in the riverbed, thereby simulating natural flow paths in a defined setting. Consortia were inoculated into the sediment and highly preserved during pre-test field trials over one week as indicated by 16S rRNA gene amplicon analyses and qPCR. In the main experiment, *in situ* mesocosms were filled with inoculated or sterile sand, and inoculated or native sediment. Metformin degradation reached 16.08 nmol l<sup>-1</sup> min<sup>-1</sup> in inoculated sand and 6.86 nmol l<sup>-1</sup> min<sup>-1</sup> with inoculated sediment, significantly exceeding non-inoculated control matrices (5.38 nmol l<sup>-1</sup> min<sup>-1</sup> and 0.12 nmol l<sup>-1</sup> min<sup>-1</sup> for sand and sediment, respectively), whereas guanyurea degradation was generally very low across both matrices. Nevertheless, guanyurea exhibited a consistent removal trend in the inoculated matrices. Overall, these findings demonstrate the potential of enriched microbial consortia as a sustainable bioaugmentation strategy for efficient pharmaceutical removal, ensuring an environmentally compatible approach to safeguarding aquatic ecosystems.

#### P-EME-006

##### Paddy soil microbial community responses to seawater intrusion across the Nile Delta, Egypt

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Climate change-driven increase in sea level is expected to cause a significant loss in crop production in Egypt by 2100. 13% of the Nile delta is expected to be submerged under seawater with a 1 m increase in sea level. Seawater intrusion into agricultural lands increases soil and groundwater salinity. The effect of soil salinization on rice has been extensively studied. Nonetheless, the impact of salinization on soil microbial communities is poorly understood, even though microbes play a significant role in maintaining soil health and fertility.

In this study, we investigate how progressive salinization affects soil microbial communities" structure and biogeochemical cycling across a spatial gradient of seawater influence. Surface and groundwater-affected soils were sampled in triplicate from three sites in Damietta, Egypt, located at distances of 3 km (site A), 5 km (site B), and 27 km (site C) from the Mediterranean coastline during the rice harvest season (October 2025). Preliminary field measurements showed that surface soil in site A, expected to be heavily affected by seawater intrusion, had a relatively lower pH ( $\sim 6.2 \pm 0.3$ ) compared to the other two sites. Surface soil had temperatures ranging between  $\sim 27.4 \pm 0.3$  and  $29 \pm 2.1^\circ\text{C}$ , while groundwater-affected soils had lower temperatures ( $\sim 24\text{--}26^\circ\text{C}$ ).

Ongoing analyses include assessing physicochemical parameters (pH, electrical conductivity, total dissolved salts, potassium, sodium, total carbon, organic carbon, total nitrogen, total sulfur, phosphorus, and methane production potential) and soil texture, along with 16S rRNA gene metabarcoding (Illumina NovaSeq 6000) to determine microbial community diversity and structure. We are also using quantitative PCR of 16S rRNA, *mcrA*, *dsrB*, and *rpoB* genes to measure the absolute abundance of methanogens and sulfate reducers.

We believe that our data will provide insights on how paddy soil microbial communities are affected by seawater intrusion. By linking microbial community structure to soil physical and chemical parameters, our work will provide a microbial perspective on the resilience and vulnerability of the Nile Delta ecosystem under climate change-driven sea level rise.

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#### P-EME-008

##### **Spatial and Environmental Modulation of Microbiome in Ash Leaves**

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Forests are key for climate mitigation but are increasingly threatened by climate change and pathogen invasions, such as *Hymenoscyphus fraxineus* causing ash dieback in *Fraxinus excelsior* L. The plant microbiome can enhance host health and pathogen resistance, making the understanding of the ash microbiome crucial for effective management strategies. We investigated the leaf bacterial microbiome of *F. excelsior* across 12 regions in Germany to identify its core microbiome and major environmental and host factors shaping it. Leaf and soil chemistry, site parameters, and tree age were assessed for 30 trees of each forest plot. The microbiome of *F. excelsior* is characterized by a very high variety of genera variable between samples and regions. A total of 755 bacterial genera were detected, with *Hymenobacter*, *Massilia*, and *Sphingomonas* being predominant across all sites. Seven genera formed the core leaf microbiome of *F. excelsior*. Microbiome composition and network complexity varied significantly between sites and were mainly influenced by geographic position, precipitation, and leaf chemistry. Network analyses identified six hub taxa essential for local core microbiomes, indicating that less abundant taxa can play key structural roles. Our results demonstrate a consistent yet locally adapted ash leaf core microbiome, modulated by (a)biotic factors. Understanding these microbial networks may support strategies to enhance ash resilience against dieback disease.

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#### P-EME-009

##### **Marine bacterial foraging mechanisms shift with pulsed complex polysaccharide inputs**

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Polysaccharides, which constitute a high percentage of marine organic matter, are major substrates for marine microbial communities.

Heterotrophic bacteria can degrade polysaccharides by selfish uptake, in which a polysaccharide is bound, partly hydrolysed, and transported into the periplasm, minimizing diffusive loss. Or via external hydrolysis, producing low molecular weight hydrolysis products in the environment.

The balance between selfish uptake and external hydrolysis can determine the quantity of low molecular weight hydrolysis products available to fuel other members of microbial communities. This balance has been observed to vary widely in the ocean, but the underlying factors driving such differences are not yet well understood.

In part, the balance between external hydrolysis and selfish uptake may be influenced by substrate availability and structural complexity.

In order to investigate the effects of structurally-complex substrates on the balance between selfish uptake and external hydrolysis, we incubated surface and bottom water (depth > 4000 m) from a productive and an oligotrophic site in the North Atlantic Ocean with comparatively low levels of two complex polysaccharides, fucoidan and arabinogalactan, and compared results to unamended mesocosms. We measured selfish uptake and external hydrolysis of six different polysaccharides, and also tracked bacterial community composition.

External hydrolysis rates and patterns varied by station and depth. At the productive station, both amendments led to a broader spectrum of polysaccharide hydrolase activities in surface water, but not in bottom water. At the oligotrophic station, both amendments had little effect on external hydrolysis rates and only simple polysaccharides were degraded externally.

Selfish uptake was detectable for all polysaccharides at both stations, in all mesocosms. At the productive station, the fucoidan-amended surface water mesocosm showed an enhanced level of selfish uptake of fucoidan. This priming effect can be observed in the bottom waters of the oligotrophic station, which shows a lower selfish uptake overall.

The findings show the shift of foraging mechanisms with pulsed inputs of complex polysaccharides and underscore the complex interplay between environmental factors and microbial community dynamics in marine ecosystems.

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#### P-EME-010

##### **Decoding the functional role of the calcium ATPase YloB in microbially induced calcite precipitation and sporulation in *Solibacillus silvestris***

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Calcium homeostasis is critical for bacterial physiology, but its regulation in specialized processes such as microbially induced calcite precipitation (MICP) and sporulation remains poorly understood. We investigated the sole annotated P-type calcium ATPase in *S. silvestris*, YloB, previously found to be upregulated under MICP conditions. Structural modeling confirmed that YloB harbors the conserved domains and motifs characteristic of calcium-binding P-type ATPases. Surprisingly, deletion of *yloB* did not impair growth under high calcium conditions nor reduced MICP yields, indicating that YloB is dispensable for calcium detoxification and biomineralization. In contrast,  $\Delta yloB$  cells formed only partially dehydrated spores with drastically reduced heat resistance, a phenotype fully rescued by complementation. Fluorescent YloB-mNeonGreen fusions localized specifically to the forespore membrane during early engulfment and

persisted throughout sporulation, while a *PyloB-msfGFP* reporter showed that *yloB* transcription was induced early in sporulation but unresponsive to calcium. These findings reveal that *YloB* does not function in global calcium homeostasis but specifically pumps calcium into the forespore to support Ca-dipicolinic acid accumulation and proper spore maturation. Our work uncovers a specialized role for a bacterial calcium ATPase in sporulation, distinguishing calcium transport for endospore development from biomineralization.

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## P-EME-011

### Microbial Necromass Decomposition Dynamics and Pathways in Soil Enrichment Cultures

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Soils are among the most microbially populated habitats on Earth. Yet a large fraction of this microbial matter is present as necromass, consisting of non-viable cellular biomass and extracellular compounds. Microbial necromass is a reservoir of carbon and macronutrients, sustaining soil organic carbon cycling and supporting ecosystem functioning. Understanding how soil prokaryotic communities recycle necromass is essential for assessing its stabilization and long-term persistence in soils.

Our study examines the metabolic functions and dynamics of soil enrichment cultures in decomposing microbial necromass macromolecules under contrasting redox conditions. Aerobic and anaerobic soil microorganisms from a temperate deciduous forest (New England, MA, USA) are enriched on individual macromolecules (carbohydrates, lipids, nucleic acids, and proteins) as the primary carbon and energy sources.

We implement stable carbon isotope labeling to track carbon fluxes into gaseous and aqueous pools, quantify transformation products, estimate turnover rates, and determine the potential half-lives of the different macromolecules. Furthermore, cell counts, fluorescence *in situ* hybridization, 16S rRNA gene metabarcoding, shotgun metagenomics, and protein-based stable isotope probing will provide insights into growth dynamics, microbial lineages, metabolic traits, and secretomes of populations associated with macromolecule decomposition.

We hypothesize that the specific necromass macromolecules and the underlying redox conditions select for distinct microbial populations with substrate-specific functional traits that shape microbial growth, as well as the spectrum of metabolic intermediates and products. Here, we present initial insights into community differentiation and growth dynamics from our enrichment cultures, which show up to a two-fold increase in cell abundance relative to unamended controls and distinct CO<sub>2</sub> productions depending on the macromolecule substrate. Together, this study will contribute to our understanding of the microbial mechanisms that potentially contribute to carbon sequestration.

## P-EME-012

### Microplastic–Microbiome Interactions in the MyBioScope Bioreactor: A Multi-Parameter Analysis of Metabolic and Taxonomic Shifts

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Microplastic particles (MPs), defined as plastic fragments <5 mm, are increasingly detected in the human body and linked to various health risks. While animal studies suggest MPs can alter gut microbiota, their impact on the human gut microbiome remains poorly characterized. Understanding MP–microbiome interactions is critical for assessing potential health consequences, especially those related to metabolic and inflammatory pathways.

This study aimed to investigate how five common MP types, polystyrene (PS), polypropylene (PP), low-density polyethylene (LDPE), poly(methyl methacrylate) (PMMA) and polyethylene terephthalate (PET), affect gut microbial composition and metabolism, using commercially available beads and cryogenically fragmented particles from daily-use plastics to increase environmental relevance.

Our *ex vivo* bioreactor model MyBioScope was used and inoculated with stool samples from healthy donors. Cultures were maintained anaerobically for five days with daily nutrient addition. MPs were added at concentrations mimicking estimated human exposure and at elevated doses to assess dose-dependence. The methods were adapted to accommodate the plastic setup, including optimization of the agitation speed and selection of appropriate control particles. Technical read-outs included continuous measurements of pH and daily total and viable bacterial cell counts. Microbial composition was analysed using 16S rRNA gene sequencing, and metabolic changes were assessed through targeted metabolomics. Plastic-type-specific effects were evaluated using linear mixed effect models and correlation analyses.

Only PMMA, but not the other plastic types, increased total or viable bacterial counts. All MP-treated cultures showed a consistent pH decrease, indicating altered microbial metabolism. Sequencing revealed plastic-type-dependent shifts in microbial composition, particularly within the phylum Bacillota. Metabolomic analysis identified changes in 5-aminopentanoic acid, valeric acid and lactic acid, some correlating with pH shifts.

This study demonstrates that MPs can modulate gut microbial metabolism and composition without affecting overall bacterial abundance in short term. The bioreactor model enabled controlled, reproducible analysis of MP–microbiome interactions, revealing plastic-specific effects on microbial function. These findings highlight the need for further investigation into chronic MP exposure and its potential role in disease development.

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## P-EME-013

### Microbial Diversity and Community Shifts in a Petroleum Reservoir under Production: Effects of Water Breakthrough and Anthropogenic Alterations

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Subsurface petroleum reservoirs host indigenous microorganisms that survive extreme conditions and long-term isolation. Microbial activity in these environments can contribute to adverse effects such as oil biodegradation and reservoir souring. Unlike the broader deep biosphere, oil reservoirs are frequently subjected to anthropogenic disturbances, particularly during production, when processes like water injection introduce external microbes and electron acceptors.

In this study, we investigated microbial diversity, community structure, and the impact of water breakthrough using 16S rRNA gene and metagenomic sequencing of produced fluids, production water, and injection water samples from the Edvard Grieg oil reservoir offshore Norway.

We found clear regional heterogeneity in community composition, characterized by overall low diversity, dominated by thermophilic, anaerobic, and halotolerant taxa. The southern region (wells A13, A17, A18, and A19) exhibited lower diversity, while the microbial community composition of well A07 showed a distinct signature. The prevailing genera included the strictly anaerobic bacterium *Thermoanaerobacter* and the hyperthermophilic archaeon *Thermococcus*. Water breakthrough triggered shifts in community structure, not because of widespread replacement by injected microbes, but due to the increase in sulphate-reducing bacteria. Comparison of sequence data from production fluids and water samples enabled the identification of microbial signatures that can serve as cost-effective tools for monitoring oil reservoir processes and integrity.

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## P-EME-014

### Microbiota and microbial transmission dynamics associated with bramble (*Rubus spp.*) flowers

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Microbes are functionally essential players for both plant and insect health. As plants interact ubiquitously with insects in diverse ecological systems, both beneficial and pathogenic microbes are frequently transmitted between them, and one of the primary microbial transmission routes is via pollination. Plants that host diverse pollinators are considered hubs within plant-insect networks, and these highly visited hub plants may also serve as key nodes for the transmission of microbes. However, the microbial transmission dynamics via

flowers remain insufficiently studied. In this study, we characterized the floral microbiota of bramble (*Rubus spp.*), a plant that produces highly nutritious pollen and is visited by a diverse group of insects. By comparing open flowers, insect-excluded bagged flowers, and flower buds, we aimed to better understand the microbial transmission processes.

Bramble flowers, including open flowers, bagged flowers, and flower buds, were collected around the Giessen area in July 2024. Bacterial and fungal microbiota composition was examined by Ion Torrent sequencing of the 16S rRNA gene (V4-V5) and fungal ITS2 from the metagenomic DNA. Microbial load and diversity analysis, functional annotation, differential abundance analysis, and co-occurrence network analysis were performed in R, SpeSpeNet, and Cytoscape.

Our results showed that bramble flower buds had the fewest microbial genera and also the fewest unique genera among the flower groups, and flowering and insect visitation significantly altered floral microbiota structure and microbial load. Insect visitation enriched fermentative and probably also pathogenic bacteria, including *Spiroplasma*. While bagged flowers exhibited distinct microbiota structure and more internally connected microbial networks, open flowers displayed greater within-group variation and more centralized networks, suggesting that insect visitation may function to stabilize the existing microbial community structure, despite its potential to introduce diverse bacteria to flowers. In addition, we identified several hub taxa in the microbial network that may help consolidate existing plant-pollinator interactions and contribute to microbiota structure stabilization.

In conclusion, our findings highlight the dominant role of insect visitation in shaping floral microbiota and underscore the ecological significance of hub plants in microbial transmission across plant-pollinator networks.

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## P-EME-015

### Correlating FISH techniques and SEM for exploring ecophysiology of uncultivated microbes

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Not-yet-cultivated microbes are abundant in oligotrophic environments such as sediments, cave deposits, and groundwater, yet their spatial organization and morphological diversity remain poorly resolved. While metagenomics reveals taxonomic and functional potential, there exists a major disconnect between sequencing data and electron-microscopy based imaging. On the other hand, FISH allows the localization of specific nucleic acids often inferred from sequencing data but lacks the ultrastructural resolution. Recently, we developed a correlative workflow to visualize virus-host associations in natural microbial communities using virusFISH combined with scanning electron microscopy (SEM) (Banas *et al.* 2023, ISME J), yet its applicability to other ecosystems and combination with other FISH techniques remain unexplored. Here, we extend this correlative FISH-SEM strategy beyond virus detection and visualize bacterial and archaeal cells using CARD-FISH, aiming to provide a versatile and broadly applicable method for studying diverse uncultured microorganisms directly in

environmental samples. Our goal was to adapt the workflow to different matrix types, improve reproducibility across challenging low-biomass systems, and establish an approach that is accessible to microbial ecologists without the need for specialized correlative light and electron microscopy (CLEM) infrastructure. Environmental samples including groundwater biofilms, cave deposits, and sediments were fixed, hybridized with CARD-FISH probes, and processed for SEM imaging. We optimized FM sample processing and imaging for downstream SEM. Correlating fluorescence micrographs with electron micrographs enabled the assignment of phylogenetic identity to morphologically preserved cells. The adapted workflow reliably distinguished archaeal and bacterial cells *in situ* across different environmental matrices. Spatial cell arrangements remained stable during SEM preparation, and morphological preservation enabled detailed visualization of microbial structures. This work establishes a correlative CARD-FISH–SEM workflow for detection and visualization of prokaryotic microorganisms, enabling growth-independent detection and spatially resolved identification of uncultured bacteria and archaea. This powerful tool for microbial ecologists will bridge sequence-based community data with *in situ* structural and ecological context.

#### P-EME-016

##### Environmental Monitoring of Aquaculture Effluents: Impacts on Microbial Communities and Antibiotic Resistance Genes in Water, Sediment and Algae

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Aquaculture is expanding globally to meet rising food demands of a growing population. However, inappropriate antibiotic use beyond therapeutic purposes in aquaculture raises concerns about antimicrobial resistance, particularly in pathogenic bacteria. This poses risks to aquatic life, environmental health, and ultimately humans.

This study presents an exploratory approach to assess the environmental impact of an aquaculture farm in Brazil as a case study, given the country's rapid aquaculture growth but limited antibiotic regulation. The goal was to investigate downstream effects on microbial communities in water, sediment and algae, focusing on taxonomic composition and microbial resistance.

The aquaculture farm located in Cravinhos, São Paulo, comprises open ponds stocked with the hybrid fish Patinga (*Piaractus mesopotamicus* × *Piaractus brachipomus*). The ponds are supplied and drained by the Tamanduazinho River, which flows through rural settlements, local facilities, and agricultural areas before reaching the farm. Water, sediment, and algae samples were collected along a spatial gradient—from an upstream control site, through the fish pond outlets, to locations up to 6 km downstream and analyzed using metagenomics short read based sequencing. Data was processed with SqueezeMeta (v1.6.5), followed by descriptive and multivariate statistical analyses in R (v4.4.2).

Results revealed distinct shifts in both water and algae samples collected at effluent outlets and further downstream.

These shifts were characterized by increased abundances of potentially pathogenic bacterial families (*Flavobacteriaceae*, *Enterobacteriaceae*, *Mycobacteriaceae*). A variety of resistance genes—including multidrug resistance genes (*emrB*), specific antibiotic resistance genes (*tetA*, *otrB*), and heavy metal resistance genes (*czcA*)—accumulated downstream, particularly in water and algal samples. Some of these genes were associated with potentially pathogenic taxa such as *Pseudomonas* and *Aeromonas*.

This study reveals downstream effects of aquaculture on microbial community composition and the spread of resistance genes in adjacent freshwater environments. Algae, as non-target organisms, emerged as potential novel hotspots for resistance gene dissemination. These findings highlight potential risks to environmental, animal, and human health, emphasizing the need to evaluate and manage veterinary antibiotic use through a One Health approach.

#### P-EME-017

##### Differences of Flavobacteria in glycan degradation of free-living and particle-attached bacteria during a spring phytoplankton bloom

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Marine phytoplankton (microalgae) account for about 50% of the global photosynthetic carbon fixation [1], most prominently during massive phytoplankton blooms. These blooms release substantial amounts of polysaccharides, a significant portion of which undergo rapid remineralization by heterotrophic bacteria. Among these bacteria, *Bacteroidota*, specifically *Flavobacteriia*, demonstrate considerable potential to degrade various algal polysaccharides [2]. We investigated the diversities, activities, and functional potentials of polysaccharide-degrading bacteria across different size fractions during a diverse spring phytoplankton bloom at Helgoland Roads (southern North Sea) at high temporal resolution with microscopic, physicochemical, biodiversity, metagenome, and metaproteome approaches [3]. Particle-attached bacterial communities exhibited a notably greater diversity compared to free-living bacterial communities. The top five abundant flavobacterial genera in free-living (0.2 - 3 µm) bacterial communities comprised *Aurantivirga*, the NS4 and NS5 marine groups, *Cd. Prosilicoccus*, and "Formosa". In contrast, *Polaribacter*, the NS9 and NS5 marine groups, *Algibacter*, *Cd. Prosilicoccus* and *Tenacibaculum* dominated *Flavobacteriia* in particles (3-10 µm and >10 µm). A total of 305 species-level metagenome-assembled genomes (MAGs) were obtained, with 43 belonging to *Flavobacteriaceae*. Particle-attached MAGs, on average, featured larger MAGs with higher proportions of polysaccharide utilization loci. These loci were predicted to target a broad spectrum of polysaccharide substrates, from readily soluble, simple-structured storage polysaccharides

(e.g., laminarin,  $\alpha$ -glucans) to less soluble, complex structural or secreted polysaccharides (e.g., xylans, cellulose, pectins). Our findings suggest that numerous active and abundant flavobacterial clades played a pivotal gatekeeping role in the solubilization and subsequent degradation of various essential classes of algal glycans.

## References

1. Falkowski PG, Barber RT, Smetacek VV. Biogeochemical controls and feedbacks on ocean primary production. *Science*. 1998;281(5374):200-6.
2. Teeling H, Fuchs BM, Bennke CM, et al. Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms. *eLife*. 2016;5:e11888.
3. Wang FQ, Bartosik D, Sidhu C, et al. Particle-attached bacteria act as gatekeepers in the decomposition of complex phytoplankton polysaccharides. *Microbiome* 12, 32 (2024).

## P-EME-018

### Integrating microbiome responses across warming experiments in coastal marshes

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Coastal marshes are effective carbon sinks, because soil microbial activity is often low under flooded oxygen-deficient conditions. Previous *ex-situ* warming experiments at the Climate Change Marsh Mesocosm Facility (CCMMF) in Hamburg, Germany, showed that warming can alter soil microbial communities, but responses vary with environmental context. Building on these recent findings, we will expand our analysis by microbial 16S rRNA gene sequencing data sets from two *in-situ* coastal marsh warming experiments: MERIT ("Marsh Ecosystem Response to Increased Temperatures") in northern Germany and SmartX ("Salt Marsh Accretion Response to Temperature eXperiment") in a brackish marsh on Chesapeake Bay, USA. By this we combine genetic microbial data of coastal marshes from different ecosystem age, vegetation type, and tidal regime. Using multivariate analysis that accounts for the compositional structure of sequencing data, we will assess whether warming-induced microbial shifts are consistent across coastal ecosystems or instead shaped by local conditions. Based on previous results, we expect microbial responses to experimental warming to vary strongly with vegetation composition and ecosystem age. With this meta study we aim to identify key factors controlling microbial responses to increased temperatures to better understand how climate change reshapes microbial composition and thereby carbon dynamics in coastal wetlands.

## P-EME-019

### Effects of stressor hierarchies on river microbial communities during degradation and recovery from drought

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Anthropogenic influences are increasingly threatening urban river systems by exposing aquatic life to stronger and more frequent occurring stressors, such as temperature and salinity increase, ultimately leading to the deterioration of the ecological status of urban rivers. Our previous research suggests that a hierarchy among stressors exists, which means that stressors such as temperature and salinity exert their effect on biota once the limitation from the superior stressor fades away. This superior stressor could be energy availability in the form of organic carbon. Here, we want to investigate this stressor hierarchy hypothesis using microbial communities from the sediment of an urban river in Germany. Energy availability can be modulated by manipulation of hyporheic flow velocity and acetate supplementation while salinity (0-7 g/L) serves as the secondary stressor. The stressor impact on sediment microorganisms will be evaluated by consideration of *in situ* microbial activity (CO<sub>2</sub> production, O<sub>2</sub> depletion), growth of cell numbers, acetate and dissolved organic carbon depletion, and compositional change of the microbial community (16S rRNA sequence analysis).

## P-EME-020

### Does future climate and agricultural farming system affect the fungal plastisphere of different biodegradable plastics at the early stage of field degradation?

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The use of biodegradable mulch films has an advantage over non-biodegradable ones, as it offers degradation by microbes under environmental conditions. Nevertheless, less is known about the microbial colonization of different biodegradable plastics under different agricultural systems and climate change conditions. In the current study, the plastic degradation experiment was conducted at the Global Change Experimental Facility platform, specifically in conventional and organic farming systems, both under ambient and future climatic conditions. In this study, we investigated the early fungal colonizers associated with polybutylene-succinate (PBS) and polybutylene adipate-co-terephthalate (PBAT) with polyethylene (PE) as a reference in comparison to the initial soil fungal community. We found a distinct pattern between soil and plastisphere fungi. Soil fungi were dominated by Sordariomycetes (mainly *Gibellulopsis*, *Fusarium*, and *Gibberella*), and fungi in plastics were dominated by Dothideomycetes (mainly *Mycosphaerella*, *Alternaria*, and *Cladosporium*). These microbes were previously reported as plastic colonizers and potential plastic degraders. We found that agricultural systems affect both fungal richness and community composition of the plastisphere. Plastic type significantly affected the fungal richness, but not the fungal community composition. The two different agricultural systems undergo different treatments, including crop rotation and fertilization, which in turn impact the fungal colonization of the biodegradable plastics. This study provides new insights into factors that affect early fungal colonization of different biodegradable plastics under real field conditions using high-

throughput sequencing. These data are of high relevance to evaluate the plastic composition for adjusted rate of plastic biodegradation for upcoming mulch film products.

#### P-EME-021

##### Plant Growth Promoting Bacteria-Driven Root Community Assembly Shapes Seed Metabolites of Wheat in a Hydroponic Vertical Farming System

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**Introduction:** With a growing global population, sustainable food production is essential. Vertical farming offers a promising solution, enabling year-round cultivation with higher yields and up to 90% reduced water usage. However, plant-microbe interactions in these systems remain underexplored. Plant growth-promoting bacteria (PGPB) can enhance crop performance by stimulating plant productivity, shaping root and seed microbiomes, and suppressing pathogens<sup>1</sup>.

**Goals:** We studied how PGPB inoculation affects microbial community assembly, yield, and grain quality in wheat grown under vertical farming conditions.

**Materials & Methods:** We analyzed microbial communities, metabolites, plant nutrients and yield in treatments with *Bacillus velezensis* FZB42, *Pseudomonas* sp. RU47, or a mixture of both, and compared it to an autoclaved inoculum and non-inoculated control. Root samples for community analysis were collected before treatment application, 24 hours after inoculation and before harvest. DNA was extracted from roots and seeds by phenol-chloroform extraction. The V4 region of the 16S rRNA gene was targeted for metabarcoding using Illumina MiSeq. Taxonomic assignment was done with the SILVA database, data were pre-processed using the DADA2 pipeline, and normalized using cumulative sum scaling. All statistical tests and further analysis were conducted using dedicated R packages. Wheat seed metabolite profiles were analyzed using UPLC-ESI-TOF MS with simultaneous acquisition of low- and high-collision energy mass spectra (MS<sup>e</sup>). Obtained data were processed in Progenesis, and metabolites were tentatively identified by comparing MS fragments with an in-house database.

**Results:** Both PGPB strains successfully colonized wheat roots and altered the associated microbial communities, leading to a more deterministic community assembly. These shifts were mirrored in the seed metabolite profiles, with distinct clustering of treatments in the untargeted metabolomics data. The combined inoculation caused the strongest changes in seed secondary metabolism, while yield and grain nutrient content remained unaffected.

**Summary:** Microbiomes can be successfully established in hydroponic systems, where they influence plant secondary metabolism. These findings highlight the potential of targeted microbial applications to improve vertical farming systems and contribute to sustainable food production strategies.

**Reference:** 1 <https://doi.org/10.1038/s41598-025-16204-0>

#### P-EME-022

##### Characterization of exopolysaccharide formation of *Segatella bryantii*

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Ruminants and their microbiome are largely in a close symbiotic relationship with each other. The microbial community benefits from a constant supply of nutrients, particularly carbohydrates and proteins derived from the host's diet, while providing the host with fermentation end products such as short-chain fatty acids (SCFA)<sup>1,2</sup>. Among the most abundant members of the ruminant microbiome are bacteria belonging to the family Prevotellaceae<sup>1</sup>. Members of this family are known to play a key role in carbohydrate metabolism and rumen homeostasis<sup>3</sup>.

In the past, the ionophore antibiotic monensin has been used as a growth promoter in cattle production. Previous studies have focused on the influence of monensin on SCFA profiles and exopolysaccharide (EPS) formation in the model organism *Segatella bryantii*, a representative of Prevotellaceae<sup>4</sup>. EPS production is a well-known microbial strategy to withstand environmental stressors, e.g. fluctuations in pH, osmotic pressure, and antimicrobial compounds<sup>5</sup>. However, despite the long-standing recognition of this adaptive mechanism, the molecular pathways and structural diversity of EPS within the Prevotellaceae family remain poorly understood.

In this study, EPS formation in *S. bryantii* and related Prevotellaceae was investigated using a combination of complementary microscopic techniques (CLSM and SEM), alongside biochemical analysis of the EPS. These approaches allowed a detailed characterization and visualization of the EPS matrix, its spatial organization, and its response to monensin exposure. The results provide new insights into the morphological characteristics and potential protective functions of EPS in Prevotellaceae, highlighting their role in microbial resilience within the rumen ecosystem.

#### References

1. Deusch, S. *et al.* A Structural and Functional Elucidation of the Rumen Microbiome Influenced by Various Diets and Microenvironments. *Front. Microbiol.* **8**, 1605 (2017).
2. Ungerfeld, E. M. Metabolic Hydrogen Flows in Rumen Fermentation: Principles and Possibilities of Interventions. *Front. Microbiol.* **11**, 589 (2020).
3. White, B. A., Lamed, R., Bayer, E. A. & Flint, H. J. Biomass Utilization by Gut Microbiomes. *Annu. Rev. Microbiol.* **68**, 279–296 (2014).
4. Trautmann, A. *et al.* A shift towards succinate-producing *Prevotella* in the ruminal microbiome challenged with monensin. *Proteomics* **23**, 2200121 (2023).



5. Netrusov, A. I. *et al.* Exopolysaccharides Producing Bacteria: A Review. *Microorganisms* **11**, 1541 (2023).

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#### P-EME-023

##### Overlooked Giants: The Widespread Presence of Giant Bacteria in Gut Microbiome

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Giant bacteria greatly exceed the dimensions of ordinary bacteria, with some reaching centimeter-scale lengths, and exhibit extreme polyploidy containing hundreds to tens of thousands of genome copies per cell. Such extreme polyploidy is thought to enable them to adapt to diverse environmental conditions, overcome diffusion limitations, and sustain complex life strategies. *Epulopiscium* spp., (or *Epulonipisium*) first discovered in tropical fishes, are bacteria with several giant morphotypes, some reaching up to 800 µm in length and 80 µm in diameter. *Epulopiscium* harbors tens of thousands of chromosome thought to be released into the host gut in a coordinated manner every day, to be partially incorporated by the germinating spores to eliminate the potential for the Müller ratchet effect. Although *Epulopiscium* spp. have been reported in association with tropical surgeonfishes, and have been sporadically witnessed in other fish, their actual host range and biogeographical distribution have not been systematically investigated. We integrated publicly available 16S rRNA gut microbiome datasets from NCBI and confirmed the presence of *Epulopiscium* spp. across a wide range of animals, including house companions, zoo, and wild animals. In some cases, *Epulopiscium* made up more than 20 % of the total sequences. Interestingly, among house pets Ferrets had the highest sequence abundance of *Epulopiscium*, while the genus was nearly absent from cats. Lambs and chickens had the highest abundance among farm animals, and were as high as zoo animals with showed little variation between different species. Our findings expand the known host range of *Epulopiscium* spp. beyond tropical fishes and uncover the wider occurrence of giant bacteria in natural environments. This study suggest that pre-filtering gut samples for debris removal, may remove significant members of the community. By showing the presence of giant bacteria in animal gut, we fill an important gap in gut microbiome research and provide new insights into host-associated ecology.

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#### P-EME-024

##### Monitoring and reporting on antimicrobial resistance (AMR) in surface waters

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#### Introduction

The occurrence and proliferation of antibiotic-resistant bacteria (ARB) and the transmission of resistance through gene transfer can be of great importance to human health. However, the role of the environment, particularly the introduction of ARBs and antibiotic resistance genes (ARG)

into surface waters, is not yet fully understood within the One Health framework, necessitating further research in this area.

#### Goals

Therefore, it is important to evaluate technical process chains regarding ARG and ARB retention and develop a well-founded data basis for the occurrence of AMR in comparison to the general bacteria load in the different seasons of the year, investigating different sampling sites in Germany. The species which are relevant in the monitoring are clinically representatives of ESBL-producing Enterobacteriaceae and VRE-producing enterococci [1].

#### Methods

Both clinically relevant representatives of ESBL-producing Enterobacteriaceae and VRE-producing enterococci were examined on CHROMagar™ ESBL and CHROMagar™ VRE media. Furthermore, the positive colonies were analysed according to EUCAST (European Committee on Antimicrobial Susceptibility Testing) resistance profile. For the quantification of total bacteria abundance qPCR methodology (16S rDNA gene) was used and specific resistant gene levels (bla-KPC, bla-TEM, bla-CTX, ermB, vanA, aadA1, intl) were determined.

#### Results and conclusion

The current studies are still in progress to achieve a comprehensive data basis on AMR in surface waters. We successfully developed a harmonized methodology encompassing monitoring objectives, sampling locations (surface water, rivers downstream of waste-water-treatment points), target indicators (*E. coli*, ESBL-*E. coli*, coliforms bacteria, ESBL-coliforms bacteria, enterococci, VRE-enterococci and eight core ARGs with optional additions), and analytical methods (culture-based and qPCR).

In conclusion, monitoring AMR in surface waters using reliable and robust methods is of great importance to establish environmental quality criteria for AMR abundance. Furthermore, the present studies show valuable insights into the challenges and complexities of environmental AMR surveillance.

#### References

- [1] Exner, M, Schwartz, T, Schmithausen, R. 2020. Synthese- und Abschlussbericht "Hygienisch-medizinische Relevanz und Kontrolle Antibiotika-resistenter Krankheitserreger in klinischen, landwirtschaftlichen und kommunalen Abwässern und deren Bedeutung in Rohwässern"

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#### P-EME-025

##### The fate of phytate: diversity and distribution of bacterial phytase producers and inositol degraders

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Phytate (or phytic acid, InsP6) is the main storage form of phosphorus and minerals in plants and is present in plant tissues such as bran and seeds of legumes and cereals, including oil seeds and nuts. Its stepwise dephosphorylation

to *myo*-inositol (*myo*-Ins) is catalyzed by phytases, a group of enzymes found across bacteria, fungi, plants, and animals. Despite the ecological and agricultural relevance of this process, a comprehensive survey of bacteria contributing to phytate metabolism - and thus to the global phosphorus cycle - remains lacking. We conducted a large-scale analysis of all 115,379 validated bacterial genome sequences currently available in public databases to resolve the distribution and diversity of phytase-producing bacterial species in environmental and intestinal habitats. Phytase genes were identified in 6,281 out of 25,105 species (25%), including 2,330 species that also possessed the genetic potential to utilize *myo*-Ins. This co-occurrence suggests a selective advantage of linking the two metabolic properties. Phytases were predominantly found in members of the phyla Pseudomonadota, Bacteroidota and Cyanobacteriota, but were comparatively rare in Bacillota and Actinomycetota. Phytase genes were present in 28% of food- and rhizosphere-associated bacteria, 15% of rumen species, and approximately one-fifth of human and swine gut commensals. Notably, numerous keystone members of the gut microbiota, along with enteropathogenic species, were identified as phytase-positive, pointing to a significant functional role of intestinal bacteria capable to dephosphorylate phytate. Together, these findings highlight an unexpectedly widespread bacterial potential for phytate dephosphorylation, suggesting that microbial phytase activities play a crucial role in environmental and host-associated phosphorus flux. The inositol catabolic gene cluster (*lolCatGC*) for *myo*-Ins degradation was detected in 7,384 (29.8%) of all species for which genome sequences were available. *lolCatGC*-positive species were particularly found among Actinobacteria and Proteobacteria, and to much lesser extent in Bacteroidetes. *lolCatGC* are very diverse in terms of gene number and functions, whereas the order of core genes is highly conserved on the phylum level. We predict 111 animal pathogens, more than 200 commensals, and 430 plant-pathogens or rhizosphere bacteria to utilize MI, underscoring that *lolCatGC* provide a growth benefit within distinct ecological niches.

#### P-EME-026

##### Ethylene- and Ethephon-Mediated Suppression of Methanogenesis in Anoxic Rice Soils

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In anoxic rice paddy soils, the greenhouse gas methane (CH<sub>4</sub>) is mainly produced by acetotrophic and hydrogenotrophic methanogenic archaea, with methyl-coenzyme M reductase (encoded by *mcrA*) catalyzing the final step of CH<sub>4</sub> formation. Ethylene (C<sub>2</sub>H<sub>4</sub>) reversibly suppresses methanogenesis in sediments, yet its mechanisms remain unclear. To evaluate its potential for CH<sub>4</sub> mitigation in rice agroecosystems, anoxic microcosm incubations were conducted with increasing concentrations of C<sub>2</sub>H<sub>4</sub> and ethephon (2-chloroethyl phosphonic acid), a common C<sub>2</sub>H<sub>4</sub>-releasing compound and P-fertilizer. CH<sub>4</sub> fluxes and expression of the methanogenic marker gene *mcrA* were determined. Both C<sub>2</sub>H<sub>4</sub> and ethephon markedly inhibited CH<sub>4</sub> production at ≥0.4 μmol g<sup>-1</sup> concomitant to a decrease in *mcrA* expression. CH<sub>4</sub> production was nearly eliminated at higher concentrations, suggesting a higher C<sub>2</sub>H<sub>4</sub> sensitivity of the mature Mcr or preceding enzymes than *mcrA* gene expression. CH<sub>4</sub> precursors like acetate accumulated dose-dependently with increasing C<sub>2</sub>H<sub>4</sub> concentration. Following C<sub>2</sub>H<sub>4</sub> removal, CH<sub>4</sub> production rapidly recovered or exceeded that of controls likely due to

accumulated CH<sub>4</sub> precursors, despite stable *mcrA* expression levels. These results indicate that ethylene and ethephon reversibly suppress methanogenesis at both enzymatic and transcriptional levels, and that low-dose ethephon may serve as a practical, transient methanogenesis inhibitor during high-emission periods in flooded rice cultivation.

#### P-EME-027

##### Antagonistic Potential of *Streptomyces* spp. from the Microbial Strain Collection of Latvia for Biocontrol of Tree Pathogens

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*Streptomyces* spp. are renowned producers of bioactive secondary metabolites with broad-spectrum antimicrobial activity. Sixty-one strains were screened *in vitro* for inhibition of the tree pathogenic fungus *Hymenoscyphus fraxineus* and the bacteria *Gibbsiella quercinecans* and *Brenneria goodwinii* using dual-culture assays. Antagonistic activity was quantified by measuring inhibition zones and statistically evaluated using Kruskal-Wallis and Dunn's tests. Several strains, including *Streptomyces* sp. MSCL 440, MSCL 469, and MSCL 1723, showed significant and consistent inhibition against several microorganisms.

Building upon these findings, ongoing work employs whole genome sequencing to identify secondary metabolite biosynthetic gene clusters and antagonistic mechanisms underlying these interactions. The reconstructed genomes were analysed using antiSMASH and its associated databased MIBiG, Pfam, smCOG, and ClusterBlast. Across all sequenced samples, terpene (isorenieratene, geosmin, hopene, albaflavenone) is the most abundant cluster, followed by polyketides (alkylresorcinol, naringenin, angolamycin) and RiPP. When compared to other well-performing strains, MSCL 469 stands out as a strain with a higher number of clusters associated with direct antimicrobial activity.

This study demonstrates the biotechnological potential of MSCL *Streptomyces* strains as sustainable biocontrol agents.

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#### P-EME-028

##### Antibacterial Effects of wood: From Extracts in liquid to surfaces

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**Introduction:** Wood is an eco-friendly and renewable resource, with certain species possessing intrinsic antibacterial properties. Owing to these characteristics, wood

has gained increasing attention to a sustainable alternative material for applications in packaging and furniture. However, its antibacterial performance is influenced by several factors, including wood species, surface contact, and environmental conditions, which complicate its consistent application in real-world settings. This study aimed to investigate how these variables affect antibacterial efficacy to identify optimal wood species and conditions for practical applications.

**Materials & Methods:** Wood extracts were obtained by incubating wood in different media. Bacterial growth in these extracts was monitored. The effect of pine extracts on biofilm formation was evaluated by crystal violet assay. Bacterial survival on wood surfaces was assessed by counting colony-forming units (CFU).

**Results:** Tryptic Soy Broth (TSB)-based extracts from pine and spruce significantly inhibited *E. hirae* growth, with inhibition rates of 66.63% and 43.66%, respectively. No inhibition was observed for *P. aeruginosa*. The antibacterial effect of pine extracts persisted for four consecutive extractions, though it gradually declined. Further analysis identified  $K_2HPO_4$  as a key component of TSB in extracting antibacterial compounds from wood, with water alone being insufficient. Surprisingly, biofilm formation by both *E. hirae* and *P. aeruginosa* was significantly higher in TSB with pine extracts than in TSB alone. Spruce surfaces maintained consistent antibacterial activity only when dry. In contrast, beech extracts exhibited only weak antibacterial activity, yet surfaces demonstrated stronger inhibition than spruce or pine, achieving complete elimination of *E. hirae*.

**Conclusion:** These findings reveal that the antibacterial effects of wood are dependent on both wood and bacterial species, with pine and spruce extracts showing stronger inhibition against *E. hirae*. In contrast, beech surfaces demonstrated superior bactericidal performance, indicating that different woods may be more suitable for different applications. The reduction in antibacterial activity with repeat extractions suggests a limited long-term sanitising effect of wood surfaces. This study highlights the complexity of wood's antibacterial behaviour and helps to guide the utilization of natural materials in real-world hygienic applications.

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#### P-EME-029

##### **A Customizable Procedure for Predicting Antibiotic Resistance Selection on Novel Antimicrobial Coatings**

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**Introduction** The global burden of antimicrobial resistance (AMR) continues to rise, causing an increasing number of deaths attributable to antibiotic-resistant bacteria (ARB). Preventing the spread of ARB in hospital environments, particularly via high-touch surfaces, is a critical component of infection control. Alongside routine disinfection and prevention protocols, antimicrobial coatings (AMC) are being developed to reduce microbial transmission. However, prolonged use of such coatings may, like antibiotics, select for resistant bacteria. To address this, predictive test methods are needed to evaluate potential AR selection before AMC implementation.

**Goal** Within the Horizon Europe project "STOP" (Grant Agreement ID: 101057961), we developed a customizable high-throughput procedure to assess whether novel AMCs exert selective pressure on ARB.

**Methods** Two bacterial libraries, each comprising 80 strains of *Escherichia coli* and *Staphylococcus aureus*—species strongly linked to AMR-attributable mortality—were established, maintaining a 1:1 ratio of antibiotic-resistant and -sensitive isolates (third-generation cephalosporin-resistant *E. coli* and methicillin-resistant *S. aureus*). A modified ISO 22196-based method was implemented using copper as a benchmark antimicrobial surface and stainless steel as a control to infer potential survival advantages of resistant phenotypes. Metadata, genomic information, and antibiotic susceptibility testing data were collected to ensure diversity in sequence types, resistance gene profiles, and geographical origins. The method was calibrated on copper, a benchmark antimicrobial material, and reproducibility was verified through independent operators and replicates.

**Results and Conclusions** Screening of the libraries can identify possible associations between resistance phenotypes and survival rates on the tested antimicrobial surface, with three possible outcomes anticipated: (i) higher survival of resistant strains, (ii) higher survival of sensitive strains, or (iii) no significant difference. Subsequent statistical analyses will explore genetic traits linked to survival advantages. This standardized and adaptable test provides AMC developers with a predictive tool to evaluate selective pressures toward ARB before real-world application, supporting safer innovation in antimicrobial surface technologies.

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#### P-EME-030

##### **Composition of vermicomposting substrates determines the community composition of gut microbiota in *Eisenia fetida* and *Dendrobaena veneta***

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Vermicomposting is a sustainable approach to organic waste management, enhancing soil quality and mitigating environmental degradation through activities of earthworms and their associated microbiota. Depending on the different nutrient quality of applied organic waste, the influence on the gut microbiota of earthworms differs and impacts their performance within the vermicomposting process.

This study aims to investigate the diet-induced shifts in gut microbiota composition and function in *Eisenia fetida* and *Dendrobaena veneta*. The study hypothesizes that the diet is driving microbial community structures, particularly influencing correlation network topology with higher connectedness under low C/N diet.

A microcosm experiment was established with 4 replicates, using 1.2-L glass containers filled with 1 L of standardized worm soil and supplied with two plant-derived food substrates, grass clippings (GC) (C/N  $\approx$  25:1), barley straw (ST) (C/N  $\approx$  80:1) and a commercial worm feed as control (C/N  $\approx$  30:1) for a total of 14 weeks. Each microcosm housed adult earthworms (7 and 5 individuals for *E. fetida* and *D. veneta*, respectively) under controlled conditions (20 °C, 70% soil moisture). In three sampling events (3, 11 & 14 weeks),

gut content was collected. Prokaryotic community composition was assessed by 16S rRNA gene metabarcoding of extracted DNA, targeting the hypervariable V3-V4 region. Library preparation was performed using the "16S Metagenomic Sequencing Library Preparation" protocol (Illumina). Data analysis will be done using the DADA2 (1.38.0) pipeline and NetCoMi (1.2.0) for network analysis.

First data on earthworm performance showed a strong dependency on the substrate used, as well as the time point of analysis. In the frame of the poster, we will present data on the responses of the gut microbiome towards differences in diet and address the question if the changes in microbial gut composition are explaining the differing performance of the studied earthworms.

This research will advance understanding of how diet shapes the gut microbiome of vermicomposting species, contributing to sustainable biowaste management in agricultural systems.

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### P-EME-031

#### **Influence of minor hydrocarbon seepage on microbial activity and geochemical signals in marine subsurface sediments**

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All hydrocarbon (HC) reservoirs leak to some extent. When small quantities of HCs escape from offshore reservoirs and migrate through overlying organic-poor marine sediments towards the surface, these HCs are often completely metabolized by microorganisms before reaching the sediment-water interface. However, these inconspicuous HC fluxes still influence microbial community composition and activity as well as the geochemistry of near-surface sediments. Using pore water geochemistry, inorganic and organic solid phase analyses as well as metagenomic and metatranscriptomic sequencing, we evaluate the differential impact of low-intensity seepage and determine which parameters can act as potential fingerprints of inconspicuous HC fluxes. We examined how localized HC seepage affects microbial activity, particularly sulfate reduction, in organic-poor sediment from the SW Barents Sea, focusing on three areas overlying known HC reservoirs and two reference areas that are not affected by HC seepage. The analysis of 50 gravity cores revealed significant variability in the predicted sulfate depletion depth, ranging from 3 to 12 m below the seafloor. We observed nearly linear pore water sulfate and alkalinity profiles, alongside low rates of sulfate reduction ( $\text{pmol} \times \text{cm}^3 \times \text{d}^{-1}$ ).

Multivariate statistical analysis of Fourier-transform ion cyclotron resonance mass spectrometry data revealed slight differences in organic matter composition of the sediment organic fraction. In contrast, X-ray fluorescence elemental analysis of the inorganic fraction showed clear indication of authigenic minerals formation, such as carbonates and sulfides, linked to microbial reductive processes. Specific major and trace element concentrations in bulk sediments suggest that microbial HC degradation can imprint distinct

geochemical signatures in mineral fractions that are more subject to dissolution-precipitation redox reactions.

These findings were supported by sequencing, metagenomic and metatranscriptomic data indicating active sulfate reduction and anaerobic oxidation of methane (AOM), with marker genes revealing metabolic activities from sulfate-reducing bacteria and ANME archaea that utilize energy from trace amounts of HCs in the sediment.

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### P-EME-032

#### **Close encounters on a micro scale: Interactions of microplastics, associated contaminants, and biofilms in aquatic ecosystems**

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In systems as dynamic as the aquatic ecosystem, microorganisms are found throughout many different compartments, playing a central role in the healthy functioning of the ecosystem. Simultaneously, they exist alongside a vast number of pollutants that pervade these environments. Microplastics, for instance, are one such pollutant. Given its unique physical properties, these synthetic substrates can circulate freely throughout aquatic ecosystems, accumulating and carrying with them a wide range of other persistent pollutants as well. Simultaneously, microplastics offer nutrition and protection to diverse microbial communities and their theatre of activity, representing a new ecological niche. In our work, we investigate the interactions of microplastics with other persistent pollutants in aquatic systems and characterise the effects of these interactions on associated microbial communities to better understand how these substrates might impact surrounding ecosystems. Demonstrating no specificity to polymer type, microplastic biofilms are shaped more by the strong influence of spatial and temporal factors. Microplastic sorption of polycyclic aromatic hydrocarbons (PAHs), in contrast, appear to be more strongly dictated by substrate type, with different polymers observed to sorb varying levels of different PAHs. These interactions between the different emerging contaminants were found in our study to have a potential effect on associated substrate biofilms, their community composition and structure. Elevated levels of specific 3- and 4-ring PAHs on polyethylene and polystyrene were found to coincide with higher proportions of specific taxa reportedly capable of hydrocarbon utilisation, as well as an overall reduced diversity among biofilm communities. The findings in our study illustrate the importance of investigating the collective effect of pollutants in combination and their complex interactions in assessing their impact on the environment.

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### P-EME-033

#### **Temporal dynamics of archaeal and bacterial communities in liquid dairy manure associated with methane emission**

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In dairy farms, manure is often stored in covered tanks as slurry for several months before field application. This form of liquid manure management creates an anoxic environment, supporting the degradation of organic matter and leading to significant emissions of non-CO<sub>2</sub> greenhouse gases. Of relevance in these systems is the emission of methane (CH<sub>4</sub>), which is produced by methanogenic archaea from CO<sub>2</sub> and H<sub>2</sub> and/or small organic molecules. So far, microbial communities in liquid manure storage have been investigated almost exclusively in batch systems, contrary to the real scenario on farms, where manure is added to storage tanks on a daily basis. Moreover, the fine scale temporal dynamics of the microbial communities in these systems is not well resolved. To address these knowledge gaps, dairy manure was collected from a farm and added on every working day to a novel laboratory system for liquid manure storage, which consists of four tanks with a diameter to height ratio typical for a practical storage tank in a farm. Samples from both the manure feed and the digestate were taken weekly over 43 weeks. Sequencing of the V1-V3 or the V3-V4 regions of the 16S rRNA gene (DNA-based, total communities) and transcripts (RNA-based, growing communities) was performed for archaeal and bacterial communities, respectively. Bacterial communities in fresh manure were consistently dominated by members of the phylum *Bacteroidota* which are known fermenters and common inhabitants of manure. In the digestate, however, communities were dominated at early stages by the genera *Acinetobacter* and *Pseudomonas*, which were partially replaced by *Corynebacterium* and members of the phylum *Fibrobacterota* as time progressed. These organisms have the ability to degrade complex organic matter and therefore likely support anaerobic digestion. In fresh manure, archaeal communities had low abundances and were fundamentally distinct from the ones present in the storage tanks. Despite the initial high relative abundance of the genus *Methanobrevibacter*, archaeal communities in the tanks were dominated by the genera *Methanosarcina* and *Methanotherix*, indicating acetoclastic and hydrogenotrophic methanogenesis, and accompanied an increase in CH<sub>4</sub> production. Our study allowed tracing the temporal dynamics of microbial communities in dairy manure, stored under farm conditions, and will assist in the design of more efficient manure management strategies to mitigate global methane emissions.

#### P-EME-034

##### Metabolic Responses of *Clostridioides difficile* in Soil-Like Environments

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Soil is one of the most diverse ecosystems on earth, where physical, chemical and biological interactions are shaped by countless microorganisms. Among them is the rod-shaped, motile and Gram-positive bacterium *Clostridioides difficile*, a major cause of antibiotic-associated diarrhea worldwide. Despite its strictly anaerobic lifestyle, *C. difficile* can persist

in aerobic environments due to its ability to form metabolically dormant endospores, which play a central role in its infection and transmission cycle. However, the role of vegetative cells outside the host remains poorly understood. It is still unclear how they respond to environmental stress and whether they contribute to infection following host secretion.

Investigating *C. difficile* stress in soil-like environments is challenging, not only due to demanding sample preparation caused by the strong interaction of charged molecules with soil components, but also because of the diverse resistance profiles among isolates affecting both, spores and vegetative cells. Here, isolates are tested for ethanol and heat resistance to capture the full resistance spectrum of *C. difficile*. The influence of soil components on intracellular metabolite recovery is assessed using artificial mineral soils, while sand exposure serves as a simplified model to study metabolic responses in vegetative cells.

By exposing *C. difficile* to soil-related factors, these approaches provide insights into its metabolic responses in non-host environments.

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#### P-EME-035

##### Seasonal dynamics and potential interactions of free-living bacterioplankton during phytoplankton blooms at Helgoland Roads

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Marine bacterioplankton play a crucial role in global carbon cycling by decomposing microalgal biomass generated during phytoplankton blooms. This study aims to investigate the seasonal dynamics and ecological interactions of bacterioplankton at Helgoland Roads, over a full year (2022–23). Metagenome and metatranscriptome analyses were performed on 44 and 36 samples, respectively. Preliminary analysis generated 855 representative non-redundant metagenome-assembled genomes (MAGs). Analysis of abundant and highly expressed MAGs revealed dynamic shifts in community composition, gene expression, and activity patterns that closely correlated with chlorophyll *a* concentrations. These patterns suggest distinct seasonal responses among bacterial clades (temporal niche partitioning). i.e., adaptations to changing conditions and ecological roles associated with spring, summer, and autumn phytoplankton blooms. Key active groups included members of *Bacteroidia* (e.g., *Aurantivirga*), *Alphaproteobacteria* (e.g., *Pelagibacter*) and *Gammaproteobacteria* (e.g., SAR92). We further hypothesize that increased bacterial cell densities occurring during summer blooms enhance both cooperative and competitive interactions among bacteria and with algae. These interactions, likely mediated by secretion systems and biosynthetic gene clusters, may lead to higher bacterial mortality rates, potentially influencing the balance between organic matter remineralization and carbon sequestration. Our findings contribute to a deeper understanding of microbial functionality and ecological relationships in dynamic coastal marine ecosystems.

## P-EME-036

### Establishing a culture-independent workflow to assess abundance and diversity of *Clostridioides difficile* in the environment

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*Clostridioides difficile* is an anaerobic, spore forming bacterium that colonizes the gastrointestinal tract of animals and humans. It is a nosocomial pathogen and well-known for causing the majority of antibiotic treatment-associated diarrhea in human patients and livestock [1]. Investigating *C. difficile* in the environment is essential to better understand its epidemiology, ecology, genetic variability and widespread distribution beyond the clinical context. As opposed to culture-dependent approaches, this study aims to directly analyze environmental DNA, collected in the vicinity of pig farms where *C. difficile* is widespread, in order to explore its abundance and diversity in various surroundings.

Quantifying the number of bacterial cells in different environmental samples would indicate the potential reservoirs for either vegetative cells or spores of *C. difficile*. Given the bacterium's strict anaerobic nature, its abundance outside a host organism is expected to be very low and therefore will be difficult to quantify. In this study, we used a primer, targeting the *Chaperonin-60* gene [2], with three different quantitative methods (qPCR with SYBR Green, qPCR with TaqMan fluorescence probe and droplet digital PCR) to develop a quantitative assay sensitive enough to work reliably in different types of complex environmental samples. Both TaqMan-based qPCR and droplet digital PCR showed a sensitivity of detection <10 gene copies/reaction, whereas the former demonstrated higher accuracy in retrieving exact copy numbers in high-abundance samples.

To be able to not only reliably quantify *C. difficile* in varying environments, but also assess its genetic diversity, we explored the potential of the internal transcribed spacer (ITS) region between the 16S/23S rRNA genes as a target region for amplicon sequencing. Results show an adequate resolution of the intraspecific genetic lineages of *C. difficile*, using 5 distinct ITS copies. This culture-independent workflow provides an approach to directly analyze environmental DNA for distribution and genetic heterogeneity of *C. difficile* without relying on the cultivation of isolates.

## References

[1] Alexiou, S. *et al.* Microorganisms. 2025, 496. <https://doi.org/10.3390/microorganisms13020429>

[2] Kohler, C. M. *et al.* Microbiological Methods. 2022, 196. <https://doi.org/10.1016/j.mimet.2022.106458>

## P-EME-037

### Hidden Genomes in the Mountains: Microbial Diversity and Environmental Dynamics in Black Lake, Montenegro

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## Introduction

Lakes are crucial freshwater ecosystems and sensitive indicators of climatic and ecological change, providing valuable insights into the genomic potential and adaptive capacity of microbial communities within them. This study focused on Black Lake, a glacial lake located in the Durmitor mountain region of northern Montenegro at an altitude of 1416 m near the town of Žabljak. Black Lake consists of two interconnected basins, Small Lake and Large Lake, which together form the largest and most prominent of Durmitor's 18 glacial lakes.

## Goals

The aim of this study was to investigate the microbial community composition, diversity, and functional potential of the Black Lake system, and to assess the influence of physicochemical factors on microbial distribution and structure.

## Methods

Epilimnetic water samples were collected monthly between 2021 and 2022. Samples for chemical and molecular analyses were collected simultaneously and short-read metagenomes were generated to characterize microbial diversity and functional potential within the lake system.

## Results

Moderate to high sequencing coverage (ranging from ~ 60% to > 90%) was observed across all samples and comparable diversity estimates between samples suggested sufficient sequencing depth for reliable community profiling. Taxonomic profiling based on normalized *rps3* relative abundance indicated dominance of the phyla *Actinomycetota*, *Pseudomonadota*, *Planctomycetota*, *Bacteroidota*, *Verrucomicrobiota*, and *Bacillota\_A*, showing temporal variation across samples. Metagenome-assembled genomes (MAGs) were reconstructed to provide insights into the genomic potential of dominant taxa. Spearman correlation analysis showed that the dominant phyla exhibited distinct relationships with measured physicochemical parameters. Multivariate analyses (PCA and NMDS) further confirmed clear spatial and temporal separation of bacterial communities.

## Conclusion

This study represents one of the first high-resolution metagenomic investigations of microbial community structure

and environmental variability within the Black Lake system of Montenegro, providing a foundation for understanding the genomic potential and ecological functioning of glacial freshwater ecosystems.

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#### P-EME-038

##### **Mechanisms of fructan utilization by marine *Gammaproteobacteria***

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Fructans are polyfructose chains, in which fructosyl units are  $\beta(2,1)$ - or  $\beta(2,6)$ -linked to sucrose, referred to as inulin or levan, respectively. Inulin and levan are common in terrestrial ecosystems, where they serve as storage compounds or osmolytes in plants and as exopolysaccharides in bacteria [1]. In comparison, the role of fructans in the marine environment remains largely unexplored.

We used physiological, proteogenomic, biochemical and computational structural analyses to reconstruct, for the first time, a pathway for the utilization of fructans by the Antarctic gammaproteobacterium *Pseudoalteromonas distincta*.

In *P. distincta*, the degradation of inulin and levan is enabled by two glycoside hydrolases from family 32 (GH32), one of which is encoded in a polysaccharide utilization locus (PUL). Unexpectedly, this PUL also encodes an inulin-binding SusD-like protein and a SusC-like TonB-dependent transporter (TBDT) for glycan import. SusC/D-like proteins evolved to form a complex [2], however, this was considered unique to *Bacteroidota*, while *Gammaproteobacteria* instead use "classical" TBDTs. In a large-scale *in silico* analysis, we detected SusC/D-like proteins also in other *Gammaproteobacteria*, mostly together with GH32s. This analysis also showed the global distribution of bacterial fructan-degrading activity.

Our study thus challenges the paradigm that only *Bacteroidota* use SusC/D-like proteins. It further suggests that fructans contribute to the marine glycan pool and are targeted by specialized bacterial communities.

[1] Versluys M, Kirtel O, Toksoy Öner E, Van den Ende W. The fructan syndrome: Evolutionary aspects and common themes among plants and microbes. *Plant Cell Environ.* 2018;41(1):16-38; doi:10.1111/pce.13070.

[2] Glenwright AJ, Pothula KR, Bhamidimarri SP, Chorev DS, Baslé A, Firbank SJ, et al. Structural basis for nutrient acquisition by dominant members of the human gut microbiota. *Nature.* 2017;541(7637):407-11; doi:10.1038/nature20828.

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#### P-EME-039

##### **Marine *Bacteroidota* isolated from the North Sea possess a so far undescribed polysaccharide utilization locus (PUL) targeting $\beta$ -1,2-glucans**

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Utilization of diverse polysaccharides by marine bacteria plays a crucial role in carbon turnover during algal spring bloom events. As polysaccharides belong to the most diverse biomolecules, heterotrophic bacteria require a set of highly adapted enzymes for the efficient decomposition of complex substrates from algal-derived organic matter. Specialized clusters of co-regulated genes, so called Polysaccharide Utilization Loci (PULs), are responsible for sensing, transport and degradation of a specific glycan substrate.

Previous analyses showed that glycans such as  $\alpha$ -glucans or  $\beta$ -1,3-glucans are important substrates for marine bacteria during North Sea bloom events (Krüger *et al.*, 2019; Kappelmann *et al.*, 2019). However, we now discovered that a high proportion of these bacteria target  $\beta$ -1,2-glucans, a previously unknown substrate for the marine environment. Therefore, we investigated the degradation of  $\beta$ -1,2-glucans using our model strain *Christiangramia forsetii* KT0803<sup>T</sup>.

Proteome analyses showed that the abundance of PUL-encoded proteins is increased in  $\beta$ -1,2-glucan-grown cells, which supported their role in  $\beta$ -1,2-glucan use. Additional characterization of all PUL-encoded glycoside hydrolases (GHs), using reducing sugar assays and ANTS-FACE, confirmed activity on  $\beta$ -1,2-linked substrates. Based on these findings and *in silico* prediction of subcellular localization of the PUL proteins we were able to propose a putative degradation pathway for  $\beta$ -1,2-glucans by marine *Bacteroidota*. In addition, preliminary analyses indicated that *C. forsetii* degrades  $\beta$ -1,2-glucans produced by another marine isolate, *Gillisia* sp. Hel\_I\_86. This suggests that bacterial  $\beta$ -1,2-glucans contribute to the marine  $\beta$ -1,2-glucan pool.

Our findings thus demonstrate the relevance of  $\beta$ -1,2-glucan in the marine environment and provide further insight into marine carbon cycling.

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#### P-EME-040

##### **A Metagenomic Approach to Investigate Antibiotic Resistance Gene Patterns in an Anthropogenically Impacted Estuary and the Southern Baltic Sea Coast**

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**Introduction** Antimicrobial resistance is among the World Health Organization's top global health threats. Aquatic habitats can serve as reservoirs and distribution vectors for antibiotic resistance genes (ARGs). Understanding spatial and temporal patterns of ARGs and their bacterial hosts in such habitats is essential for assessing environmental risks and developing effective prevention strategies.

**Goal** This study aims to characterize the diversity, abundance, and mobilization potential of ARGs along the transition from freshwater to brackish habitats within the Baltic Sea region. We specifically investigate how spatial, seasonal, and anthropogenic influences shape ARG distribution in an estuarine ecosystem affected by urban, industrial, and touristic activities.

**Methods** Over the course of one year, weekly water samples were collected from ten stations along the Baltic

Sea coast near Rostock and within the Warnow Estuary — a highly anthropogenically impacted system. In total, 522 metagenomic samples were generated, providing a dataset with high temporal and spatial resolution. ARGs were identified and characterized regarding their hosts and plasmid association to explore patterns, e.g. in ARG abundance, diversity or potential mobility.

**Results and Conclusion** Initial analyses revealed distinct patterns in the ARG distribution across freshwater, estuarine, and coastal sites. We found first indications that the most prevalent ARGs are similar across all habitat types, while the main ARG-carrying bacteria vary between habitats. Latest comparative results across sites and seasons will be presented at the conference.

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#### P-EME-041

##### **Rhizosphere microbiome structure and function in response to root exudates of *Zea mays* L. landraces and modern hybrids under drought**

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Drought and heat stress are significant contributors to global crop yield stagnation, with daunting trends anticipated for maize in Germany. A promising strategy to address drought-induced crop yield losses involves identifying shifts in soil bacterial communities under water scarcity and harnessing specialized microbes and their functions for improved drought resilience. Beneficial plant-microbe interactions are essential for plant performance and stress resistance, yet their complexity remains poorly understood. As key mediators of plant-microbial communication, root exudates play a critical role in promoting beneficial microbiomes and facilitating adaptation to drought conditions. Here, we propose that fundamental distinctions may exist between older landraces and modern hybrids of maize in their harnessing of these interactions. We investigate the influence of maize genotypes on root exudation and related composition and activity of rhizosphere microbiota under drought.

In this ongoing experiment, we investigate the drought response of three traditional and three modern maize varieties in two different soils under three different irrigation strategies. Rhizosphere microbes are tracked via 16S rRNA gene metabarcoding. Initial results demonstrate an enrichment of drought-responsive taxa, including *Actinobacteria*, *Gammaproteobacteria*, and *Alphaproteobacteria*, under drought conditions. This enrichment was most pronounced in dry silty loam. In contrast to our hypothesis, plant variety or genotype did not appear to be a dominating driver of microbiome response under drought. In ongoing work, microbial activity in the rhizosphere is monitored via microbial exoenzymes using MUF substrates. Understanding the drought-related interactions between plants and microorganisms in the rhizosphere is highly relevant for developing strategies to promote microbiome services for sustainable crop production.

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#### P-EME-042

##### **Soil bacterial diversity under contrasting agricultural management across European biogeographical regions**

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Soil biodiversity supports ecosystem functions that sustain human well-being. Intensive agricultural management, a key anthropogenic driver, strongly shapes soil bacterial communities, with critical consequences for soil health and sustainability. However, little is known about how agricultural practices influence soil microbiomes under contrasting climatic and biogeographical conditions, limiting our understanding of soil biodiversity responses at a continental scale and subsequently the development of tailored mitigation strategies.

Within the BIOServicES project, this study examines how agricultural management affects bacterial diversity across five European regions (Alpine, Atlantic, Boreal, Continental, and Mediterranean). We assessed the effects of real-world cropping intensities to identify large-scale patterns of soil biodiversity.

Five agricultural sites were selected, one per region and including three management intensities: high, medium, and low. Soil samples (0-20cm) were collected in April 2024 (n=150; 10 per intensity). DNA was extracted and sequenced for the 16S rRNA gene on an Illumina NovaSeq. Sequence processing and taxonomic assignment were performed in R using DADA2 and SILVA database. Statistical analyses on CSS-normalized data included PERMANOVA and Kruskal-Wallis tests ( $p < 0.01$ ).

Management intensity significantly influenced bacterial community composition across all regions. Alpha diversity differed in the Atlantic region, where higher management intensity reduced richness and diversity. Environmental conditions, mainly soil texture, modulated responses elsewhere. Dominant phyla were *Actinomycetota*, *Acidobacteriota*, and *Gemmatimonadota*, with subtle genus-level shifts. Regional patterns showed *Desulfuromonas* (Alpine), *Priestia* (Atlantic), *Neobacillus* and *Niallia* (Boreal, Continental) and *Arthrobacter* and *Blastococcus* (Mediterranean), reflecting distinct ecological strategies.

This continental-scale study reveals regional variability in bacterial diversity responses to management intensity. *Arthrobacter* and *Blastococcus* were linked to stress tolerance under nutrient-poor conditions, whereas *Priestia*, *Neobacillus*, and *Niallia* dominated under higher intensity, reflecting copiotrophic taxa adapted to nutrient-enriched soils. *Desulfuromonas* suggests regional adaptation to redox conditions. These findings highlight the need for tailored soil management strategies to maintain soil quality under climate and global change pressures.



## P-EME-043

### Lignin degradation over a land use gradient in German grasslands

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#### Introduction

Understanding the process of organic matter decomposition in soils is crucial for unraveling the mechanisms of carbon cycling and nutrient availability. Plant-derived, phenolic-rich compounds like lignin and phenolic acids represent a major component of organic matter input in grassland soils. Due to its aromatic polymer structure, the degradation of lignin is slow, and only a limited spectrum of microbial taxa can do so. The reconciliation hypotheses address the timing of lignin degradation during litter decomposition in relation to labile C sources. However, how land use intensity affects the microbial community composition and its potential to degrade lignin remains poorly understood.

#### Goals

In this study, we aim to assess how a land use gradient and associated soil biogeochemical factors influence the microbial community composition regarding its functional potential to degrade phenolic rich compounds.

#### Material and Methods

We sampled 150 (50 per region) grassland topsoil across three German regions in the frame of the Biodiversity Exploratories, differing in nutrient concentrations and climate. A continuous land use index (LUI) quantifies management based on mowing, grazing and fertilization intensity on each plot. All samples were 150paired-end (PE) shotgun sequenced (NovaSeq X Plus), then assembled and annotated using the SqueezeMeta Pipeline v1.6.5. KEGG-annotated genes linked to lignin degradation were filtered and categorized into three degradation steps: depolymerization, aromatics catabolism, and ring cleavage. We analyzed the influence of environmental factors like phenolic concentration, C:N ratio and LUI on the functional abundance and diversity of microbial degraders, accounting for site differences.

#### Results

Metagenomic analysis revealed significant differences in lignin degradation across three sites. We observed an increase in bacterial degrader abundance when comparing depolymerization to aromatics catabolism steps. Additionally, there was a shift in the degraders' community composition resulting in an increase in Pseudomonadota and Acidobacteriota and a decrease in Actinomycetota. These functional and taxonomic changes appeared to be driven more by environmental factors like soil type than by land use intensity.

#### Summary

Our findings enhance understanding of how land use intensity and soil environmental factors affect lignin degradation in grasslands, which is essential for predicting global carbon cycling and storage.

## P-EME-044

### Investigation of *Listeria monocytogenes* colonizing corn salad

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*Listeria monocytogenes* is one of the most important foodborne pathogens due to its high hospitalization and mortality rates. While meat, fish, and dairy products are recognized as common sources, leafy greens such as corn salad (also known as lamb's lettuce) have also been identified as vectors for *L. monocytogenes*. A major concern is the pathogen's ability to attach to plant surfaces and form biofilms, which allows it to persist over time. In response to this, the new EU Regulation 2024/2895 tightens the detection limit for *L. monocytogenes* at the end of shelf life to zero cfu per 25 g of food, emphasizing the need to reassess food hygiene measures and storage practices.

This study aimed to investigate the attachment, colonization, and biofilm formation of *L. monocytogenes* on corn salad and to identify the genes involved in these processes. Three isolates, two of plant origin and one clinical, were characterized by whole-genome sequencing. Biofilm formation was quantified using crystal violet assays in different media (BHI, LB, and corn salad extract), revealing peak biofilm development after 12–14 hours. Ongoing RNA-seq shall reveal the genes involved in attachment and colonization.

To visualize colonization, *L. monocytogenes* strains were fluorescently labeled using a chromosomally integrated plasmid vector, which is phage derived and host specific. This labelling system was also utilized to create reporter fusion constructs of genes which are involved in virulence or hypothetically in attachment to the plant surface. Colonization and biofilm formation on corn salad leaves were analyzed using confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM). CLSM and SEM revealed that *L. monocytogenes* predominantly colonizes the stomata of corn salad leaves. Dense clusters and string-like structures were observed in the stomatal regions, while microcolony and biofilm formation was also detected on surrounding surfaces. Three-dimensional CLSM imaging of the GFP-expressing strain, combined with quantitative analysis using the "BiofilmQ" software, showed that the architectural characteristics of the biofilm differed depending on the growth medium.

These findings suggest that stomatal colonization may offer *L. monocytogenes* protection from external stressors, posing a specific challenge to food safety, particularly for ready-to-eat salads.

## P-EME-045

### Mitigating nitrous oxide emissions from wastewater treatment systems using nitrous oxide respiring bacteria

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Atmospheric nitrous oxide (N<sub>2</sub>O) has a much higher greenhouse gas potential than CO<sub>2</sub> and plays a crucial role in climate change. N<sub>2</sub>O emissions from wastewater treatment plants (WWTPs), resulting from biological processes within these facilities, are an increasingly relevant concern. This study explores the potential of using suspensions of N<sub>2</sub>O-respiring bacteria for bioaugmentation to mitigate N<sub>2</sub>O emissions from WWTPs.

Samples of activated sludge from a communal WWTP were inoculated with N<sub>2</sub>O-respiring cells (*Comamonas* sp., *Pseudomonas* sp. cor *Neobacillus vireti*) and incubated under anoxic conditions. Dissolved N<sub>2</sub>O turnover was measured by microrespirometry, both immediately after inoculation and after a 24-hour incubation period at 30 °C in the presence of an N<sub>2</sub>O-containing atmosphere. Control experiments using autoclaved or pasteurised activated sludge samples were also conducted.

Control measurements using autoclaved or pasteurised activated sludge showed no N<sub>2</sub>O reduction up to 20 hours, confirming the inactivation of the native microbiome. In contrast, using different cell concentrations, the bioaugmented autoclaved or pasteurised samples exhibited significant N<sub>2</sub>O consumption rates, both immediately after inoculation and after 24 (or 48 h) hours of incubation. The native (non-bioaugmented) samples also revealed N<sub>2</sub>O reduction, presumably due to the indigenous microbial community. However, the N<sub>2</sub>O consumption rates were approximately doubled when the native microcosms were bioaugmented. Furthermore, bioaugmented samples that were incubated for 24 hours in the presence of N<sub>2</sub>O showed increased N<sub>2</sub>O consumption rates in comparison with the preincubated non-bioaugmented samples, indicating that the added cells remained metabolically active.

The findings provide proof of concept that introducing N<sub>2</sub>O-respiring bacteria into anoxic activated sludge systems can effectively enhance N<sub>2</sub>O reduction. This approach provides a promising basis for future research into long-term implementation of this process in laboratory-scale and real-world wastewater treatment bioreactor systems. Similar experiments are planned at different temperatures and dissolved oxygen levels.

#### P-EME-046

##### Exploring Environmental Persistence of Enterococci in Agricultural Soils under Controlled Abiotic and Biotic Conditions

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Potential zoonotic pathogens can persist and evolve in non-clinical environments, although this requires physiological and ecological mechanisms for survival outside human and animal hosts. This research focuses on Enterococci as model organisms to understand how soil properties in agricultural systems can influence the environmental persistence and potential adaptation of bacteria originating from livestock faeces. Specifically, we aim to disentangle abiotic (mineralogy, land use management) and biotic (community diversity) controls on enterococcal persistence in agricultural soils, linking field observations with mechanistic microcosm experiments.

Soil samples representing distinct management practices were collected from two pig farms, one free-range and the other under conventional practices. Abiotic soil properties were determined, and *Enterococcus* abundance was quantified using a genus-specific TaqMan qPCR. Microbial community composition is being assessed through 16S rRNA gene amplicon sequencing to characterize the ecological context in which Enterococci would have to colonize and survive *in situ*. Representative soils from both sites were used in sterile microcosm experiments to determine whether Enterococci can establish and persist in the absence of competition with other autochthonous microorganisms under *in vitro* conditions. Future experiments will apply microbial diversity gradients of the native community as well as targeted dual microcosm setups with candidate inhibitor species to dissect potential biotic suppression mechanisms. Controlled variation of soil parameters will further define abiotic boundaries for enterococcal survival.

The free-range soil was dominated by sand, whereas the conventional site was richer in silt. Enterococci were detected across several management zones, with higher abundances in areas with recent pig activity. In sterile microcosms, both auxotrophic *E. lactis* and prototrophic *E. sulfureus* proliferated in sandy and silty soils, indicating that abiotic soil conditions alone do not inhibit growth. These findings suggest that biotic controls, such as microbial competition or predation, are major constraints on enterococcal persistence in natural soils. Ongoing analyses will integrate qPCR-based abundance data with 16S rRNA gene community profiles to identify key taxa potentially involved in biotic exclusion or antagonism, informing subsequent microcosm experiments with strains isolated from these soils.

#### P-EME-047

##### Symbiont-specific responses of the lung lichen holobiont to excess light and nitrogen

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**Introduction:** *Lobaria pulmonaria* (L.) Hoffm. is a bioindicator of old-growth forests. This lichen is highly sensitive to increased light intensity from activities such as forest thinning and air pollution, particularly reactive nitrogen compounds (NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup>), which may disrupt symbiotic nitrogen metabolism.

**Goals:** Our study investigates how the *L. pulmonaria* holobiont responds to varying light conditions and levels of nitrogen enrichment, hypothesizing that these stressors impact different lichen components - the fungal partner, photobiont, cyanobiont, and associated bacterial microbiome - differently.

**Methods:** Using a combination of amplicon sequencing and metaproteomic analyses, we examined changes in taxonomic composition and function of the holobiont in response to experimental manipulations: transplantation to alter light conditions and simulated precipitation-driven nitrogen deposition.

**Results:** Increased light exposure led to higher alpha diversity of both bacterial and eukaryotic communities, along with shifts in bacterial community composition. While nitrogen enrichment did not significantly affect overall community composition, it was associated with symbiont-specific metabolic shifts: cyanobiont glutamate synthase (GlnA) and mycobiont ammonium transporters increased under high nitrogen, indicating prioritization of nitrogen assimilation over fixation. Additionally, diverse eukaryotic associates of unknown ecological roles - including protists such as Gregarinomorpha (Apicomplexa) and microfauna within Tardigrada - were detected across all treatments.

**Conclusion:** These findings highlight *L. pulmonaria* as a dynamic and adaptive holobiont, where abiotic factors have distinctly different effects on microbial community structure and function.

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#### P-EME-048

##### Using total RNA sequencing to assess the non-target effects of nitrification inhibitors nitrapyrin and DMPP on soil microbial communities

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Nitrification inhibitors (NIs) such as nitrapyrin and DMPP are widely used to mitigate the environmental impacts of excessive fertilizer use, particularly by reducing emissions of the greenhouse gas N<sub>2</sub>O. While most research has focused on NI effects on their primary target organisms, ammonia-oxidizing bacteria and archaea, their broader impacts on the soil microbial community remain poorly understood. To address this gap, we will simultaneously analyze rRNA and mRNA to identify NI-induced changes in the active microbial community and its functional profile.

Field trials were conducted over a six-month period in two Danish agricultural soils cultivated with spring barley, with field doses of nitrapyrin and DMPP applied. Soil samples were collected at five time points and subjected to total RNA extraction and sequencing.

rRNA profiles indicated no significant changes in the active microbial community in response to NI application. Major community shifts were caused by the addition of manure, potentially favoring fungal carbon degraders. At a lower taxonomic level, slight NI-induced effects were observed, including the suppression of nitrifying archaea.

While rRNA analysis did not reveal significant effects of NI application, ongoing analysis of the mRNA pool will be used to trace potential impacts on key ecosystem functions as low-abundance microbial groups, which are often overlooked in taxonomic assessments, can perform important functions.

This study highlights the potential of integrating total RNA analysis into environmental risk assessments, enabling the identification of subtle ecological effects and supporting more sustainable agricultural practices.

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#### P-EME-049

##### Genome-wide identification of genes associated with root colonization in *Pantoea agglomerans*

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Plasmids are key drivers of evolutionary transformations and ecological adaptations in prokaryotes. The contribution of plasmids to the adaptation of a host-associated lifestyle has been described in several bacterial taxa. Examples are virulence plasmids in *Salmonella* and symbiosis plasmids in *Rhizobia*, where essential traits for the interaction with the host are plasmid-encoded. *Pantoea agglomerans* belongs to the most abundant colonizers of plants and is characterized through two vertically inherited [KR1] plasmids: the "large *Pantoea* plasmid" (LPP) and pPag1. Recent studies suggest that the LPP is crucial for the *Pantoea* plant-associated lifestyle, while the function of pPag1 remains unclear. In this study, we demonstrate that the LPP is beneficial for plant colonization, while pPag1 has no effect on plant colonization. We generated four genotypes by curing LPP and pPag1 from *P. agglomerans* R1, a stable colonizer of the wheat rhizosphere: *P. agglomerans* R1 wild-type, ΔLPP, ΔpPag1 and the plasmid free strain ΔLPP/ΔpPag1. Comparing the fitness of the LPP-cured strains ΔLPP and ΔLPP/ΔpPag1 to the wild-type and ΔpPag1 strains, shows that the loss of LPP has a stark negative fitness effect on *P. agglomerans* R1 under *in planta* and *in vitro* conditions. Additionally, *P. agglomerans* ability to utilize diverse carbon and nitrogen sources is abolished in the absence of the LPP. Thus, we conclude that the LPP encodes traits that are beneficial for plant colonization and therefore is a lifestyle plasmid. We expect that our results will be of use in identifying traits that are essential for *P. agglomerans* R1 colonisation of roots and persistence in the plant over time.

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#### P-EME-050

##### Boosting Oceanic CO<sub>2</sub> Uptake: Microbial Responses to Alkalinity Enhancement in Sediments Exposed to Oxidic and Low-Oxygen Bottom Waters

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Rising atmospheric CO<sub>2</sub> levels driven by industrialization continue to warm the planet, with oceans acting as major buffers by absorbing both heat and CO<sub>2</sub>. This uptake results in oceanic warming, oxygen depletion and acidification, with cascading effects acting on marine life and biogeochemical cycles. To mitigate climate change, carbon dioxide removal (CDR) strategies are being explored, with ocean alkalinity enhancement (OAE) via enhanced benthic weathering (EBW) proposed as a promising technology. In EBW, alkaline minerals are added to surface sediments, where mineral dissolution releases alkalinity that promotes oceanic CO<sub>2</sub> uptake from the atmosphere. Yet, feedbacks from sedimentary microbial communities and associated shifts in biogeochemical cycling remain poorly understood.

In this study, alkaline minerals (calcite and dunite) were added to surface sediments and incubated under oxic and low-oxygen conditions to assess microbial responses. While overall benthic community structure remained relatively stable in experiments, specific taxa, such as *Candidatus* Electrothrix, increased under oxygen-limited conditions where calcite dissolution was most pronounced. Genetic data, metabolic rate measurements and porewater analyses indicated that after calcite addition sulfate reduction, sulfide

oxidation and denitrification were stimulated. This underlines the importance to test the long-term effects of EBW in field trials.

#### P-EME-051

##### Investigating the diversity of genomic structural variants present in prokaryotic and viral communities from freshwater samples

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Prokaryotic microbes (bacteria and archaea) constitute the majority of microorganisms in most ecosystems. Due to their wide-ranging metabolic capabilities, they serve as key drivers of biogeochemical cycles. Interactions between prokaryotic hosts and their viruses play crucial roles in shaping the structure of microbial communities. Previous amplicon-based and metagenomic studies revealed that microbial diversity in freshwater environments is influenced by various stressors. However, the factors influencing the microdiversity (i.e. intraspecies genomic variation) of freshwater microbes remain largely unexplored, leaving major gaps in our understanding of how these communities respond to environmental changes.

In this study, we investigate how environmental stressors shape microbial microdiversity in river sediments, and how genomic variations relate to population resilience. To achieve this, we developed a MetaCarvel-based bioinformatics pipeline for the detection of structural variants (SVs; e.g., insertions/deletions > 50bp) and applied it to over 250 river sediment metagenomes obtained from four ExStream mesocosm experiments conducted in Germany, New Zealand, and Ireland (2022-2023). Applied stressors included reduced flow velocity, elevated temperature, and increased salinity, and samples were collected after exposure to and after release from these stressors. So far, fewer than 130 SVs were identified per sample, with metagenomes containing larger prokaryotic fractions yielding more SVs. To examine other potential factors influencing SV detection, a set of metagenomes from drinking water reservoirs in Germany was analyzed. The results show that between 136 and 1,887 SVs were detected in this dataset, substantially exceeding those in ExStream samples, likely reflecting that the simpler community composition of the reservoir metagenomes facilitates the detection of within-species variation. Further analysis of microbial composition will reveal whether the low SV detection in ExStream samples is due to high community richness and whether it could be improved in future samples, for example by increasing sequencing depth. This work opens the door for in-depth characterization of microbial SVs in freshwater ecosystems and their responses to multiple stressors, providing insights into ecosystem resilience under changing environmental conditions.

Keywords: microbial microdiversity, structural variants, metagenomics, river sediments

#### P-EME-052

##### Interactive effects of drought and temperature on soil methane uptake and microbial community dynamics in an upland forest soil

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Aerated soils, especially forest soils, are a sink for atmospheric methane, oxidizing on average 30 to 40 Tg (bottom-up and top-down estimates) of this powerful greenhouse gas yearly. Methane oxidizing microorganisms, i.e., methanotrophs, mediate this methane sink. Soil methane uptake (SMU) largely depends on environmental parameters such as soil water content and temperature. Climate change is expected to alter these soil properties, affecting SMU, but more research is needed to understand how SMU will respond to combined changes in temperature and precipitation.

To investigate how soil water content and temperature interact to regulate SMU and methanotroph abundance, we established a rain exclusion experiment in an upland forest soil on the Telegrafenberg campus, Potsdam (Germany) and monitored SMU and microbial community. Soil methane uptake was measured biweekly with a chamber-based method. Microbial abundances were assessed by qPCR (*pmoA*, *mcrA* and 16S rRNA gene) and sequencing of the 16S rRNA genes. Additionally, we measured methane uptake and microbial gene abundances of soil samples from the same location in laboratory incubations at varying water contents and temperatures.

Preliminary findings from the incubation experiment revealed that SMU was highest at 35% and 65% of the maximum water holding capacities. The incubations with 100 and 130% WHC even switched to methane production. The changes in SMU were accompanied by differing abundances of microbial genes (*mcrA*, *pmoA*). Our research will provide valuable insights into how climate change may impact SMU and the associated microbial community in upland forest soils.

#### P-EME-053

##### Phenotypic and genotypic pathogenicity risk assessment of environmental *Pseudomonas aeruginosa* isolated from water bearing systems

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Clean water with drinking water quality is essential for hygiene such as personal hygiene and health care and as cleaning agent for removing dirt in industrial and food washing processes. *Pseudomonas aeruginosa* is a widespread environmental bacterium that occurs in almost moist habitats and mainly in cold water. As a common opportunistic human pathogen, the proof of its existence in water is relevant from ecological and clinical perspectives especially because of its high adaptability and development of various strategies to survive.

The aim of the study was to characterize environmental *P. aeruginosa* isolates with respect to their phenotypic expression and their molecular virulence factor profiles to assess their pathogenicity potential.

A total of 139 *P. aeruginosa* and 10 non-*aeruginosa* pseudomonads were isolated from the environment and water-bearing systems. Isolates were evaluated in terms of swarming and twitching activity, pigmentation, morphology, fluorescence property, hemolysis and exoenzymatic activities after cultivation on various agar plates as well as with regard to the ability of biofilm formation in 96-well plates determined by crystal violet staining. Genotypically, the presence of ten *P. aeruginosa*-specific virulence genes (*lasB*, *toxA*, *plcH*, *phzM*, *exoS*, *exoU*, *ecfX*, *exoY*, *plcN*, *exoT*) was investigated using PCR assays.

All analyzed *P. aeruginosa* isolates demonstrated elastase and protease activities, hemolysis, twitching and swarming motility and biofilm formation. A particularly large number of isolates exhibited strong elastase activity (78.3 %), very strong protease production (82.6 %) and pronounced swarming behavior (65.3 %). Genotypically, 47.8 % of the strains carried eight and 52.2 % nine of the ten analyzed virulence genes, including *lasB*, *toxA* and *plcH*. In direct comparison with the 10 non-*aeruginosa* pseudomonads, *P. aeruginosa* demonstrated a higher virulence potential than the other species, particularly in terms of biofilm formation, the presence of virulence genes and the expression of exoenzymes.

The results show that environmental isolates of *P. aeruginosa* carry numerous pathogenicity factors. This highlights that *P. aeruginosa* isolated from the environment and water-bearing systems should not be neglected in terms of pathogenicity.

#### P-EME-054

##### Arctic phytoplankton dynamics are impacted by zoosporic fungi belonging to chytrids

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Chytrids (Chytridiomycota) are unicellular fungi that comprise lineages with saprotrophic, parasitic or intermediate lifestyles. Their host taxa are most diverse, including cyanobacteria, diatoms, dinoflagellates, plants, or animals. Phytoplankton-infecting chytrids are assumed to terminate algae blooms, thereby impacting food availability and carbon cycling. Yet, while their ecological significance is mostly researched in freshwaters, observations from marine habitats are scarce. This is particularly true for the Arctic Ocean, where chytrids are highly abundant and hypothesized to control phytoplankton dynamics. Here, we used metabarcoding surveys from two expeditions in the subarctic oceans and the ice-covered Arctic Ocean (*Tara* Polar Circle, MOSAiC), the latter providing a dense sampling transect across one year from both the sea ice and the under-ice water column. Chlorophyll *a* concentrations remained high in pockets that persisted throughout the year in the sea ice, while seasonal changes indicating blooms were discovered in bottom-ice and under-ice water samples. Both habitats were dominated by diatoms. Chytrids

dominated fungal communities and of the combined phytoplankton and chytrid communities, they accounted for >2% in pelagic samples and 66% in sympagic (ice-associated) samples, revealing them as major players in the sea ice habitat. Uncovered species-specific interactions between chytrid taxa and dominant Arctic phytoplankton taxa corroborate the chytrids' critical role in controlling phytoplankton blooms.

#### P-EME-055

##### Diversity analyses of microbial communities in Armanis gold-polymetallic mine: A metagenomics approach

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**Introduction:** Environmental pollution is among the most critical environmental concerns and is considered to be one of the significant challenges in the modern era. Acid mine drainage causes massive environmental concerns worldwide. Goals: In this study, modern molecular approaches (metagenomic) allowed a more complete revealing of the composition of microbial diversity in the Lori Region of Armanis gold-polymetallic mine (Armenia).

**Methods:** The samples were analyzed for heavy metal content using the Agilent 5800 VDV ICP-OES. DNA extraction for metagenomic studies was performed according to the Fast DNATM Spin Kit for Soil protocol (MP Bio-Medicals, Santa Ana, CA, USA) according to the manufacture's instructions. Taxonomic classification was performed using Diamond against the NCBI NR database.

**Results and Conclusion:** The microbial diversity of the acid mine drainage was investigated by using culture-independent techniques, including DGGE and high-throughput sequencing. A total of 103,493,310 and 112,789,352 bases were read. Data analysis identified 28 phyla, 63 classes, 129 orders, 264 families, and 738 genera. *Proteobacteria* were found to be the most abundant phylum in all the sampling sites, with the highest abundance (95%) in all samples, followed by *Actinobacteria* and *Bacteroidetes*. It was shown that water outflow samples of AMD contained the following genera: *Leptospirillum*, *Acidithiobacillus*, *Sulfobacillus*, as well as heterotrophic iron-reducing bacteria. The genus *Oscillatoria* and *Cyanobacteria* were the most abundant blue-green algae. However, algae can be used to treat AMD by removing toxic heavy metals (Cu, Zn, Pb, Hg) through a process called bioaccumulation, and by increasing the pH of the acidic water, which helps metals precipitate out of the solution. Thus, the study of diversity and dissemination of microorganisms in ore deposits of Armenia, as well as their activity in AMD generation is very important and relevant from the point of view of their dangerous effects on the environment.

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**References:** Fashola MO, Ngole-Jeme VM, Babalola OO (2016) Heavy metal pollution from gold mines: environmental effects and bacterial strategies for resistance. *Int J Environ Res Public Health* 13:1047. Khachatryan A, Vardanyan N, Willscher S, Sevoyan G, Zhang R, Vardanyan A (2024) Bioleaching of Chalcopyrite by a New Strain *Leptospirillum ferrodiazotrophum* Ksh-L Isolated from a Dump-Bioleaching

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#### P-EME-056

##### Microplastics and their role in the survival of foodborne pathogens under routine disinfection conditions

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Microplastics, and the dangers it poses to human and animal health, as well as to the environment, has become a topic of great urgency. With the environment being the main focus until recently, most research on the spread of microplastics has been conducted in soil, water systems, and sewage. Based on evidence that microplastics act as microbial vectors, we hypothesized that they may promote bacterial survival in clinical and food production environments by affecting disinfection efficiency. To test this, two well-known foodborne pathogens, *Listeria monocytogenes* and *Escherichia coli*, were exposed to different disinfection methods — physical (heat), chemical (lactic acid and benzalkonium chloride) and UV— in presence of microplastics. Two experimental groups were tested and subsequently compared with the control group: bacteria incubated overnight in presence of 10mg/ml microplastics, and bacteria mixed with 10mg/ml microplastics shortly before exposure to disinfectant methods. Our study included two different types and sizes of microplastics: 1-5µm thermoset amino-formaldehyde (AF) polymers and 425-500µm polyethylene (PE). Disinfection efficiency was determined from the log<sub>10</sub> reduction in bacterial counts after 10, 30 or 60 minutes of exposure to physical and chemical disinfectants, and after 60 minutes of exposure to UV. Additionally, we analyzed the surface morphology of the two different microplastics and the attachment of bacteria to their surface using scanning electron microscopy (SEM).

Findings of this research suggest that microplastics can aid the survival of the *L. monocytogenes* and *E. coli* in presence of benzalkonium chloride and under exposure to UV light. The thermoset amino formaldehyde polymer was observed to neutralize the antimicrobial efficacy of benzalkonium chloride. Our research points out the necessity in taking microplastics into account when assessing disinfection methods efficiency.

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#### P-EME-057

##### Impact of Oxygen Availability on Host State and Phage – Host Dynamics in Multi-Phage Environments

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Bacteriophages are the most abundant biological entities on Earth, driving microbial turnover, gene transfer, and nutrient cycling. Phage–host interactions depend on host physiology

and environment, with oxygen availability being critical. Aerobic–anaerobic transitions alter metabolism, affecting phage adsorption, replication, and assembly, and can trigger SOS responses that could induce prophages. Despite its importance, the effect of oxygen availability on infection dynamics in complex viral communities is poorly understood, as most studies focus on single phage–host systems. Understanding this is key to predicting phage behavior in fluctuating redox environments like wastewater and sediments. In this study, we examined how oxygen availability affects infection outcomes when *Serratia plymuthica*, carrying four putative prophages, is exposed to a sewage-derived virome.

We studied lytic infection, prophage activation, and phage diversity under four oxygen regimes: aerobic growth and infection (AP), aerobic growth with anaerobic infection (AAN), anaerobic growth with aerobic infection (ANA), and anaerobic growth and infection (ANP). Plaques from overlay assays were purified, sequenced (MiSeq), and assembled *de novo* to assess infection outcomes.

Under aerobic conditions, plaques were morphologically diverse, and assemblies yielded four complete phages (three with Myovirus and one with Siphovirus morphotype), showing efficient lytic infections. Under oxygen transitions (AAN, ANA), plaques were uniform and revealed one wastewater-derived phage with three host prophages, suggesting strong induction under redox stress. Under strict anaerobic conditions (ANP), the host remained stable and resistant to most external phages; two phage types were recovered—a host prophage and the same wastewater-derived phage, which persisted across all conditions, indicating its ability to infect stressed or metabolically constrained hosts.

Oxygen availability controls whether infection proceeds via external phages or prophages. Aerobic conditions favor lytic infections, while oxygen limitation or transition triggers prophage induction. The host is stable under anaerobic conditions, limiting external infections except for a highly adaptive phage capable of infecting under all oxygen regimes. These results highlight oxygen availability as a key regulator of phage–host dynamics. Future work will focus on this persistent phage and the host's oxygen-dependent infection responses.

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#### P-EME-058

##### The Soil Microplastics Observatory (SMO): unravelling the impacts of plastic inputs by different land use types on microbes in agricultural soil and rhizosphere

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Microplastic (MP) particles are a growing concern, found in ecosystems globally, yet their impact on soil and rhizosphere microbes remains poorly understood. MP enters arable soils primarily via agricultural practices and atmospheric deposition. A complex interaction of abiotic and biotic factors influences how microplastics affect the rhizosphere and plants. MP distribution is non-uniform, postulated to accumulate in the rhizosphere due to root growth and water uptake.

Our study aims to address critical research questions regarding the effect of MP in soil. We established the Soil

Microplastic Observatory (SMO) at the Ecological Botanical Garden of the University of Bayreuth. The experiment involves 32 randomized plots, with strawberries or a grass mixture representing two different land-use types. In MP-treated plots, environmentally relevant MP particles of different polymers (PS, PE, PET, and PP, size <125 µm) were added to the topsoil at a low dose to simulate atmospheric deposition. Additionally, fragments of PBAT and LDPE films were added to strawberry plots to simulate agricultural mulching.

Soil temperature, moisture, and water potential are continuously monitored by sensors. Depth-resolved mobilization, accumulation, and degradation of MP will be quantified by particle analytics and TD-GC-MS/MS. Microbiota in bulk and rhizosphere soil, as well as the microbial plastisphere on recovered MP, will be analyzed through fluorescence microscopy and amplicon sequencing.

First results of this ongoing work show potential physiological effects of MP presence on leaf chlorophyll content. Our study will allow us to better understand how microplastic pollution interacts with plant roots and soil microbial communities under realistic field conditions.

This knowledge is essential for evaluating the long-term implications of microplastic contamination for soil health and crop productivity, and for guiding more sustainable agricultural and plastics management strategies.

#### P-EME-059

##### Mathematical modeling of cyanotoxin biodegradation in lakes

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Cyanotoxins, including the microcystin (MC) hepatotoxins, pose a significant human health risk. Mathematical models are critical tools for managing lake systems (e.g. evaluating nutrient reduction scenarios, climate change) and they support research (e.g. hypothesis testing). A number of mechanistic models have been developed that simulate production of MC by *Microcystis* considering the effects of temperature, light and nitrogen (Hellweger et al., 2022; Schampera and Hellweger, 2025). However, loss of MC is still simulated via simple first-order decay, although there are several mechanisms, including biodegradation by planktonic and zooplankton gut bacteria, photodegradation and adsorption/sedimentation that may exhibit non-linear kinetics. For example, MC-degrading bacteria (*mlrA*+) must first grow to sufficient numbers before they can impact the toxin concentration. Here, a model for MC loss considering biodegradation, photodegradation and sedimentation is presented. The model includes state variables for phytoplankton and extracellular MC and the above-mentioned processes. Parameterization is supported by a comprehensive literature review (e.g. temperature optima observed in laboratory studies) and an automatic calibration routine (Nelder-Mead method). The model is applied to three systems (Dianchi Lake, San Juan Reservoir, Gongendo Reservoir) and can reproduce the main observed patterns, including the time-lag between the appearance of dissolved MC and MC-degrading bacteria. The model ranks the importance of loss processes: biodegradation in the zooplankton gut > biodegradation by planktonic bacteria > sedimentation > outflow > photolysis. A model with first-order decay cannot reproduce the observed temporal pattern.

Results of management (adding *mlrA* bacteria to the lake) and climate change (+3°C) simulations are presented. This model constitutes an advance in mechanistic modeling of cyanotoxins. Major challenges include the uncertainties in quantifying MC-degrading bacteria concentrations and the effects of other dissolved organic carbon (DOM) substrates on their dynamics.

Hellweger, F.L., Martin, R.M., Eigemann, F., Smith, D.J., Dick, G.J., Wilhelm, S.W., 2022. *Science* 376, 1001-1005.

Schampera, C., Hellweger, F.L., 2025. *Ecological Modelling* 504, 111094.

#### P-EME-060

##### Synergistic effects of palm shell mulching and biogenic silicon nanoparticles derived from *Streptomyces* sp. on enhancing water productivity and yield in tomato cultivated in sandy soils in the United Arab Emirates

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Addressing water scarcity in agriculture requires innovative methods to improve water-use efficiency (WUE). This study investigates the effects of four irrigation levels (100%, 75%, 50%, and 25% of crop evapotranspiration [ET<sub>c</sub>]) alongside palm shell mulching (M), biogenic silicon nanoparticles (BSiNPs) derived from *Streptomyces* sp., or their combination (M + BSiNPs) on water use efficiency (WUE), evapotranspiration, physicochemical properties, yield, and net profit of tomato plants employing a deep drip irrigation system. Irrigation requirements during the growth phase ranged from 80.16 to 815 mm/day, subsequently declining to 16.22 to 2.2 mm/day at 25% ET<sub>c</sub>. The M and M + BSiNPs treatments increased fruit weight by 31-62%, whereas BSiNPs enhanced yield by 29% at the lowest irrigation rate (IR 25%). The combination of M and BSiNPs treatments increased carbohydrate, protein, and moisture levels, significantly reduced ET<sub>c</sub>, and raised WUE. The yield response factor remained below the FAO (2002) standard, signifying resilience to water stress. The energy savings from M + BSiNPs treatments ranged from 35.33% to 69.18%. The use of IR at 25%, alongside M and BSiNPs, improved marketable yield, yielding relative increases of 44-61% compared to the control group. The reduction in irrigation costs increased net benefits by 35%. The application of palm shell mulching and BSiNPs with reduced irrigation (IR 25%) offers a sustainable strategy to enhance water-use efficiency, increase production, and maximize economic returns for tomato plants cultivated in sandy soils in the United Arab Emirates.

#### P-EME-061

##### Biogenic Silver Nanoparticles from *Streptomyces* sp. Enhance Cucumber Immunity Against Damping-off Disease for Sustainable Agriculture in the United Arab Emirates

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Cucumber is a crucial global vegetable crop, but its cultivation is significantly hindered by damping-off disease, predominantly caused by *Pythium aphanidermatum*, resulting in substantial economic losses. Chemical fungicides provide excellent control but pose significant risks to human health and the environment, underscoring the urgent need for sustainable alternatives. This study examined, for the first time, the efficacy of zinc (Zn) nanoparticles (ZnNPs) generated by *Streptomyces* sp. as an environmentally sustainable approach to combat damping-off disease in cucumbers. The biosynthesized ZnNPs were spherical, with an average size of 45 nm and a surface charge of -23.8 mV. At a concentration of 100 µg/mL, they demonstrated significant antioxidant activity, neutralizing 94% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, and effectively inhibited the *in vitro* growth of various phytopathogenic fungi, including *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, and *P. aphanidermatum*. The application of biogenic ZnNPs at a dosage of 50 mg/L increased cucumber seed germination by 95% in the presence of *P. aphanidermatum*. *In vivo* investigations revealed that biogenic ZnNPs treatment (50 mg/L) as a soil drench diminished the damping-off disease index by 95%, concurrently enhancing fruit weight and overall yield by 54% and 69%, respectively. Biogenic ZnNPs markedly elevated expression of essential defense-related genes (Lox, WRKY, LecRK, and PR1a) in infected plants, thereby enhancing the accumulation of growth hormones, phenolics, flavonoids, and systemic acquired resistance (SAR). Moreover, the treatment with biogenic ZnNPs improved antioxidant activity and elevated chlorophyll and carotenoid levels. Soil drenching with biogenic substances restored antioxidant enzyme levels (peroxidase, polyphenol oxidase, and catalase) to near-normal levels and increased chlorophyll content by 225% and carotenoid content by 91% relative to the control group. The application of 50 mg/L ZnNPs as a soil drench with *P. aphanidermatum* was significantly more effective in alleviating damping-off than all other treatments, with efficacy comparable to that of the metalaxyl chemical fungicide treatment. The findings underscore the promise of biogenic ZnNPs produced by *Streptomyces* sp. as a sustainable, eco-friendly alternative to chemical fungicides for the management of damping-off disease in cucumber agriculture.

#### P-EME-062

##### High-Resolution Temporal Dynamics of Methanotrophs and Methane Fluxes in a Rewetted Temperate Peatland

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Peatlands hold nearly a third of global soil carbon while covering only ~3% of Earth's land. When they are drained, accelerated decomposition of the stored organic matter leads to the release of vast amounts of CO<sub>2</sub> in the atmosphere. Rewetting, an increasingly used restoration strategy, mitigates CO<sub>2</sub> release but elevates methane (CH<sub>4</sub>) emissions. Methanotrophs, meaning CH<sub>4</sub>-oxidizing organisms, are the only known biological filter of CH<sub>4</sub>, yet their temporal development and resilience to environmental disturbances post-rewetting remain poorly understood.

This study (within the framework of WETSCAPES 2.0—a DFG TRANSREGIO collaborative research centre on peatland rewetting and its ecological, hydrological, and

biogeochemical consequences) focuses on the Zarnekow percolation fen—a long-term monitoring site of the TERENO Northeast German Lowland Observatory. Field campaigns were conducted twice a month over the summer and autumn 2025 to capture the seasonal variability of CH<sub>4</sub> fluxes, porewater geochemistry and methanotrophic community composition, abundance and activity. DNA- and RNA-based analyses are being used to link high-resolution temporal changes in hydrology, porewater chemistry, methane fluxes, soil temperature and methanotroph dynamics, aiming to contribute to a predictive understanding of carbon budgets in rewetted temperate peatlands. Preliminary data across multiple sampling dates indicate a consistent decline of dissolved CH<sub>4</sub> concentrations in porewater with increasing depth, suggesting strong depth-dependent controls on peat CH<sub>4</sub> concentrations.

#### P-EME-063

##### Isolation and characterization of pharmaceutical-degrading bacteria

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Advances in medicine and the resulting demographic change have contributed to an increase in the consumption of pharmaceuticals and, consequently, also to an increased release of such compounds into the environment. As pharmaceuticals are often insufficiently degraded in wastewater treatment plants (WWTPs), the North-West Europe Interreg program "Prevent water pollution by pharmaceuticals" (PREWAPHARM) aims to address the issue of medicine residues in water resources through a transnational collaboration, fostering removal of high priority pharmaceutical directly at the source. Within the framework of this initiative, we plan to develop bioaugmented filter systems for improved pharmaceutical removal from water in decentralized water treatment facilities at healthcare institutes and remote communities to make microbial and technical water purification processes compatible and synergistic.

Using bioelements and activated sludge from a municipal WWTP, along with hospital wastewater samples, we started enrichment cultures to isolate bacteria capable of biological degradation of pharmaceuticals. For initial studies, metformin (MET) and diclofenac (DCF) were selected due to their global usage, insufficient degradation in conventional WWTPs, and occurrence in surface waters at concentrations that are potentially toxic to aquatic life.

We were able to observe reproducible growth in transferred cultures, accompanied by a decrease in the concentrations of both substances. Further analysis of transformation products formed during degradation is still pending.

These initial results present a promising step towards establishing a repository of bacteria that can be used in bioaugmented filter systems. Current efforts are focused on the isolation, identification, and further characterization of the DCF- and MET-degrading bacteria.



## P-EME-064

### Degradation of cholate by *Novosphingobium* sp. strain Chol12 suggests the existence of two classes of 7 $\alpha$ -hydroxysteroid dehydratases among bile acid-degrading bacteria

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Bile acids (BA) are amphipathic steroids produced in the digestive tract of vertebrates. After excretion, they can serve as carbon and energy sources for environmental bacteria. Degradation of BA proceeds via formation of  $\Delta$ 1,4-intermediates in Actinobacteria and in  $\gamma$ - and  $\beta$ -proteobacteria. In contrast, sphingomonads such as *Sphingobium* sp. strain Chol11 use a variant that involves the formation of  $\Delta$ 4,6-intermediates from 7-hydroxylated BA via water elimination at C7 catalyzed by the 7 $\alpha$ -hydroxysteroid dehydratase (Hsh2), the key enzyme of this variant [1].

The novel isolate *Novosphingobium* sp. strain Chol12 also degrades 7-hydroxy BA via  $\Delta$ 4,6-intermediates but showed different  $\Delta$ 4,6- and  $\Delta$ 1,4-intermediate formation kinetics compared to strain Chol11. This study aimed to compare the Hsh2 activity from both strains using  $\Delta$ 4-3-ketocholate and 12 $\beta$ -DHADD (with and without acyl-side chain, respectively) as substrates. Purified Hsh2Chol12 showed activity with 12 $\beta$ -DHADD but, unlike Hsh2Chol11, no activity with  $\Delta$ 4-3-ketocholate was observed, suggesting that the presence of an acyl-side chain affects the Hsh2 activity in strain Chol12.

A phylogenetic tree of Hsh2 homologs from different BA degraders showed that Hsh2 from strains Chol12 and Chol11 formed distinct clades. Hsh2Chol12 clustered with homologs from other *Novosphingobium* species, such as *N. aromaticivorans* DSM12444, while Hsh2Chol11 grouped with Hsh2 from *Sphingobium* species like *S. herbicidovorans* DSM11019. Functional analysis of *hsh2* using *Pseudomonas stutzeri* Chol1 $\Delta$ kstD1 $\Delta$ stdA1 as an expression platform confirmed that Hsh2 from both *Novosphingobium* strains produced different intermediates compared with homologs from *Sphingobium*, *Caenibius*, *Azoarcus*, and *Dietzia*. Cross-complementation of Chol11 $\Delta$ hsh2 with intact copies of *hsh2* from these genera are currently in progress.

However, Hsh2Chol12 and Hsh2Chol11 showed a high structural similarity.

Our results provide novel insights into the substrate specificity of hydroxysteroid dehydratases and might be relevant for the biotechnological production of pharmaceutical steroid compounds.

[1] Feller FM, Holert J, Yücel O, Philipp B (2021). Degradation of bile acids by soil and water bacteria. *Microorganisms* 9:1759.

## P-EME-065

### Microbially influenced corrosion in monopiles of offshore wind power systems: key players and potential protection measures

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In the maritime sector microbially influenced corrosion (MIC) is a widespread issue affecting the service life of steel-made structures and thus causing economic damage. Support structures of offshore wind turbines and especially monopiles are particularly prone to MIC as the cylindrical hollow piles are driven into the seabed and trap seawater with only limited exchange with the ambient water masses. These special conditions play a pivotal role in shaping the microbial activity and biogeochemical processes inside monopiles and thus also influence MIC. So far, no published studies of MIC in monopiles are available and commonly used corrosion protection measures only address electrochemical corrosion.

In the interdisciplinary MiCorFe project we aim at developing effective, environmentally friendly and economically feasible protection measures against MIC in offshore monopiles. In the first phase, we focused on obtaining a solid understanding of the environmental conditions, microbial communities and mechanisms involved in MIC inside the monopiles. For this baseline study, in situ incubations of commonly used mild steel inside a monopile in the North Sea were carried out for 3 and 12 months, respectively, and accompanied by repeated geochemical profiling. First results show a strong seasonal stratification of the water column inside the monopile with the absence of oxygen and accumulation of biogenic methane and hydrogen sulfide (up to millimolar concentrations) in bottom waters at certain times of the year. Despite storm-related ventilation events of the water column, RNA-based taxonomic profiles of biofilms from steel samples incubated at 1 m above the seafloor exhibited an enrichment in sulfate reducing Bacteria and methanogenic Archaea, demonstrated to be relevant players in MIC under anoxic conditions. Steel-associated communities at 6 m water depth, hallmarked by oxic conditions, were enriched in Fe-oxidizing Bacteria after 3 months of incubation. In the 12 months incubation, however, this effect was less prominent and certain heterotrophic and even sulfate-reducing Bacteria were observed in high abundances in biofilms of the 6 m incubation. Our current and future work involves lab-based and in situ incubations of suitable coated steel and steel alternatives as well as the development of local inhibition measures to identify the most efficient measures for MIC inhibition inside monopiles.

## P-EME-066

### Presence and role of non-culturable *Listeria monocytogenes* in food production environment

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Transmitted mainly via food, *Listeria monocytogenes* the causative agent of listeriosis, can cause fatal infections threatening human health and thus its presence in food and production environments is strictly regulated and routinely monitored. To date, all detection methods are growth based, but recent research highlights *L. monocytogenes* ability to enter a viable but non culturable (VBNC) state which enables the pathogen to persist in production environments undetected (Domínguez-Medina et al., 2020).

In the current study we investigated the presence of non-culturable *L. monocytogenes* cells in food production environments struggling with persistent contaminations.

Over the course of a year, we collected and analysed >250 swab samples in two food processing facilities by a combined detection approach including direct qPCR and enrichment to detect and differentiate culturable and non-culturable cells. Additionally, we evaluated published *L. monocytogenes* PCR protocols to develop a reliable viability PCR assay based on the PMAxx™ system and established evaluation protocols.

Screening results via the combined enrichment approach microbiological results showed a positivity rate of over 5%, consistent with previous reports from Austria. Via qPCR, the detection rate was above 40%, revealing a recurrence of bacterial load which could not be cultivated.

Samples taken before and after disinfection revealed that *L. monocytogenes* could successfully persist in production environments despite intensive cleaning and disinfection efforts. We also found that even after disinfection bacteria can still be detected and reappear on previous negative sampling sides indicating occurrence of VBNC cells, although presence of dead cells could not be completely excluded. First results of the primer set evaluation for vPCR clearly demonstrated that existing assays are not readily applicable for vPCR and optimisation steps are necessary.

In summary this study shows that VBNCs can play a role in non-sufficient disinfection and relapsing outbreaks of *L. monocytogenes*. To support these findings, we develop a vPCR to distinguish between DNA artefacts and potentially threatful persisting cells.

Domínguez-Medina et al. (2020). *Aquatic environment drives the emergence of cell wall-deficient dormant forms in Listeria*. <https://doi.org/10.1038/s41467-019-13981-0>

## P-EME-067

### Exploring the Altiarchaeal CO<sub>2</sub> Fixation Pathway: An Uncultivated Archaeon from the Deep Biosphere

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The deep subsurface biosphere hosts unique microbial communities thriving under extreme conditions, such as CO<sub>2</sub>-driven cold-water geysers, where groundwater erupts from CO<sub>2</sub>-rich aquifers. These ecosystems are dominated by autotrophic microorganisms utilizing the Wood-Ljungdahl (WL) pathway for carbon fixation. The archaeon *Candidatus Altiarchaeum hamiconexum*, dominant in biofilms at the Mühlbacher Schwefelquelle (MSI) in Regensburg, possesses a distinctive, factor 420-independent variant of the WL

pathway that may employ both tetrahydrofolate (H<sub>4</sub>F) and tetrahydromethanopterin (H<sub>4</sub>MPT) methyl branches for CO<sub>2</sub> reduction. However, *Altiarchaea* remain to be uncultivated in the lab due to its unknown electron donors and acceptors responsible for the growth. To elucidate the enzymatic functions and cofactor specificities of key proteins involved in the altiarcheal WL pathway through heterologous expression and biochemical characterization.

Candidate genes involved in altiarcheal WL pathway were expressed in *E. coli* BL21. Recombinant proteins were purified, and enzyme assays were performed to determine substrate affinities and kinetic parameters for H<sub>4</sub>F- and H<sub>4</sub>MPT-dependent reactions. The gene Alti\_1516, initially annotated as a methylenetetrahydrofolate dehydrogenase, showed high specificity for H<sub>4</sub>MPT. Kinetic analysis revealed a *K<sub>m</sub>* of 29 µM and a *V<sub>max</sub>* of ~2000 U/mg for methylene-H<sub>4</sub>MPT, while activity toward methylene-H<sub>4</sub>F was <0.05 U/mg. These results confirm mis annotation and identify Alti\_1516 as a methylenetetrahydromethanopterin dehydrogenase. This work provides the first biochemical evidence for a factor 420-independent, H<sub>4</sub>MPT-dependent enzyme in *Altiarchaeum*, revealing a unique adaptation of the WL pathway in subsurface archaea. Ongoing studies aim to validate additional candidate enzymes and analyze native biomass to better understand carbon fixation and metabolic strategies in CO<sub>2</sub>-driven ecosystems.

## P-EME-068

### From Green to Gray: How Exposomes Drive Non-Linear Microbiome Responses in Urban Environments

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**Introduction:** Urban green spaces are vital for biodiversity, human well-being, and ecosystem resilience within the One Health framework. Yet, rapid urbanization through land occupation, pollution, and habitat fragmentation alters these environments in complex ways. The resulting gradients of chemical, physical, and biological exposures create a dynamic urban exposome driving non-linear biodiversity responses.

**Goals:** This study aims to understand how soil and sediment microbiomes in urban environments adapt to anthropogenic influences. Levels of human impact were defined using remote sensing-based Local Climate Zones (LCZs) and a long-read metagenomics approach.

**Methods and Materials:** Based on LCZs data, 36 samples (24 soil, 12 sediment) were collected along the Schwabinger Bach in Munich's English Garden across four sites ranging from high to low anthropogenic impact (autumn 2023). DNA was extracted with the PowerSoil Pro Kit (Qiagen, Germany), and sequencing was performed on a PromethION P2P

(Oxford Nanopore Technologies, UK). Data were processed using Dorado v1.1.1, Porechop v0.2.4, Chopper v0.4.4, NanoPlot v1.41.0, and SqueezeMeta v1.7. Resistance genes were identified with AMRfinderPlus v3.12.8. Datasets were downsampled to 300k reads per sample, and analyses conducted in RStudio v4.3.1.

**Results:** Long-read sequencing produced 276 Gb of data (mean 6 kb), providing strong coverage. Sediment samples showed higher taxonomic richness than soils, suggesting sediments act as microbial reservoirs. Both habitats shared a core microbiome dominated by *Bradyrhizobium*, *Methyloceanibacterium*, and *Nocardioides*. PERMANOVA and PCoA revealed spatial clustering: soils grouped by transect orientation, sediments by site. Alpha diversity varied significantly among sites, indicating non-linear responses to urbanization, with major shifts at site 3. Functional profiles showed greater abundance of metal resistance (*ars*, *mer*, *czc*, *cop*) and antimicrobial resistance genes in urbanized areas, suggesting adaptation to anthropogenic stressors.

**Summary:** Urban exposome gradients shape microbial diversity and function across soil and sediment habitats. Understanding these adaptive patterns is key to predicting ecosystem resilience and designing greener, healthier cities that harness microbial ecosystem services.

**References:** Nugent, A. & Allison, S. D. (2022). A framework for soil microbial ecology in urban ecosystems. *Ecosphere*, 13(3), 3968.

## P-EME-069

### Effects of downstream oxygenation on the microbial community in sewage sludge treatment

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**Introduction:** Residual sludge generated during wastewater treatment is a significant economic and environmental challenge. The company Pro-Entec developed a patented process (PEGA, "Pro-Entec Gärrest-Aufbereitung"), a technology that reduces residual sludge volume and nitrogen-load of the waste water to CH<sub>4</sub> by downstream intermittent oxygenation of the residual sludge. The treated sludge is then returned to the digester.

**Goals:** The aim of this study was to analyze the influence of the PEGA process on the microbial community structure in PEGA-treated vs. regular treated digested sewage sludge.

**Methods:** Bacterial and Archaeal 16S rRNA gene amplicons were amplified from gDNA from sludge of both fermenters and submitted for sequencing.

**Results:** The most abundant bacterial phyla included *Bacteroidota*, *Firmicutes*, *Actinobacteria* and *Chloroflexi*. Among the dominant taxonomic groups, representatives of the genus *Candidatus Microthrix* and the family *Anaerolineaceae* were identified - microorganisms previously reported in anaerobic sludge. In the fermenter with the integrated PEGA stage, denitrifying microorganisms, including *Comamonas*, were additionally detected, which were absent in the reference fermenter without aerobic treatment. This suggests that the PEGA process promotes the occurrence of denitrifying populations.

Among the Archaea, the phylum *Halobacterota* was dominant, with numerous methanogenic archaea, including *Methanosaeta*, *Methanolinea*, and *Methanospirillum* being detected, all of which play a central role in biogas formation.

Overall, the sludge microbiome exhibited high diversity. Distinct differences in the relative abundance of specific bacterial phyla, particularly *Actinobacteriota* and *Firmicutes*, were observed between the two fermenters. In the PEGA-treated digester, the relative abundance of *Actinobacteriota* decreased slightly, whereas that of *Firmicutes* increased. In contrast, no major differences were observed among the archaeal communities, indicating that the aerobic intermediate treatment primarily affects the bacterial community, while the abundance of methanogenic archaea remains largely stable.

Conclusion: These findings provide evidence that treating digested sludge with low amounts of oxygen induces microbiological shifts in digested sludge, which potentially contributes to enhanced biogas yield and nitrogen reduction observed before.

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#### P-EME-070

##### Genomic and Proteomic Insights into Adaptive Phosphonate Degradation by *Delftia* sp. UMB14

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Phosphonates are organophosphorus compounds characterized by a stable carbon–phosphorus bond that confers resistance to chemical and biological degradation. They are widely employed as complexing agents in detergents, water treatment, and industrial cleaning to prevent scale formation and enhance cleaning efficiency. However, their chemical persistence raises environmental concerns, as phosphonates contribute to phosphate accumulation in aquatic systems, potentially triggering eutrophication and disrupting wastewater treatment processes. Consequently, understanding their biological degradation is essential for developing sustainable mitigation strategies.

The bacterial strain *Delftia* sp. UMB14, isolated from biofilms in domestic washing machines, has been identified as a phosphonate degrader. In degradation assays, repeated passaging of *Delftia* sp. UMB14 led to accelerated phosphonate breakdown, indicating adaptive genetic mechanisms may be involved. We will test whether the enhanced degradation observed in *Delftia* sp. UMB14 results from physiological adaptation or genomic changes, integrating proteomic analyses to correlate genomic alterations with changes in protein expression and enzymatic activity.

Whole-genome sequencing of *Delftia* sp. UMB14 was performed using Oxford Nanopore technology to detect mutations and structural rearrangements associated with improved phosphonate metabolism. Orbitrap-based proteomic analysis revealed distinct changes in protein expression profiles across passages 0, 6, and 15. These variations indicate a dynamic metabolic adaptation over time, characterized by a reduction in overall protein diversity and a relative increase in enzymes associated with phosphonate utilization. This suggests a progressive optimization of metabolic pathways during prolonged exposure to phosphonates. Complementary proteomic analyses across

successive passages aim to link genomic adaptations with changes in protein expression and enzymatic activity. These results are preliminary and will require further quantitative proteomic and genomic analyses. This study integrates genomic and proteomic data to elucidate the molecular basis of rapid microbial adaptation and its effect on phosphonate biodegradation. The findings will inform the development of optimized microbial systems for efficient biotechnological remediation and wastewater treatment, addressing environmental pollution with persistent organophosphorus compounds.

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#### P-EME-071

##### Establishment of a valid method for detection of ESBL-producing Enterobacteriaceae and VRE-producing Enterococci

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#### Introduction

The occurrence, persistence and spread of antibiotic-resistant bacteria (ARB) and the transfer of resistance through gene transfer is of significant importance to human health, making this issue one of the greatest global threats to the health of humans, animals, plants, and ecosystems. To achieve this appropriate, standardized and valid cultural methods must be developed to detect ARB. These insights are necessary to constrain the ABR spread cycle and to enhance awareness and education on the use of antibiotics.

#### Goals

For improving the comparability of ARB bacteria in the aquatic environment, it is necessary to establish a valid cultural detection method.

#### Methods

Clinically relevant representatives of ESBL-producing Enterobacteriaceae (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter* spp., *Escherichia coli*, *Citrobacter* spp.) and VRE-producing enterococci were examined on CHROMagar™ ESBL and CHROMagar™ VRE. Presumptive colonies on the selective agars were confirmed by identification the species with MALDI-TOF-MS (Matrix-assistierte Laser-Desorption/Ionisation Flugzeit-Massenspektrometrie) and the antibiotic resistance profile according to EUCAST (European Committee on Antimicrobial Susceptibility Testing). For the validation of the primary method, secondary tests were necessary (CCA and oxidase reaction for species, reverse hybridization for resistance). The performance data were collected in accordance with DIN EN ISO 13843 (2018) [1].

#### Results and conclusion

As part of the validation, the performance characteristics sensitivity, specificity, false positive rate, false negative rate, efficiency, selectivity, robustness, upper limit and precision of the methods were determined.

A harmonized methodology for the detection of ESBL-producing Enterobacteriaceae and VRE-producing Enterococci in surface water was successfully developed.

**References** [1] DIN EN ISO 13843:2018-03. 2018 Wasserbeschaffenheit - Anforderungen zur Bestimmung von Leistungsmerkmalen von quantitativen mikrobiologischen Verfahren, Beuth-Verlag

## P-EME-072

### Mosses and Nostocs: an update from the Fichtel Mountains

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Symbioses among bryophytes and cyanobacteria are of increasing interest since their rediscovery, particularly *Nostoc*-Bryophyte symbioses. Here we summarize the results of our recent survey of mosses in the Fichtengebirge region for *Nostoc* in forest mosses. We report several new hosts and new *Nostoc* symbionts. We also report a possible endophytic life-stage of cyanobacteria in these mosses. We present our cultivation and nanopore sequencing pipeline for sequencing of 16S and *nifH* barcodes, as a potentially useful method for other rare, slow-growing, plant-associated microorganisms.

## P-EME-074

### Analysis of the unusual Phycoerythrin (PE-III) in *Prochlorococcus*

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*Prochlorococcus*, the most abundant oxygenic phototroph on Earth, is a key contributor to global primary production and carbon cycling. In contrast to its close relative *Synechococcus* and other cyanobacteria, *Prochlorococcus* does not possess phycobilisomes (PBS) and harvests light using divinyl-chlorophylls and chlorophyll binding proteins (Pcbs). Interestingly, remnants of genes for phycoerythrin, a phycobiliprotein that is typically found within the PBS, can be found within different species and ecotypes of *Prochlorococcus*. While low light (LL-) ecotypes of *Prochlorococcus* possess both the genes correlating to the  $\alpha$ - and the  $\beta$ -subunit of PE-III, high light (HL-) ecotypes show an even more reduced form with only the genes for the  $\beta$ -subunit being present within these organisms.

While previous studies suggest only a minor role for PE-III in energy transfer, its precise function in *Prochlorococcus* remains unclear. Within this study, different in vitro and in vivo experiments are combined to investigate PE-III of two different *Prochlorococcus marinus* strains (SS120 (LL) and MED4 (HL)).

[1] Chisholm S., Olson R., Zettler E., Goerick R., Waterbury J., Welschmeyer N. 1988. A novel free living prochlorophyte abundant in the oceanic euphotic zone. *Nature* 334, 340–343. [2] Flombaum P., Gallegos J. L., Gordillo R. A., Rincón J., Zabala L. L., Jiao N., Karl D. [3] Moore L. R. R. Rocap G., Chisholm S. W. 1998. Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. *Nature* 393:464–67 [4] Partensky F., Blanchot J., Vaultot D. 1999. Differential distribution and ecology of *Prochlorococcus* and

*Synechococcus* in oceanic waters: A review. . In *Marine Cyanobacteria*, ed. L. Charpy, A. Larkum, pp. 457–75. Monaco: Musée Océanographique [5] Waterbury J. B., Rippka R. 1989. The order Chroococcales. In: Kreig NR, Holt JB (eds) *Bergey's manual of systematic bacteriology* vol 3. Williams and Wilkins, New York, pp 1728–1746 [6] Hess W. R., Steglich C., Lichtlé C., Partensky F. 1999. *Plant Mol. Biol.*, 40, 507– 521. [7] Hess W. R., Partensky F., van der Staay G. W., Garcia-Fernandez J. M., Börner T., Vaultot D. 1996. *Proc. Natl. Acad. Sci. USA*, 93, 11126– 11130. [8] Penno S., Campbell L., Hess W. R. 2000. *J. Phycol.*, 36, 723– 729. [9] Steglich C., Mullineaux C., Teuchner K., Hess W., Lokstein H. 2003. Photophysical properties of *Prochlorococcus marinus* SS120 divinyl chlorophylls and phycoerythrin in vitro and in vivo, *FEBS Letters*, 553

## P-EME-075

### Irrigation water quality and soil type shape antibiotic resistance gene (ARG) and mobile genetic element (MGE) profiles and bacterial community composition in soil-plant systems

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**Introduction:** The reuse of wastewater for agricultural irrigation is increasingly adopted in water-scarce regions to optimize water use and recycle nutrients, thereby enhancing crop productivity and reducing reliance on synthetic fertilizers. However, wastewater contains bacteria and micropollutants, including antibiotics, biocides, and antibiotic-resistant bacteria, which can be incorporated by crops or persist on their surfaces, potentially entering the food chain and posing health risks.

**Goals:** This study investigated how irrigation water quality influences the soil and plant resistome and shapes the bacterial community composition in soil-plant systems.

**Method:** A greenhouse soil column experiment was conducted using Leptosol and Vertisol soils from the Mezquital Valley, Mexico, planted with cilantro (*Coriandrum sativum*) and irrigated for eight weeks with treated or untreated wastewater, either spiked or unspiked with antibiotics and disinfectants. Brilliant blue dye was added during the final irrigation to visualize water flow paths. Soil and plant samples were collected for DNA analysis by qPCR and 16S rRNA gene amplicon sequencing.

**Results:** ARG and MGE profiles differed significantly between stained and unstained soil, with stained soil showing higher ARG abundances. Irrigation with spiked wastewater significantly affected ARG and MGE profiles in soil. In the rhizosphere, soil type predominantly determined ARG and MGE distribution, with Leptosol showing higher relative abundances than Vertisols. Spiked wastewater irrigation increased the relative abundances of class 1 integron integrase gene (*intI1*), sulfonamide (*sul1*, *sul2*), and tetracycline (*tetA*) resistance genes in soil and rhizosphere, as well as erythromycin (*ermA*) and fluoroquinolone (*qnrA*) resistance genes in the phyllosphere.

Bacterial community composition in stained soil and rhizosphere was primarily shaped by soil type but also by the spiking of the irrigation water, whereas water type influenced only the rhizosphere microbiome.

**Conclusions:** Antibiotics and disinfectants in irrigation water significantly increased the relative abundance of various ARGs in the soil-plant system, while the microbial community composition was primarily shaped by soil type followed by spiking level. These findings highlight the pivotal role of wastewater-borne micropollutants in determining the antibiotic resistance profile and shaping microbial community dynamics across the soil-plant continuum.

## P-EME-076

### Algae on the menu: Searching for ulvan and porphyran-degrading bacteria and pathways in rumen fluid and faeces of dairy cows

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Marine macroalgae from the Baltic Sea represent an underexploited biomass resource [1]. Within the *Alg4Nut* project, their use as feed additives for dairy cows is being explored, focusing on the effects on the rumen and gut microbiota, as well as on algal polysaccharide (PS) degradation under anaerobic, fermentative conditions.

Therefore, we are currently working on isolating novel algal PS degraders from rumen fluid (RF) and faeces (F) samples, obtained from cows fed with *Ulva* sp. or *Porphyra* sp. (10% dry mass of total mixed ratio). After identification of the isolates, the ability to utilize the algal PS ulvan and porphyran is further being investigated. For isolation, a defined minimal medium (DMM) containing trace elements and vitamins and supplemented with 0.2 g/L yeast extract and either 5 g/L *Ulva* extract (soluble fraction, >90% ulvan) or *Porphyra* (purified, 80-90% porphyran estimated) was used. The isolates were identified by 16S Sanger sequencing and BLAST-based taxonomic assignment. Growth experiments on DMM, supplemented with either glucose, yeast extract or ulvan and yeast extract, with HPLC analysis of metabolite profiles, provided insight into their ability to degrade these PS and the metabolites produced.

A total of 16 isolates has been obtained and identified so far, including one isolate from porphyran medium (*Shigella sonnei*). Isolates from ulvan medium comprised *Streptococcus* sp. (RF n = 3; F n = 5), *Escherichia* sp. (RF n = 2, F = 1), *Proteus* sp. (RF = 2), *Enterocloster* sp. (F), and *Paenibacillus* sp. (RF). Only the *Escherichia* isolates showed growth on DMM, supplemented with ulvan and yeast extract. Further analyses are planned to get better insights into ulvan utilization.

In summary, first isolates from DMM supplemented with ulvan or porphyran extracts and inoculated with RF or F have been obtained. However, only three *Escherichia* isolates demonstrated growth on media containing ulvan extract. Based on these findings, further optimization of the isolation strategy is planned. In parallel, *Bacteroidia* strains, which may play a key role in the ruminal degradation of complex algal polysaccharides [2], will be screened for their ability to grow on monosaccharides, such as galactose, rhamnose, xylose and glucuronic acid that occur as structural subunits of ulvan and porphyran.

[1] Hasselström et al. (2020) *Sci Rep* 10

[2] Pudlo et al. (2022) *Cell Host Microbe* 30

## P-EME-077

### Following the lipids: insights into the ecology of symbiotic uncultured prokaryotes

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Among the uncultured microbial diversity uncovered by metagenomics over the past two decades, small-celled prokaryotes are predominant, particularly in freshwater ecosystems. The difficulty in obtaining these small prokaryotes in pure culture is likely due to their dependence on a host. In particular, the lack of lipid biosynthesis genes suggests a tight and obligatory symbiotic interaction through which they acquire host-derived lipids for cell membrane synthesis. However, due to the sparsity of cultured representatives, our knowledge of their membrane lipid composition remains limited. To investigate the membrane composition of small prokaryotes and potentially identify their hosts and mechanisms of membrane synthesis, we studied the microbial communities of three freshwater ecosystems: a high-CO<sub>2</sub> geyser, a groundwater system, and a lake, where the small, potentially symbiotic prokaryotes represent important community members. We compared the lipidomic and genomic composition of two cell-size fractions (>0.2 µm and >0.1 µm) by applying untargeted mass spectrometry analysis to characterize the lipids and integrating our results with metagenomic data. Beyond the community-driven differences in lipidomes among the three ecosystem types, we identified clear differences in the composition of the annotated lipids between the two cell-size fractions, which have not been reported before. Notably, we observed a clear enrichment of phosphatidylcholine (PC) in the small cell-size fraction of shallow lake samples, co-occurring with an enrichment of Saccharibacteria representatives within the prokaryotic community. However, no PC synthesis genes were linked to any metagenome assembled genomes (MAGs) classified as Saccharibacteria, nor to their putative actinobacterial hosts. This suggests that saccharibacterial cells may acquire lipids from an unknown host or at the community level. Additionally, we identify an enrichment of ceramide lipids in the small cell-size fraction of the geyser samples, which is noteworthy given the established role of these lipids in bacteria-eukaryote interactions. Yet, only ~4% of the metagenomic sequences were eukaryotic, suggesting ceramide lipids may instead play a role in prokaryote-prokaryote interactions in this environment. Overall, these findings provide a new perspective on the lipid composition of uncultured and potentially symbiotic prokaryotes and their ecological significance.

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## P-EME-078

### Residence of diverse nitrifiers with unstudied anaerobic metabolic potential in the hindgut of millipedes

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Nitrifiers are globally spread in diverse habitats, where they are classically considered to be aerobes responsible for aerobic transformation of reactive nitrogen, an ecosystem function linked to nutrient cycling and global change. However increasingly, they are detected in low oxygen and anoxic systems challenging this current understanding. Recently, we have identified diverse nitrifiers in gut compartments of different in-house reared species of millipedes (*Diplodota*). Direct redox measurements of the hindguts showed very reduced conditions of -199 mV to -230 mV, demonstrating the absence of standing concentrations of oxygen and other oxidised molecules. The relative abundance of nitrifiers of the *Nitrosospora*, *Nitrososphaera*, *Nitrolancea*, and *Nitrobacter* genera was shown to be comparable to common soil by 16S rRNA gene sequencing of DNA extracted from millipede mid- and hindguts. In contrast, the feed and sand used for rearing did not contain any detectable nitrifiers. Using a millipede faecal pellet as inoculum, we further cultured two separate nitrifiers: one pure culture of a new strain variant of *Nitrobacter vulgaris* and one enrichment culture with a new member of *Nitrosospora*, which is the nitrifier group with the highest relative abundance in anoxic millipede hindguts. A closed genome obtained by PacBio sequencing of the *Nitrobacter* strain surprisingly showed the presence of a complete *nos* (nitrous oxide reductase) operon with all genes necessary to produce the enzyme that converts nitrous oxide to di-nitrogen in the last step of denitrification. Intriguingly, the *nos* operon is flanked by several transposases and transferases, implying a possible horizontal gene transfer event as the explanation for its presence in the genome. Since the new *Nitrosospora*-like culture was obtained very recently, it still awaits a more detailed genomic analysis, which is ongoing. Together our data so far suggest that diverse nitrifiers could be stable community members in anoxic hindgut of millipedes performing little understood anaerobic metabolisms. Such nitrifier functions are likely to be more widespread than previously assumed and their environmental function in low-oxygen systems may need to be more thoroughly evaluated.

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## P-EME-079

### Enhanced metagenomic characterization of microbial communities in hyperarid subsurface habitats of the Atacama Desert

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Introduction: The hyperarid Atacama Desert represents one of the most extreme terrestrial ecosystems and serves as a Mars analog for astrobiological research. While near-surface microbial communities have already been characterized,

deeper subsurface habitats (>1 m) remain largely unexplored. Previous 16S rRNA studies identified persistent communities down to 4.20 m depth, but functional characterization of these extremophilic microorganisms was lacking.

Goal: This study extends our knowledge through deep metagenomic sequencing for functional characterization of microbial communities along a soil profile in the Atacama Desert. The objective is to identify metabolic strategies enabling microbial persistence under hyperarid isolation, high salt stress, and nutrient limitation.

Materials & Methods: Sediment samples from up to 420 cm depth were collected to obtain horizons representing surface (2, 10, 20 cm), intermediate (200 cm), and deeper subsurface (260, 420 cm) samples. Extremely deep metagenomic sequencing (>200 mio reads per sample) was performed using Illumina short-reads to analyze even low abundant taxa. A metagenomic analysis including QC, assembly, binning and MAG generation, gene functional annotation, and a gene-centric analysis was performed to study the full genomic potential.

Results: Metagenomic analysis identified more than 60 high-quality MAGs. Surprisingly, the gene-centric approach revealed more than 1.5 mio genes with a vast breadth of functions. With respect to the extreme conditions, our analysis focused on the core part of the energy metabolism as well as genes involved in high salt stress and nutrient limitation. We observe a clear difference between surface and subsurface samples, underscoring chemolithoautotrophic survival strategies in the absence of photosynthetic energy input.

Summary: The metagenomic data reveal specialized metabolic niches in hyperarid subsurface habitats: while halotolerance and osmoregulation enable survival in salt-rich surface layers, deeper subsurface colonization relies on chemolithoautotrophic metabolic pathways utilizing inorganic substrates (H<sub>2</sub>, CO<sub>2</sub>). These findings expand our understanding of desert biodiversity and provide genomic signatures for the search for life in extraterrestrial hyperarid environments.

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## P-EME-080

### Identifying microbial drivers of leaf litter decomposition in forests with contrasting mycorrhizal types

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Trees are the dominant primary producers in forest ecosystems and play a central role in global carbon (C) cycling. A substantial fraction of tree-assimilated C is shed as leaf litter and decomposed by soil microorganisms. Whether C from litter is rapidly mineralized to CO<sub>2</sub> or stabilized as soil organic matter largely depends on microbial activity and functional traits. Understanding the factors shaping litter-decomposing microbiomes is therefore essential for predicting the capacity of forests to act as C-sinks.

Most trees form symbiotic associations with either arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) fungi. Litter from AM-associated trees typically contains more accessible

nutrients and decomposes faster than more recalcitrant litter from ECM-associated trees. ECM fungi possess broad enzymatic repertoires and, in some taxa, oxidative mechanisms for nutrient mobilization, whereas AM fungi largely rely on interactions with other microbial taxa. Although these contrasts are well established, the multipartite interactions among trees, mycorrhizae, and other soil microorganisms, and particularly the functional differences of microbiomes in AM- and ECM-dominated forests, remain insufficiently understood.

To address this, we conducted an *in-situ* incubation experiment with *Acer saccharum* (AM-associating) and *Quercus alba* (ECM-associating) litter in AM- and ECM-dominated forest stands. We assessed the change in decomposer microbiomes with progressing litter decay and seasonal dynamics through analyzing litter and adjacent soil by metabarcoding of the 16S rRNA and ITS2 regions after 1, 3, 6, and 12 months. Additionally, we performed metatranscriptomic analyses for decomposer communities after 3 months. Combined with existing metagenomic data, these analyses will enable us to identify microbial taxa and processes driving litter decomposition, C- and nutrient processing in contrasting mycorrhizal contexts.

First results indicate that early decomposition of AM-litter was dominated by fast-growing *Aspergillaceae*, while *Sordariomycetes*, capable of degrading recalcitrant compounds, were more abundant in ECM-litter. These patterns align with our expectations and demonstrate the potential of our setup to uncover the functions of microbiome members beyond mycorrhizal fungi. Ultimately, this study will advance our understanding of the key microbial taxa critical for C cycling in forests and how microbiome dynamics are shaped by the mycorrhizal type.

## P-EME-081

### Mechanistic modeling of cyanobacteria growth and cyanotoxin production in Lake Mendota

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Toxins produced by cyanobacteria in freshwaters may lead to several problems such as dog deaths [1], human intoxications [2] or drinking water supply interruptions [3]. Mechanistic models may help us to understand the environmental processes driving this toxin production and to evaluate management measurements.

In order to understand better the toxin production we have used an existing mechanistic model [4] that simulates the growth of both toxigenic and non-toxigenic cyanobacteria *Microcystis* and the production of toxin Microcystin (MC). In this model, the growth of *Microcystis* cells depends on their phosphorus and nitrogen quota, light availability and temperature. The synthesis of MC depends on the nitrogen quota, light availability and temperature. The intracellular MC might become extracellular due to excretion, grazing or death of the cells. A general benefit provided by MC to *Microcystis* is included by making the growth rate a function of MC quota.

Here we present the extension and application of this model to Lake Mendota (Wisconsin, USA), where we simulated *Microcystis* and MC concentration from 2013 to 2018 [5]. The model can reproduce the observed seasonal patterns over 5 years. The year 2016 remains more challenging in terms of

modeling due to factors not explicitly included and related to the zebra mussel invasion that took place that year.

This application includes several extensions/improvements to the previous model applications:

- Dynamic simulation of sediment nutrient fluxes

- Simulation of grazing based on herbivore zooplankton concentration

- Simulation of zebra mussel grazing

- Simulation of additional non-*Microcystis* phytoplankton species

- Microcystis* overwintering process based on water temperature

## References

[1] McCarron, P., Rafuse, C., Scott, S., et al. Toxicon 2023, 227, 107086 <https://doi.org/10.1016/j.toxicon.2023.107086>

[2] Giannuzzi, L., et al. Mar. Drugs 2011, 9(11), 2164–2175 <https://doi.org/10.3390/md9112164>

[3] McKindles, K.M., et al. Appl. Environ. Microbiol. 2020, 86(19), e01397- <https://doi.org/10.1128/AEM.01397-20>

[4] Schampera, C.; Hellweger, F.L. Ecol. Model. 2025, 504, 111094. <https://doi.org/10.1016/j.ecolmodel.2025.111094>

[5] Magnuson, J.J., Carpenter, S.R., Stanley, E.H. Environmental Data Initiative 2022, North Temperate Lakes LTER: Phytoplankton - Madison Lakes Area 1995. <https://doi.org/10.6073/pasta/bd03330e83fc52a5442bb39e131c95e9>

## P-EME-082

### Characterization of Bacteriophages Infecting *Burkholderiaceae* Isolates as Model Systems for Phage-Bacterium Interactions

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Bacteriophages are the most abundant biological entities on Earth, infecting bacteria and shaping microbial populations and ecosystem processes. They display remarkable diversity in morphology, genome composition, and habitat preference. Beyond their ecological significance, phages serve as tools in medicine and biotechnology, from antibacterial therapies to the discovery of CRISPR/Cas systems. Despite their importance, phage–host interactions remain insufficiently understood, highlighting the need for well-characterized model systems.

This study aimed to establish model systems for phage–bacterium interactions by identifying bacterial isolates with few or no detectable prophages and isolating and



characterizing their infecting phages. These systems will support future studies on how phages influence bacterial growth, genomic plasticity, and transcriptional responses.

Two *Burkholderiaceae* isolates, B1 (*Paraburkholderia* sp. B1; 8.88 Mbp) and P1 (*Pandoraea* sp. P1; 5.4 Mbp) were obtained from the aquatic fern *Azolla filiculoides* and fully characterized. Bacterial analyses included morphology, growth kinetics, metabolic profiling, and prophage region prediction. B1- and P1-specific lytic phages were isolated from environmental samples and characterized using TEM, restriction pattern analysis, nucleic acid assessment, and host-range determination.

Bioinformatic analyses revealed that B1 lacks prophages, whereas P1 harbors a single, highly active and spontaneously inducible prophage (45 kb). These results identify B1 as an ideal model for studying prophage integration and phage-mediated horizontal gene transfer (HGT), while P1 represents a suitable system for CRISPR/Cas9-based prophage removal. Both bacterial strains and their associated phages have been fully characterized, providing a foundation for controlled investigations of phage-bacterium dynamics.

This work establishes two complementary *Burkholderiaceae* model systems for studying phage-mediated effects on bacterial hosts. The prophage-free strain B1 and the prophage-containing strain P1, together with their respective phages, provide versatile models for investigating how phages influence bacterial physiology, genome evolution, and HGT. These systems form a foundation for controlled studies of phage-host dynamics and will facilitate deeper insights into phage-driven adaptation in plant-associated microbiomes, with potential applications in microbial ecology, biotechnology, and synthetic biology.

### P-EME-083

#### Planktonic archaea in coastal water and sediment of the North Sea

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Planktonic archaea represent a major fraction of marine microbial communities and contribute to marine biogeochemical cycles. For instance, ammonia-oxidizing archaea of the family *Nitrosopumilaceae* (initially MGI) are ubiquitous chemolithoautotrophic members of the oceanic picoplankton and play a key role in the global nitrogen cycle. The second most abundant group of planktonic archaea belongs to the order *Poseidoniales* (also MGII) and has not yet been cultured. However, metagenomic approaches suggest a chemoorganoheterotrophic lifestyle and an important role in the degradation of complex, high-molecular-weight organic compounds. To study the ecophysiology of both groups in coastal environments of the North Sea, we combined 16S rRNA-based analyses of their spatial and temporal distribution with metagenomic and selective cultivation approaches.

Water-column 16S rRNA gene surveys conducted between June 2022 and May 2023 around the islands of Spiekeroog and Helgoland showed that MGII, rather than MGI, represents the predominant planktonic archaeal group. The occurrence of MGII exhibited pronounced seasonal

dynamics, with relative abundances peaking in September (>7% of all prokaryotes) and correlating positively with chlorophyll *a* concentrations originating from phototrophs. Comparative genomic analyses of nearshore and offshore MGII metagenome-assembled genomes (MAGs) revealed lineage-level differentiation, including metabolic signatures associated with cofactor biosynthesis (MGIIa) and central carbon metabolism (MGIIb), highlighting habitat-driven functional diversification.

While MGI play only a minor role in North Sea coastal waters, they were highly abundant down to 28 cm in sediments near Spiekeroog, where they represented the main archaeal population, accounting for up to 25% of the microbial community. From a subsequent selective cultivation approach using oxygen-depleted surface sediment, we enriched an ammonia-oxidizing archaeon of the genus *Nitrosarchaeum*, thus providing experimental support for its ecological relevance as a nitrifier in coastal sediments.

Together, these results suggest niche partitioning between MGI and MGII populations and emphasize the influence of environmental gradients and seasonal variability on archaeal community structure. Our findings contribute to the understanding of archaeal ecology in dynamic coastal systems and underscore their roles in linking carbon and nitrogen cycling.

### P-EME-084

#### Ectomycorrhizosphere interactions in a former uranium mining site

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Mycelial networks form enormous surface areas providing three-dimensional interaction structures with soil particles, soil pollutants, microorganisms and plant roots, and play crucial roles in mycoremediation. Particularly processes in the ectomycorrhizosphere bear a potential to remediate contaminated soils. To develop strategies for soil remediation we work with soil from our test fields on a former uranium mining site near Ronneburg, Thuringia, Germany.

Distinct morphotypes of mycorrhizal root tips were identified from the test field, with dominating types and a relatively low diversity. We assume, that soil pollution and acid mine drainage selected for species that were capable to adapt to these challenging conditions. An element analysis of fruiting bodies from the test field showed distinct accumulation patterns. Some rhizosphere bacteria supported mycelial growth of ectomycorrhizal fungi. Further plans include metatranscriptome analysis of test field soil and transcriptome analysis of lab-based interaction experiments.

In a microcosm experiment we analyzed mycorrhizosphere interactions with soil particles. Here, autoclaved test field soil was inoculated with the long-range exploration type ectomycorrhizal fungus *Tricholoma vaccinum*, with and without co-inoculation with its host tree *Picea abies*. For comparison, another fungus able to survive in contaminated soil, the white rot model organism *Schizophyllum commune*, was applied in axenic soil cultures. After 3 months, a rain event was simulated to study the effects of the symbiosis on soil aggregation. Visually, for both fungi mycelial colonization

of the soil was observed after that time. Microscopically, the inoculated tree roots showed a very high mycorrhization rate, and the mycelium contributing to soil aggregation by holding together soil particles.

These first results of an entirely new experimental design indicate that ectomycorrhizal treatments bear potential for the remediation of former mining sites and might mitigate challenges coming with climate change, such as periods of drought alternating with heavy rain events causing enhanced soil erosion.

#### P-EME-085

##### Assessing the Impact of Poor Sanitation on Public Health in Nigerian Rural Communities of Abia and Imo States

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**Introduction:** Sanitation remains a cornerstone of public health and environmental sustainability. This study evaluated sanitation practices, microbial contamination, and related health outcomes in selected rural communities of Abia and Imo States, Nigeria.

**Goal:** Primarily the goal was to evaluate the effects of inadequate sanitation on the health of rural populations or dwellers and identify potential interventions.

**Methodology:** Using a mixed-methods cross-sectional design, data were collected from 200 households through questionnaires, focus-group discussions, and environmental assessments conducted between April and September 2025. Water and soil samples were analyzed microbiologically following APHA (2017) standards.

**Results and Conclusion:** Results revealed that 54% of households used traditional pit latrines, 28% practiced open defecation, and only 18% had improved toilet facilities. Indiscriminate waste disposal was widespread, with 61% of respondents dumping refuse in bushes or streams. Microbial analysis showed high total coliform counts ( $2.3 \times 10^4$ – $4.5 \times 10^4$  CFU/ml) in wells, streams, and soil near latrines, exceeding WHO permissible limits. *Escherichia coli*, *Salmonella* spp., and *Pseudomonas* spp. were detected in most samples, indicating fecal contamination. Disease incidence was significantly associated with open defecation practices ( $\chi^2 = 12.7$ ;  $p < 0.05$ ), with diarrhea and typhoid being the most prevalent illnesses, particularly among children under five. The study underscores the urgent need for strengthened sanitation infrastructure, behavioral-change programs, and local-government capacity-building to reduce environmental contamination and disease transmission. Implementing Community-Led Total Sanitation (CLTS), promoting hygiene education, and investing in low-cost sanitation technologies are recommended to advance Nigeria's progress toward Sustainable Development Goal 6 (Clean Water and Sanitation).

#### References:

Ezeugwunne, I. P., Nnamonu, E. I., & Okechukwu, M. C. (2021). Microbiological quality assessment of community water sources in southeastern Nigeria. *African Journal of Microbiology Research*, 15(9), 456–465.

Ibe, S. N., Okafor, C. U., & Nwosu, I. (2019). Bacteriological and physicochemical quality of drinking water sources in Abia State, Nigeria. *Nigerian Journal of Microbiology*, 33(2), 2205–2215.

#### P-EME-086

##### A proteomics view on aerobic defluorination by *Labrys portucalensis* F11

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Fluorinated organic compounds, including per- and polyfluoroalkyl substances (PFAS), pose a major environmental challenge, as many are highly persistent, globally distributed, and potentially toxic. *Labrys portucalensis* F11, an alphaproteobacterium previously isolated from sediment at a polluted site in northern Portugal, can metabolize multiple fluorinated compounds with stoichiometric fluoride release under aerobic conditions. However, the molecular mechanisms underlying this activity remain largely unclear. The recently published genome of *L. portucalensis* F11 now enables proteomic analyses.

This study aims to gain insight into the metabolism of fluorinated organic compounds in *L. portucalensis* F11 using a comparative proteomics approach. Cells were cultivated on fluorinated and non-fluorinated substrates under controlled aerobic conditions, and expressed proteins were analyzed by bottom-up shotgun proteomics with label-free quantification (LFQ). This approach allows identification of proteins expressed under different substrates, providing evidence beyond genomic potential, and highlights differences in abundance that may indicate proteins involved in fluorinated compound transformation.

This work provides a proteomic view on defluorination by aerobic microorganisms and may inform the development of biotechnological strategies for remediating persistent fluorinated pollutants.

#### P-EME-087

##### Environmental controls on benthic microbial sulfide production in seasonal hypoxic locations of Kiel Bight

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The Kiel Bight is particularly affected by various anthropogenic activities resulting in prolonged seasonal stratification. These have caused warmer water temperatures and intensified algal blooms with higher organic matter (OM) flux to the seafloor. Amplified respiration rates enhance deoxygenation of bottom waters. Due to the water column stratification, oxygen depleted bottom waters cannot be replenished with oxygenated surface waters and hydrogen sulfide can start to accumulate in the bottom water. This turns the seafloor environment toxic for most species. Specific atmospheric conditions favor upwelling of this toxic sulfidic bottom water causing fish kill events near shore.

Over three years we collected sediment and water column data from the Kiel Bight region during seasonal hypoxia to

provide insights into the benthic microbial community and their contributions to sulfide production, with sulfate reducing bacteria (SRB) being key degraders of OM. We identified putative (SRB) that exhibited spatial and temporal variation in microbial sulfate reduction rates (SRR) i.e., sulfide production. SRR were below the detection limit in the water column but substantial SRR were measured within the sediment. Hence, the sulfide accumulating at the seafloor is formed in the sediment and is subsequently released into the bottom waters. Interestingly, abundant SRB in the sediments do not necessarily correlate with high SRR or sulfidic bottom water enrichment, indicating that additional processes play an important role for maintaining sulfidic bottom waters in the study area in late summer. We hypothesize that although sedimentary production by SRB is the principal source of bottom water sulfide, local hydrographic and geochemical conditions ultimately determine whether sulfide accumulates or is reoxidized at the seafloor.

#### P-EME-088

##### From Farm-to-Fork to National Genomic Landscape: Dissemination of *tet(X4)* and *mcr-1.1* in *Escherichia coli* in Pakistan

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**Introduction:** Antimicrobial resistance is a critical One Health challenge and its surveillance and monitoring is essential. We analyzed *Escherichia coli* isolates from integrated poultry production chain, from day-old chicks to retail meat and enriched the study by *E. coli* genomes carrying *mcr-1.1* and *tet(X4)* genes reported from Pakistan.

**Goals:** To assess tigecycline/colistin resistance dissemination and compare genomic characters within a single integrated farm and on a national scale in human, animal, and environment. **Methodology:** 150 samples were collected from the poultry farm (day-old chicks, broiler breeders, 30day broilers, retail meat). *E. coli* isolates were screened on tigecycline/colistin media. Resistant 17 strains were subjected to antimicrobial susceptibility, biofilm, and adhesion assays. Short read sequencing and long-reads for selected resistant strains were performed. All *E. coli* genomes from Pakistan carrying *mcr-1.1* or *tet(X4)* (n = 214) were retrieved. Multi-omics (resistome, virulome, adhesiome, mobilome) and pangenome analyses inferred the dissemination dynamics, genetic context, and stability of *mcr-1.1* and *tet(X4)*. **Results:** In the integrated farm, 17 *E. coli* isolates were resistant to colistin and tigecycline; 11 were co-resistant to fosfomycin, one carried all three. *mcr-1.1* was localized on IncI2 plasmids. *tet(X4)* was encoded on large conjugative megaplasms (IncFIA–IncFIB–IncFII), enhancing dissemination. Analysis of 231 genomes revealed 156 with *mcr-1.1*, 39 with *tet(X4)*, and 36 co-harboring both. Sequence typing revealed distinct structuring: *mcr-1.1* predominated in ST-48, ST-206, ST-1011; *tet(X4)/fosA4* were frequent in ST-351, ST-69, ST-6751. Triple-positive isolates (*mcr-1.1*, *tet(X4)*, *fosA4*) were identified across ST-155, ST-69, ST-4388, ST-48, ST-398, and ST-694. *mcr-1.1* was widespread (poultry, meat, wild birds, wastewater); *tet(X4)* was mostly concentrated in wild birds and poultry. Synteny analysis showed minimal insertion sequences near *mcr-1.1* but high flanking region variability, suggesting structural adaptation. *tet(X4)* bearing plasmids showed high IS diversity linked to MDR modules. **Conclusion:** Alarming detection of last-resort resistance across one farm suggests wider unseen spread. *mcr-1.1* is undergoing genomic fossilization (chromosomal stabilization), while the expanding

co-localization of *tet(X4)*, *fosA4*, and other genes on megaplasms is a critical evolutionary step, forming the basis for future 'superbug' lineages.

#### P-EME-089

##### Effects of Nitrification and Urease Inhibitors on Microbial Communities in Aquatic Mesocosms

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The increasing use of nitrification and urease inhibitors (NUI) in agriculture aims to improve nitrogen fertilizer efficiency and reduce greenhouse gas emissions. NUI slow down the microbial conversion of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  and urea to  $\text{NH}_3$ , thereby decreasing gaseous emissions of  $\text{NH}_3$  and  $\text{N}_2\text{O}$  and reducing  $\text{NO}_3^-$  leaching into groundwater. This practice supports climate change mitigation and sustainable nutrient management. Concerns arise regarding possible impacts on non-target organisms, especially in aquatic ecosystems exposed to NUI through surface runoff or drainage following rainfall or irrigation events. These pathways play a crucial role in aquatic systems. We investigate the short-term effects of selected NUI on microbial community structure and function in aquatic mesocosms. We hypothesize that exposure to NUI will cause noticeable shifts in microbial community structure. An aquatic mesocosm experiment was carried out at the German Environment Agency's artificial pond facility in Berlin. Small artificial pond were treated with both high and environmentally relevant concentrations of specific NUI to mimic runoff conditions, alongside an untreated control mesocosm. Water and sediment samples were collected at defined time intervals during the exposure period to track temporal changes in microbial community responses. DNA and RNA were extracted from water and sediment samples to evaluate active and total microbial communities. We will conduct full-length 16S rRNA gene and cDNA sequencing using Oxford Nanopore Technology. Additionally, qPCR assays targeting functional genes linked to key nitrogen cycle processes will be conducted on selected samples. We aim to identify how nitrification and urease inhibitors impact aquatic microbial communities and nitrogen turnover rates. It will assess if the benefits of these inhibitors in agriculture outweigh the risks to aquatic ecosystems. The findings will guide policymakers on the safe use of inhibitors in agricultural areas near water bodies.

#### P-EME-090

##### Comparative Evaluation of Low-Cost Water Purification Techniques for Rural Households in Abia State, Nigeria

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**Introduction:** Access to safe drinking water remains a major public health challenge in many rural areas of developing nations. This study evaluated the effectiveness of four low-cost household water purification methods—boiling, solar disinfection (SODIS), activated charcoal filtration, and Moringa oleifera seed coagulation on the microbial and physicochemical quality of contaminated water.

**Goal:** The goal of this research is to assess and compare the effectiveness, affordability, and sustainability of various water treatment methods in removing contaminants and improving water quality, and also identifying the most suitable for rural communities.

**Methodology:** Surface water samples were collected from a local stream in Uturu, Abia State, Nigeria, and artificially inoculated with *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* ( $10^4$  CFU/ml). Each treatment was applied under controlled laboratory conditions, and reductions in total heterotrophic bacterial count (THBC), total coliform count (TCC), pH, and turbidity were assessed following APHA (2017) standard methods.

**Results and Conclusion:** Results showed that boiling achieved complete microbial elimination (100%), while SODIS, Moringa coagulation, and activated charcoal filtration produced 92%, 85%, and 65% bacterial reductions, respectively. Boiling and SODIS were most effective in reducing turbidity to below WHO limits ( $\leq 5$  NTU). Although boiling remained the most reliable purification technique, SODIS and Moringa seed treatments were identified as affordable and sustainable alternatives suitable for low-resource communities. The findings highlight the potential of integrating locally available materials and simple technologies to improve rural water safety, thereby supporting the attainment of Sustainable Development Goal 6.

#### References:

Nwankwo, E. C., Okorie, C. P., & Ugboaja, O. (2020). Assessment of bacteriological quality of rural drinking water sources in southeastern Nigeria. *Journal of Public Health and Epidemiology*, 12(3), 85–93.

Reller, M. E., Nelson, E. J., & Greenough, W. B. (2018). Simple household water treatment: Practical options for developing countries. *American Journal of Tropical Medicine and Hygiene*, 98(2), 395–402.

Ubong, I. J., Etukudo, U. I., & Effiong, N. S. (2021). Effectiveness of solar disinfection on microbial quality of rural drinking water. *Nigerian Journal of Environmental Health*, 17(1), 21–29.

#### P-EME-091

##### Impact of cationic and anionic surfactants on methane production in an anaerobic digester sludge

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Surfactants are widely used as detergents, wetting agents and emulsifiers. More than 70% of the surfactants produced are used in household cleaning agents and personal care products and the majority ends up in residential wastewater. Wastewater treatment is an energy-intensive process. To recover a part of this energy, excess activated sludge is used for biogas production in an anaerobic digester. Surfactants readily adsorb onto activated sludge and the sludge dewatering step concentrates the adsorbed surfactants further. Therefore, sludge introduced into the digester has a higher concentration of surfactants than the original sewage.

We determined the effect of linear alkylbenzene sulfonates (LAS), which are commonly present in household detergents, and dodecyl trimethyl ammonium chloride (DTAC), which is found in a variety of antimicrobial products such as antimicrobial fabric softeners, on the methanogenic microbial community.

Sludge obtained from an anaerobic digester at the Teaching and Research Wastewater Treatment Plant (Büsnau, Stuttgart area) was loaded into 7 lab-scale digester reactors together with LAS at concentrations 0, 1, 10 and 100 mg/L or DTAC at the same concentrations. CH<sub>4</sub> production was monitored on-line using optical CH<sub>4</sub> sensors and low-rate gas flow meters. DTAC in a concentration of 100 mg/L decreased the CH<sub>4</sub> production rate, whereas 100 mg/L of LAS and 10 or 1 mg/L of either surfactant didn't significantly affect the CH<sub>4</sub> production rate.

To investigate the direct effect of surfactants on methanogenic organisms, a pure culture of a representative of the genus previously found in the digester sludge via 16S rRNA gene sequencing, *Methanosarcina barkeri*, was cultivated anoxically with the addition of LAS or DTAC in concentrations 0, 1, 10 and 100 mg/L in triplicate. The headspace pressure and CH<sub>4</sub> concentration were measured daily to assess CH<sub>4</sub> production. Methanogenesis in *M. barkeri* was not affected by 1 mg/L of surfactants, but was totally inhibited by 100 mg/L of each surfactant. 10 mg/L DTAC completely inhibited CH<sub>4</sub> production, whereas 10 mg/L LAS prolonged the lag phase by approximately 48 hours.

Methanogenesis in the digester sludge is more resistant to surfactants compared to a pure methanogenic culture. This can be explained by the presence of matrix that adsorbs surfactants and hydrolytic microorganisms capable of degrading LAS and DTAC. DTAC exerts a higher inhibitory effect on methanogenesis than LAS in both experiments.

#### P-EME-092

##### Fertilization with microalgal biomass produced in wastewater treating high-rate algae ponds (HRAPs) – effects on the wheat root microbiome

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High-rate algae ponds (HRAPs) efficiently remove nitrogen and phosphorus from wastewater. The produced microalgal biomass can be used as NPK-rich fertilizer in a sanitation–agricultural circular economy. The effects of microalgal based organic fertilizer on plant growth are well documented, but the knowledge of effects on the plant microbiome and the transmission of wastewater derived bacteria into the agricultural system is scarce. We analysed the microalgal biomass used as fertilizer and the rhizosphere and root microbiome of wheat plants seven weeks after fertilization. The microalgal biomass was harvested from a HRAP pilot plant used for municipal wastewater treatment. Plants fertilized with a NPK mineral fertilizer and unfertilized control plants were studied in parallel. Three fertilizer levels, 60, 120, and 180 kg N ha<sup>-1</sup>, were compared. The microalgae biomass showed at higher N application levels improved physiological performance, growth, yield, and nutrient uptake compared to the unfertilized control plants (Mückschel et al., 2023). Fertilization with microalgal biomass increased the bacterial abundance in roots compared to control and

mineral fertilizer treated plants, but the diversity and composition of the root microbiome hardly changed compared to control plants (Ollo et al. 2025). In contrast, the mineral fertilizer reduced the bacterial abundance and diversity in roots. The microalgal biomass had a very specific microbiome containing high abundances of potentially plant growth promoting but also potentially pathogenic bacteria. *Escherichia coli*, 3rd generation cephalosporine resistant *E. coli*, and enterococci were cultivated efficiently from the microalgal biomass and plants fertilized with it, while fast growing bacteria tolerant to biocidal compounds and antibiotic resistance genes (ARGs) were detected in all samples. Although microalgae-based organic fertilizer provides a sustainable source of nutrients, its contribution to the spread of antibiotics, biocidal compounds, and antimicrobial resistance in agricultural systems comparable to biosolids produced in conventional wastewater treatment plants.

Mückschel et al. (2023) Nitrogen use efficiency of microalgae application in wheat compared to mineral fertilizer. *J Plant Nutr Soil Sci.* 186:522–531.

Ollo et al. (2025) Fertilization with microalgal biomass of wastewater treatment high-rate algae ponds (HRAP): Effects on the wheat root microbiome. *Tot Environ Microbiol.* 1:100033.

#### P-EME-093

##### Still under-researched: diversity and ecological role of aquatic fungi

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Microbes are essential components of food webs and play an important ecological role. In aquatic ecosystems, microbiologists have focused primarily on primary and secondary production, while the decomposition of organic matter, especially by bacteria, has been less studied. Among eukaryotic microbes, fungi, both saprotrophs and parasites, can play a major role in the decomposition process and nutrient cycling in aquatic ecosystems, but their contribution to biodiversity and ecosystem functioning is still largely unknown. Recent advances in molecular technology have provided important insights into the taxonomic and functional diversity of aquatic fungi. Although studies have attempted to assess the diversity of aquatic fungi, much of the "dark matter" of fungi, i.e., a large, uncultivated, and undescribed fungal diversity, remains to be described for many understudied aquatic ecosystems. Linking metagenomics with metatranscriptomics and other OMICS data may be a smart way to investigate the contribution of fungi to the pelagic carbon cycle and shed light on their important ecological role not only for terrestrial but also for aquatic environments. In my presentation, I will discuss the biodiversity patterns of fungi and fungus-like organisms, as well as potential environmental factors that influence these communities and their potential ecosystem functions. Using a series of examples from our current research, I would like to illustrate that fungi and fungus-like organisms (either as parasites or saprotrophs) should not be neglected in aquatic microbiology research, especially in our rapidly changing world.

#### P-EME-094

##### Taking a microbial sample as simple as making a coffee

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Filtration is probably the most common method to prepare and take microbial samples, whether in the laboratory or during field expeditions. Often, the membrane filter is retained and used for sequencing, microscopy, or chemical analysis. Despite the central role of filtration to the field of microbiology, the process itself but also sample management and labelling is often error prone and require specialized personal. We from Thalassys, a startup project from the Max Planck Institute for Marine Microbiology, are happy to present our new filtration device. Our development is an easy-to-use all-in-one device, which allows any layperson to take high-quality scientific filters. The device works with our newly developed smart filters, which automatically store all relevant metadata. Therefore, the traceability of the samples is permanently possible, dooming unknown samples a feature of the past.

#### P-EME-095

##### Stratified microbial communities and biogeochemical features of Baltic Sea surface slicks

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#### Introduction

The sea-surface microlayer (SML) represents the dynamic boundary where the ocean meets the atmosphere, mediating key processes in gas exchange and nutrient cycling. Coastal slicks are thickened patches of the SML that often concentrate microbial communities<sup>1</sup>.

#### Goals

We aimed to understand how slicks affect microbial communities and biogeochemical processes in nearshore environments.

#### Methods

SML samples were collected using a glass plate sampler, and underlying water (ULW) was sampled from 1 m depth. Microbial abundance was determined using flow cytometry for prokaryotes, cyanobacteria, and pico-/nano/microphytoplankton, while microscopy characterized the >100 µm fraction. Surfactants were quantified via voltammetry, and dissolved organic carbon and total dissolved nitrogen using high-temperature catalytic combustion. O<sub>2</sub> dynamics were assessed using a needle-type O<sub>2</sub> sensor to determine gross primary production (GPP),

respiration (R), and net community production (NCP). Enrichment factors were calculated as the ratio of SML to ULW concentrations.

## Results

Slicks contain higher surfactant loads and microbial densities than adjacent waters. O<sub>2</sub> measurements revealed that heterotrophic respiration frequently outweighed photosynthetic production, leading to net O<sub>2</sub> consumption, although certain ULW sites showed positive NCP. The SML was enriched in small photoautotrophs such as pico- and nanophytoplankton, while microphytoplankton were more abundant in the ULW, indicating vertical ecological partitioning. Microscopic observations identified ciliates like *Vorticella*, and early-stage macroalgae as constituents of the slick >100 µm community. Correlations between microbial groups, surfactants, and O<sub>2</sub> dynamics suggest that slicks structure both biological and biogeochemical processes at the sea surface.

## Conclusion

Overall, our results<sup>2</sup> highlight the role of slicks as biogeochemical hotspots that shape plankton community structure and O<sub>2</sub> dynamics in nearshore marine environments.

## References

1. Voskuhl, L., & Rahlff, J. (2022). Natural and oil surface slicks as microbial habitats in marine systems: A mini review. *Front Mar Sci*, 9, <https://doi.org/10.3389/fmars.2022.1020843>
2. Peter, C., Giebel, H. A., Chai, B. C., Serafim, T. S., Lehnert, C., Wurl, O., Osterholz H. & Rahlff, J. (2025). Biogeochemical function of slicks in coastal surface waters of the Baltic Sea. *bioRxiv*, <https://doi.org/10.1101/2025.06.25.660598>

## Infection biology

### P-IB-001

#### Elucidating the mode of action of the allopeptimicin compound class

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Selection pressure exerted by the overuse and misuse of antibiotics in both healthcare and agriculture has led to an increasing emergence of multidrug-resistant pathogens, raising the urgency to discover and characterize novel antimicrobial compounds. Most antibiotics in clinical use were originally derived from natural sources such as soil bacteria and fungi. The rich biodiversity of natural products continues to offer untapped potential for the discovery of novel compounds with unique and potentially new mechanisms of action.

The allopeptimicin compound class, isolated from an *Actinoallomurus* spp. consists of eight congeners, which

share a structural core of a C20 polyketide chain attached to a cyclic depsipeptide containing four non-proteinogenic amino acids (Iorio et al, 2022). Although allopeptimicins were described to be bactericidal against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), the mechanism of action remains elusive. In this ongoing study, we demonstrate that allopeptimicins exert their activity at the bacterial cell envelope. More specifically, allopeptimicins rapidly compromise the integrity of the cytoplasmic membrane of Gram-positive bacteria, leading to cell death. Notably, the membrane-disruptive properties of allopeptimicins are not limited to bacterial cytoplasmic membranes, as they can compromise the integrity of artificial liposomes consisting of a variety of lipid compositions. Looking forward, we will aim to shed further light into the molecular interaction of allopeptimicins with the cell envelope as well as the overall structure-activity relationship of the compound class.

### Literature:

Iorio M., Gentile A., Brunati C., Tocchetti A., Landini P., Maffioli S. I., et al. (2022). Allopeptimicins: unique antibacterial metabolites generated by hybrid PKS-NRPS, with original self-defense mechanism in *Actinoallomurus*. *RSC Adv.* 12 (26), 16640–16655. doi: 10.1039/D2RA02094G

### P-IB-002

#### Cysteine-stabilized αβ-defensins as promising antimicrobial agents against drug-resistant bacteria

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Antimicrobial resistance (AMR) has emerged as one of the biggest global health challenges of our time. The lack of newly approved antibiotics, combined with the rapid rise of resistant pathogens, has created an urgent need for new antimicrobial strategies. Antimicrobial peptides (AMPs), produced by both prokaryotic and eukaryotic organisms, have gained attention as promising candidates for the development of new antibiotics. Among these, cysteine-stabilized αβ-defensins (CSαβ-defensins) form a structurally conserved class of AMPs, defined by a characteristic α-helix and β-sheet motif, with activity against a broad range of bacteria, including AMR strains. Here we focus on two major subfamilies of CSαβ-defensins: the GXGCP and XTCD subgroups. Plectasin and actifensin are members of the GXGCP subgroup, both binding the cell wall precursor lipid II and showing high structural similarities, although differ in mechanistic aspects (Sugrue et al., 2025). Within the XTCD subgroup, lucifensin—also interacts with lipid II, while sapecin has shown affinity for cardiolipin. This highlights a gap in understanding of mechanism-linked structural differences between the two groups of peptides. Here we elucidate the structural and mechanistic aspects and the potential role of CSαβ-defensins in the fight against antimicrobial resistance.

## P-IB-003

### Compound-specific redox responses of farnesyltransferase inhibitors in *Staphylococcus*: implications for antibacterial activity

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## Introduction

Drug repurposing provides a pragmatic route to new antimicrobials. Farnesyltransferase inhibitors (FTIs), originally developed for oncology, display activity against several gram-positive and gram-negative pathogens [1, 2]. Given the absence of canonical farnesyltransferase in bacteria, indirect redox-linked effects may dominate. As reactive oxygen species (ROS) can contribute to antibiotic action and resistance trajectories [3], we probed whether FTIs elicit compound-specific redox phenotypes linked to antibacterial effects.

## Methods

Methicillin-susceptible and -resistant *S. aureus* and *B. subtilis* served as models. Susceptibility testing followed CLSI/EUCAST microdilution standards. Intracellular ROS were quantified by DCFH-DA and CellROX Deep Red in kinetic and endpoint formats. ROS involvement was challenged with N-acetylcysteine (NAC). Membrane potential and envelope stress were assessed by DiOC<sub>2</sub>(3) and SYTOX Green uptake. Sub-MIC colistin was used as a redox-active comparator and as a potentiator in combination studies.

## Results

FTIs triggered distinct redox signatures in *S. aureus*: tipifarnib produced consistently higher DCFH-DA and CellROX signals than lonafarnib, which remained at or below control for 3 hours. Sub-MIC colistin caused a pronounced early ROS rise in matched runs and, when co-applied, synergized with FTIs against *S. aureus* (checkerboard/time-kill). NAC attenuated tipifarnib's antibacterial effect more strongly than lonafarnib's, consistent with a larger ROS contribution to tipifarnib activity. Envelope-associated readouts indicated membrane involvement for both FTIs.

## Conclusion

FTIs induce compound-specific ROS dynamics in *Staphylococcus*: tipifarnib shows a stronger, NAC-reversible redox component than lonafarnib. Together with envelope stress signals and colistin synergy, these data suggest that redox perturbation contributes to FTI activity and may bear on resistance development [4]. Future evolution studies will clarify how FTI-induced redox stress influences resistance trajectories.

[1] Weber et al. 2019. PMID: 34917041

[2] Klose et al. 2025. PMID: 40911556

[3] Dwyer et al. 2009. PMID: 19647477

[4] Choi et al. 2020. PMID: 32041713

## P-IB-004

### Decoding *Staphylococcus aureus* in cutaneous T-cell lymphoma: antimicrobial synergy and insights into Protein A variability

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Mycosis fungoides (MF), the most common form of cutaneous T-cell lymphoma (CTCL), represents a rare subtype of non-Hodgkin lymphoma that primarily affects the skin and causes various lesions (1). MF patients can be classified as either SA-positive or SA-negative depending on the predominant bacterial colonizer. In SA-positive patients, *Staphylococcus aureus* is the dominant skin colonizer, leading to a markedly altered microbiome composition, whereas SA-negative lesions are mainly colonized by other *Staphylococcus* or *Cutibacterium* species. Clinically, the presence of *S. aureus* correlates with increased disease severity and a higher rate of secondary complications (2). We isolated multiple multiresistant *S. aureus* strains from MF plaques and patches, underpinning the urgent need for new antimicrobial strategies. Screening of natural compounds extracted from diverse fungal species revealed several drug candidates displaying inhibitory activity against *S. aureus* MFMZ isolates. In addition, we could observe synergistic effects for combinations of  $\beta$ -lactams, aminoglycosides, and quinolones against these multiresistant strains. Further, genomic analyses of these isolates identified, among other virulence factors, diverse *Staphylococcus* protein A (SpA) subtypes, enriched with unique octapeptide repeat domain patterns, in the MF-derived isolates. Since SpA plays a pivotal role in immune evasion by interacting with host immunoglobulins and modulating inflammatory responses, these subtype variants may highly influence pathogenicity. Ongoing biochemical analyses focus on understanding their structural diversity and potential impact on immune modulation in MF lesions. In summary, our findings highlight the clinical and molecular relevance of *S. aureus* in MF, demonstrate promising antimicrobial and synergistic treatment options, and emphasize the importance of detailed SpA subtype analysis for understanding immune evasion mechanisms in this disease.

(1) Kempf, W. and Mitteldorf, C. (2021), Hematol Oncol

(2) Licht, P. and Dominelli, N. (2024), NPJ Biofilms Microbiomes

## P-IB-005

### Novel drug targets in Actinobacteria essential for polyamine metabolism: glutamine synthetase-like enzymes

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The actinobacterial model organism *Streptomyces coelicolor* and other taxonomically related actinobacteria have to cope with limited nitrogen availability in their ecological niches. A complex nitrogen metabolism of these bacteria allows the



utilization of various compounds as *N*-sources. Our investigations showed that assimilation of polyamines can allow the survival of *S. coelicolor* under nitrogen limitation conditions. We analyzed the function of three proteins previously annotated as glutamine synthetase-like enzymes (GS-like): GlnA2<sub>Sc</sub>, GlnA3<sub>Sc</sub> and GlnA4<sub>Sc</sub> and demonstrated that in *S. coelicolor* GlnA2<sub>Sc</sub> and GlnA3<sub>Sc</sub> are involved in the catabolism of polyamines<sup>1</sup>. Transcriptional, phenotypical, structural and biochemical analysis of these enzymes demonstrated a dual function of GlnA2<sub>Sc</sub>, GlnA3<sub>Sc</sub> in ensuring both nutrients availability (C- and N-source) and resistance against high polyamine concentrations, which are toxic for bacteria<sup>1</sup>. Based on our studies we were able to elucidate the polyamine utilization pathway in *S. coelicolor*<sup>1</sup>. Further analysis revealed the presence of GlnA2 and GlnA3 enzymes in other actinobacteria including human pathogens like *Mycobacterium tuberculosis*. Our structural and biochemical studies demonstrated the glutamylation of the polyamine spermine by the mycobacterial GlnA3<sub>Mt</sub><sup>2</sup>. Since GS-like enzymes like GlnA3<sub>Mt</sub> ensure survival of mycobacteria in their host, inhibition of GlnA3<sub>Mt</sub> might be an effective therapeutic strategy that is safe and specific. Our recent studies aimed further characterization of polyamine metabolism in *Actinobacteria* as well as the development of novel anti-tubercular drugs based on the validation of GlnA3<sub>Mt</sub> as a target. Recent results in cooperation with other research groups allowed structure-based design of first inhibitors of bacterial polyamine metabolism<sup>3</sup>.

1. Krysenko et al. (2017) Gamma-Glutamylpolyamine Synthetase GlnA3 Is involved in the first step of polyamine degradation pathway in *Streptomyces coelicolor* M145. *Front Microbiol* 8: 726.
2. Krysenko et al. (2025). GlnA3<sub>Mt</sub> is able to glutamylate spermine but it is not essential for the detoxification of spermine in *Mycobacterium tuberculosis*. *Journal of bacteriology*, e0043924. Advance online publication. <https://doi.org/10.1128/jb.00439-24>
3. Krysenko et al. (2023). A novel synthetic inhibitor of polyamine utilization in *Streptomyces coelicolor*. *FEMS microbiology letters*, 370, fnad096. <https://doi.org/10.1093/femsle/fnad096>

#### P-IB-006

##### Speed Matters: Accelerated Pharmacokinetics Reduce Persisters in *Escherichia coli*

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Persister cells are bacterial cells that form small subpopulations and are refractory to antimicrobials. Importantly, those cells can cause the relapse of an infection and also provide a starting point for antimicrobial resistance evolution [1]. By now, several mechanisms of persister formation have been studied. For example, it has been shown that a short exposure to sublethal concentrations of antimicrobials can induce persister formation [2]. Given this, we hypothesised that the pharmacokinetics, the temporal changes of drug concentrations under treatment, could impact persister formation. We predicted that the longer bacteria spend under sublethal drug concentrations, the more persisters would form. Using a set-up with small chemostats and the antimicrobial peptide Pexiganan, we indeed found that faster pharmacokinetics resulted in lower persister numbers compared to slower pharmacokinetics. This finding provides a proof of principle that

pharmacokinetics, which can be influenced by treatment procedures, has consequences for persister formation. In the current project, a new approach is used as a proxy to determine the level of persister and tolerant new cells that have divided between 2 and 20 min earlier. The reporter system enables us to distinguish between tolerant and persisting cells. By constructing a reporter based on the promoter of the elongation factor Tu (EF-Tu), one of the most abundant proteins in fast growing *E. coli* cells and tagging it with GFP [3], and a second reporter containing a ds-Red monomer under a fully dependent promoter of RpoS corresponding to the gene *dps* is inserted. DPS is an unstable protein that is highly abundant in metabolically inactive cells [4]. This allows us to detect covariation between persister cells (red) and time after division of metabolically active cells (green) and to determine the influence of priming by AMPs on the induction of persister cells and tolerant cells. Following the transforming of the reporter into the pGEMTeasy and doing the preliminary testing of the system, we will start the priming experiments. If, as expected, this covariation occurs, the role of priming and bacterial stage and their possible interactions in inducing persistence and/or tolerance can be established.

#### P-IB-007

##### Metal meets Microbe: Cobalt Ion-Induced Adaptation and Antibiotic Resistance in PJI-Associated Pathogens

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#### Introduction/Goals

Cobalt-chromium alloys are widely used in orthopedic implants [1]. The corrosion-related release of cobalt ions (Co<sup>2+</sup>) has been linked to altered host immune responses [2]. However, the impact on key contributors to prosthetic joint infections (PJIs) is not well understood. Therefore, we investigate the effect of cobalt ions on the growth, viability, biofilm formation and antibiotic susceptibility of PJI pathogens *S. aureus*, *S. epidermidis* and *E. coli* [3] *in vitro*. This study is part of the DFG Research Training Group 2901 SYLOBIO.

#### Methods

Bacteria were adapted to different Co<sup>2+</sup> concentrations by repeated inoculations in CoCl<sub>2</sub> enriched media. Growth was assessed via OD<sub>600</sub> and CFU/mL. Antibiotic susceptibility was analyzed in MIC and checkerboard assays. Biofilms were analyzed using crystal violet staining, electron microscopy, and viable counts. Proteomic changes were analyzed in *S. aureus* cells during adaptation to CoCl<sub>2</sub> to identify mechanisms helping the bacteria to overcome adverse effects of cobalt stress.

#### Results

Exposure to ≥1000 μM Co<sup>2+</sup> initially impaired growth of all species, but serial passaging in Co<sup>2+</sup>-enriched medium led to strain-specific recovery. *S. epidermidis* formed biofilm-like aggregates in planktonic cultures under cobalt stress. Biofilm formation in *E. coli* and *S. epidermidis* was initially reduced by Co<sup>2+</sup> but recovered within 72 h. Adaptation to Co<sup>2+</sup> strongly decreased susceptibility to gentamicin in *S.*



*aureus*. This may be caused by transiently increased efflux pump activity under Co<sup>2+</sup> stress.

## Conclusion

PJI pathogens can overcome adverse effects of Co<sup>2+</sup> by adaptation processes that can lead to reduced susceptibility towards selected antibiotics. This may complicate PJI treatment. Further research is needed to understand the underlying mechanisms.

## References

- [1] A. F. Mavrogenis et al., *Journal of long-term effects of medical implants* 21, 349–358 (2011); doi: 10.1615/jlongtermeffmedimplants.v21.i4.80.
- [2] A. H. Hosman et al., *Acta orthopaedica* 81, 526–534 (2010); doi: 10.3109/17453674.2010.519169.
- [3] D. B. G. Tai et al., *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 28, 255–259 (2022); doi: 10.1016/j.cmi.2021.06.006.

### P-IB-008

#### Bacterial Cell Age, Persistence and Antimicrobial Resistance

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Bacterial sub-populations can resist antimicrobial killing over short durations. These sub-populations, known as persisters, arise in isogenic populations and the mechanisms driving them are distinct from genetically encoded resistance. However, phenotypic resistance is increasingly recognised as a cause of treatment failures in infection – due to mismatches between antimicrobial pharmacokinetics and pharmacodynamics – with the persisting cells able to resume growth and re-colonise. A wide variety of mechanisms and processes have been implicated in the switch of a sub-population of bacterial cells to the persister phenotype but most investigations have focused on persister formation in response to a stimulus and not the constitutive persister sub-population. Population age has been shown to influence the persister fraction; stationary phase cultures have a higher proportion of persisters than those in exponential phase. We employed the *Escherichia coli* Baby Cell Column to produce *E. coli* cultures which were synchronised in age and comprised of newly divided daughter cells. These cultures were used to investigate the impact of both daughter age and parent age on persister formation. For mid-log phase parent cultures, enumeration of the persister fraction of their daughter cells showed the persister sub-population to be absent in newly divided cells immediately after cell division. The persister fraction of these cells increased over time until they matched that of their parent culture. By contrast, daughter cells of stationary phase parent cultures were able to form persisters immediately upon production by cell division. These findings demonstrate that the constitutive formation of antimicrobial resistant sub-populations in *E. coli* is a function of both cell age and parental age. Investigations into the underlying mechanisms of the switch of a proportion of the population to persistence are ongoing and these results will potentially allow for the development of new drug

targets and treatment strategies for phenotypic antimicrobial resistance.

### P-IB-009

#### Bacteriophage-mediated decolonization of *Klebsiella pneumoniae* KPC st512 in *Galleria mellonella* gut colonization model

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**Introduction:** The gut serves as a critical reservoir for multidrug-resistant *Enterobacteriaceae*, particularly carbapenemase-producing *Klebsiella pneumoniae* (KPC-KP). Ethical and cost constraints of vertebrate models highlight the need for alternative systems to study gut colonization and phage therapy efficacy. Bacteriophage therapy shows strong potential for decolonization due to its high specificity and effectiveness against particular bacterial strains while preserving the microbiome. Moreover, phages can be evolved against specific bacterial isolates, providing enhanced efficacy and a reduced likelihood of resistance emergence.

**Goals:** To establish a reproducible *Galleria mellonella* gut colonization model using a clinical KPC-producing *K. pneumoniae* ST512 isolate and to evaluate the decolonization potential of non-evolved and evolved bacteriophages.

**Materials & Methods:** Larvae were force-fed with bacterial suspensions (10<sup>6</sup> CFU/larva) at 0, 24, and 48 h. After stable colonization, larvae received a one-time oral treatment with an evolved phage, UKJKp\_7. CFU counts and survival were analyzed using Kruskal–Wallis and log-rank (Mantel–Cox) tests.

**Results:** Colonization at 10<sup>6</sup> CFU/larva achieved ≥ 80 % survival and stable gut colonization. Phage treatment reduced *K. pneumoniae* load by approximately 5 log<sub>10</sub> CFU/larva, surpassing the reduction observed with gentamicin, without affecting larval viability. Fluorescence microscopy confirmed both colonization and decolonization of the larval gut.

**Summary:** This novel *G. mellonella* gut model provides a reliable and ethical platform for evaluating antimicrobial and phage-based interventions against carbapenem-resistant pathogens, supporting the 3Rs principle and advancing preclinical phage therapy research.

### P-IB-010

#### New insight in endocarditis: Do biofilms impact antibiotic therapy success?

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## Introduction:

Infective endocarditis is a life-threatening disease associated with high mortality. To date, antibiotic therapy is solely adapted to the pathogen species and its sensitivity to antibiotics according to the ESC / EACTS guidelines. Neither the biofilm status, bacterial activity nor the in situ effect of previous antibiotic therapy on the bacteria are considered so far.

## Goals:

This study aims to determine whether the combination of Fluorescence in situ Hybridization (FISH) and 16S rRNA gene PCR with sequencing (FISHseq) can provide complementary diagnostic insights to support future antibiotic therapeutic decision-making.

## Methods:

We used FISHseq to visualize and identify the infectious agents in heart valve tissues from endocarditis patients. The FISH signal intensity is proportional to the ribosomal content of the bacteria, which correlates with microbial metabolic activity. Thus, FISH shows not only the spatial organization of bacteria and biofilm formation, but also their metabolic activity at the time of surgery. We statistically correlated bacterial biofilm formation, amount, activity and preoperative antibiotic therapy of these endocarditis patients.

## Results:

FISH revealed a wide variability in bacterial biofilm formation. In some cases, the suspected diagnosis of endocarditis was confirmed, but microorganisms appeared scattered and partially degraded within the tissue; in others, microcolonies or highly active, mature biofilms were detected. Notably, bacterial activity correlated significantly with biofilm formation, independent of the duration of preoperative antibiotic therapy.

We are currently developing an endocarditis score based on biofilm staging by FISH to enable patient risk stratification and individualized antibiotic therapy. This approach may support evidence-based decisions for therapeutic de-escalation or escalation with biofilm-active antibiotics, as well as for or against early oral switch therapy — ultimately improving patient safety and outcomes.

## Conclusion:

FISHseq results from endocarditis patients revealed marked variability in infection "landscapes", with bacterial activity correlating with biofilm formation. Developing a biofilm-based severity score may support future personalized antibiotic treatment strategies in infective endocarditis.

### P-IB-011

#### Not All Alpha Hemolysins Are Equal: Variant-Dependent Cytotoxicity in *E. coli*

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**Introduction:** We sequenced a collection of 78 hemolytic *E. coli* from various hosts and different clinical statuses encoding alpha hemolysin operon *hlyCABD*. Alpha-hemolysin genes can vary in sequence, as confirmed by the 19 *hlyA* variants in our 78 hemolytic isolates.

**Goals:** An important step in colonization and infection is adhesion to host cell surfaces, which may enhance the cytotoxicity of alpha-hemolysin by increasing toxin concentrations at cell membranes, and *E. coli* express a variety of adhesins to facilitate this process. It is hypothesized that the pathogenic effects of alpha-hemolysin depend on *hlyA* gene variants and are influenced by other adhesins variants.

**Methods:** The cytotoxicity of all 78 strains has been evaluated against various cell line i.e., LoVo, 5637, Caco-2, IPECJ-2 etc. The variants have been selected which exhibit most exfoliation, least exfoliation, most prevalence and host cell specificity have been selected to generate *hlyA* deletion mutants. Furthermore, specific gene variants of three main adhesins i.e., *fimH*, *csgA* and *ecpD*, have been selected to determine the role of adhesin in the hemolytic ability. All the mutants will be complemented with respective gene by reintroduction of the same gene either by pBAD33 or pACYC177. *hlyCABD* expression of multiple variants by qPCR at different time points during exfoliation of 5637 and LoVo cells is also being analyzed.

**Results:** Isolates show different exfoliation ability of different isolates even within the same *hlyA* variants. For instance, variant 7 (n=35) in 28 isolates causes high cytotoxicity while the remaining 7 isolates with the same variant exhibit medium to low cytotoxicity. In contrast, all variant 11 (n=7) isolates exhibit very high exfoliation across all cell lines. Highly cytotoxic *hlyA* variant 01 has been successfully deleted in two different isolates and both lost the ability to exfoliate on 5637 and LoVo cells as well as failed to induce hemolysis on the blood agar. Further generation of deletion mutants of both *hlyA* and adhesins is ongoing. Time based expression analysis has also showed differential expression of the operon of various variants against diverse host cells.

**Conclusion:** Hemolytic *E. coli* carry a broad variety of hemolysin gene variants along with diverse adhesin variants. The current data indicate that different *hlyA* variants have varying cytotoxic effects and the role of alpha hemolysin has been quantified in both exfoliation as well as hemolysis.

### P-IB-012

#### Beyond colonization: towards linking virulence, metabolism, and dysbiosis in *Staphylococcus aureus* from mycosis fungoides plaques

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The most encountered form of cutaneous T-cell lymphoma (CTCL) is mycosis fungoides (MF), which predominantly affects skin tissue causing lesions. Patients subsequently experience dysbiosis and are therefore classified in SA-negative and SA-positive. This depends on the bacteria primarily colonizing the lesions. SA-negative patients display

a more various skin microbiome colonized by different *Staphylococci* or *Cutibacterium* species. SA-positive patients reveal a high abundance of *S. aureus* specifically on plaques, which is clinically relevant as they are more prone to experience complications within disease progression. Various strains from diverse MF patients were isolated to understand virulence and pathogenicity driving mechanisms in microbe-host interactions. Therefore, the strains were genomically investigated. Indeed, in more virulent strains isolated from plaques, diversity within the *ent* gene clusters, harboring enterotoxin or leukocidin coding genes, were observed. Additionally, these strains harbor specific stress adaptation mechanisms, in particular GroES/ GroEL and Clp proteases. Furthermore, antiSMASH analyses revealed gene clusters involved in synthesis of different metabolites such as the lanthipeptide Lactococcin or various lysins. These compounds produced by CTCL-associated *S. aureus* strains are putatively involved in bacterial competition as well as in weakening the patients skin barrier. In addition, via GC-MS analyses secondary metabolites like urocanic acid that may promote dysbiosis, could be detected. In MF patients, this imbalance can be further explained by the presence of the *agr* type III system, which we observed in the MF-associated *S. aureus* isolates. This is particularly linked with higher skin colonization and inflammation. In summary, specific virulence factors occur in plaque associated *S. aureus* strains that might indicate its role in host-microbe interaction and possible effects on disease progression and, consequently, patient fitness.

#### P-IB-013

##### First insights into quorum quenching fungal compounds targeting multispecies oral biofilms for the prevention of peri-implantitis

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Bacterial biofilms provide a substantial obstacle in healthcare settings, resulting in huge economic losses and health concerns worldwide. Notably, biofilm development on medically relevant surfaces, specifically dental implants, drives peri-implantitis, which affects one out of every five individuals who were rehabilitated with implants. Existing preventive measures involving the mechanical removal of biofilms alongside antibiotics are constrained, often ineffective, and have the potential to increase bacterial resistance. The composition and activity of oral biofilms has a profound impact on their formation, progression, and pathogenicity in the mouth. The presence or absence of certain bacteria determines the biofilm's role in health or disease (dental caries, periodontitis, and peri-implantitis). The shift from a healthy eubiotic oral microbiome, in which majority of the bacteria are aerobic and nonpathogenic e.g., *Streptococcus* and *Actinomyces* genera, to dysbiotic stage predominantly includes pathogenic anaerobes such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* leading to inflammation, bleeding, bone loss and ultimately implant failure.

Biofilm formation and pathogenicity relies on bacterial communication systems like quorum sensing (QS). While these systems are well studied for intra-species communication, little is known about inter-species communication during infection, or interkingdom signalling

(IKS) with host cells. We suspect that several key species in this multispecies oral biofilm influence the shift from a symbiotic to dysbiotic biofilm. In our studies, we used a periodontal bacterial biofilm model composed of 6 species to investigate how the absence of one bacterial species impacts the formation of biofilms. Thus far, we have observed that the presence of *Actinomyces naeslundii* significantly enhances biofilm formation in this consortia. This is a first step to better understand the microbial community and indicates that a treatment method specifically targeting the QS system of *A. naeslundii* has potential to diminish the overall biofilm production.

In our future studies, we aim to explore the nature of these molecules and interrupt the communication by inhibiting, capturing or degrading the communication molecules which is termed as quorum quenching (QQ). We aim to screen an extensive library of fungal extracts for their QQ activity and coat the QQ compounds onto the dental implants to inhibit biofilm formation.

#### P-IB-014

##### Overcoming Resistance via Reactivation – Mode of Action of Vancomycin conjugates

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Glycopeptide antibiotics (GPAs) are drugs of last resort to treat infections caused by (multi-) resistant Gram-positive pathogens. They inhibit peptidoglycan biosynthesis mainly by binding to the d-alanyl-d-alanine terminus of the lipid II peptide stem, thereby blocking transglycosylation and transpeptidation reactions in a sterical manner. Vancomycin (VAN) and teicoplanin (TEICO) represent the prototype GPAs in clinical use today. However, widespread GPA resistance has become a serious threat to global health. Resistance resulting from alteration of the PGN precursor, e. g. by exchange of the terminal d-alanine to d-lactate or d-serine, is conferred by the acquisition of a van-resistance operon. Enterococci, which harbour either a *vanA* or a *vanB* cluster, are most significant in the clinical setting. Current research is focusing on structural modification of glycopeptide antibiotics to overcome resistance. Lead candidate FU002, a vancomycin-hexa-arginine derivative with high potency against vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA), was generated by conjugation of polycationic peptides to VAN. Cell-based and in vitro assays were used to elucidate the precise mechanism of action to build a rational basis for targeted structure optimization. The results showed that differences in potency were not based on interaction with a secondary binding site on lipid II and remain still elusive.

## P-IB-015

### Deciphering non-canonical protein export via the accessory SecA2 system in *Streptococcus pneumoniae*

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**Introduction:** The accessory Sec (aSec) system represents a specialized protein secretion pathway predominantly found in Gram-positive bacteria such as streptococcal and staphylococcal species. Central to this system is the ATPase SecA2, which functions independently of the canonical SecA1 to export a subset of proteins, including large serine-rich repeat adhesins essential for bacterial adherence and pathogenicity. Despite its critical role, the molecular mechanisms and physiological significance of the aSec pathway in *Streptococcus pneumoniae* remain incompletely characterized. **Goal:** This study aims to identify and characterize the SecA2-dependent secretome across clinically relevant pneumococcal strains: the invasive serotype 4 strain TIGR4, and the colonization-associated serotype 19F strain EF3030. **Materials & Methods:** Genetically engineered  $\Delta$ secA2 and  $\Delta$ secA2 $\Delta$ lytA mutants, lacking SecA2 and in the latter case the major autolysin LytA, will be employed to analyze both the secreted and cell-associated proteins following detailed morphological and physiologic characterization. Cultures will be harvested at defined growth phases and both, bacterial pellets and supernatants will be subjected to quantitative mass spectrometry for protein identification and comparison. Proteins are evaluated for secretion signals, with particular emphasis to candidates lacking canonical export motifs, which will reveal novel secretion mechanisms. Complementary bioinformatic analyses using the Global Pneumococcal Sequencing Project (GPSP) database will enable correlation of SecA2-dependent proteins with strain-specific virulence or colonization phenotypes. **Summary:** This project will generate a comprehensive, functionally annotated catalog of SecA2-dependent pneumococcal surface and secreted proteins, thereby advancing the mechanistic understanding of accessory Sec-mediated secretion and informing future therapeutic or preventive strategies targeting this pathway.

## P-IB-016

### Host-microbe-microbe interactions during bacterial systemic co-infection in *Drosophila melanogaster*

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Microbes rarely exist in single colonies within their natural environments or in the host organisms. While extensive research has been done on how individual microbes behave *in vitro* and *in vivo* contexts, the complex interactions within polymicrobial infections and their hosts remain inadequately understood. In this context, we have established a natural co-infection model utilizing *Drosophila melanogaster* and further dissect the pathogen-pathogen interactions in such model. The fly-killing capacity of several bacterial combinations have been assessed. Notably, two *Providencia* strains have demonstrated to act synergistically when co-infected with *Enterococcus faecalis*. The synergistic effect is seen in male flies from two different background fly lines, but not in female flies, suggesting that the host is important for

the outcome of co-infection. When *Providencia* strains was co-infected with heat-killed *E. faecalis*, the synergistic effect was not observed, suggesting *E. faecalis* must be alive to have the greater fly-killing capacity. This finding suggests that *E. faecalis* is the agent contributing to the interaction between these three parties. The inhibition of the melanisation response observed during co-infection may provide a mechanistic explanation for the increased mortality observed in co-infected male flies. The interplay between two interacting pathogens and their modulation of the host immune responses are still under investigation through transcriptomic and proteomic approaches.

## P-IB-017

### Antibiotic pretreatment with ampicillin or streptomycin can differentially affect the course of *Salmonella*-induced intestinal inflammation

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*Salmonella enterica* serovar Typhimurium (S. Tm) is a widely used model organism to study enteric pathogenesis. In natural infections, S. Tm competes with the resident gut microbiota, which confers colonization resistance. To overcome this barrier in mouse models, high-dose streptomycin pretreatment is commonly applied to facilitate gut colonization and reproducible infection kinetics. More recently, alternative pretreatment regimens using broad-spectrum antibiotics such as ampicillin have been employed, particularly for infections with type III secretion system 2 (TTSS-2)-deficient S. Tm (S. Tm<sup>SPI2</sup>). However, the differential impact of streptomycin versus ampicillin pretreatment on key infection parameters such as inflammation dynamics and S. Tm colonization, remains poorly characterized. Here, we directly compared infection outcomes of S. Tm<sup>SPI2</sup> in streptomycin- versus ampicillin-pretreated mice across different genetic backgrounds. Ampicillin pretreatment elicited a markedly stronger intestinal inflammatory response and more severe histopathology than streptomycin, despite comparable S. Tm<sup>SPI2</sup> colonization levels. These differences correlated with distinct antibiotic-induced changes in microbiota composition. Moreover, the altered gut environment in ampicillin-pretreated mice was linked to enhanced activation of early TTSS-1 virulence programs in S. Tm, which mediate epithelial invasion. This comparative approach highlights how different antibiotic pretreatments shape the gut environment, thereby influencing infection kinetics, pathogen behavior, and host responses. It also provides valuable guidance for selecting appropriate pretreatments in experimental models. Together, these findings emphasize that the choice of antibiotic pretreatment is a major determinant of *Salmonella* pathogenesis in mice and should be carefully considered when designing infection studies to accurately capture host-pathogen-microbiota interactions.

## P-IB-018

### Generation of monoclonal antibodies against synthetic oligosaccharides resembling *Francisella tularensis* capsular polysaccharides

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*Francisella tularensis* (*F. tularensis*) is a Gram-negative bacterium that causes the highly infectious and zoonotic disease tularemia. Due to ease of aerosolization, low infectious dose and potential use as a bioweapon, it is classified as a Tier 1 Select Agent. Type B strains are less virulent, whereas an infection with *F. tularensis* subspecies *tularensis* (Type A) leads to a mortality rate of 30-60% if untreated. Although antibiotics are available, there is a risk of natural occurring or intentionally generated antibiotic resistance and the cure rate is variable. Despite the existing research in vaccine development, there is currently no licensed vaccine. Our research focuses on the development of preventative and therapeutic strategies against *F. tularensis* infections using well-defined, synthetic glycans resembling strain 15 O-antigen capsular polysaccharides as antigen epitopes. Screening sera from tularemia-infected and non-infected animals on glycan microarrays with these synthetic glycans identified two disaccharides as glycoconjugate vaccine candidates. After mice immunization with either of these glycans, successful antibody production was revealed and monoclonal antibody production through hybridoma technology was achieved. Four monoclonal antibodies were obtained which bound strongly to the synthetic glycans. Interestingly, ELISA revealed that one monoclonal antibody exhibited robust binding to several *F. tularensis* species. This study describes the generation of a monoclonal antibody with specificity toward different *F. tularensis* strains that may provide a new tool for diagnostic and/or therapeutic research.

## P-IB-019

### Antimicrobial control first: Fibroin scaffold is not enough in infected wounds

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Chronic and/or bacteria-infected wounds remain refractory because biofilm development hinders fibroblast migration and reepithelialization. In contrast, non-infected wounds can heal if the physiological microenvironment is stabilized. Silk fibroin is a promising, additive-free biodegradable biomaterial whose morphology may differentially support repair: dense membranes could favor reepithelialization, while nanofibrous fleeces may promote dermal integration. We compared both matrices in a standardized human *ex vivo* wound model under non-infected and infected (chronic-like) conditions.

Full-thickness human skin punches (8 mm) received a central 3 mm wound and were maintained at an air-liquid interface. Disks of cast fibroin membrane or electrospun fleece were press-fit into the wound, while controls received

no material. In infected cohorts, *Staphylococcus aureus* or *Pseudomonas aeruginosa* inocula (low CFU) were applied. Tissues were harvested over 20 days for blinded histology (H&E, Gram). The investigated parameters were reepithelialization, the location of bacteria within the tissue, and infection dynamics with and without fibroin matrices.

In non-infected wounds, both constructs accelerated healing significantly versus controls. Membranes consistently supported rapid, near-continuous reepithelialization, forming a smooth migratory substrate and early barrier. Fleeces yielded more dermal interaction, with cellular infiltration along fibers; however, epithelial closure was slower than in membranes. Under infected, chronic-like wound conditions, neither format overcame microbial burden: epithelial closure was not achieved, and Gram stains indicated persistent colonization.

Silk fibroin morphology dictates context-specific benefits: membranes act as "runways" for keratinocytes, while fleeces function as permissive 3D scaffolds for ingrowth. However, both effects collapse under infection, underscoring that bioburden control is a prerequisite to scaffold success. These data argue for morphology-guided material selection in clean wounds (membrane to close, fleece to integrate) and for combining fibroin constructs with effective antimicrobial strategies when chronic infection is present.

## P-IB-020

### Towards the exploitation of T-box riboswitches (TBRS) as targets for new anti-infectives against Gram-positive pathogens

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Antimicrobial resistance (AMR) of human bacterial pathogens is a serious public health issue that urgently requires action by identifying novel antibacterial targets and anti-infective compounds. Our work currently focuses on T-box riboswitches (TBRS) as putative drug targets in common Gram-positive pathogens such as multiresistant staphylococci and enterococci. TBRS represent unique bacterial RNA transcription control platforms that interact with tRNAs as ligands to regulate downstream gene expression. They are widespread among Gram-positive bacteria where they strictly control many essential pathways involved in amino acid and tRNA metabolism. Importantly, TBRS are specific to prokaryotes and have no counterpart in the eukaryotic cell, rendering these structures highly selective antibacterial targets. The aim of the project is to establish robust *in vitro* and *in vivo* reporter assays for future anti-TBRS drug screening endeavours. In our previous work, we functionally characterized a methionyl-tRNA-specific (Met)-TBRS that controls *de novo* biosynthesis of methionine in *Staphylococcus*. Mutational analyses demonstrated that the system is not only a specific anti-staphylococcal drug target, but is also suitable for studying TBRS functions in general. Here, we report the construction of a suicide vector system in *Corynebacterium glutamicum* as host bacterium. The vector harbours the Met-TBRS from staphylococci fused to the *sacB* gene from *Bacillus subtilis* as well as the met-tRNA from *S. aureus* as the Met-TBRS-specific ligand whose binding enables anti-terminator formation and *sacB* expression. As *sacB* encodes a levansucrase that is toxic to *Corynebacterium glutamicum* in the presence of sucrose

(Jager et al., 1992), tRNA-mediated TBRS activation would lead to cell death, while inhibition of the system, for example by TBRS-targeting small molecules, will enable bacterial growth. We show that the system is functional and suitable to determine either the terminator or anti-terminator status of the TBRS. Moreover, the vector can be employed to study tRNAs and their specific interactions with the TBRS.

## P-IB-021

### Carriage of Multidrug-Resistant *Streptococcus pneumoniae* 19F in a COVID-19 Pediatric Patient in Yogyakarta, Indonesia: Genomic, Phenotypic, and Clinical Characterization

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**Introduction** Multidrug-resistant *Streptococcus pneumoniae* is a major concern due to its ability to acquire antimicrobial resistance and virulence determinants that support persistence and adaptation. Understanding pneumococcal carriage during SARS-CoV-2 infection in children provides valuable insights into bacterial coexistence and resistance dynamics in the respiratory tract.

**Methods** Nineteen pediatric patients with suspected COVID-19 were screened for SARS-CoV-2 infection using RT-PCR. Nasopharyngeal swabs from SARS-CoV-2-positive patients were further examined for pneumococcal carriage. Any *S. pneumoniae* isolates were characterized by serotyping, sequence typing, antimicrobial susceptibility testing, and whole-genome sequencing (WGS) to identify antimicrobial resistance genes, virulence factors, and functional gene categories.

**Results** Fourteen of nineteen children tested positive for SARS-CoV-2 and most presented fever, cough, and nasal congestion. One COVID-19-positive patient carried *S. pneumoniae* serotype 19F, sequence type (ST) 320. The isolate showed resistance to  $\beta$ -lactams, macrolides, tetracyclines, and sulfonamides. WGS revealed resistance genes *ermB*, *mefA*, and *tetM*, and point mutations in *folA* and *folP*, consistent with phenotypic resistance. Virulence profiling identified capsule biosynthesis genes (*cps4A*, *cps4D*), the *rfa* pathogenicity islet, pilus islet 2 (PI-2), autolysins, and neuraminidases. Functional categorization showed enrichment in genes related to translation, metabolism, and cell wall biogenesis, indicating bacterial adaptation to the host environment.

**Conclusions** This study provides genomic and phenotypic insights into a multidrug-resistant *S. pneumoniae* 19F (ST320) isolate carried by a pediatric COVID-19 patient. The findings highlight the importance of genomic surveillance for monitoring pneumococcal carriage, antimicrobial resistance, and virulence evolution in pediatric populations.

## P-IB-022

### Investigating interactions between endosymbiont *Spiroplasma poulsonii*, the microbiota and *Drosophila melanogaster* in the context of gut immunity

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## Introduction

Insects are naturally colonised by a plethora of microorganisms. The resulting interactions can range from transient to stable and have a wide variety of effects on host physiology. Endosymbiotic microbes form particularly close long-term associations with insect hosts and can affect host nutrition and immunity (Corbin et al. 2017). *Spiroplasma poulsonii* MRSO, a facultative endosymbiont which naturally infects *Drosophila melanogaster*, has been the focus of recent studies. It has been shown to modulate the nutrients available to the host (Herren et al. 2014) and to affect host immune response after systemic infection (Hrdina et al. 2024). However, the microbiota of *Drosophila* may similarly impact host immunity and metabolism (Yu and Iatsenko 2025) (Douglas, 2018). As recent findings suggest that presence of the microbiota can impact endosymbiont density (Hrdina et al. 2024), we hypothesise that the microbiota may play a key role in the interactions between *S. poulsonii* MRSO and the host.

## Goals

Our aim is to study the interactions between endosymbiont *Spiroplasma poulsonii*, the microbiota and *Drosophila melanogaster* in the context of gut immunity.

## Materials & Methods

To assess the effect of the endosymbiont on the host, *Pseudomonas entomophila*, a known *Drosophila* pathogen, was used to orally infect flies harbouring the endosymbiont (Spiro+) and flies which did not (Spiro-) and host survival was monitored. Furthermore, to explore the effect of the endosymbiont on the remaining microbiota, the density of culturable microbiota was assessed in Spiro+ and Spiro- at different host ages.

## Results

In Spiro+ flies, survival upon oral infection with *P. entomophila* was significantly increased compared to Spiro- flies. Furthermore, *S. poulsonii* MRSO impacted the density of culturable microbiota in a host age dependent manner: in 5-day old flies the density was significantly increased Spiro+ flies compared to Spiro- on mannitol media, but this effect disappeared with fly ageing. On MRS, the density of culturable microbiota did not significantly differ in younger flies, but was significantly decreased in 15-day old Spiro+ flies.

## Summary

Our data suggests that *S. poulsonii* MRSO impacts both host immunity and microbiota density. Further work will focus on deciphering the impact of the endosymbiont on gut microbiota composition, and explore potential effects of host immunity and the gut microbiota on the endosymbiont density and protein expression.

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### P-IB-023

#### A Single Amino Acid Variation in Pneumococcal Pneumolysin Alters Platelet Binding and Cytotoxicity

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**Introduction.** *Streptococcus pneumoniae* is a major human pathogen responsible for diseases such as pneumonia, meningitis, otitis media, and sepsis. Pneumolysin (PLY), a cholesterol-dependent cytolysin, is a key virulence factor that contributes to host cell damage through pore-formation. PLY also disrupts platelet's function, potentially promoting vascular leakage and bacterial dissemination. Although highly conserved, minor sequence variations among PLY isoforms may alter its cytolytic activity. Notably, PLY derived from two widely studied pneumococcal strains, TIGR4 and D39, differs by a single amino acid substitution in the D4 domain at amino acid D380.

**Goals.** This study aimed to assess the platelet-binding capacity and lytic activity of PLY derived from pneumococcal serotype 4 strain TIGR4 and serotype 2 strain D39.

**Materials & Methods.** Recombinant *Strep-tagged* PLY from TIGR4 and D39 were heterologously expressed in *E. coli* DH5 $\alpha$ . Protein purity was confirmed by Coomassie staining and immunoblotting using anti-PLY antibodies. Platelet binding was assessed by flow cytometry with primary anti-PLY antibodies and FITC-conjugated secondary antibodies. Platelet death was evaluated using FITC-conjugated Annexin V.

**Results.** TIGR4-derived PLY bound to human platelets in a concentration-dependent manner and exhibited higher mean fluorescence intensity compared to D39-derived PLY at equivalent concentrations ( $p < 0.05$ ). Consistent with these results, TIGR4-derived PLY induced greater platelet death compared to D39 PLY, as reflected by increased Annexin V positivity.

**Summary.** These results demonstrate that minor sequence variations in amino acid 380 of D4 domain of PLY can significantly alter its platelet binding and cytotoxicity properties. TIGR4-derived PLY shows enhanced platelet toxicity compared to D39-derived PLY, highlighting the importance of strain-specific PLY-differences in shaping *Streptococcus pneumoniae* virulence and pathogenic potential.

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### P-IB-024

#### Environmental cues shape the biogenesis and function of extracellular vesicles in *Streptococcus pneumoniae*

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**Introduction:** The Gram-positive human pathogen *Streptococcus pneumoniae* adapts its metabolism to the environment during host invasion. During this process, extracellular vesicles are produced by *S. pneumoniae* (Sp-EVs), but the exact interplay between metabolic adaptation and vesicle formation remains elusive.

**Goals:** In this study, we aim to elucidate the role of host and environmental cues in shaping Sp-EVs' production and composition (DNA and protein content) and to investigate the potential involvement of Sp-EVs in metabolic adaptation and inter-species interactions.

**Methods:** We characterized EVs isolated from *S. pneumoniae* D39, grown under various environmental conditions such as human serum, temperature, pH and glucose availability, using nanoparticle tracking analysis. We assessed biofilm formation to investigate the role of Sp-EVs in intra- and inter-species communication. To study the cargo of Sp-EVs, a multi-omics approach was conducted (whole genome sequencing and proteomics profiles).

**Results:** We demonstrate that exposure to normal human serum and low glucose availability significantly increased the production of Sp-EVs. Temperature and pH also appeared to be critical factors for the formation of Sp-EVs: 37°C supported optimal production, while bacterial exposure to either basic or acidic environments slowed down their biogenesis. Functionally, Sp-EVs promoted biofilm formation in both *S. pneumoniae* and *Streptococcus pyogenes*. Glycolysis and general carbon metabolism pathways were enriched in the proteome of Sp-EVs. Multi-omics results revealed that the pathways related to metabolic and biosynthetic processes were tentatively upregulated on glucose-related EVs in comparison with the control.

**Summary:** In exploring how environmental conditions and nutrient availability shape the production of pneumococcal EVs and how these vesicles contribute to biofilm formation and inter-species interactions, our study contributes to the understanding of how *S. pneumoniae* applies complex strategies to persist and spread, with potential future applications in infection control and therapies.

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### P-IB-025

#### Functional analysis of putative cargo genes on Integrating Conjugative Elements in *Helicobacter pylori*

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*Helicobacter pylori* is a gram-negative bacterium, which colonizes the stomach of nearly half of the human population. Characteristic features of this organism include its high genetic variability due to high mutation rates, natural transformation competence, and efficient recombination, which help the bacteria persist in the stomach. *H. pylori* strains frequently contain mobile genetic elements like phages, plasmids and/or Integrating Conjugative Elements (ICEs), which may enhance diversity by horizontal gene transfer. In *H. pylori* two different ICEs are known which encode type four secretion systems (type four secretion system 3 and type four secretion system 4) termed as ICEHptfs3 and ICEHptfs4 respectively.

Comparative genome analysis of different *H. pylori* strains revealed variations in the presence of both ICEs as well as

their respective gene content. These elements mainly differ in the presence of certain variable genes, which potentially have additional functions besides the basic ICE functions, and may therefore be considered cargo genes. In order to study these genes further, we utilized a split-luciferase system fusing presumptive cargo proteins with a small luciferase fragment (HiBiT) which interacts at high affinity with the larger fragment (LgBiT) to form a functional luciferase, enabling localization and secretion studies. Our findings reveal that the presence of a C-terminal sequence motif influences the subcellular localization of the cargo proteins. Moreover, we could also detect a subset of these cargo proteins in the culture supernatants, indicating an active secretion mechanism. Preliminary infection experiments of a human gastric cell line with *H. pylori* strains producing these proteins demonstrated potential interaction with host cells that has to be investigated further.

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#### P-IB-026

##### **Glucose fuels the resistance of *Staphylococcus aureus* to host-derived antimicrobial fatty acids**

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Antimicrobial fatty acids (AFAs), considered as important components of the innate immune system on human skin, strongly inhibit the proliferation of *Staphylococcus aureus*, one of the most conspicuous bacteria due to its highly pathogenic potential. Unsurprisingly, *S. aureus* has evolved numerous adaptation strategies to thrive in AFA-rich environments, which are yet to be fully understood. Here, we used various rich media to probe the growth of *S. aureus* in the presence of physiologically relevant concentrations of AFAs. While AFAs completely prevented *S. aureus* growth in glucose-free media, the bacterium could replicate in glucose-replete tryptic soy broth (TSB) despite AFA supplementation. The susceptibility of *S. aureus* to AFAs in TSB without glucose phenocopied that of the bacterium in other glucose-free media, including a chemically defined medium. Glucose also rendered *S. aureus* biofilms recalcitrant to AFAs. Inhibition of glucose metabolism enabled AFAs to strongly inhibit or even eradicate *S. aureus* in glucose-rich TSB. Mechanistically, glucose-aided AFA resistance required the quorum-sensing regulatory Agr system, controlling most AFA resistance factors described to date. A mutant lacking one of these factors, wall teichoic acid (WTA), was very susceptible to PA irrespective of glucose, suggesting WTA as the primary strategy used by *S. aureus* to boost AFA resistance in the presence of glucose. Besides, *S. aureus* was outcompeted by *S. epidermidis* in co-culture experiments in glucose-free medium supplemented with AFAs. Together, our data suggest a model in which elevated glucose availability enables *S. aureus* to resist host defenses like AFAs and thrive in otherwise inhospitable polymicrobial communities.

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#### P-IB-027

##### **Prevalence and Molecular Characterization of *Stenotrophomonas maltophilia* Isolated from Hospital Environments and Clinical Samples in Duhok City, Kurdistan Region, Iraq**

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**Background/Objective(s):** *Stenotrophomonas maltophilia* is a Gram-negative, multidrug-resistant opportunistic pathogen increasingly linked to hospital-acquired infections, particularly in immunocompromised patients. This study investigated the prevalence, antimicrobial susceptibility, and virulence gene profiles of *S. maltophilia* isolated from hospital environments and clinical samples in Duhok, Iraq.

**Materials and Methods:** Between November 2024 and February 2025, 155 environmental samples (sink drains, medical devices, containers, surfaces, and tap water) and 126 clinical specimens (sputum, bronchial washings, blood, urine, CSF) were collected. Isolates were identified by culture, biochemical assays, species-specific PCR, and 16S rRNA sequencing. PCR was used to detect virulence genes (*smf-1*, *rpfF*, *plcN1*, *hlyIII*) and resistance determinants (*blaL1*, *blaL2*). Phylogenetic analysis based on 16S rRNA was conducted to assess relatedness between environmental and clinical strains.

**Results:** *S. maltophilia* was isolated from 25 environmental samples (16.1%) and 12 clinical samples (9.5%), with sink drains and sputum as major sources. Infants under one year had the highest infection rate. Most isolates harbored multiple virulence genes, particularly *smf-1* and *plcN1*. Antimicrobial testing showed complete resistance to ceftazidime (100%) and high resistance to ticarcillin-clavulanic acid (>90%), whereas trimethoprim/sulfamethoxazole remained the most effective but indicated emerging resistance. Resistance genes were frequent: *blaL1* was found in 56% of environmental and all clinical isolates, while *blaL2* was detected in 60% and 91.7%, respectively. Phylogenetic analysis of 37 isolates revealed marked genetic heterogeneity, forming four clades. The clustering of environmental and clinical isolates in the same subgroups suggests potential common reservoirs, while unique clades (e.g., En22) indicated distinct evolutionary lineages.

**Conclusion:** This study demonstrates a notable prevalence of multidrug-resistant *S. maltophilia* in Duhok hospitals, especially from sink drains and sputum. The high frequency of resistance genes and virulence factors underscores the urgent need for reinforced infection control, continuous surveillance, and targeted antimicrobial therapy to mitigate the risk posed by this pathogen.

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#### P-IB-028

##### **Transcriptomic Profiling to Identify Novel Targets for *E. faecalis* Biofilm Inhibitors**

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*Enterococcus faecalis*, a common gut commensal and major cause of hospital-acquired infections, poses serious treatment challenges due to its strong biofilm-forming ability and antibiotic resistance. As up to 80% of human infections are biofilm-associated, new therapeutic strategies targeting biofilm formation are urgently needed.

The primary objective of this study is to identify novel molecular targets and effective inhibitors for *E. faecalis* biofilm formation. Our approach focuses on discovering potential drug targets through comparative transcriptomic analyses between planktonic and biofilm states, as well as under biofilm inhibition conditions induced by novel inhibitor



compounds as well as the established antimicrobial surface AGXX, which has been also demonstrated to effectively reduce biofilm formation (Vaishampayan et al., 2021).

Transcriptomic profiling will be employed to identify new candidate genes involved in biofilm formation. The selected targets will be analyzed and characterized using protein chemistry techniques, including interaction studies and site-directed mutagenesis to elucidate critical binding regions. *E. faecalis* knockout mutants will be used to evaluate the functional relevance of each target. Complementary *in-silico* molecular docking studies support target-based inhibitor development. In parallel, a biochemical screening platform will be established to evaluate compound libraries using target-specific activity assays. The efficacy of identified targets and inhibitors will be further assessed in static biofilm assays and flow-cell biofilm systems. We will present transcriptomic data demonstrating differential gene expression patterns between planktonic and biofilm states of *E. faecalis*. In addition, preliminary *in-silico* molecular docking analyses will be shown, illustrating predicted interactions between selected targets and candidate inhibitory compounds that may interfere with biofilm-associated functions.

As no current biofilm therapies have successfully advanced beyond clinical trials identifying novel drug targets remains crucial. Employing integrated transcriptomic, biochemical, and computational approaches provides a robust framework for the discovery of next-generation biofilm inhibitors against multi-resistant nosocomial *E. faecalis* infections.

Vaishampayan, A. et al. Transcriptomic analysis of stress response to novel antimicrobial coatings in a clinical MRSA strain. Materials Science and Engineering: C 119, 111578 (2021).

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#### P-IB-029

##### **Systematic classification of canonical antibiotic resistance mutations from the AMRfinder database with a CRISPR screen in *Escherichia coli***

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Bacteria acquire antibiotic resistance either through the uptake of resistance genes or by mutations within their genome that lead for example to amino acid changes in the drug target, in transporters or their regulators. Comprehensive databases as AMRfinderPlus (Feldgarden et al. 2021) collect such canonical resistance mutations across diverse bacterial species. While some of these mutations are experimentally validated, they have not been systematically tested in the same genetic background. To systematically evaluate the impact of 1510 amino acid changes listed in the AMRfinderPlus database, we designed oligonucleotides to introduce them into the *Escherichia coli* strain MG1655, using a pooled CRISPR-based approach (Lubrano et al. 2025). For each mutation, two sgRNAs were designed, and the parent codon was substituted with all possible child codons for the corresponding amino acid. This resulted in a library with 5,806 *E. coli* mutants, each with one mutation from the AMRfinderPlus database. The library was treated with the 3<sup>rd</sup> generation cephalosporins ceftazidime and ceftriaxone, the fluoroquinolone ciprofloxacin, and the last-resort polymyxin colistin. We observed modest increases in minimum inhibitory concentrations (MICs) for the

cephalosporins relative to the wild type, whereas ciprofloxacin and colistin showed four- and ten-fold MIC elevations, respectively, indicating that the impact of SNPs on resistance is antibiotic class-dependent. Pooled screening in combination with next-generation sequencing of barcode abundances will enable a quantitative ranking of these mutants according to their effects on antibiotic resistance and facilitate the analysis of how genetic background influences resistance phenotypes. This comprehensive validation and categorization of AMRfinderPlus SNPs will lead to a better understanding of how single-point mutations in the *E. coli* genome can contribute to the evolution of bacterial antibiotic resistance.

#### References

Feldgarden, Michael, Vyacheslav Brover, Narjol Gonzalez-Escalona, et al. 2021. "AMRfinderPlus and the Reference Gene Catalog Facilitate Examination of the Genomic Links among Antimicrobial Resistance, Stress Response, and Virulence". Scientific Reports 11 (June): 12728.

Lubrano, Paul, Fabian Smollich, Thorben Schramm, et al. 2025.

"Metabolic Mutations Reduce Antibiotic Susceptibility of *E. Coli* by Pathway-Specific Bottlenecks". Molecular Systems Biology, January 2, 1–20.

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#### P-IB-030

##### **Establishing a CRISPRi Platform to Dissect Virulence and Survival Mechanisms in *Salmonella* Typhimurium**

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*Salmonella enterica* rapidly adapts to the diverse and dynamic environments it encounters during infection, yet the regulatory mechanisms that drive its swift transcriptome remodeling remain poorly understood. Many genes required for survival within the host also contribute to the invasion process, which limits the use of classical knockout approaches for studying invasion-associated or essential genes. CRISPR interference (CRISPRi) offers a powerful alternative by enabling targeted, reversible downregulation of gene expression without generating lethal mutants.

In this work, we aim to establish a robust CRISPRi platform in *Salmonella enterica* serovar Typhimurium (STM) to investigate genes involved in pathogen virulence, including invasion, intracellular survival and evasion of host immune defenses. To achieve this, we are constructing a genome-wide sgRNA library targeting all annotated STM genes and integrating it into a strain expressing inducible dCas9. High-throughput CRISPRi screening will be used to identify genetic determinants of *Salmonella* pathogenesis, providing a comprehensive framework for dissecting the regulatory networks underlying infection.

## P-IB-031

### Membrane Dyes as Tools to Investigate Cell Envelope Integrity in Gram-Negative Bacteria

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Fluorescence microscopy is a powerful tool in bacterial cell biology, enabling the study of diverse physiological processes. While fluorescent proteins are widely used, they cannot label molecular structures such as peptidoglycan or lipid bilayers. Small fluorescent probes that diffuse into living cells and selectively stain defined cellular components therefore provide a valuable complementary approach. Although various membrane dyes have been successfully applied in Gram-positive bacteria such as *Bacillus subtilis*, their use in Gram-negative bacteria, including *Salmonella enterica*, remains challenging due to the additional outer membrane barrier.

In this study, we systematically characterize a defined set of membrane dyes and evaluate their ability to label the inner (IM) and outer membranes (OM) of *Salmonella enterica*. We assess membrane specificity, determine conditions for reliable and reproducible staining, and establish imaging parameters suitable for live-cell fluorescence microscopy. Using these dye-based tools, we aim to investigate the role of OM integrity in flagella-mediated motility, as well as the effects of innate immune system factors that target the OM.

## P-IB-032

### Recovery of the gut microbiome upon challenge with multiresistant *E. coli*

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Antimicrobial resistance is a critical global health threat, with extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* (ESBL-*E. coli*) among the most widespread multidrug-resistant pathogens (1). These strains colonize the intestinal tract, facing the host microbiota providing colonization resistance. Understanding how the microbiome (epi)genetically, transcriptionally, and metabolically adapts to and recovers from ESBL-*E. coli* colonization is essential to explore mechanisms of resilience and therapeutic targets.

This study applies an integrated multi-omics framework, combining metagenomics, epigenetics, transcriptomics, and metabolomics, to characterize the gut microbiome's dynamic response to ESBL-*E. coli* colonization. Specific-pathogen-free C57BL/6 mice harboring a complex microbiota were challenged orogastrically with a clinical ESBL-*E. coli* isolate (2) and transiently treated with ceftriaxone to facilitate colonization. Fecal samples were collected daily, and cecal content at days 0, 2, 3, and 5 post-infection for comprehensive molecular profiling.

The infection model showed reproducible colonization dynamics, with *E. coli* levels peaking on days two to three and declining thereafter, indicating microbiome recovery. Native, PCR-free long-read sequencing enables strain-resolved metagenomic and methylation analyses, while parallel transcriptomic and metabolomic datasets capture system-level shifts in gene expression and metabolism. We will use these datasets to profile coordinated molecular

responses, functional reorganization, and community adaptations during colonization and clearance.

Combining long-read sequencing with epigenetic, transcriptomic, and metabolomic profiling, this work will provide a multidimensional view of gut microbiome perturbation and recovery following multidrug-resistant *E. coli* challenge. These insights advance understanding of microbiome resilience and identify molecular pathways supporting recovery from antibiotic-associated dysbiosis.

#### References:

1. Dokuta, S. et al. Sci. Rep. 15, 23886 (2025). <https://doi.org/10.1038/s41598-025-06410-1>
2. Schweitzer, M. et al. Microbiol. Res. 2023, 14, 177–189. <https://doi.org/10.3390/microbiolres14010015>

## P-IB-033

### Contact-catalytic AGXX<sup>®</sup> surfaces retain rapid pathogen-killing activity under high bacterial challenge

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The global rise of antibiotic resistance has intensified the search for novel antimicrobial strategies. Surface technologies have emerged as a promising approach to prevent microbial colonization and pathogen transmission in healthcare environments. AGXX<sup>®</sup>, a contact-catalytic antimicrobial surface based on silver and ruthenium, effectively eliminates pathogenic microorganisms, including multidrug-resistant bacteria, fungi, and viruses through generation of reactive oxygen species (ROS). Although AGXX<sup>®</sup>'s broad-spectrum potential has been well established, its antibacterial efficacy has not previously been quantified.

Here, we quantitatively assessed the bactericidal efficacy of AGXX<sup>®</sup> and uncoated silver surfaces against pathogenic *Staphylococcus aureus* (MRSA) and *Escherichia coli* strains using a modified ISO 22196 assay. While both materials achieved complete bacterial eradication within 24 hours, time-resolved analysis revealed distinct kinetic differences in antimicrobial activity strongly dependent on the initial bacterial load. Under standard conditions (low inoculum,  $\approx 10^6$  CFU/cm<sup>2</sup>), both materials performed comparably, achieving total bacterial elimination within 60 minutes of incubation. However, with increasing bacterial challenge ( $10^7$ – $10^8$  CFU/cm<sup>2</sup>), AGXX<sup>®</sup> maintained rapid bactericidal activity, achieving 3-log reductions in viable cell counts by 90 minutes and total bacterial eradication ( $>5$ -log reduction) by 180 minutes of incubation, whereas silver surfaces only reached a 1-log reduction in the same period.

These results indicate that AGXX<sup>®</sup> retains strong catalytic activity even under extreme microbial challenge, while the bactericidal efficiency of silver decreases with rising contamination levels. The data suggest that the contact-catalytic mechanism of AGXX<sup>®</sup>—mediated by ROS generation—becomes increasingly relevant under high microbial pressure. This study provides the first quantitative evaluation of AGXX<sup>®</sup>'s bactericidal efficacy and underscores the critical influence of inoculum size on measured antimicrobial performance. Elevated bacterial

loads - while not representative of realistic contamination levels - serve as analytically stringent conditions that accentuate material-specific performance differences. AGXX® thus emerges as a highly potent antimicrobial surface with strong potential for application in healthcare infection control.

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#### P-IB-034

##### **Soda–lime–silicate glass composition shapes early colonization by *Staphylococcus aureus* and *Klebsiella pneumoniae***

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#### **Introduction**

Biomaterials-associated infections (BAIs) occur when bacteria colonize the surface of biomaterials [1]. BAIs begin with rapid bacterial attachment, and the formed biofilms are notoriously difficult to eradicate. Accordingly, soda–lime–silicate (SLS) glasses provide a controlled system to study how composition (50–75 mol% SiO<sub>2</sub>) shapes early microbial responses [2]. Depending on silica content, SLS glasses can act as biostable materials (BSG; 75 mol% SiO<sub>2</sub>) or biodegradable materials (BDG; 70 mol% SiO<sub>2</sub>), enabling diverse biomedical applications.

#### **Goals**

This study aimed to determine whether biodegradable SLS glass (50/60/70 mol% SiO<sub>2</sub>) alters *Staphylococcus aureus* attachment and viability within 24 h, quantified by fluorescence and colony-forming units (CFU), and to test whether replacing CaO (12.5 mol%) in biostable SLS glass (75 mol% SiO<sub>2</sub>) by ZnO affects bacterial colonization by *S. aureus* or *Klebsiella pneumoniae*.

#### **Materials and Methods**

SLS granules were provided by collaborators. For BDG, standardized inocula of GFP-labelled *S. aureus* were incubated with glass granules; fluorescence was recorded over 24 h, and adherent/planktonic fractions collected at predefined times were plated for CFU. For BSG, *S. aureus* and *K. pneumoniae* were incubated with BSG or BSG-ZnO, followed by CFU quantification after 24 h.

#### **Results**

In BDG experiments, the control (bacteria only) showed the highest fluorescence (biomass/viability). At 12 h, biomass/viability was lower than control by ~40 % (50 % SiO<sub>2</sub>), ~24 % (60 %), and ~17 % (70 %), indicating a silica composition-dependent delay. At 6 h, viable counts were reduced relative to control by ~6.8-log<sub>10</sub> (50 %), ~3.4-log<sub>10</sub> (60 %), and ~1.6-log<sub>10</sub> (70 %).

When comparing BSG containing either 12.5 mol% of CaO (control) or ZnO, *K. pneumoniae* viability was significantly reduced using BSG-ZnO, while *S. aureus* was not affected.

#### **Summary**

BDG composition governs early *S. aureus* colonization: lower-silica BDG suppresses initial biomass/viability and survival, whereas higher-silica BDG facilitates earlier settlement – indicating that silica content is an adjustable parameter for limiting early attachment.

In BSG, replacing CaO with ZnO inhibits *K. pneumoniae* but not *S. aureus*. Future work will explore higher ZnO contents to identify effective concentrations against different bacteria.

#### **References**

[1] Kasapgil et al., *WIREs Nanomedicine and Nanobiotechnology* 2024; 16

[2] Jin et al., *J Mater Chem B*. 2025; 13

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#### P-IB-035

##### **A Murine Pressure Ulcer Model for Evaluating Persistence and Treatment of *Staphylococcus aureus* Infection**

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**Introduction** Pressure ulcers are significant healthcare challenges, especially in the elderly population. Pressure ulcers are susceptible to bacterial colonization and infection, with *Staphylococcus aureus* being one of the most prevalent pathogens (1). *S. aureus* infections can significantly impair wound healing and lead to complications, including sepsis, highlighting the need for effective treatment strategies and reliable experimental models. However, developing reliable murine models has been challenging due to self-limiting nature of infection and onset of sepsis (2).

**Goals** This study aimed to provide an experimental murine model of chronically infected pressure ulcers to achieve real-time, non-invasive monitoring of infection dynamics and evaluate treatment efficacy.

**Methods** Mice model was established by using a single cycle of magnet-induced ischemic injury combined with infection by bioluminescent *S. aureus*. Wound photography and histology analysis were performed to assess wound healing. Bacteria CFU analysis and *vivo* imaging system (IVIS) were performed to evaluate bacteria load and confirm *S. aureus* infection. To evaluate treatment efficacy on this model, the wounds were treated with a hydrogel either containing or lacking a host defense peptide, followed by wound photography, CFU analysis, and IVIS imaging.

**Results** Wound photography and histology showed that this experimental method produces reproducible pressure ulcer wounds with significant tissue changes over a 14-day period. CFU analysis and IVIS showed persistence of infection and the dynamics of *S. aureus*. IVIS also revealed *S. aureus* infection was limited to wound area and surroundings without systemic spread or sepsis. The Comparison of treated and untreated wounds demonstrated the antimicrobial efficacy of the peptide.

**Conclusion** This mice model offers a non-invasive monitoring approach for investigating therapeutic strategies and mechanisms of bacterial persistence in pressure ulcers. Future studies using this model could explore host-pathogen

interactions and novel treatment combinations, ultimately contributing to improved clinical management of pressure ulcer infections.

1. Dunyach-Remy C, et al. Pressure Ulcers Microbiota Dynamics and Wound Evolution. *Sci Rep* (2021) 11(1):18506. Epub 20210916.
2. Fila G, et al. Murine Model Imitating Chronic Wound Infections for Evaluation of Antimicrobial Photodynamic Therapy Efficacy. *Front Microbiol* (2016) 7:1258. Epub 20160809.

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#### P-IB-036

##### **Impact of the Cefazolin Inoculum in Methicillin-Susceptible *Staphylococcus aureus* infections**

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The Cefazolin inoculum effect (CzIE) is observed to exhibit unique characteristics within *Staphylococcus aureus* (MSSA) infections. To date, certain strains expressing the *blaZ* gene show significantly reduced Cefazolin activity in high bacteria inoculums, which can result in suboptimal drug exposure and therapeutic failure, particularly in deep-seated infections such as endocarditis or bacteremia.

This study aims to characterize the cefazolin inoculum effect by performing standard and high-inoculum minimum inhibitory concentration (MIC) testing using the broth microdilution method to compare changes in MIC values across clinical isolates. To establish a gold-standard approach for detecting the inoculum effect, cefazolin susceptibility will be assessed in microplates alongside cell viability assays (Alamar Blue). Furthermore, specific  $\beta$ -lactamase enzyme types (A–D) associated with strong inoculum effects will be analyzed using in-house sequencing, RT-qPCR, and Nitrocefin-based quantification assays. The feasibility of  $\beta$ -lactamase inactivation methods for potential diagnostic applications will also be evaluated.

By integrating molecular and microbiological methods, this study provides crucial insight into the mechanisms underlying cefazolin treatment failure. Microdilution and cell viability testing enable identification of MSSA strains with reduced susceptibility at high bacterial loads, while  $\beta$ -lactamase profiling elucidates the genetic determinants of resistance. Incorporating genomic analysis of virulence factors and toxin profiles further refines our understanding of the inoculum effect.

Ultimately, the development of a gold-standard method for detecting the cefazolin inoculum effect in MSSA will facilitate early recognition of high-risk infections, support optimized dosing strategies, and improve the effectiveness of  $\beta$ -lactam therapies. Comparative evaluation of cefazolin with other  $\beta$ -lactams (e.g., ceftriaxone, nafcillin, oxacillin) under high-inoculum conditions may identify superior therapeutic options, thereby reducing treatment failure and mortality in critically ill patients.

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#### P-IB-037

##### **NAD(H)-depletion activated phospholipase facilitates bacterial killing by fluoroquinolones**

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**Introduction:** We recently uncovered the unusual mechanism controlling the activity of the *Legionella pneumophila* phospholipase and virulence factor PlaB (PMID: 34074754). Physiological concentrations of NAD(H) stabilize inactive PlaB tetramers, but low NAD(H) triggers tetramer deoligomerization into highly active protein dimers. Active PlaB dimers are a threat to *Legionella* since its cell membranes are no longer protected from PlaB's phospholipase activity. Bacterial NAD(H) can be targeted and degraded by NAD hydrolyzing enzymes, such as NADases. Recent work showed that genotoxic stress conferred by the fluoroquinolone antibiotic ciprofloxacin can activate the GndRX NADase toxin-antitoxin module of *L. pneumophila*, efficiently depleting cytosolic NAD(H) (PMID: 40825874). Based on these combined findings, we hypothesized that efficient killing of *L. pneumophila* by fluoroquinolones depends on PlaB, putting the phospholipase into interest as a potential drug target.

**Goals:** Does presence and activation of PlaB upon fluoroquinolone exposure improve clearance of *L. pneumophila* from cultures and infected host cells?

**Methods and Results:** Consistent with NAD(H) being central for regulation of PlaB's activation status, in gene co-occurrence studies, we found conservation of *plaB* with genes encoding NAD(H) consumers, suggesting a functional link. Indeed, co-expression of a NADase toxin and PlaB in *E. coli* and *L. pneumophila* revealed accelerated cell death when PlaB was present and NADase transcription was induced as well as cytosolic NAD(H) depletion. Lipid analysis of *E. coli* expression cultures demonstrate that activation of cytosolic PlaB results in membrane phospholipid digest, suggesting toxin driven NAD(H) depletion can kill by inducing self-digest when PlaB is present. Gene expression analysis in *L. pneumophila* uncovered co-regulation of *plaB*, toxin, and antitoxin genes, all of which being induced upon fluoroquinolone exposure, as well as upon prolonged stress. Our results support a model in which PlaB is functionally linked with an NADase effector module as part of its stress response. Our combined findings suggest that activation of PlaB could be exploited for sensitizing *L. pneumophila* for better clearance by antibiotic regimens in the future.

**Summary:** PlaB can act as a cell death effector when cytosolic NAD(H) is low, making it an interesting future drug candidate. PlaB co-occurs with NADase in – but not limited to – *Legionella pneumophila*.

# Host-pathogen interactions

## P-HPI-001

### Genome analyses of the potato-associated 16SrXII-A 'Candidatus Phytoplasma solani' strain POT, transmitted by the vector *Hyalesthes obsoletus*

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## Introduction

'Candidatus Phytoplasma solani' poses an increasing threat to sugar beet and potato production across Europe. Polyphagous vectors efficiently transmit the pathogen from reservoir plants to new crop populations, enabling the pathogen to spread rapidly. While both 16SrXII-A and -P strains have been reported, a lack of genomic data for the A-type hinders our understanding of its diversity and epidemiology in Germany.

## Goals

In this study, the complete genome sequencing of the 'Ca. P. solani' strain POT – isolated from potato and transmitted by the planthopper *Hyalesthes obsoletus* – aims to close this gap and provide new insights into the genomic diversity, evolutionary adaptations, and deviating virulence factors of these phytopathogens.

## Methods

The genome of the 'Ca. P. solani' strain POT was determined by SMRT sequencing, *de novo* assembled, manually annotated, and functionally reconstructed. Comparative and phylogenetic analyses further elucidated genomic traits underlying host adaptation and virulence.

## Results and Conclusion

Phylogenomics based on a conserved ortholog set delineated the taxonomic boundaries between and within the 16SrXII-A and -P subgroups. Despite a largely conserved core metabolism, differences in mobilome and associated effectors, and membrane-associated proteins indicate that lineage-specific variation shapes host range and transmission efficiency, providing a genomic basis for the distinct epidemiology in Germany.

## P-HPI-002

### Hippo Pathway Activation Drives Fibrogenic Remodeling in Influenza A Virus-Infected Lung Fibroblasts

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**Introduction:** Pulmonary fibrosis is a progressive and often fatal interstitial lung disease with largely undefined etiology. Alveolar macrophages and lung fibroblasts play key roles in

maladaptive tissue remodeling. However, the cellular and molecular mechanisms underlying their coordinated fibrotic responses are still incompletely understood. Increasing evidence suggests that respiratory viruses such as influenza A virus (IAV) may contribute to fibrosis progression, yet the precise mechanisms by which viral infections modulate fibrotic signalling pathways remain elusive.

**Goal:** In this study, we aim to investigate how IAV-induced alterations in the Hippo signalling pathway drive fibrotic remodeling in lung fibroblasts, thereby uncovering mechanisms through which respiratory viral infections may exacerbate pulmonary fibrosis.

**Methods:** Following IAV infection, viral load, cytokine release, and transcriptomic changes were analysed. To assess indirect effects on fibrosis, IMR-90 fibroblasts were treated with conditioned media from infected alveolar macrophage-like (AML) cells. Direct IAV infection of IMR-90 cells was additionally performed to evaluate changes in the Hippo signalling pathway and fibrotic marker expression using microarray and transcriptomic approaches.

**Results and Conclusion:** For the first time, AML cells were successfully infected with IAV, validating this model for studying pathogen-driven exacerbation of lung fibrosis. Transcriptomic and protein-level analyses revealed that IAV promotes fibrogenesis in fibroblasts through two distinct mechanisms: (I) indirectly, via pro-inflammatory and pro-fibrotic mediators released by infected macrophages, and (II) directly, through viral-induced modulation of the Hippo signalling pathway, resulting in upregulation of key fibrotic markers such as CTGF, fibronectin, and collagen I.

These findings provide novel mechanistic insights into macrophage–fibroblast crosstalk in the context of viral infection and fibrosis. They suggest that dysregulation of these interactions contributes to the susceptibility of the lung to fibrotic remodeling following respiratory viral infections and may inform future therapeutic interventions targeting Hippo pathway signalling.

## P-HPI-003

### Application of a transposon-directed insertion site sequencing approach to identify bacterial factors involved in intracellular survival of *Yersinia enterocolitica*

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Pathogenesis of yersiniosis comprises cell-invasive events in which the bacteria subvert the endocytic-autophagolysosomal pathway of infected cells to create an intracellular niche for bacterial replication. The *Yersinia*-containing, proliferative vacuoles are protected against acidification and display autophagy-related features.

To decipher chromosomal genes of *Yersinia enterocolitica* that play a role in enabling the multiplication in infected epithelial cells we performed a high-throughput transposon-directed insertion site sequencing approach (TraDIS).

In this screen, we identified several putative virulence factors with either predicted metabolic activities or with functions in determining the composition of the *Yersinia* outer membrane and surface. The deduced processes involve glutamine synthesis, energy generation, the folding of proteins by disulfide bonds, and the synthesis of carbohydrates. Individual knockout mutants of the identified genes are impaired in intracellular bacterial survival and replication. These mutants furthermore differentially affect autophagy and acidification of the *Yersinia*-containing vacuoles in infected cells.

Our results suggest that *Yersinia* outer membrane components and metabolic pathways communicate in manifold ways with the host cell to enable efficient intracellular bacterial multiplication. We now focus our research on the molecular characterization of the crosstalk of the identified *Yersinia* factors with the interacting host cell compartments.

#### P-HPI-004

##### **Characterization of a *Salmonella* Typhimurium ST19 clade associated with bloodstream infections and enhanced invasiveness**

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**Introduction:** In Germany, *Salmonella enterica* is mainly associated with foodborne infections and largely causes self-limiting gastroenteritis. In contrast, invasive non-typhoidal *Salmonella* (iNTS) infections are particularly prevalent in Sub-Saharan Africa, with *Salmonella* Typhimurium strains of specific MLST sequence types (ST), primarily ST313 and ST19, being the main culprits behind iNTS cases in the region. At the German National Reference Centre for *Salmonella* (NRC), a rise in *Salmonella*, including *S.* Typhimurium, bloodstream infections has been detected during the last years. However, the specific *Salmonella* subtypes, mechanisms and genes of the pathogens driving invasive infections are largely unknown.

**Goals:** We aimed to identify genomic signatures and *in vitro* infection phenotypes of *S.* Typhimurium strains associated with invasive disease.

**Materials and Methods:** Ninety-six clinical *S.* Typhimurium isolates (2020 to 2023) from Germany and from Sub-Saharan Africa were analyzed using bioinformatic tools to estimate their invasive potential. Based on a predicted invasiveness index, isolates were classified and subsequently analyzed in *in vitro* infection experiments to determine bacterial invasion and intracellular replication. Comparative genomic analyses, including gene presence/absence profiling, were used to identify candidate genes or genomic regions potentially associated with increased invasion capacity.

**Results:** Within *S.* Typhimurium phylogeny, a distinct *S.* Typhimurium ST19 clade showed a higher invasiveness index and higher cell invasion and/or intracellular replication capacity compared to other ST19 strains. Interestingly, this clade contained both German and African isolates. Gene presence/absence analyses identified several genomic loci

potentially associated with this phenotype, providing a basis for further investigation through gene knockout experiments.

**Summary:** Within the phylogeny of *S.* Typhimurium ST19, we identified a clade containing strains from Germany and Africa that show higher invasiveness and intracellular replication capacity. Ongoing functional validation aims to pinpoint specific genetic signatures providing new insights into the evolution and virulence of non-typhoidal *Salmonella*.

#### P-HPI-005

##### **Synergistic Phage-β-Lactam Co-Treatment Eradicates *Klebsiella* Biofilms and Suppresses Resistance**

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Biofilms formed by *Klebsiella pneumoniae* and related species exhibit high tolerance to conventional antibiotics, leading to persistent, difficult-to-treat infections. Bacteriophages (phages) represent a promising alternative, particularly in combination with antibiotics, where phage-antibiotic synergy (PAS) can enhance antibiofilm activity.

This study used light sheet fluorescence microscopy (LSFM) for real-time analysis of PAS in *K.* quasipneumoniae biofilms treated with lytic phage vB\_KpUKJ\_2<sup>1</sup>, ceftazidime (CAZ), or both over 24 h. Biofilm viability was measured metabolically<sup>2</sup>, and LSFM data were processed with custom ImageJ macros. Confocal microscopy (CLSM) was employed to assess EPS polysaccharide changes post-phage treatment, and phage-resistant mutants were identified and characterized.

Quantitative LSFM analysis and viable cell counts revealed that high-dose phage (10<sup>8</sup> PFU/mL) combined with CAZ at 0.25× minimum inhibitory concentration (MIC) induced a rapid and sustained reduction in viability over 24 hours. Area-under-the-curve (AUC) analysis confirmed pronounced synergistic activity. CLSM imaging revealed progressive degradation of EPS polysaccharides post phage exposure, suggesting depolymerase-mediated disruption of biofilm. Notably, the combined regimen of phage and CAZ completely suppressed the emergence of phage resistant mutants in both planktonic and biofilm states. In contrast, phage mono-treatment yielded resistant isolates but with fitness trade-offs, including reduced growth rate and impaired biofilm formation. Whole-genome sequencing of the phage resistant isolates revealed mutations spanning outer membrane receptors (*fhuA*), purine biosynthesis (*purA*), and transcriptional regulation (*rpoC*), suggesting diverse resistance mechanisms.

These findings highlight the therapeutic potential of PAS for both eradication of *Klebsiella* biofilms and suppression of resistance, and establish LSFM as a powerful platform for quantitative, time-resolved evaluation of antimicrobial strategies in biofilms.

#### References:

1. Mirza KA, Tchatchiashvili T, Marquet M, Nietzsche S, Pletz MW, Makarewicz O. Characterization and genome analysis of novel *Klebsiella pneumoniae* phage vBkPukJ\_2 isolated from hospital sewage water. BMC Microbiol. 2025 Feb 26;25(1):96.

2. Tchatchiashvili T, Jundzill M, Marquet M, Mirza KA, Pletz MW, Makarewicz O, et al. CAM/TMA-DPH as a promising alternative to SYTO9/PI for cell viability assessment in bacterial biofilms. *Front Cell Infect Microbiol.* 2025 Jan 21;14:1508016.

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## P-HPI-006

### Uncovering Alternative ER-SCV Contact Sites: A High-Throughput Screen of Membrane Trafficking Proteins Exploited by *Salmonella*

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Infectious diseases are among the greatest threats to human health and causing significant morbidity and mortality worldwide. Membrane contact sites (MCSs) emerged as important key players in cellular signaling, energy metabolism and cellular defense mechanisms during pathogenic infections. Additionally, MCSs are exploited by pathogens to redirect important metabolites and promote their survival and proliferation. *Salmonella* forms a specialized contact site between the *Salmonella*-containing vacuole (SCV) and the endoplasmic reticulum (ER) to redirect cholesterol trafficking, resulting in cholesterol accumulation in the SCV, mTOR activation and autophagy inhibition. While this process has been linked to the OSBP-VAP pathway, emerging evidence reveals alternative mechanisms: the ER-mitochondria contact site protein PDZD8 has been found at the SCV, and the *Salmonella* effector SteA was determined to promote ER-SCV MCSs, with its depletion impairing SCV division and bacterial proliferation.

Using a high-throughput microscopy screening approach this study aims to unravel the exploitation of host membrane trafficking proteins at the ER-mitochondria contact site by *Salmonella*. Host cells expressing fluorescent markers for ER-mitochondria MCSs will be infected with fluorescent-labelled *Salmonella* and transfected with a siRNA library targeting human membrane trafficking proteins. Positive hits will be further characterized to elucidate the molecular mechanisms, the impact on cellular trafficking and how *Salmonella* enhance its survival by exploiting these contact sites. A comprehensive understanding of these interactions will not only expand our knowledge of host cell biology and pathogen exploitation strategies but also contribute to the identification of novel therapeutic targets for combating intracellular bacterial infections.

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## P-HPI-007

### Impact of extracellular serine proteases on the pneumococcal proteome

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**Introduction:** *Streptococcus pneumoniae* (the pneumococcus) employs a diverse set of extracellular serine proteases to adapt to different host environments and to maintain protein homeostasis at the cell surface. The four known pneumococcal extracellular serine proteases, namely PrtA, SFP, HtrA, and CbpG, have been implicated in

colonization and virulence, with HtrA being the most extensively studied. However, their collective roles in bacterial physiology and global proteome regulation remain poorly understood.

**Goals:** This study aimed to elucidate how pneumococcal extracellular serine proteases influence the physiology and global proteome of *S. pneumoniae* under nutrient-limited, host-mimicking conditions using a chemically defined minimal medium (CDM+).

**Materials and Methods:** Isogenic single and multiple serine protease mutants were constructed in *S. pneumoniae* strains EF3030 (serotype 19F) and TIGR4Δcps (serotype 4). Cultures were grown to mid-logarithmic phase in CDM+ (De et al., 2025; DOI: 10.3389/fcimb.2025.1606161). Supernatant proteins were precipitated by trichloroacetic acid, and total cellular proteins were obtained. Both fractions were analyzed by data-independent acquisition LC-ESI-MS/MS-based quantitative proteomics. Morphological phenotypes were assessed by field-emission scanning electron microscopy (FESEM).

**Results:** Loss of extracellular serine proteases induced substantial metabolic and surface remodeling. In EF3030, protease-deficient mutants displayed increased abundance of arginine transporters and zinc-binding proteins, suggesting altered nutrient uptake and metal ion regulation. In TIGR4Δcps, complete protease deficiency redirected metabolism from homolactic to mixed-acid fermentation and reduced pilus and adhesion proteins, correlating with decreased epithelial adherence. Conversely, lipoproteins and Pht family proteins accumulated, indicating compensatory surface remodeling. FESEM confirmed no morphological differences between mutants and wild-type.

**Summary:** Extracellular serine proteases globally modulate the pneumococcal proteome, influencing metabolism, metal homeostasis, and surface architecture. These enzymes act as key regulators balancing adaptation and virulence under nutrient limitation, underscoring their importance in pneumococcal colonization and persistence.

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## P-HPI-008

### The *Yersinia enterocolitica* type III secretion system induces cytosolic Ca<sup>2+</sup> responses that shape cell-death morphology in human macrophages

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**Introduction:**

Macrophages of the gut-associated lymphoid tissue constitute a critical first barrier against *Yersinia enterocolitica* infection. A hallmark of pathogenic *Yersinia* spp. is their type III secretion system (T3SS), which translocates effector proteins (Yops) into macrophages to modulate immune responses such as inflammasome-triggered cell death. While a contribution of intracellular Ca<sup>2+</sup> changes to inflammasome activation has been reported, the role of bacteria-induced intracellular Ca<sup>2+</sup> changes in cell death remains unclear.

**Goals:**

We aimed to characterize the role of cytosolic Ca<sup>2+</sup> dynamics

in inflammasome-mediated cell death of human monocyte-derived macrophages (hMDMs) infected with *Y. enterocolitica*.

#### Methods:

Using live-cell fluorescence imaging,  $\text{Ca}^{2+}$  responses in primary hMDMs infected with a *Y. enterocolitica* strain harboring the T3SS but lacking all effector Yops were analyzed. Inflammasome activation and pyroptotic cell death were monitored by visualizing apoptosis-associated speck-like protein containing a CARD (ASC) speck formation, plasma membrane integrity and morphology. Pharmacological inhibition of gasdermin D (GSDMD) was used to assess the contribution of GSDMD pores to extracellular  $\text{Ca}^{2+}$  entry and membrane changes.

#### Results:

The employed *Y. enterocolitica* strain triggered a pronounced and sustained cytosolic  $\text{Ca}^{2+}$  increase resulting from  $\text{Ca}^{2+}$  influx from the extracellular space, which followed ASC speck formation and preceded plasma membrane rupture.  $\text{Ca}^{2+}$  increases could be inhibited by the GSDMD inhibitor disulfiram, suggesting that GSDMD pores serve as the entry route for extracellular  $\text{Ca}^{2+}$ . The GSDMD-mediated  $\text{Ca}^{2+}$  entry promoted plasma membrane blebbing and prevented a ballooning cell death phenotype.

#### Conclusion:

Our findings demonstrate that GSDMD-dependent  $\text{Ca}^{2+}$  entry is a consequence of *Y. enterocolitica*-induced inflammasome activation and actively alters plasma membrane morphology during pyroptotic cell death of hMDMs. This identifies GSDMD-dependent  $\text{Ca}^{2+}$  entry as a key link between bacteria-induced inflammasome activation and the resulting plasma membrane reorganization.

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#### P-HPI-009

##### Structural and functional characterisation of a membrane associated anti-phage defence system

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Bacteria have evolved a number of diverse strategies to evade and resist phage infection, however the detailed mechanisms for many of these remain to be understood. In particular, fewer membrane associated anti-phage defence systems have been described in detail, especially ones functioning through a non-abortive mechanism. Here we investigate a two-protein defence system where one protein is predicted to be membrane associated and the other lacks any homology with known domains or existing structures. Using cryo-electron microscopy we show the predicted transmembrane protein forms a dimer, lacking any canonical membrane-spanning helices. Additionally, we obtained a high-resolution map of the two proteins in complex, revealing extensive conformational changes relative to the homodimer. Finally, through functional characterisation and fluorescence microscopy, we provide further insights towards the localisation of each protein during phage infection as well as the overall mechanism of defence.

#### P-HPI-010

##### Investigating Glp12 as a Candidate Receptor for Host-Derived Nutrient Signals in *Ustilago maydis*

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Fungal pathogens are a threat to crop productivity, infecting a wide range of economically important crops. They influence the health, nutritional acquisition, and overall growth and development of these crops. Some fungi live closely associated with their hosts and keep them alive during infection. These are known as biotrophic fungi. They reprogram host metabolic pathways to redirect resources for their survival and proliferation. While these processes are critical for establishing and progressing infections, the underlying nutrient-perception and regulatory mechanisms remain poorly understood. In the model biotrophic fungus *Ustilago maydis*, previous studies suggest that a combination of glucose and organic acids supports proliferation and late-stage development during host colonization. However, the molecular mechanisms by which these nutrients are perceived and translated into metabolic responses remain unclear. In other model filamentous fungi such as *S. cerevisiae*, G-protein-coupled receptors (GPCRs) have been shown to play crucial roles in regulating these processes, where they activate cell signaling pathways leading to changes in cellular behaviour. In the case of *U. maydis*, such knowledge remains limited.

This thesis addresses this gap by investigating Glp12, a putative G-protein-coupled receptor (GPCR) predicted to function in glucose and organic acid perception. Here, we employ a combination of genetic, cell biological, and biochemical approaches to characterize Glp12 function, including the generation of deletion and complementation strains, subcellular localization analysis, and assessment of virulence in maize infection assays. We predicted potential downstream signaling interaction partners of Glp12 using AlphaFold, evaluated the influence of *glp12* deletion on fungal growth and virulence, and used a *S. cerevisiae*-based heterologous system to screen for possible ligand candidates. Fluorescence tagging was further applied to determine the subcellular localization of Glp12 in *U. maydis*. By elucidating the role of Glp12 in nutrient perception, this work aims to provide new insights into how *U. maydis* integrates the host's nutrient signals to regulate growth and pathogenic development.

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#### P-HPI-011

##### Surgical Reconstruction Shapes the Biliary Microbiome and Biofilm Formation After Liver Transplantation

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Introduction Biliary tract infections, especially cholangitis, are major causes of morbidity and mortality after liver transplantation. While most studies emphasize systemic or immunological factors, local factors such as surgical reconstruction and microbial biofilms also play key roles. This study examines how biliary reconstruction affects post-transplant infection risk, focusing on bile duct catheter biofilm formation and microbial composition.

Objectives We aimed to determine how reconstruction technique influences the incidence and microbial ecology of post-transplant cholangitis. Duct-to-duct anastomosis (D2DA) and bilioenteric anastomosis (BEA) were compared regarding catheter biofilms, microbial diversity, and antibiotic resistance. In vitro dual-species biofilm models were used to study colonization mechanisms and potential therapies.

Materials & Methods A prospective study included liver transplant recipients with either D2DA or BEA. Postoperative biliary catheters were analyzed using 16S rRNA sequencing, scanning electron microscopy, and microbial culture. Microbial communities were assessed for diversity, co-occurrence, and  $\beta$ -diversity. Clinical isolates of Enterobacteriaceae and Enterococcaceae were used to form dual-species biofilms, evaluating integration, antibiotic resistance, and catheter material influence. Antibiotic lock therapy was tested for biofilm eradication.

Results D2DA patients showed lower cholangitis incidence and microbial colonization than BEA. Biofilm microbiomes resembled gut flora, dominated by Enterobacteriaceae and Enterococcaceae. BEA catheters showed reduced diversity and distinct, dysbiotic structures. In vitro, Enterobacteriaceae promoted Enterococcaceae integration and persistence within mixed-species biofilms, independent of strain or resistance type. Catheter material had no effect on colonization. Antibiotic lock therapy effectively reduced biofilm-associated bacteria.

Summary Surgical technique and biofilm ecology strongly influence post-transplant infection risk. D2DA limits biofilm formation and maintains microbial balance, reducing cholangitis. The similarity between biliary and gut microbiomes suggests microbial translocation. Dual-species biofilms showed that Enterobacteriaceae support Enterococcaceae persistence and antibiotic tolerance. Integrating microbiome insights into surgical planning and applying biofilm-targeted strategies such as antibiotic lock therapy may improve transplant outcomes.

## P-HPI-012

### Trade-offs between polymyxin B resistance and virulence in *Ecc15*

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#### 1. Introduction

Antimicrobial peptides (AMPs) are key effectors of innate immunity in multicellular organisms. For a pathogen to establish infection, it must overcome this defence. Accordingly, AMP resistance is considered an important virulence factor. In *Drosophila melanogaster*, AMP resistance is a major virulence factor for *Providencia alcalifaciens* infection (Shaka et al. 2022). Interestingly, *Erwinia carotovora carotovora* 15 (*Ecc15*), a natural

*Drosophila* pathogen, was found to be highly sensitive to the artificial AMP polymyxin B *in vitro* (Arias-Rojas et al. 2023). This raises the question of why *Ecc15* has not evolved AMP resistance. We hypothesise that the gain of AMP resistance comes with an evolutionary cost, either via increased sensitivity to other immune effectors, as seen in *Staphylococcus aureus* infection of mealworm beetles (El Shazely and Rolff 2025) or through reduced bacterial fitness.

#### 2. Goals

Our aim is to determine the cost of AMP resistance in *Ecc15*.

#### 3. Methods

Experimentally evolved polymyxin B-resistant *Ecc15* strains were used for systemic infection of *Drosophila*. Host survival was monitored to assess pathogenicity. To identify potential trade-offs with different immune branches, we infected *Drosophila* lines deficient in specific immune pathways, including those affecting AMPs, phagocytosis, and iron sequestration.

#### 4. Results and Conclusion

In wild-type flies, survival did not differ between infections with wild-type and polymyxin B resistant *Ecc15*. In AMP-deficient flies, mortality caused by the resistant strains was significantly lower than that caused by wild-type bacteria, indicating reduced virulence independent of AMP immunity. Similarly, flies with impaired phagocytosis or iron sequestration survived better following infection with most resistant *Ecc15* strains. *In vitro* assays further revealed increased sensitivity of the resistant mutants to reactive oxygen species (ROS), suggesting an additional fitness cost that remains to be validated *in vivo*.

In summary, polymyxin B-resistance in *Ecc15* is associated with reduced virulence *in vivo* and heightened ROS sensitivity *in vitro*, implying a trade-off that may prevent its natural evolution. Future work will test the involvement of melanisation and ROS pathways *in vivo*, assess overall fitness costs, and identify the genetic basis of polymyxin B-resistance through whole-genome sequencing.

## P-HPI-013

### Staphylococci remodel lipids at the host – microbe interface

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Lipids are essential components of the skin where they play key roles especially in restricting the proliferation of opportunistic microorganisms like *Staphylococcus aureus*. This human pathogen has evolved several adaptation strategies to resist the host-derived antimicrobial fatty acids (AFAs) and has been reported to compromise epidermal lipid profiles. However, how *S. aureus* manipulates host lipids is not yet fully understood. Recently, we have discovered that *S. aureus* utilizes its lipase Lip2 to esterify and detoxify AFAs with cholesterol. Considering the promiscuity of Lip2, we reasoned that many more Lip2 substrates and products may await discovery. Here, we describe fatty alcohols as new substrates for *S. aureus* lipases Lip2 and Lip1. Both lipases

generated wax esters upon esterification of AFAs with fatty alcohols, suggesting that wax ester production is a widespread feature of staphylococcal lipases. Indeed, heterologously expressed GehD of *S. epidermidis* or SsL of *S. simulans* was able to complement a lipase-deficient *S. aureus* mutant ( $\Delta lip1/2$ ) and protect this bacterium from toxic AFAs by producing wax esters. When cocultured with *S. simulans* in rich medium supplemented with AFAs,  $\Delta lip1/2$  was eradicated. In contrast, the cocultivation of  $\Delta lip1/2$  and *S. simulans* in the presence of AFAs and fatty alcohols protected  $\Delta lip1/2$  from otherwise toxic AFAs in an SsL dependent manner, suggesting that the lipid composition of the environment may be critical for a community composition. Given the importance that lipid diversity seems to have on the survival of some bacteria, we explored lipase-mediated changes in AFA-derived lipid species and uncovered a production of new lipids by *S. aureus* that might be released or carried by bacteria to interact with host cell membranes. By leveraging *S. aureus* mutants and lipidomics, we are currently investigating the production pathway of these novel lipids and the biological effect they might exert on the host during infection. Collectively, our study suggests that *S. aureus* lipases generate underestimated lipid compounds, thereby modifying the lipid landscape, with potential implication for the microbiome composition, colonisation and host defence.

#### P-HPI-014

##### TTSS-2 virulence drives inflammatory destruction of the gut epithelial barrier and modulates inflammatory response profiles in the *Salmonella*-infected mouse gut

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*Salmonella enterica* serovar Typhimurium (*S. Tm*) employs type III secretion system 1 and 2 (TTSS-1 and TTSS-2) to infect the host's tissues. In orogastric infections, both TTSSs manipulate host responses, increasing mucosal pathogen loads and eliciting inflammation. However, we still do not fully understand how virulence and inflammatory enteropathy are interconnected. In our study, we investigate whether TTSS-2-dependent virulence contributes to epithelial barrier disruption and delineate its role in shaping inflammatory response profiles in the mouse gut. Using wild-type and TTSS-2 mutant *S. Tm* strains in antibiotic-pretreated mouse models, we demonstrate that intestinal epithelial destruction is promoted by TTSS-2 virulence. This effect is observed in both wild-type and immune-deficient C57BL/6J mice. Transcriptomic profiling together with immunofluorescence microscopy analysis reveals that wild-type *S. Tm* triggers a distinct, yet amplified immune response compared to a TTSS-2 mutant which is characterized by enhanced phagocyte recruitment and a unique transcriptional signature. These findings underscore the role of TTSS-2-mediated virulence in *S. Tm* gut infection, shaping distinct inflammatory microenvironments with potential implications for host-pathogen interaction studies.

#### P-HPI-015

##### Multi-omics approach to evaluate Bacteria-Driven Tumor Adaptation

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Tumor-resident intracellular microbiota is a tumor component present in a variety of cancer types including breast cancer, that can impact many cancer-related functions. In a murine breast cancer model, intracellular bacteria suppressed the accumulation of tumor infiltrating T cells, enhanced survival of circulating tumor cells by cytoskeleton reorganization, promoted metastasis and influenced the chemotherapy effect. Interestingly, *Staphylococcus xylosus* is among the tumor-resident intracellular bacteria capable of invading tumor cells. Triple negative breast cancer (TNBC) displays a more aggressive behavior and lacks effective targeted therapies compared to other breast cancer subtypes, making the identification of novel targets and development of new treatment regimens for these patients urgent.

Since recent studies reported that the perturbation of intracellular bacteria can reduce metastasis and tumor exacerbation, the aim of this project is to explore the host-bacteria interaction in TNBC using a multi-omics approach, in order to reveal molecular mechanisms and identify novel therapeutic targets.

To this end, an *in vitro* co-culture model was established using mouse TNBC derived organoids and viable intracellular *S. xylosus*. Spatial transcriptomic revealed that *S. xylosus* colonization drives transcriptional reprogramming of tumor cells, characterized by suppression of luminal differentiation programs and induction of a basal-like phenotype. Importantly, Electron microscopy and Immunofluorescence staining showed evidence that intracellular *S. xylosus* exists in a cell wall altered form, suggesting an adaptive bacterial state that enables persistence within tumor cells by evading host clearance mechanisms. Integrated metabolomic and metaproteomic analyses further demonstrated that *S. xylosus* modulates tumor-cell metabolism toward a stress-resistant state, suggesting a role in promoting tumor plasticity.

Collectively, these findings suggest that *S. xylosus* can modulate TNBC cell states and metabolism, providing new insights into bacteria-driven tumor adaptation and potential avenues for therapeutic intervention.

#### P-HPI-016

##### NLR4-inflammasome activation by *Y. enterocolitica* phagosome disruption in primary human macrophages

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Pathogenic *Yersinia* spp. activate canonical and non-canonical inflammasomes in macrophages, causing cytokine release and pyroptosis. Inflammasome activation is initiated by distinct *Yersinia* activities, including disruption of phagosomal membranes and sensing of needle components of the bacterial type 3 secretion system (T3SS). We investigated the spatiotemporal dynamics of NLRC4 inflammasome activation in macrophages following phagocytosis of mutant *Yersinia* strains that overproduce the T3SS but contain no effectors. These strains cause strongly increased phagosome disruption and inflammasome activation. Interestingly, formation of ASC-containing specks, dense condensates of multiple inflammasomes, occurred only after phagosome disruption. NAIP, that recognizes T3SS needle proteins and activates NLRC4, but neither NLRC4 nor ASC were detected at the bacteria-containing disrupted phagosomes. In comparison, NAIP, NLRC4 and Pro-Caspase 1 were all found in ASC specks that were located in some distance from the phagosomes. This indicates that NAIP migrates from disrupted phagosomes to the specks after its activation by the bacterial T3SS needle proteins and stimulates NLRC4 there or on its way there. Superresolution MINFLUX and STED microscopy documented the nanometer scale suborganisation of NAIP, NLRC4, Pro-Caspase1, NLRP3 and ASC in the specks and at the disrupted phagosomes. Our data provide novel information on the spatiotemporal dynamics of NLRC4 activation by the *Yersinia* T3SS as well as the organisation of inflammasomes and their components within primary human macrophages.

#### P-HPI-017

##### Quantitative and Biophysical Analysis of Phage-Induced Outer Membrane Vesicles in *Salmonella*

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Functional membranous structures such as extracellular vesicles (EVs) are found across all kingdoms of life. In bacteria, extracellular vesicles (BEVs) can reduce the number of infective bacteriophage particles within a population. Accordingly, bacteria have evolved multiple surface-associated defense strategies to limit phage infection. Among these, the release of outer membrane vesicles (OMVs) serves as an effective decoy mechanism to modulate phage populations and protect bacterial cells<sup>1</sup>. However, the molecular mechanisms linking phage exposure to vesiculation remain largely unexplored. Understanding these processes is broadly relevant, as OMVs influence microbiome-host interactions<sup>2</sup> and may be helpful for rational design of effective bacteriophage therapies where OMVs might act as additional virulence factors<sup>1</sup>.

In this project, we investigate the *in vivo* vesicular response of *Salmonella* upon phage challenge and aim to characterize OMV formation and composition during infection. In this work podovirus P22 is used as a model phage specific for *S. Typhimurium*, and by using advanced fluorescence microscopy, single-particle profiling, and biochemical approaches, we will quantify OMV production under different infection conditions and assess how phage presence alters vesicle composition including lipopolysaccharide and O-antigen structures. Furthermore, we will do the biophysical characterization of fusion behavior of phage loaded OMVs with bacterial outer membranes.

This work will help clarify how OMVs fuse with the outer membranes of Gram-negative bacteria. By examining differences between phage-loaded OMVs and free OMVs, we seek to identify unique interaction profiles with host cells and link these results to *in vivo* observations where OMVs transfer phage genes to other bacteria.

1. Stephan, M. S., Broeker, N. K., Saragliadis, A., Roos, N., Linke, D., & Barbirz, S. (2020). In vitro Analysis of O-Antigen-Specific Bacteriophage P22 Inactivation by Salmonella Outer Membrane Vesicles. *Frontiers in Microbiology*, 11. <https://doi.org/10.3389/fmicb.2020.510638>
2. Toyofuku, M., Schild, S., Kaparakis-Liaskos, M., & Eberl, L. (2023). Composition and functions of bacterial membrane vesicles. *Nature Reviews Microbiology*, 21(7), 415–430. <https://doi.org/10.1038/s41579-023-00875-5>

#### P-HPI-018

##### Uncovering bacteriophage-LPS interactions with Gram-negative model membranes

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*Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) is a Gram-negative enterobacteria of public health concern. The lipopolysaccharide (LPS) leaflet of the Gram-negative outer membrane (OM) has a defensive role and modulates the interactions between the bacterial cell and the microenvironment. Due to the increasing numbers of antimicrobial resistant bacteria, the interest in alternative treatments, such as phage therapy, has grown. Thus, understanding the mechanisms of phage-host interaction become increasingly important. The bacteriophages *Lederbergvirus P22* (P22) and *Nonanavirus nv9NA* (9NA) infect *S. Typhimurium* after binding and depolymerizing the O-antigen portion of LPS<sup>1</sup>. *In vitro* studies have shown that a complete LPS structure (lipid A, core, and O-antigen) is essential for P22 and 9NA's DNA release<sup>2,3</sup>. However, the dynamics of the early steps that precede infection, in which phages locate, recognize and cleave the LPS O-antigen before DNA entry, remain largely unresolved. To address this, we developed LPS-enriched supported lipid bilayers (LPS-SLBs) as model membranes that mimic the *S. Typhimurium* OM, showcasing its usability as versatile biomimetic platforms for tracking microbial virus interactions. In addition, we investigated phage-surface interactions by tracking single phage particles on distinct LPS-SLB surfaces using total internal reflection fluorescence (TIRF) microscopy. This allowed us to monitor phage diffusion on the model membrane surface and characterize the dynamic events leading to the start of the infection. Our results provide mechanistic insight into how the OM composition of Gram-negative hosts guides the efficiency of bacteriophage infection.

#### References

1. Broeker, N. K. & Barbirz, S. *Mol. Microbiol.* **105**, 353–357 (2017).

2. Andres, D. *et al. Mol. Microbiol.* **83**, 1244–1253 (2012).

3. Stephan, M. S. *et al.* Preprint at <https://doi.org/10.1101/2024.08.19.608551> (2024).

## P-HPI-019

### Characterization of the outer membrane protein HopZ in *Helicobacter pylori*

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**Introduction.** *Helicobacter pylori* is a Gram-negative pathogenic bacterium that localizes in the human stomach and causes severe infections such as ulcer disease or gastric cancer. Main virulence factors of *H. pylori* are the Cag type IV secretion system (CagT4SS) and outer membrane proteins (OMPs). *H. pylori* possesses a large family of about 30 OMPs. Some OMPs have been studied well, but not much is known about HopZ and its involvement in *H. pylori* infection. HopZ exists in two allelic variants and is regulated by a phase-variable dinucleotide repeat resulting in an ON or OFF status. *hopZ* exhibits high variability and within-host diversity. We recently discovered that HopZ interacts with proteins of the CagT4SS and the host receptors ceacam1 and integrin  $\alpha 5 \beta 1$  and also plays a functional role in the context of the CagT4SS.

**Aims.** Our study aims to discover how HopZ is involved in host interaction, either alone or together with other virulence factors, and which function the switch and the two allelic variants have.

**Methods and Results.** In order to discover the function of HopZ during infection, we genetically engineered two *H. pylori* strains to switch the *hopZ* gene constitutively ON. During co-incubation with gastric epithelial cells, the constitutive ON mutants showed reduced induction of IL-8 production in infection assays and a reduced hummingbird phenotype in AGS cells, demonstrating a dampening function on the CagT4SS. Furthermore, we performed plate-binding assays with epithelial cells to investigate binding behavior of HopZ to proteins and cells under different conditions such as varying pH. HopZ binds to gastric epithelial cells in a concentration-dependent manner, with improved binding at pH 6 compared to pH 7. Despite our results that HopZ interacts with human ceacam1 and integrin  $\alpha 5 \beta 1$ , our experiments suggest more binding partners. To identify them, we performed pulldowns of His-tagged HopZ with epithelial cell lysates and subsequent mass spectrometry. We also isolate bacterial outer membrane vesicles from different strains to assess whether HopZ influences OMV formation and protein content. Mass spectrometry results and potential new binding partners will be presented.

**Summary and Conclusion.** In summary, HopZ exerts a reducing effect on host cell activation. It interacts with various host receptors and Cag proteins. The mechanisms of HopZ activities and how different HopZ variants diverge in their functionalities are currently under investigation.

## P-HPI-020

### Functional Analysis of the Type III Secretion System Effector BipC from *Burkholderia thailandensis* E264 and Its Interactions at Host Membranes

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## Introduction

Human pathogenic bacteria within the *Burkholderia pseudomallei* group are partly responsible for causing infectious diseases such as melioidosis. One key factor in their pathogenicity is their intracellular lifecycle, involving host cell invasion and phagosomal escape into the cytosol. Cytosolic bacteria evade the host's LC3-associated autophagy, enabling survival and replication. The bacteria utilize actin-based motility to invade adjacent cells via membrane protrusions and membrane fusion, allowing the bacteria to infect neighboring cells with minimal immune response. The Bsa Type III secretion system, including *Burkholderia* invasion proteins (Bip), is a major virulence factor. The translocator BipC is homologous to *Salmonella* SipC and *Shigella* IpaC; it likely forms a translocon pore within the host cell membrane and may act as a translocated effector, probably promoting intracellular survival through uncharacterised interactions at the host membrane.

## Methods

Bioinformatics analyses were performed to predict protein structure. In vitro binding assays with recombinant protein identified interactors. CRISPRi-mediated gene knockdown strains of the model organism *B. thailandensis* were used in macrophage infection assays, supplemented by proteomics analyses.

## Results

Bioinformatics revealed only ~40% sequence similarity and limited structural resemblance between BipC and its homologs, with no clearly defined conserved motifs or transmembrane regions in BipC. In vitro pulldown assays and affinity purifications from primary human macrophage lysates showed BipC interacts with F-Actin and other yet unidentified binding partners. Recombinant BipC interacts with F-actin and binds to host endocytosis proteins, membrane-associated host proteins, and small GTPases. Infection of macrophages with *B. thailandensis* bipC knockdown strains resulted in no significant impact on bacterial invasion but significantly impaired the bacterium's ability to escape from the phagosome.

## Conclusions

These findings indicate that BipC plays a crucial role in phagosome escape and likely interacts within a local microdomain at the phagosomal membrane, potentially involving host membrane contact sites. Although the BipC sequence and structure show limited similarity to its homologs, its interactions imply a different function, possibly modulating host phagocytosis pathways, in contrast to its homologs.

## P-HPI-021

### Parasite Life History Trade-offs and virulence evolution: A Test of the Trade-off Hypothesis

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Parasites are predicted to evolve intermediate levels of virulence by balancing the harm they cause their hosts and their need for transmission. However, this hypothesis is controversial in part due to limited empirical evidence. One explanation may be that other parasite life history trade-offs and ecological factors such as interaction of the parasite with the host's microbiota also contribute significantly to the parasite's evolution and therefore play a role in shaping virulence evolution. Here, we investigate how parasite life history trade-offs and host microbiota influence the evolution of virulence using *Tribolium castaneum* and *Metarhizium brunneum*. We are currently examining whether virulence, transmission, recovery rate and other parasite life history traits are correlated and how these correlations are influenced by microbiota. Next, we will establish whether evolutionary change in virulence results in correlated changes in these traits by experimental evolution. We will then sequence ancestral and evolved populations to identify genomic differences underlying observed phenotypic changes. Finally, we will use our empirical data to mathematically describe our system and understand the role of the two-step growth process and the presence of an intact or disrupted microbiota on virulence evolution. With this work, we aim to provide empirical data on trade-offs between transmission, virulence, recovery rate and other life history traits while clarifying the role of hosts' microbiota in virulence evolution.

## References

Anderson, R. M., and R. M. May. 1982. "Coevolution of Hosts and Parasites." *Parasitology* 85 (2): 411–26. <https://doi.org/10.1017/S0031182000055360>.

Armitage, Sophie AO, Elke Genersch, Dino P McMahon, Charlotte Rafaluk-Mohr, and Jens Rolff. 2022. "Tripartite Interactions: How Immunity, Microbiota and Pathogens Interact and Affect Parasite Virulence Evolution." *Current Opinion in Insect Science* 50 (April): 100871. <https://doi.org/10.1016/j.cois.2021.12.011>.

Goldhill, Daniel H, and Paul E Turner. 2014. "The Evolution of Life History Trade-Offs in Viruses." *Current Opinion in Virology, Antivirals and resistance / Virus evolution*, 8 (October): 79–84. <https://doi.org/10.1016/j.coviro.2014.07.005>.

## P-HPI-022

### Unravelling Fungal Antimicrobial Defence as a Driver of Niche Adaptation in *Magnaporthe oryzae*

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*Magnaporthe oryzae*, the causal agent of rice blast, is a major threat to global food production and a model for studying fungal adaptation in complex microbial environments. In the barley root microbiome, the fungus encounters diverse microorganisms that release antimicrobial compounds to compete for ecological niches. Such interactions have driven the coevolution of

antimicrobial production and defence, shaping fungal strategies to tolerate or neutralize toxic metabolites. While *M. oryzae* is well studied for its virulence toward host plants, the molecular mechanisms that enable resistance to antimicrobials from coexisting microbes remain poorly understood. Understanding these defences is essential for explaining fungal persistence and ecological niche adaptation, and for identifying new antifungal targets.

This project aims to elucidate the molecular basis of *M. oryzae* self-protection against antimicrobial activities of barley-associated microbes by combining microbial confrontation assays, genome-wide mutagenesis, and transcriptomic analyses. Confrontation assays between *M. oryzae* and barley root-associated bacteria identified inhibitory and non-inhibitory strains. To dissect the genetic basis of fungal defence, a transposon-based mutagenesis system was established in which a dual-resistance cassette (hygromycin and fenhexamid), flanked by inverted terminal repeats, serves as the substrate for transposition. Following transient delivery of purified PiggyBac or Sleeping Beauty transposases into fungal protoplasts, single transposition events relocate the cassette to random genomic sites, generating unique insertion mutants. The resulting transformants will be pooled into a genome-wide library and tested in negative selection screens to identify mutants sensitive to antimicrobial stress. Functional screening and insertion-pool sequencing (iPool-Seq) will reveal genes essential for fungal defence, complemented by transcriptomic analyses during microbial confrontation and plant infection.

Stable cassette integration and purification of both transposases were achieved. Transformation protocols were optimized, and confrontation assays differentiated inhibitory from non-inhibitory bacterial interactions, providing the foundation for mutant library generation and functional screening. Together, this approach will identify key genes and pathways that enable *M. oryzae* to tolerate antimicrobial stress and adapt within competitive plant-associated microbiomes.

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## Membranes and transport

## P-MT-001

### The functionally essential TatA membrane anchor and its adjacent hinge region adapt to specific membrane systems

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The Tat system translocates folded proteins across energized membranes [1]. To achieve membrane permeabilization, the associations of multiple TatA components destabilize the membrane with clusters of short membrane-spanning regions in conjunction with laterally aligned amphipathic helices, which together cause a drastic membrane-thinning and membrane lipid disorder [2]. Association of transported proteins causes orientational changes of the amphipathic helices, which likely precedes transport [3]. The flexibility of the hinge region connecting the two helices has been the subject of discussions. Here we present data showing that the hinge can be highly flexible and that both, the transmembrane helix as well as the hinge region are used by Tat systems to adapt their functionality to distinct membrane systems.

[1] Natale P., Brüser, T. and Driessen A.J.M. (2008) *Biochim Biophys Acta* 1778:1735-56. doi: 10.1016/j.bbame.2007.07.015

[2] Mehner-Breitfeld D., Ringel M.T., Tichy D.A., Endter L.J., Stroh K.S., Lünsdorf H., Risselada H.J., Brüser T. (2022) *J Biol Chem* 298:102236. doi: 10.1016/j.jbc.2022.102236

[3] Hou B., Heidrich E.S., Mehner-Breitfeld D., Brüser T. (2018) *J Biol Chem*. 293:7592-7605. doi: 10.1074/jbc.RA118.002205

#### P-MT-002

##### Uncovering the Periplasmic Protein Network underlying Pyoverdine Recycling in *Pseudomonas alloputida*

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Iron is an essential metal for bacteria. Due to its low bioavailability under aerobic conditions, bacteria have evolved systems to acquire iron. These include siderophores, low molecular weight molecules with extremely high affinity for ferric iron [1].

The only siderophore produced by the Gram-negative soil bacterium *Pseudomonas alloputida* KT2440 is the fluorescent pyoverdine (PVD) [2]. It is secreted into the environment to scavenge iron and subsequently imported back into the periplasm. Here, the Fe-PVD interacts with several membrane and periplasmic binding proteins leading to the dissociation of PVD and iron [1]. While the iron is imported into the cytoplasm, apo-PVD is likely bound by the periplasmic FpvF, which presumably transfers it to the tripartite efflux pump PvdRT-OpmQ [3, 4]. PVD is suggested to interact with the inner membrane component PvdT as well as the periplasmic adaptor protein PvdR and is then secreted back into the environment through the outer membrane porin OpmQ to scavenge further iron [4, 5]. Our aim is to characterize the molecular processes involved in PVD secretion and recycling, including kinetic and thermodynamic parameters, using biophysical methods.

To this end, the involved proteins were overproduced in *P. alloputida* or *E. coli* and subsequently purified by metal affinity and size exclusion chromatography. PVD was produced in a constitutive producer strain and purified by HPLC. The interactions among selected binding partners were then analyzed. We demonstrated that apo-PVD is bound by FpvF dimers with low-micromolar affinity, using microscale thermophoresis and isothermal titration calorimetry. Furthermore, surface plasmon resonance and DRaCALA revealed an interaction of PVD with PvdRT. However, no direct contact could be demonstrated yet using only the periplasmic domain of PvdT, which contains the putative binding site. Future investigations focus on this as well as other interactions involved in PVD recycling.

Our results provide physical evidence for the involvement of the periplasmic binding protein FpvF in PVD recycling and reveal, for the first time at the biochemical level, a direct interaction between PvdRT and PVD.

[1] Schalk, I.J. & Perraud, Q. (2023) *Environ Microbiol* 25 (4), 811–831

[2] Ringel, MT & Brüser, T (2018) *Microb Cell* 5, 424-437

[3] Brillet, K. et al. (2012) *ACS Chem Biol* 7 (12), 2036-2045

[4] Bonneau, A. et al. (2020) *Sci Rep* 10, 120

[5] Stein, N.V. et al. (2023) *FEBS Lett* 597, 1403-1414

#### P-MT-003

##### The role of cyclic di-GMP as a second messenger in *Thermus thermophilus*

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**Introduction:** *Thermus thermophilus* is a model organism to study the structure and function of natural transformation systems (1). The DNA transporter is linked to the type 4 pilus which is powered by a hexameric ATPase PilF (2). PilF contains three successive GSPII domains (general secretory pathway type II domain) which can potentially bind c-di-GMP (3,4). Cyclic-di-GMP is a second messenger involved in signal transduction and plays a key role in several intracellular processes. The c-di-GMP level is regulated in response to internal and environmental cues and is modulated by diguanylate cyclases (DGC) and phosphodiesterases (PDE).

**Goals:** The first aim was to get insights into the role of c-di-GMP in the function of the ATPase PilF and the role in natural transformation of *T. thermophilus*. Moreover, the goal was to get insights into the role of c-di-GMP as a global second messenger in *T. thermophilus*.

**Materials & Methods:** The role of c-di-GMP in *T. thermophilus* was investigated by mutant studies and analysis of c-di-GMP levels in dependence of growth conditions and in PDE and DGC mutants via HPLC.

**Results:** *T. thermophilus* harbours nine proteins, potentially involved in controlling the level of c-di-GMP. Deletion of a putative diguanylate cyclase led to a lowered cell-cell adhesion and less adhesion to solid surfaces. Deletion of a protein with both a DGC and PDE domain led to the decrease of twitching motility but enhanced adhesion to solid surfaces. Deletion of the putative diguanylate cyclase or the dual domain protein led in the exponential growth phase to reduced and increased levels of c-di-GMP, respectively. Natural transformation was not affected in any of the mutant strains. Analysis of the c-di-GMP concentration during growth of *T. thermophilus* revealed that the c-di-GMP levels are growth phase dependent. Highest levels were detected in the stationary growth phase while the lowest levels were detected in the exponential growth phase.

**Summary:** Here we report on the role of c-di-GMP as a second messenger in *T. thermophilus* and provide insights into the regulation of c-di-GMP levels in *T. thermophilus*.

[1] Averhoff, B. (2009). *FEMS Microbiol. Rev.* 33, 611-626

[2] Salzer, R., Joos, F., Averhoff, B. (2014) *Appl. Environ. Microbiol.* 80, 644-652

[3] Neißner, K., Frohnapfel, C., Keller, H. et al. *Chem. Bio. Chem.* 26, e202400959

[4] Neißner, K., Keller, H., Kirchner, L. et al. *J. Biol. Chem.* 301, 108041

#### P-MT-004

##### Role of Tripartite Efflux Pumps in Siderophore Secretion by *Pseudomonas allopuntida* KT2440

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*Pseudomonas allopuntida* KT2440 is a Gram-negative, rod-shaped model organism found in various environments that usually have limited iron bioavailability. To access iron, *P. putida* developed many strategies, including the secretion of the siderophore pyoverdine (PVD) to acquire ferric iron (Fe<sup>3+</sup>) from the environment[1]. At the same time, several tripartite efflux pumps were involved in PVD transport, including PvdRT-OpmQ and MdtABC-OpmB[2].

In this study, we investigate the tripartite efflux pumps PvdRT-OpmQ and MdtABC-OpmB, in conjunction with the pyoverdine-mediated iron transport system represented by the *fpv* gene clusters of *P. allopuntida*, focusing on the genetic and functional characterization of their key components. The *fpv* gene cluster codes for several periplasmic proteins and membrane proteins that play roles in PVD uptake, ferric iron reduction, PVD recycling, and secretion. Our aim is to study the roles of the tripartite efflux pumps PvdRT-OpmQ and MdtABC-OpmB, the PVD-Fe<sup>3+</sup> receptor FpvA, and the periplasmic proteins FpvCDEF and FpvGHJK in PVD transport and recycling[3].

We deleted the corresponding individual genes and gene clusters, and compared the phenotypes of the resulting mutants with those of the wild-type strain. Under iron-deficient conditions, growth defects were observed upon deletion of *fpvA* (PVD-Fe<sup>3+</sup> receptor), *fpvGHJK* and *fpvCDEF* encoding periplasmic binding proteins, the ferric iron reductase complex and a ferrous iron uptake system, confirming the key role of *fpv*-encoded proteins in iron uptake. Furthermore, the deletion of the genes of the tripartite efflux pumps *pvdRT-opmQ* and *mdtABC-opmB* inhibited growth under iron-limiting conditions.

Regarding pyoverdine secretion, additional deletion of *fpvF* in a mutant with deletion of both *pvdRT-opmQ* and *mdtABC-opmB* further inhibited PVD secretion, suggesting a participation of the periplasmic binding protein FpvF in PVD recycling and/or secretion. Together, our findings highlight the roles of the tripartite efflux pumps PvdRT-OpmQ and MdtABC-OpmB, the PVD-Fe<sup>3+</sup> receptor FpvA, and the periplasmic and inner membrane proteins FpvCDEF and FpvGHJK in *P. allopuntida*, impacting not only growth but also other phenotypes such as PVD secretion, motility, and biofilm formation.

1. Schalk, I.J. and Q. Perraud, 2023. **25**(4): p. 811-831.
2. Henríquez, T., N.V. Stein, and H. Jung, 2019. **11**(2): p. 98-106.
3. Vigouroux, A., et al., FEBS J, 2020. **287**(2): p. 295-309.

#### P-MT-005

##### RND efflux pumps modulate phenotypic heterogeneity in *Acinetobacter baumannii*

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**Introduction** *Acinetobacter baumannii* is a highly adaptable opportunistic pathogen notorious for its multidrug resistance and persistence in healthcare settings. Efflux pumps and their regulatory systems play pivotal roles in antibiotic tolerance and phenotypic heterogeneity, yet their physiological contributions to population diversity and drug susceptibility remain poorly understood.

**Goals** This study aimed to (i) examine adaptive resistance and collateral susceptibility following sub-MIC tigecycline exposure, and (ii) investigate phenotypic heterogeneity and subpopulation dynamics in *A. baumannii* under antibiotic stress, focusing on the formation and characteristics of distinct morphotypes.

**Methods** Isogenic RND efflux pump mutants were constructed in *A. baumannii* ATCC19606. Antibiotic susceptibility was tested across multiple drug classes. Colony morphotypes were characterized on LB agar with or without tigecycline. Distinct subpopulations were isolated as wild-type opaque (WT<sup>O</sup>) and transparent (WT<sup>T</sup>) morphotypes. Stability and growth phenotypes were assessed by repeated subculturing. Whole-genome sequencing (WGS) was used to evaluate genetic variation, while growth kinetics and population analysis profiling (PAP) were applied to characterize phenotypic diversity.

**Results and Conclusion** Tigecycline exposure induced a slimy, hypermucoid-like phenotype in *Acinetobacter baumannii*. On antibiotic-free media, two morphotypes WT<sup>O</sup> and WT<sup>T</sup>, emerged; WT<sup>O</sup> occasionally reverted to WT<sup>T</sup>, whereas WT<sup>T</sup> remained stable, indicating asymmetric switching. Whole-genome sequencing revealed no genetic differences except a synonymous SNP shared with the parental strain, supporting a non-genetic basis. WT<sup>T</sup> showed reduced tolerance to carbapenems,  $\beta$ -lactamase inhibitors, and vancomycin without growth defects. Deletion of RND efflux pumps increased tigecycline susceptibility, confirming roles for AdeAB and AdeJK in intrinsic resistance. Remarkably, all strains, including RND mutants, developed adaptive tigecycline tolerance after sub-MIC exposure, accompanied by increased polymyxin B susceptibility, consistent with membrane perturbation. RND mutants segregated into antibiotic-tolerant and -sensitive subpopulations without genomic variation, highlighting non-genetic heterogeneity. These findings reveal stable subpopulation structures and RND-mediated diversification, underscoring efflux regulation and adaptive responses as key drivers of *A. baumannii* persistence.

#### P-MT-006

##### Loss of RND Efflux Pumps Triggers Oxidative Adaptation in *Acinetobacter baumannii*

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**Introduction** Efflux pumps are central to antibiotic resistance in *Acinetobacter baumannii*, actively extruding diverse antimicrobial compounds. Beyond this canonical role, emerging evidence suggests they also modulate bacterial physiology and stress adaptation. However, their contributions to oxidative stress tolerance remain poorly defined.

**Goals** This study aimed to (i) investigate the transcriptomic and phenotypic responses of *A. baumannii* ATCC19606 mutants lacking the AdeAB, AdeIJ, or CraA efflux pumps under LB and chloramphenicol stress, and (ii) assess how efflux pump deletion influences growth and susceptibility under oxidative (H<sub>2</sub>O<sub>2</sub>) and heavy metal (Cu<sup>2+</sup>, Mn<sup>2+</sup>) stress.

**Methods** RNA-seq analyses identified differentially expressed genes in wildtype (WT) and knockout strains. Strains were cultivated in LB medium containing defined H<sub>2</sub>O<sub>2</sub> or metal concentrations. Growth kinetics were monitored to determine maximum growth rate ( $\mu_{\max}$ ), lag phase, time to  $\mu_{\max}$  ( $T_{\mu\max}$ ), and maximum OD<sub>600</sub> ( $OD_{\max}$ ). Statistical analyses used linear mixed-effects modelling and Tukey's test in R 4.4.1. Growth inhibition by H<sub>2</sub>O<sub>2</sub> was further assessed on solid medium.

**Results and Conclusion** Transcriptomic profiling under untreated conditions revealed minimal changes in  $\Delta$ craA but strong metabolic reprogramming in  $\Delta$ adeAB and  $\Delta$ adeIJ, including upregulation of ribosomal genes, iron–sulfur cluster biogenesis, aromatic compound degradation, and amino acid transport systems. Phenotypically,  $\Delta$ adeIJ displayed elevated basal and H<sub>2</sub>O<sub>2</sub>-induced oxidative stress, implicating AdeIJ in redox homeostasis. While both *adeAB* and *craA* were both upregulated under chloramphenicol stress, only  $\Delta$ craA exhibited a 32-fold reduction in MIC, confirming CraA as the primary chloramphenicol efflux pump.

All strains failed to grow at 1 mM H<sub>2</sub>O<sub>2</sub>, whereas the WT was highly sensitive at 0.5 mM. Despite comparable  $\mu_{\max}$ , WT showed prolonged lag time, delayed  $T_{\mu\max}$ , and reduced OD<sub>max</sub> compared to mutants. Notably,  $\Delta$ craA demonstrated significantly greater tolerance to H<sub>2</sub>O<sub>2</sub>, implying compensatory physiological adaptations that enhance oxidative stress resistance.

Collectively, these findings highlight efflux pump-specific roles in both antibiotic resistance and oxidative stress adaptation, identifying CraA as a dominant chloramphenicol efflux pump and RND transporters as key modulators of redox balance in *A. baumannii*.

#### P-MT-007

##### Role of Membrane Raft-Associated Proteins in Hyphal Polarity and Cell Signaling of *Schizophyllum commune*

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*Schizophyllum commune*, a filamentous white-rot fungus, plays a major role in the degradation of organic matter and nutrient cycling. Its complex hyphal growth patterns make it a valuable model organism in mycology (Van Dijk et al., 2016). Polarized hyphal elongation depends on targeted delivery of proteins and lipids to the growing tip via vesicle trafficking (Riquelme et al., 2018). According to the fluid mosaic model (Singer and Nicolson, 1972), biological membranes are dynamic lipid bilayers composed of distinct microdomains.

Among these, lipid rafts—enriched in sterols, sphingolipids, and specific proteins—act as platforms for signal transduction, membrane trafficking, and polarized growth (Alvarez et al., 2007). Both the actin cytoskeleton and raft domains are crucial for establishing and maintaining hyphal polarity (Takeshita et al., 2008).

This study aimed to investigate the composition, localization, and functional role of raft domains in *S. commune*. Two raft-associated proteins, stomatin and striatin, were analyzed for their involvement in signaling, protein–protein interactions, and the assembly of signaling complexes. Sterol-enriched raft domains were visualized using Filipin staining. Stomatin was tagged with the fluorescent protein dTomato and examined by laser scanning microscopy. To elucidate stomatin's function, a deletion strain was generated, and RNA sequencing (RNA-seq) was performed to identify transcriptional changes.

Sterol-rich domains were detected at hyphal tips and septation sites. Stomatin localization shifted from young branch tips toward the apex during growth and colocalized with eGFP-labeled actin in the dikaryotic stage. Stomatin deletion affected hyphal morphology and led to upregulation of MAPKs, GPCRs, and cytoskeleton-associated genes. Raft-associated proteins therefore play a key role in hyphal growth and signaling in *S. commune*. Stomatin contributes to the organization of sterol-rich domains and actin dynamics. These findings provide new insights into raft-mediated regulation of fungal polarity and potential targets for antifungal or biotechnological applications.

#### P-MT-008

##### Identification of a sulfur transporter in the purple bacterium *Rhodobacter capsulatus*

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The Alphaproteobacterium *Rhodobacter capsulatus* exhibits remarkable metabolic versatility and can conserve energy by anoxygenic photosynthesis and aerobic or anaerobic respiration. Both growth modes can be fuelled by electrons derived from the oxidation of sulfide, which produces zero-valent sulfur. The transcriptional repressor SqrR controls not only the sulfide-dependent formation of two sulfide-oxidizing sulfide:quinone oxidoreductases (SQR), but also a candidate transporter of the RND family. When grown organoheterotrophically, a mutant lacking the transporter proved to be much more sulfide-sensitive than the wild-type. The effect was observable in the dark under aerobic conditions as well as in the light under oxygen exclusion on solid and liquid media alike. Two hours after sulfide addition under aerobic conditions, the wild-type formed extracellular sulfur that could be separated from the cells by sucrose gradient centrifugation. However, the sulfur remained with the cells of the transporter-deficient strain. When inoculated to an OD<sub>690</sub> of 0.1 in serum bottles, phototrophic batch cultures of both strains oxidized 200  $\mu$ M sulfide within 40 hours. While the wild-type grew regularly until it reached stationary phase, the mutant failed to grow even after sulfide had been depleted and the resting cells accumulated sulfur.



Finally, microscopic observation confirmed that, regardless of growth conditions, the wild-type formed sulfur globules outside the cells, whereas the transporter-deficient mutant accumulated sulfur intracellularly. Raman microspectroscopy showed that intra- and extracellular sulfur globules formed in the presence of oxygen consisted mainly of cyclo-octasulfur (S<sub>8</sub>). Database analyses revealed that bacterial species possessing homologs of the sulfur-responsive RND transporter from *R. capsulatus* also contain SQR, suggesting that the transporter functions in association with the sulfide-oxidizing enzyme and plays a role in the removal of reactive sulfur species from the cytoplasm, which may ultimately lead to extracellular sulfur globule formation. In summary, our results provide evidence for a transporter functionally linked to sulfur globule formation in a sulfide-oxidizing bacterium, offering new insights into the connection between sulfide oxidation, sulfur accumulation, and export in microbial sulfur metabolism.

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#### P-MT-009

##### Biological function of archaea-type ether lipids in Bacillales

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Bacteria and Archaea differ in many aspects, including their rRNA, transcription machinery, and cell wall composition, but perhaps the most significant difference concerns their membrane lipids. Bacteria and Eukaryotes possess membranes based on a glycerol-3-phosphate (G3P) backbone to which fatty acids are linked by ester bonds, whereas Archaea use a glycerol-1-phosphate (G1P) backbone with polyprenyl chains attached via ether bonds. Because this separation is central to the divergent evolution of the three domains of life, it has been termed the "lipid divide" and was long thought to be absolute. However, our recent studies have identified G1P-based ether lipids with polyprenyl chains in some bacterial species, including *Bacillus subtilis*, and we have found that they comprise about 0.24% of the total lipids in this species. They differ from typical archaeal lipids by their single, longer polyprenyl chain (one C35 instead of two C20 chains), and a mono- or diacetylation at the glycerol instead of a conventional polar headgroup. The biosynthetic pathway of these lipids has been described recently, but their biological function remained unclear.

To address this, we analyzed *B. subtilis* mutants lacking ether lipids to assess effects on differentiation and stress response. While no impact on differentiation or motility was observed in the mutants, our currently available data indicate a reduced tolerance to temperature and pressure stress.

In summary, our results suggest that archaea-type ether lipids might contribute to the stress resistance of Bacillales, particularly under thermal and pressure challenges. We will validate this hypothesis by investigating the ether lipid content in other Bacillales species under varying growth conditions.

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#### P-MT-010

##### Regulation of cell envelope biosynthesis in *Escherichia coli*

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The bacterial cell envelope is a dynamic structure that must maintain its integrity despite continuous remodelling and exposure to physical and chemical stressors, including antibiotics. The major components of the Gram-negative cell envelope, peptidoglycan and lipopolysaccharides, are derived from a shared pool of the precursor metabolite uridine diphosphate N-acetylglucosamine, which is generated via the hexosamine biosynthesis pathway. The hexosamine biosynthesis pathway is initiated with the synthesis of glucosamine-6-phosphate (GlcN6P) by glucosamine-6-phosphate synthase (GlmS) via fructose-6-phosphate diverted from glycolysis. This metabolic junction is tightly regulated at the level of GlmS based on the availability of GlcN6P, which can vary depending on external amino sugar availability and the rate of cell envelope remodelling. In *Escherichia coli*, GlmS is regulated post-transcriptionally by the RNA-binding protein RapZ in coordination with two small RNAs, GlmY and GlmZ. By directly binding GlcN6P, RapZ can couple the metabolite availability to the rate of GlmS translation through its interactions with the two small RNAs GlmY and GlmZ. Recent data indicate a novel protein-protein interaction between RapZ and a glycolytic enzyme occurring at the same domain with which RapZ binds GlmY and GlmZ. Genetic experiments suggest that this protein-protein interaction can increase GlmS translation, even during normal growth conditions. Thus, the recently identified interaction between RapZ and this glycolytic enzyme may serve to further regulate the partitioning of metabolites between glycolysis and hexosamine biosynthesis to ensure sufficient supply of the cell envelope components.

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#### P-MT-011

##### Influence of menthol on F<sub>o</sub>F<sub>1</sub>-ATPase and proton fluxes in *Escherichia coli* under anaerobic conditions

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#### Introduction

The growing rate of antibiotic resistance has become a global problem, and plants are considered promising sources of new antibiotic agents due to the antibacterial activity of their extracts and essential oils.

Our previous studies show that menthol, the major component (≈70%) of *Mentha arvensis* essential oil, has antibacterial activity against kanamycin-resistant *Escherichia coli* pARG-25 and wild-type *E. coli* BW25113. Menthol at MIC concentration (125 µg mL<sup>-1</sup>) inhibits the growth rate of both strains by about 40% under anaerobic conditions.

#### Goals

This study aims to understand menthol's mode of action by studying its influence on the membranes of kanamycin-resistant *E. coli* pARG-25 and wild-type *E. coli* BW25113 strains—particularly on total and DCCD-sensitive ATPase activity and proton flux rates under anaerobic conditions.

## Materials & Methods

Two *E. coli* strains were used in the experiments - *E. coli* BW 25113 (wild-type parent strain (Keio collection)) and *E. coli* pARG-25 (carrying pARG-25 plasmid (kanR) (high copy-cloning, kanamycin-resistant strain)). The strains were grown at 37°C, in liquid peptone media, pH adjusted to 7.5.

Cells were cultivated after 18–22 h growth under anaerobic conditions, harvested by centrifugation at 3500×g for 10 min and washed twice with distilled water. Proton ( $\text{H}^+$ ) fluxes were measured using a potentiometric assay with a pH/mV/ISE meter equipped with selective electrode (HI1131). Fluxes were recorded upon the addition of glucose ( $2 \text{ g L}^{-1}$ ), menthol ( $125 \mu\text{g mL}^{-1}$ ) and DCCD ( $0.1 \text{ mM}$ ).

## Results

Under anaerobic conditions, menthol at  $125 \mu\text{g mL}^{-1}$  inhibited  $\text{F}_0\text{F}_1$ -ATPase activity and proton flux in both *E. coli* strains. The total ATPase activity decreased by ~35% in the wild type and by ~60% in the kanamycin-resistant strain. The DCCD-sensitive ATPase activity was suppressed by about 35% in the wild type and 40% in the resistant strain. Similarly, proton flux declined by ~40% and ~80%, respectively, and DCCD-sensitive proton flux was inhibited by ~80%, indicating that menthol interferes with  $\text{F}_0\text{F}_1$ -ATPase function and membrane-associated proton transport under anaerobic conditions.

## Conclusion

Our data suggests that menthol MIC decreased the DCCD-sensitive ATPase activity and potassium flux mainly by inhibiting DCCD-sensitive fluxes, meaning the sites where DCCD inhibits ATPase activity. Like DCCD, menthol inhibits  $\text{F}_0\text{F}_1$ -ATPase, which is responsible for proton translocation across the membrane.

### P-MT-012

#### Crossing the Lipid Divide: Substrate Specificity of Bacterial Phospholipid N-Methyltransferases?

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The lipid divide describes the fundamental divergence between archaeal and bacterial/eukaryotic membrane architectures. While lipid head groups are predominantly conserved across all domains of life, influencing physicochemical properties like charge and curvature, the hydrophobic tails differ significantly. Archaeal membranes contain highly branched isoprenoid chains linked via ether bonds to a *sn*-glycerol-1-phosphate (G1P) backbone, whereas bacterial and eukaryotic lipids feature fatty acyl chains esterified to *sn*-glycerol-3-phosphate (G3P).

Despite these pronounced structural differences, numerous archaeal and bacterial enzymes exhibit remarkable substrate promiscuity, catalyzing reactions on non-native lipid variants. For example, the archaeal cardiolipin synthase from *Methanospirillum hungatei* can utilize bacterial phosphatidylglycerol as a substrate<sup>1</sup>; while *Bacillus subtilis* phosphatidylserine synthase can synthesize archaeatidylserine from archaeal precursors<sup>2</sup>.

In this study, we aim to test the promiscuity of bacterial phospholipid N-methyltransferases (Pmts). These cytosolic enzymes facilitate the stepwise methylation of phosphatidylethanolamine (PE) to phosphatidylcholine (PC). The activity of representatives from two distinct Pmt classes: the *Rhodobacter* (R)-type PmtA from *Rubellimicrobium thermophilum* (RtPmtA) and *Sinorhizobium* (S)-type PmtA from *Agrobacterium tumefaciens* (AtPmtA) are examined *in vitro*. Using a range of PE substrates, we demonstrate that AtPmtA and RtPmtA display different substrate specificities depending on the lipid backbone chemistry, linkage type, and hydrophobic tail composition of lipid substrates. These findings highlight both the evolutionary flexibility and the biochemical limitations of lipid-modifying machinery.

<sup>1</sup> Exterkate M, de Kok NAW, Andringa RLH, Wolbert NHJ, Minnaard AJ, Driessen AJM. A promiscuous archaeal cardiolipin synthase enables construction of diverse natural and unnatural phospholipids. *J Biol Chem.* 2021 Jan-Jun;296:100691.

<sup>2</sup> Morii H, Koga Y. CDP-2,3-Di-O-geranylgeranyl-*sn*-glycerol:L-serine O-archaeatidyltransferase (archaeatidylserine synthase) in the methanogenic archaeon *Methanothermobacter thermautotrophicus*. *J Bacteriol.* 2003 Feb;185(4):1181-9.

### P-MT-013

#### GroEL functions as Tat signal peptide chaperone in *Pseudomonas fluorescens*

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Tat systems transport folded proteins across energized biological membranes [1]. Fluorescent pseudomonads contain several Tat-dependently translocated periplasmic enzymes of the pyoverdine biosynthesis pathway, including the copper-containing tyrosinase PvdP, the PLP-containing transaminase PtaA, and the PLP-containing PvdN, which catalyzes the unusual oxidative decarboxylation under retention of the  $\alpha$ -amino group [2]. In our studies on the enzymology of periplasmic pyoverdine maturation in *Pseudomonas fluorescens*, we identified GroEL as signal peptide-interacting chaperone of PvdN. As exposed signal peptides of folded Tat substrates need to be protected from degradation prior to Tat-dependent translocation, GroEL thus likely fulfils this novel function in addition to its well-studied involvement in protein folding.

[1] Brüser T. and Hou B. (2011) *Biomol Concepts.* 2:507-23. doi: 10.1515/BMC.2011.040

[2] Ringel M.T. and Brüser T. (2018) *Microb Cell.* 5:424-437. doi: 10.15698/mic2018.10.649

### P-MBE-002

#### News from LPSN, the database for prokaryotic nomenclature

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The List of Prokaryotic names with Standing in Nomenclature (LPSN) was first published in 1997 to keep track of the large number of newly proposed bacterial and archaeal names and other rapid changes in prokaryotic nomenclature. Relaunched at the Leibniz Institute DSMZ in 2020, LPSN now encompasses over 59,000 taxon names, alongside information on nomenclatural types, taxonomic and nomenclatural status, synonymy, classification, etymology, literature and much more. In 2023, LPSN's unique importance as an expert-curated, authoritative source of scientific names of prokaryotes was recognised by its designation as a Global Core Biodata Resource (GCBR).

Recent LPSN developments include integrating thousands of cyanobacterial names, thereby harmonizing long-separated microbial nomenclatures. Other key updates from the International Code of Nomenclature of Prokaryotes (ICNP) have also been adopted, such as the introduction of the taxonomic category kingdom and the nomenclatural pro-status of *Candidatus* names. Furthermore, information relevant to medical microbiology has been added by integrating the List of Recommended Names for bacteria of medical importance (LoRN) and augmenting the pathogenicity information. Both can now be queried using an enhanced advanced search.

The LPSN Application Programming Interface (API) has been extended to include names of higher taxonomic ranks and their hierarchical relationships, facilitating efficient access to prokaryotic taxonomic information, in line with FAIR data principles. Additionally, the LPSN taxonomic hierarchy can now be browsed via TaxMap (<https://taxmap.dsmz.de/>), developed by DSMZ's SILVA team. Integrating LPSN into the DSMZ Digital Diversity Consortium enables cross-connection to complementary databases that are fundamental to the life sciences, such as BacDive, BRENDA and SILVA. LPSN is pursuing several projects to further enhance its usability and support scientists worldwide.

### P-MBE-003

#### Antimicrobial resistance evolution: concordance of *in vitro* and *in vivo* studies

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Antimicrobial resistance is a major global concern, and current measures to address the problem are new drugs, prudent use and rapid diagnostics. Recently, the idea of forecasting resistance evolution experimentally to prevent resistance *a priori* has gained attention. But how well do experimental data on antimicrobial resistance evolution reflect resistance in strains from patients? To address this, we conducted a systematic review and analysis to compare *in vitro* and *in vivo* data. We collected and analysed literature on experimental resistance evolution published between 1977 and 2024 available on PubMed. We focussed on *P. aeruginosa* and *E. coli* under ciprofloxacin (CIP) selection, as these were most frequently used in experimental evolution. CIP-selected gene mutations and amino acid substitutions, as well as the associated minimum inhibitory concentrations (MIC) in experimentally evolved *P. aeruginosa* and *E. coli* isolates, were compared with corresponding *in vivo* data. For both species, *in vitro* and *in vivo* isolates showed high concordance in the selected gene mutations and the specific amino-acid changes. Evolved MIC values differed significantly depending on the respective gene mutation and the total number of mutations, but mostly

not due to the origin of the strains – *in vitro* or *in vivo*. Our findings clearly show that experimental resistance evolution *in vitro* has the potential to inform our understanding of the dynamics of antimicrobial resistance evolution *in vivo*. This supports the idea that experimental data can be used to forecast the risks of resistance to emerge and therefore contribute to antimicrobial stewardship.

### P-MBE-004

#### Unraveling Microbial Biodiversity and Functional Dynamics in two Drinking Water Reservoirs via metagenomics

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Although freshwater ecosystems constitute only 0.01% of Earth's water, they are critical hotspots of biodiversity and ecological function, hosting an estimated 10% of known species. In these ecosystems, biodiversity is closely tied to water quality and the health of both humans and natural systems. Riverdam-mediated drinking water reservoirs, as integral parts of these systems, provide substantial amounts of drinking water and serve as complex habitats for microbial communities that underpin essential ecological functions. Amid growing anthropogenic influences and climate-driven pressures, it remains largely unknown how microbial biodiversity both taxonomic and functional and the dynamics of pathogens such as viruses and antimicrobial resistances develop over time. The implications for drinking water quality remain poorly understood.

To address this gap, we used metagenomic sequencing to assess microbial diversity and functional potential in two oligotrophic to mesotrophic German drinking water reservoirs across seasonal and depth gradients. Nineteen samples from epilimnion, metalimnion, and hypolimnion were concentrated by ultrafiltration, followed by DNA extraction, Illumina shotgun sequencing, assembly, rpS3 analysis, and genome reconstruction.

We found marked seasonal and depth-dependent shifts in microbial diversity. Cyanobacteria were strongly enriched in the metalimnion of one reservoir, accompanied by viral contigs suggesting cyanophage activity. rpS3 profiles revealed reservoir-specific Proteobacteria patterns. Phototrophic eukaryotes appeared mainly in photic layers in summer and autumn, while their winter occurrence in deeper waters reflected mixing. Functional analyses identified ecological roles, pathogenic potential, and antibiotic resistance genes (ARGs). ARGs spanned ten antibiotic classes, with Trimethoprim resistance most common; their diversity varied with stratification and anthropogenic inputs. Viral community patterns paralleled these trends, with higher abundances in surface and deep layers, indicating physicochemically driven host-virus interactions.

This study highlights the power of metagenomics to resolve reservoir microbial and functional biodiversity. Our findings offer key insights into how microbial communities, water quality, and environmental gradients interact. This knowledge supports the development of monitoring systems that help protect biodiversity and ecosystem services under climate and human pressures.

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## P-MBE-005

### Ecology and evolutionary traits in Rozellomycota for contrasting environments

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### Introduction

Rozellomycota ecology and biology are limited to a few species only. Rozellomycota are tiny eukaryotes, mostly known as parasites of microeukaryotes, and the discovery of their lineages is primarily based on environmental DNA, which has uncovered a hitherto unknown taxonomic richness that challenges the diversity of all Fungi. The phylogenetic diversity of these environmental lineages is enormous; however, they consist of dark taxa only.

### Goals

Our work aims to shed light on aquatic Rozellomycota lineages and investigate the environmental factors that drive their diversity, beyond suitable hosts. In particular, we are interested in how Rozellomycota thrive under highly reduced conditions, as redox gradients are one of the strongest environmental filters in microbial ecology.

### Methods

To achieve this, we focused our attention on engineered and natural water bodies spanning a range of differing redox conditions, as well as existing datasets on environmental Rozellomycota lineages. We further tested their establishment in an experimentally replicated reactor system and followed a 10-year time series of a peat bog lake with oscillating redox conditions to trace patterns of occurrence and diversity.

### Results and Conclusion

Our data analysis shows that the phylogenetic diversity is partly clustered by ecotone; however, environmental filtering due to differing redox conditions is not established within evolutionary lineages. In contrast, different major Rozellomycota lineages appear to have interspersed taxa from anaerobic environments, and our experimental reactor build-up a stable community under copiotrophic conditions and strong redox gradients. In the lake data series, we can find identical strains (i.e., MAGs) under anaerobic and aerobic conditions, suggesting that this taxon is tolerant to changing redox conditions. Combined, this leads us to hypothesize that this organism exhibits either a free-living, aerotolerant, fermentative lifestyle or lives in a symbiotic relationship with other planktonic members of the lake, making it redox-indifferent. Overall, our results suggest that Rozellomycota ecology is more diverse than parasitism alone, and that typical environmental filters are not strongly represented in their phylogeny.

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## P-MBE-006

### Microbial DNA decay within stalagmites preserves a molecular record of time

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Molecular palaeomicrobiology—the study of microbial ancient DNA (aDNA) preserved in natural archives—has transformed our ability to reconstruct microbial biodiversity, evolutionary change, and ecosystem dynamics over millennia. A central challenge, however, is accurately dating microbial aDNA. Post-mortem damage such as fragmentation and cytosine deamination in single-stranded overhangs are used for authentication, but their accumulation depends on environmental conditions, limiting their use as geochronological clocks. Current dating approaches rely on dating the host materials, leaving uncertainties where DNA of differing ages is intermixed.

We propose stalagmites as time-resolved archives of microbial aDNA. Formed by drip water mineralization in thermally stable cave systems, stalagmites provide a datable matrix that physically isolates microorganisms, minimizes contamination, and stabilizes DNA within the calcified matrix. This makes them a promising system to study long-term DNA decay and develop a geochronological clock based on molecular damage.

We analysed 61 subsamples of stalagmite TM-17 from Tham Doun Mai cave, Laos, spanning ~38 ka of continuous growth. Using optimized low-biomass aDNA extraction and *de novo* metagenomic assembly, we quantified DNA damage across stratigraphic layers. We focused on six genera that were consistently detected in more than 90% stalagmite assemblies, such as *Candidatus Methylophilus*, *Candidatus Nitrosotenuis*, *Conexibacter*, *Nitrosotalea*, *Nitrospira*, and *Piscinibacter*. These taxa include mainly autotrophic methane- and ammonia-oxidizers, alongside heterotrophic degraders of organic matter.

Damage increased progressively with both stratigraphic depth and COPRA-modeled age, following exponential saturation models. Fragmentation proceeded an order of magnitude more slowly than cytosine deamination. By calibrating the total 5' C-to-T substitution frequency (PyDamage  $p_{max}$ ) against age, we established a proof-of-concept molecular dating approach reaching back 24 ka with a median relative error of 26%.

These results demonstrate that stalagmites can preserve microbial aDNA under stable conditions in a temporally resolved manner, linking molecular damage directly to sample age. Stalagmites thus serve as molecular archives of microbial life, enabling new approaches to study microbial evolution, community turnover, and responses to long-term environmental change.

## P-MBE-007

### Introduction of a standardized flow system for quantitative biofilm measurement: a test system to visualize resistance development in biofilms by FISH

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#### Introduction:

Biofilm-associated infections pose a significant risk to patients, as biofilm-embedded bacteria tolerate higher antibiotic concentrations than single, planktonic bacteria. Standardized in vitro systems with robust and reproducible biofilm growth are essential to research antibiotic treatment strategies and the evolution of resistance.

#### Goals:

Our goal was to develop an in vitro system based on the ibidi flow system to produce standardized, well-defined *Staphylococcus epidermidis* biofilms. We used fluorescence in situ hybridization (FISH) in combination with digital image analysis for biofilm quantification and activity measurement. This setup was subsequently used in an evolution experiment to visualize the resistance development in biofilms compared to planktonic bacteria.

#### Methods:

Biofilms were grown under controlled conditions and compared with a static microtiter plate model. Subsequently, mature biofilms were exposed to increasing concentrations of daptomycin or gentamicin. FISH was employed to visualize and quantify biofilm thickness and bacterial metabolic activity, followed by digital image analysis.

#### Results:

The robustness of the ibidi flow system for biofilm growth was evaluated by analyzing average biofilm thickness in separate channels and in repeated independent runs under similar conditions. The biofilm profiles and the bacterial activity measurements indicated homogenous results, especially compared to the static culture. Overall intra- and inter-assay variability indicated high reproducibility in the ibidi flow system. Initial FISH results of the evolution experiment revealed persistence of cells in biofilms at high antibiotic concentrations without increased minimum inhibitory concentration (MIC).

#### Conclusion:

The results demonstrate that the ibidi flow system in combination with FISH and digital image analysis is a reliable platform for testing the antimicrobial susceptibility of biofilms and monitoring their resistance development.

## P-MBE-009

### Mating and fruiting body formation of *Fomes fomentarius* under laboratory conditions

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*Fomes fomentarius*, a basidiomycete species belonging to the order of polyporales, is an interesting organism due to the formation of perennial fruiting bodies undergoing tissue differentiation and the production of fungal composite materials using fungal biomass and agricultural side streams. Yet, the laboratory methods which can be used for this organism are still limited. Since this species originates from the subkingdom Dikarya, the natural life cycle is dominated by the dikaryotic state, indicated by the presence of clamp connections between hyphae to maintain two nuclei in each cell during cell division. Interestingly, the requirements for mating of monokaryotic strains in *Fomes fomentarius* has not been investigated yet.

We developed a simple assay to perform mating of monokaryotic *Fomes fomentarius* on solid media and compared the growth speed of mono- and dikaryotic strains obtained from these experiments. We are able to discern the presence of both genome copies in the mated strains by a set of SNPs present in both parental strains. We then investigated the cell wall composition of the <sup>13</sup>C/<sup>15</sup>N-fed mono- and dikaryotic strain using solid-state NMR experiments and found subtle differences in lipid and protein composition while the cell wall matrix remained largely unchanged. We further established a method to obtain fruiting bodies of *Fomes fomentarius* under laboratory conditions and observed fruiting body formation under cold-stress for both birch logs inoculated with *Fomes fomentarius* PaPF11, a dikaryotic isolate as well as for Pdm1/Pdm2, a newly mated strain. Further, we performed proteomics experiments and RNA-Sequencing to identify lead genes involved in browning of the fruiting bodies of *F. fomentarius*.

These methods that are prerequisite for successful establishment of *Fomes fomentarius* as a polyporales model species and are a valuable milestone that might enable studies on plant-fungal interactions of genetically altered basidiomycete strains in a laboratory.

## P-MBE-010

### The Type (Strain) Genome Server (TYGS): Recent Developments in Genome-Based Prokaryote Taxonomy

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The Type (Strain) Genome Server (TYGS) is a widely used high-throughput web platform established in 2019 to enable genome-based prokaryote taxonomy without requiring specialist knowledge. TYGS provides users with comprehensive genomic, taxonomic, and nomenclatural information derived from submitted genome sequences and offers continuously updated access to type (strain) data, nomenclature, synonymy, and taxonomic literature.

TYGS consists of three main components: (i) a workflow engine comprising independent microservices for parallelized data processing, (ii) a central TYGS database, and (iii) a user-friendly web interface with its own web database. Upon submission, user genomes are processed on a dedicated computing cluster through a multi-step workflow that includes Mash distance estimation, extraction and BLAST-based comparison of 16S rRNA genes against >23,500 type (strain) genomes, and whole-genome Mash analyses to identify the closest relatives. These are followed by Genome BLAST Distance Phylogeny (GBDP) calculations and prediction of digital DNA–DNA hybridization (dDDH) values for taxonomic delineation. The results are displayed as interactive trees annotated with metadata, dDDH values, and pre-generated text suitable for publication.

Since its launch, TYGS has attracted more than 21,800 unique users, processed over 189,000 jobs, and performed nearly 99 million genome comparisons. Recent updates include performance optimization through precomputed pairwise distances among type genomes, implementation of local identifier databases for faster sequence retrieval, and improved email notifications that facilitate user correspondence.

TYGS continues to serve as an essential resource for microbiologists and taxonomists worldwide, supporting accurate classification and the description of new microbial taxa in the era of genome-based taxonomy.

#### P-MBE-011

##### **The effect of flavonoids on cyanobacterial motility and their role in symbiotic relationship with land plants**

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Cyanobacteria have symbiotic relationships with diverse land plants, from bryophytes to ferns, gymnosperms, and angiosperms. One specific example is the symbiosis between *Azolla filiculoides* and its cyanobiont *Nostoc azollae*: the cyanobiont is vertically inherited, and both partners co-evolve, which results in full co-dependency. In *Azolla*, hormogonia are required for colonisation of new tissue and inheritance; however, the molecular cues underlying the symbiotic transfer are poorly understood. We want to test whether SA and flavonoids play a role in cyanobacterial symbiotic relationships. To do that, we investigated the effect of SA and flavonoids produced by *A. filiculoides* on cyanobacterial symbionts. We induced motility in cyanobacteria using far-red light and tested the ability of the phenolic compounds to modulate the transition into hormogonia stages. The results of the treatments were documented using light microscopy, and RNA was extracted for further analyses and then sequenced. We set up a machine-learning-based image analysis pipeline and analysed light microscopy images to quantify differentiation

into hormogonia and the amount of vegetative cells. We found that SA and the majority of flavonoids tested enhanced the hormogonia transition in a symbiotic cyanobacterium due to the upregulation of motility-related genes.

#### P-MBE-012

##### **Phylogeny and comparative genomics of *Arthromitus* nom. rev. and other lineages of segmented filamentous bacteria in arthropod guts**

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More than 170 years ago, Joseph Leidy described microbial filaments colonizing the hindgut of various arthropods as members of the genus *Arthromitus*. It was later hypothesized that the filaments, recognized as endospore-forming bacteria, constitute a life stage of *Bacillus cereus*. Since then, our lab has demonstrated that segmented filamentous bacteria (SFB) in arthropod guts comprise representatives of the clostridial order *Lachnospirales* and are therefore distinct from morphologically similar and equally uncultured *Clostridiales* found in mammalian intestines. However, the identity of Leidy's *Arthromitus* and the diversity and physiology of SFB in arthropods remain unresolved. Using whole-genome sequencing of capillary-picked single filaments and RNA-FISH, we demonstrated that the SFB in the gut of termites and millipedes represent several family-level lineages of *Clostridia*. Based on phylogenomic analysis, we formally describe the first representatives of these lineages, including *Arthromitus cristatus* Leidy nom. rev. from the millipede *Glomeris marginata* and new genera from the termite *Reticulitermes flavipes*. We are currently conducting a comprehensive genome annotation to elucidate the major metabolic pathways and cellular processes that characterize each of the lineages.

#### P-MBE-013

##### **Convergent evolution in *Nucleococcaceae* fam. nov., a novel lineage of *Opitutales* (*Verrucomicrobiota*), and other endosymbionts of termite gut flagellates**

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Cellulolytic flagellates play a key role in lignocellulose digestion within termite guts. Previous research has shown that each flagellate species harbours a diverse assemblage of symbionts from various bacterial phyla. Metagenomic studies yielded 82 metagenome-assembled genomes (MAGs) that represent a cluster of novel, family-level lineages in the order *Opitutales* (phylum *Verrucomicrobiota*), including previously reported endonuclear bacteria of the genus *Nucleococcus*. Fluorescence in situ hybridization of several phylotypes confirmed their intranuclear localization. Comparative genome analysis revealed that all lineages experienced a severe genome reduction compared to other *Opitutales* (average genome size 1.68 vs. 2.98 Mbp). Substantial gene losses in carbon and energy metabolism were compensated by the presence of transporters for the uptake of sugar phosphates, hexuronic acids, amino acids, and vitamins, which were often acquired through horizontal gene transfer. Together with the retention of an ATP/ADP antiporter in all lineages, these traits are adaptations to the

intracellular environment that were previously observed also in flagellate symbionts from other phyla, showcasing examples of evolutionary convergence. A 16S rRNA-based analysis of the bacteria associated with individual flagellates from different termite species revealed that representatives of *Nucleococcaceae* and related lineages are not consistently present in every host cell, suggesting that they are not mutualistic symbionts but parasites of their flagellate hosts.

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#### P-MBE-014

##### Conjugation promotes the segregation of novel plasmid alleles under non-selective conditions

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The emergence of multi-drug resistant bacteria has become an increasing threat to human health in recent years. Mobile plasmids are one of the key players in the spread of antibiotic resistance allele as they may enable distribution over species borders. The evolution of multicopy plasmids differs from that of monoploid chromosomes due the increase mutational supply and the effect of segregational drift. However, the effect of plasmid transfer on plasmid allele dynamics remains understudied. Establishing an experimental system for following conjugative plasmid allele dynamics, we compared the dynamics of a novel resistance allele between two conjugative plasmid types – pHHV216 and RK2 – in *Acinetobacter baylyi* under non-selective conditions. A novel resistance allele was introduced into the ancestral donor population, generating heteroplasmic hosts that segregated over time. Daily mating of donors with recipients revealed that the recipient population comprised only homoplasmic hosts, with rare heteroplasmic hosts due to plasmid fusion in the donor population. Furthermore, the recipient genotypes mirrored the novel allele frequency in the donor population. Our results demonstrate that conjugation is an alternative route for the segregation of novel plasmid alleles. Thereby, conjugation can promote plasmid allele segregation by increasing the frequency of homoplasmic hosts in the population.

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#### P-MBE-015

##### Unveiling novel *Planctomycetota* through systematic exploration of the Schlesner culture collection

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Members of the phylum *Planctomycetota* occur ubiquitously and receive attention due to various cell biological peculiarities including an uncommon form of asymmetric cell division without canonical cell division proteins<sup>1</sup>. In line with their large genomes and complex lifestyles, *in silico* genome mining analyses of planctomycetal strains yielded several putative biosynthetic gene clusters which might be responsible for the production of novel small molecules with potential antimicrobial properties<sup>2</sup>. For a long time, *Planctomycetota* were considered unculturable. Due to pioneering work of Heinz Schlesner (Kiel University), many strains belonging to the phylum have been brought into culture in the 1970s to the early 2000s<sup>3</sup>.

The Schlesner collection of microbes contains more than 250 strains of "budding bacteria" morphologically resembling members of the phylum *Planctomycetota* that were isolated mainly from aquatic habitats. We have now set out to re-cultivate and analyze the strains from this collection, which is a valuable source for extending the open collection of axenic planctomycete cultures. More than 180 planctomycetal strains were revived from the collection and were identified through 16S rRNA gene sequencing. The collection harbours strains that belong to at least 18 novel genera and 24 novel species within the phylum. Typical genome sizes of 7-10 Mb and up to 13 secondary metabolite-associated biosynthetic gene clusters per genome were obtained. The Schlesner collection will be an important driving force to expand the currently described diversity of the phylum.

#### References

1. Wiegand, S., Jogler, M., Boedeker, C., Pinto, D., Vollmers, J., Rivas-Marín, E., Kohn, T., Peeters, S. H., Heuer, A., Rast, P., Oberbeckmann, S., Bunk, B., Jeske, O., Meyerdierks, A., Storesund, J. E., Kallscheuer, N., Lückner, S., Lage, O. M., Pohl, T., . . . Jogler, C. (2020). Cultivation and functional characterization of 79 planctomycetes uncovers their unique biology. *Nat Microbiol*, 5(1), 126-140.
2. Calisto, R., Godinho, O., Devos, D. P., & Lage, O. M. (2025). "Genome-based in silico assessment of biosynthetic gene clusters in Planctomycetota: Evidences of its wide divergent nature". *Genomics*, 117(1), 110965.
3. Kallscheuer, N., Wurzbacher, C. E., Schmitz, R. A., Jogler, C. (2024). In the footsteps of Heinz Schlesner and Peter Hirsch: Exploring the untapped diversity of the phylum Planctomycetota in isolates from the 1980s to the early 2000s. *Syst Appl Microbiol*, 47(1), 126486

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#### P-MBE-016

##### Highly synchronized co-evolution of codon usage patterns in functionally related genes

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Prokaryotic genomes contain diverse gene sets characterized by varied origins, functions, and expression patterns. This genetic diversity has long raised questions about how cells optimize protein demands (i.e., product abundance) and translation fidelity. One mechanism is by optimizing codon usage. As all genes in the genome share the same genetic code, optimizing codon usage for individual genes requires careful fine-tuning, particularly in genomes with high or low GC content. The usage of synonymous codons varies along the genome, with strong biases in conserved and highly expressed genes that are optimized for translation.

The extent of codon usage adaptation within genes and co-adaptation between genes, as well as the influence of gene function on these patterns, remain important open questions. Here, we investigate how gene codon usage is optimized in diverse bacterial genomes, focusing on sixteen of the most prevalent bacterial phyla. Using a set of high-quality genomes from representative species of these phyla, we explore various codon usage bias indices and identify



patterns of functional relatedness in co-optimizing genes across genomes. We demonstrate that codon usage is highly non-random in most bacterial genes, with ~32-52% of gene families presenting synchronized codon usage evolution. We show that co-adapting genes are co-expressed, co-regulated, and metabolically and functionally associated. Together, this underappreciated regulatory dimension provides new evidence of functional relatedness with implications for proteome partitioning, metabolic modeling, microbial ecology, and synthetic biology.

#### P-MBE-017

##### **Monitoring methylation-dependent conformational changes in *Helicobacter pylori* intragenome interactions by high-throughput chromosome conformation capture (Hi-C)**

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#### Introduction

*H. pylori* is a carcinogenic Gram-negative bacterium that infects the human gastric mucosa. Its genome encodes a large number of restriction modification (RM) systems, resulting in a highly variable portfolio of active methyltransferases (MTases), making it a model organism to study genome variation and DNA methylation. MTases recognize short DNA motifs and methylate specific adenine or cytosine residues. In bacteria, MTases, as parts of RM systems, are involved in the defense against bacteriophages. MTases are also involved in other essential processes, as gene regulation, DNA replication, and repair mechanisms.

#### Goals

The aim of this study was to investigate possible roles of DNA methylation on the structural properties of the genome, utilizing selected *H. pylori* methyltransferase mutants.

#### Methods

Methylation-dependent spatial conformation changes of the *H. pylori* genome were analyzed using high-throughput chromosome conformation capture (Hi-C). The basic principle of Hi-C involves cross-linking genomic DNA, followed by fragmentation and ligation of cross-linked fragments that are in close spatial proximity. The ligated fragments are then sequenced, allowing for the identification of genomic regions that interact. Those interactions can be visualized in contact maps.

#### Results

The contact maps of multiple *H. pylori* wild-type strains show, as most prominent feature, a so-called secondary diagonal. In other species, secondary diagonals are frequently observed in genomes containing structural maintenance of chromosomes (SMC) complexes, indicating increased interactions between the two replichores during replication. Despite the presence of a second diagonal in multiple strains, *H. pylori* appears to lack any canonical SMC complexes characterized in other organisms. A link between methylation and chromosome architecture was suggested by the observation that the deletion of specific methyltransferases was associated with the loss of the second diagonal in the mutant strains.

#### Conclusion

Secondary diagonals represent one of the most pronounced long-range interactions observed in bacterial Hi-C maps. The

disruption of this conformational feature suggests that methylation may play a crucial role in maintaining chromosome organization. To further understand the mechanisms by which these enzymes modulate chromosome conformation, we will purify selected DNA-binding proteins to enable *in vitro* characterization of methylation-dependent conformational change.

## **Microbial communities**

#### P-MC-001

##### **Polyoxometalate Ionic Liquids (POM-ILs) as Protective Coatings for Industrial Infrastructure and Cultural Heritage against Microbiologically Influenced Corrosion (MIC)**

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Corrosion of stone and metal surfaces caused by acid rain and microbial activity remains a major challenge for both industrial infrastructure and cultural heritage conservation. Microorganisms, especially sulfate-reducing bacteria (SRB) and methanogenic archaea, are key contributors to Microbiologically Influenced Corrosion (MIC), accelerating metal degradation through biofilm formation and the production of corrosive metabolites such as sulfides.

To counteract such deterioration, innovative materials are needed that can resist both chemical and microbial attack. Polyoxometalate-based ionic liquids (POM-ILs) represent a new class of multifunctional nanomaterials that combine the redox tunability of polyoxometalates with the structural versatility of ionic liquids. Thin, transparent POM-IL films form stable, water-insoluble layers that act as dual chemical and biological barriers. Their intrinsic antimicrobial and acid-resistant properties have been demonstrated in previous studies, where POM-IL coatings effectively protected stone substrates against simulated acid rain and inhibited biofilm formation.

Notably, successful application against lampenflora in the Pommery Champagne cellar and promising results under outdoor environmental conditions have established their long-term performance and ecological safety, as the coatings are non-leaching and environmentally stable.

The current research extends the application of POM-IL nanocoatings to metallic substrates such as brass, carbon steel, cast iron, and bronze, with a specific focus on MIC. The project aims to optimize coating-metal adhesion, assess physicochemical stability, and evaluate protective efficacy against anaerobic microorganisms. Laboratory studies investigate SRB- and methanogen-driven corrosion under controlled conditions, focusing on biofilm inhibition, metabolic suppression, and the reduction of corrosion product formation. This presentation will highlight POM-ILs as sustainable, high-performance nanocoatings for mitigating MIC by integrating material protection with microbial control. It will discuss published case studies, ongoing



microbiological investigations, and future perspectives for applying these coatings to safeguard metal infrastructure and cultural heritage artefacts.

#### P-MC-002

##### **Towards standardization in pig microbiome research: Biases introduced by DNA extraction kits are more prevalent in 16S rRNA gene datasets compared to shotgun metagenomic profiles**

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The lack of standardization in DNA extraction procedures for pigs, an animal model of agricultural and biomedical significance, limits reproducibility and cross-study comparability.

The goal of this study was to evaluate the influence of DNA extraction kits on various 16S rRNA gene regions and shotgun metagenomic profiles from the same pig samples.

Fecal samples from 50 male German Landrace × Pietrain pigs were spiked with 25 µL of ZymoBIOMICS Spike-in Control I (High Microbial Load) and then subjected to DNA extraction using 12 commercial kits following manufacturer protocols. Eluted DNA was used to amplify the V1–V2, V1–V3, V3–V4, and V4 regions of the 16S rRNA gene, including negative controls. Amplicons were pooled equimolarly and sequenced (250 bp paired-end) on an Illumina MiSeq by Biomarker Technologies (BMK GmbH, Münster, Germany). In parallel, metagenomic libraries were prepared from 30 µL of DNA (≥ 20 ng/µL) and sequenced (150 bp paired-end, PCR-free) on an Illumina NovaSeq. Quality control was performed using DADA2 in QIIME2 [1] (16S amplicons) and Trim Galore [2] (metagenomics), followed by bioinformatic analysis in QIIME2 (amplicon and moshpit distributions v2025.4).

The choice of DNA extraction kit significantly influenced bacterial community structure in both amplicon and metagenomic data, as well as metagenomic functions. Alpha diversity was affected in amplicon data (all P-values < 0.01) but not in metagenomic data (all P-values > 0.05). The theoretical ratio (I/A ratio) of two species included in the spike-in standard, *Imtechella halotolerans* and *Allobacillus halotolerans*, was calculated to be 1 (1:1) for metagenomic samples (genome copies) and 0.43 (3:7) for amplicon samples (16S rRNA gene copy number). For commercial kits, the QIAamp PFP kit was most accurate for metagenomics (1.034) and the second most accurate for amplicon sequencing (V3–V4: 1.258; V4: 1.26).

The selection of a DNA extraction kit affects amplicon-based microbiome profiles more than metagenomic profiles, affecting diversity estimates. Standardizing methods is therefore crucial for reproducibility in animal microbiome studies.

[1] Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016;13(7):581–583

[2] Krueger F. TrimGalore [Internet]. GitHub; 2025. Available from: <https://github.com/FelixKrueger/TrimGalore>.

#### P-MC-003

##### **Developing methods for long-term preservation of complex microbial communities: Insights from soil microbiomes**

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The rapid development of microbiome research marks a paradigm shift in the field of microbiology, from the traditional focus on individual strains to that on complex communities, their interactions and functionality. However, while technology and knowledge of microbiomes rapidly advanced, methods and infrastructure to preserve complex microbial communities and their entire functionality are still lacking. While methods for preservation of axenic strains are highly refined, and the infrastructure to preserve these strains is well developed, many techniques cannot be directly transferred to microbiomes, and studies on long term-preservation of microbiomes are still scarce. This emphasizes the importance of developing supporting infrastructure and methods for the long-term microbiome preservation to ensure sustainable progress in the field of microbiome research.

In this long-term experiment, complex soil communities were preserved for one year using a broad range of methods, combining different storage temperatures with various cryoprotectants to identify the best conditions for long-term preservation of the microbiota and their functionality. To evaluate the quality of applied preservation methods, a comprehensive analysis was conducted, including DNA- and RNA amplicon sequencing, cultivation experiments and functionality assessments. Ultimately, the different methods could be rated according to their ability to preserve the original microbiota and functionality. This represents a first step toward understanding the requirements for long-term microbial preservation and its supporting infrastructure.

#### P-MC-004

##### **Inside Bilioenteric Catheter Biofilms: A Microscopic View of Microbial Community Dynamics**

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During liver transplantations microbiota may infiltrate the biliary system. This can lead to postoperative polymicrobial infections and thus cholangitis, suspected to result from bacterial colonization of bilioenteric catheters. Cholangitis can lead to systemic, life-threatening infections such as sepsis or organ failure. Therefore, understanding the dynamics of polymicrobial biofilms on bilioenteric catheter is of high importance. [1, 2]

This study therefore aimed to elucidate the inter- and intraspecies interactions between representative isolates of *Enterococcaceae* and *Enterobacteriaceae* derived from bilioenteric catheters, using a standardized *in vitro* model system. The primary focus was placed on high-resolution visualization and quantification of biofilm development through confocal laser scanning microscopy (CLSM), complemented by scanning electron microscopy (SEM). CLSM was employed to investigate spatial and temporal changes in biofilm architecture, using viability staining and species specific staining to investigate species distribution. SEM imaging provided detailed surface morphology and structural integrity analysis of bilioenteric-catheter associated polymicrobial biofilms.

To correlate imaging data with microbiological parameters, colony forming unit (CFU) counting and 16S rRNA gene sequencing were performed to assess microbial composition and community shifts. We further evaluated how the microbial dynamics between *Enterococcaceae* and *Enterobacteriaceae* differ under planktonic versus biofilm growth conditions, and how these interactions are modulated by intrinsic factors such as antibiotic resistance genes and extrinsic factors including bile acids and antibiotic exposure.

By integrating advanced CLSM and SEM imaging with microbiological and genomic analyses, this multifaceted approach aims to provide in-depth insight into the mechanisms governing polymicrobial biofilm formation in the biliary system and to identify potential strategies to prevent biofilm-associated postoperative complications in patients.

1. Shbaklo, N., et al., *Bacterial and Viral Infections in Liver Transplantation: New Insights from Clinical and Surgical Perspectives*. Biomedicine, 2022. **10**(7).
2. Hernandez Mdel, P., P. Martin, and J. Simkins, *Infectious Complications After Liver Transplantation*. Gastroenterol Hepatol (N Y), 2015. **11**(11): p. 741-53.

#### P-MC-005

##### Combating biofilm-immigration in wound dressings

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When wound dressings are left on injuries for too long without being changed, they can themselves become a reservoir for bacteria causing wound infection. This is a major problem, particularly given the current shortage of nursing staff in hospitals and the resulting shortcomings in patient care. It can lead to new or subsequent infections in poorly treated wounds, which compromise the healing process.

To address this issue, we have developed a new method of quantifying the biomass accumulated in wound dressings by incubating them on pathogenic biofilms and sample the biomass afterwards. To this end, we established an appropriate protocol based on biofilms grown in a drip-flow reactor. We also added antimicrobial agents to the dressing material to explore ways of addressing the infection problem by preventing the immigration of bacterial pathogens into the wound dressing in the first place. Using this procedure, we demonstrated that the number of colony-forming units of species such as *Pseudomonas aeruginosa* and

*Staphylococcus aureus* re-isolated from treated wound dressings could be reduced by several orders of magnitude. The established to investigate bacterial behavior in wound dressings could help develop dressings that are better suited to avoiding infections caused by pathogens in everyday clinical practice.

#### P-MC-006

##### Microbial interactions in a high-performance tubular foam-bed reactor for biomethanation

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Biomethanation can be used to convert excess electrical energy into storable biomethane. Recently, a lab-scale high-performance tubular foam-bed reactor (TFBR) was developed to increase gas transfer which is a major limitation of this process. The addition of foam-stabilizing detergents to a microbial community (MC) consisting of methanogenic archaea and associated bacteria is thought to induce stress and trigger adaptation within the MC. To investigate this adaptation, the MC of the TFBR was analyzed, focusing on both structural and functional characteristics. Combined metagenomics and metaproteomics were applied to analyze the MC of TFBR.

The MC consisted of three archaeal MAGs assigned to *Methanobacteriaceae* (40%) and 60 % bacterial MAGs, predominantly *Thiopseudomonas* (12 %), *Alkaligenes* (5%), *Natronincolaceae* (5%), *Massilibacterium* (4%), *Petrimonas* (3%). The presence of bacteria, such as *Petrimonas*, feeding on archaeal biomass was observed in the stirred tank reactor for biomethanation and was further supported by the detection of extracellular hydrolases and proteases. However, the high abundance of *Thiopseudomonas* and *Alkaligenes* was surprising. Highly expressed proteins involved in oxidative phosphorylation, including complex IV of respiratory chain, implement metabolism using O<sub>2</sub> as electron acceptor. The unexpected presence of O<sub>2</sub> was confirmed by high expression of enzymes responsible for detoxifying reactive oxygen species (ROS) in most of the members of the MC. Although H<sub>2</sub> produced by PEM electrolysis is considered to be very pure, the high abundance *Thiopseudomonas* and *Alkaligenes* clearly indicated a continuous contamination with O<sub>2</sub>. The oxidative metabolism *Thiopseudomonas* and *Alkaligenes* scavenged most O<sub>2</sub> protecting the strict anaerobic archaea from damage by ROS.

The oxidative metabolism of *Thiopseudomonas* and *Alkaligenes* protected the methanogenic archaea from damage by ROS by scavenging O<sub>2</sub> from H<sub>2</sub> supply. This process might be crucial for process stability when operating biomethanation as an industrial process.

#### P-MC-007

## Quantification of an anaerobic synthetic co-culture containing *Acetobacterium woodii* and *Clostridium drakei*

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Microbial gas fermentation offers a way to produce platform chemicals, such as mid-chain fatty acids, while using CO<sub>2</sub> as a carbon source. This study examines a synthetic co-culture of *A. woodii* and *C. drakei* that together produce butyrate and hexanoate through gas fermentation. Typically, acetate is the main product of *A. woodii*, generated from H<sub>2</sub>+CO<sub>2</sub>. The strain *A. woodii* LDHD was engineered to extend its product spectrum to make lactate by introducing a recombinant D-lactate dehydrogenase (LDHD) from *L. mesenteroides*. A further boost of lactate production should be mediated by expression of a pyruvate formate lyase (PFL) from *C. pasteurianum* in a newly constructed strain termed *A. woodii* LDHD\_PFL. These *A. woodii* strains supply lactate as substrate for *C. drakei* FP, which produces butyrate and hexanoate via reverse  $\beta$ -oxidation. One challenge in evolving a synthetic co-culture is adjusting an appropriate inoculation strategy to maximize product yields and maintain balanced growth. Therefore, cell monitoring of each species is crucial during co-cultivation.

One way to quantify the cells of each species is a quantitative PCR using gDNA. Therefore, DNA fragments of specific genes (encoding FdhF2 subunit of *A. woodii*'s hydrogen-dependent CO<sub>2</sub> reductase and phosphate butyryltransferase of *C. drakei* FP) were amplified. Two typical exponential growth phases of *C. drakei* FP were identified during growth on lactate and H<sub>2</sub>+CO<sub>2</sub>. A strong correlation was found between c<sub>q</sub> and OD<sub>600</sub> values in both growth phases (1<sup>st</sup> exp. growth phase R<sup>2</sup> = 0.95; 2<sup>nd</sup> exp. growth phase R<sup>2</sup> = 0.90). Lactate was consumed during the first growth phase of *C. drakei* FP. The respective correlation for *A. woodii* growth was determined, resulting in an R<sup>2</sup> of 0.87.

Two co-culture experiments were done with both *A. woodii* strains, but with different *A. woodii* and *C. drakei* FP start OD<sub>600</sub> ratios. First, the OD<sub>600</sub> start ratios for *A. woodii* LDHD\_PFL and *C. drakei* FP were 7:1. Second, *A. woodii* LDHD and *C. drakei* FP were inoculated with a ratio of 1:1. Surprisingly, the co-culture inoculated with the 7:1 ratio of *A. woodii* and *C. drakei* yielded higher butyrate and hexanoate titers compared to the 1:1 ratio co-culture experiment. Both co-cultures were successfully quantified via qPCR. The *C. drakei* share during growth stabilized between 20% and 40%. The balance of *C. drakei* FP share was approved for one growth experiment via fluorescence in situ hybridization, and validates the quantification via qPCR.

### P-MC-008

#### The Role of Bacterial Aggregation in the Intestinal Microbiota

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Bacterial aggregation, occurring in single or multi-species communities, is a widespread phenomenon with potential ecological significance, suggesting an important role within human-associated microbiomes. In competitive

environments such as the gut, aggregation may influence bacterial survival, spatial distribution, and interactions with the host. However, the physiological and ecological functions of bacterial aggregates in the intestinal environment remain poorly understood. In particular, how aggregation affects microbial flow and distribution in the dynamic gut environment is largely unexplored.

To address this, we isolated Enterobacteriaceae from fecal samples of patients in intensive care units (study: MS-ICU), who had received meropenem, piperacillin/tazobactam, or no antibiotics. A total of 75 isolates were characterized for aggregation capacity, growth rates, biofilm formation, and antibiotic resistance or tolerance. The influence of bile acids, key modulators of gut microbial composition, on these phenotypes was also assessed. In addition, we tested whether aggregation alters host immune responses. To explore the impact of aggregation under flow conditions resembling the gut, we applied microfluidic assays, allowing controlled observation of aggregate formation and dispersion.

Our results reveal considerable variability in aggregation among isolates, strongly modulated by bile acids. Interestingly, and contrary to previous assumptions, aggregation was negatively correlated with biofilm formation and showed no association with increased antibiotic tolerance or resistance. Bacterial aggregates did not provide protection against the host immune system; rather, they could elicit strong immune responses and were not consistently shielded from phagocytosis. Microfluidics experiments further suggest that aggregation may influence bacterial flow and spatial dynamics, although detailed analysis is ongoing.

Overall, these findings challenge common assumptions regarding the functional benefits of bacterial aggregation and emphasize its potential role in shaping microbial distribution and dynamics. Understanding how aggregation affects bacterial flow may provide important insights into the recovery and stabilization of a balanced intestinal microbiota following disturbances such as disease or antibiotic treatment. These insights can help support strategies to enhance microbiome resilience in vulnerable patient populations.

### P-MC-009

#### Synthetic ecology as playground to evaluate stability and resilience in defined but complex microbial communities

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Synthetic microbial communities provide a key strategy for the development and testing of new ecological models. Microbial interactions are extremely diverse, they encompass competition for substrates; chemical signaling for partnership/antibiosis and can be driven by environmental fluctuations that are hard to dissect in fully complex microbiomes. In this work, a semi-complex community of 18 defined microbes was assembled to generate data for community balance – disbalance – rebalance transitions, as basis for theory building and better access to modeling of natural communities. For this purpose, 3-parallel chemostats

were inoculated with 18 strains comprising core isolated members of the groundwater microbiome from Hainich National Forest, Thuringia (Germany) selected for a diverse phylogeny and range in niche breadth score range. A fully defined medium of 64 components allows for elucidation of nutrient preferences. The co-culture inoculum was prepared based on the individual growth rates of the strains in precultures to allow all strains to have the same chance to thrive in a batch phase operated for 48 h. The chemostat phase was operated at a dilution rate matching  $\approx 70\%$  of the growth rate of the slowest member to avoid loss of strains due to physical washout. Chemostats were operated identically for 2 weeks, thereafter, two fermenters were subjected to temporary or permanent salt stress to trigger balance/disbalance experiments. Population dynamics was resolved with metagenomic analysis (shotgun sequencing), which confirmed that a stable semi-complex community was assembled without stress. Although no changes were observed upon short-term salt stress pulses (1 hydraulic retention time, HRT), osmotic stress for 6 HRTs led to a shift in community composition, which however was reversible to the original community composition upon release of the stress. We are currently deciphering the individual function of the community members dominating during the non-stress and stress state. These results highlight the utility of synthetic community studies to gain insight into functional roles of microbes that can drive stability and resilience in microbiomes.

## P-MC-011

### A soil-analogue microcosm to validate mechanism of microbial dispersal

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## Introduction

Soil is opaque and structurally complex, which hinders observation of microbial dispersal in the natural environment of these communities. Most often studies used culture-based platforms to study translocation of microbes, yet invariably such approaches neglect the high diversity of microbes found in soil. With our new approach we aim to assess fungal–bacterial interactions in real soils, with a focus on fungal hyphae–mediated transport (FHMT) where bacteria use fungal hyphae as travel routes through soil.

## Goals

1. Design and test a contamination-resistant soil microcosm for tracking community translocation
2. Demonstrate how the system enables mechanistic validation of FHMT in natural communities

## Materials & Methods

PVC columns were set up comprising a source chamber containing a soil sample (sterile quartz sand for controls) and a target chamber containing initially sterilized quartz sand. These chambers were separated by a steel mesh (pore size = 70  $\mu\text{m}$ ) and 2 types of sintered borosilicate glass frits (5 mm thickness and max. pore size 250 and 100  $\mu\text{m}$ , respectively) providing an increasing complexity to pass for microbes. Gas exchange was ensured by sterile 0.2  $\mu\text{m}$  filters that also inhibited back-contamination by air exchange. The target chamber was sampled at a 1-cm resolution,

microbial abundances and community profiles measured by qPCR and amplicon sequencing.

## Results

Higher fungal and bacterial gene copies ( $10^1$ – $10^2$  copies per gram sand) were measured when soil was connected to initially sterile sand compared to controls (below detection limit), confirming passage through the barrier. With increasing complexity of the barrier, fewer gene copies were detected. A diverse fungal community entered the target chamber, while a limited set of bacterial genera appeared. Target chamber communities differed from the source community, consistent with selective translocation rather than bulk cell leakage.

## Summary

Our experimental system enables studies on microbial translocation under controlled laboratory conditions, while mimicking key features of a real soil environment. The design opens avenues to quantify distance-decay and colonization rates across centimeter scales, while enabling control of experimental factors like soil moisture, structure, chemistry and nutrient availability. Overall, the system advances experimental soil ecology by retaining soil-like constraints without the oversimplifications of culture-based platforms.

## P-MC-012

### ProBioHuhn: Dynamic changes in the organic chicken gut microbiome driven by genetic and environmental variation

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The intestinal microbiota plays a vital role in chicken performance and health [1]. However, our understanding of the organic chickens gut microbiome remains limited. This study aimed to characterize the gut microbiota of organic meat chickens in Germany. Three fattening types were included (slow-growing broilers, dual-purpose cockerels, and male layers), with five sampling time points throughout their lifespan. Meconium samples were collected on the first day of life directly from transport boxes. Cloacal swab samples were collected at four subsequent time points: approximately 12 and 30 days of age (indoor phase), two weeks after gaining outdoor access, and at the end of the fattening period. The V1-V2 region of the 16S rRNA gene was sequenced and analyzed. QIIME2 and the R programming language were used for bioinformatic analysis. The results revealed that alpha diversity was significantly different between meconium and other time points (cloacal samples) and between periods when chickens were housed indoors versus those with outdoor access. The hatchery was the main factor in shaping the initial microbiota. Farm and fattening type gained importance over time. Outdoor access appears to enrich the gut microbiome with diverse environmental and animal-associated bacteria, including potential pathogens, while chickens kept indoors before 30 days of age correlates with a higher relative abundance of specialized anaerobic gut microbes involved in energy metabolism. During outdoor access periods, the type of housing system was also identified as an influencing factor. Mobile stables tended to have higher alpha diversity compared to stationary stables. This research provides novel insights into the gut microbiota of organic chickens,

indicating the diverse factors that influence it at different life stages and under different management practices.

[1] Broom LJ, Kogut MH 2019. Anim. Feed. Sci. Technol. 204:44–51.

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#### P-MC-013

##### Regulation of the orsellinic acid gene cluster in *Aspergillus nidulans*

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Fungi possess a remarkable potential for producing a diverse array of pharmacologically active compounds. Genome sequencing has shown that many antibiotic-producing fungi contain far more gene clusters for natural products than previously thought. However, some of these genes remain inactive under standard laboratory conditions, representing a largely unexplored reservoir for novel drug discovery. *Aspergillus nidulans*' orsellinic acid gene cluster remains silent under laboratory conditions. Co-cultivation of *Aspergillus nidulans* with bacteria from the genus *Streptomyces* triggers the production of orsellinic acid in *Aspergillus nidulans* by modifying its epigenetic landscape. The SAGA/ADA complex in *Aspergillus nidulans* acetylates histones in the *ors* gene cluster, enabling the activation of the transcription factor BasR and the subsequent production of orsellinic acid. Due to the pivotal role that BasR plays in the activation of the *ors* gene cluster, we further investigated the mechanisms of activation of the *ors* gene cluster by BasR. By conducting DNA-pulldown assays, we identified a putative binding sequence for BasR in the orsellinic acid gene cluster. Additionally, through expression analysis and LC-MS studies, we found that SirE is the HDAC that represses the expression of the *ors* gene cluster. Our data shed light on the molecular regulation of bacterial–fungal interactions and reveal the complex epigenetic mechanisms underlying these processes.

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#### P-MC-014

##### Insights into the microbial community of parchment

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Written artefacts made from parchment represent an important part of our cultural heritage. Since the collagen structure of parchment can deteriorate under various conditions, the preservation of these historically and culturally valuable documents has become a major challenge. In addition to environmental conditions, the influence of microorganisms is considered to be the main cause of deterioration. The biological decomposition of parchment is due to the activity of microorganisms with extracellular enzymes that enable them to metabolically break down proteins. The organisms with these abilities are able to degrade the collagen fibers of parchment through hydrolysis with collagenases.

In order to better counteract microbial decay, it is important to gain an understanding of the microbiota on parchment. Therefore, 16 samples of parchment showing symptoms of

decay were analyzed by Illumina amplicon sequencing to examine the microbial community, with a special focus on microorganisms that could be involved in degradation. The five most abundant genera, in each case of fungi and bacteria, are represented by *Aspergillus*, *Alternaria*, *Plectosphaerella*, *Wallemia*, and *Fusarium*, as well as by *Bacillus*, *Skermanella*, *Streptococcus*, *Metabacillus*, and *Cytobacillus*. The key organisms that can contribute to the decay of written artefacts are those that can form collagenases. The main genera in this context, *Bacillus* and *Clostridium*, were both found within the microbiota of the parchment samples analyzed, notably with *Bacillus* even being the most common genus. The investigation underlines the importance of understanding the microbiota of parchment in order to counteract the microbial decay of parchment.

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#### P-MC-015

##### Engineering cross-kingdom synthetic microbial communities powered by cyanobacteria

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In nature, different microorganisms form highly complex communities in which every species has a distinct role. To increase our understanding of the complex structures and interactions within microbial communities and the role of each microbial partner, the design and analysis of simplified microbial model communities is essential.

In this collaborative project, our goal is the *de novo* design of a cross-kingdom synthetic microbial community based on the well characterized and genetically amenable model organisms *Synechococcus elongatus* PCC 7942 (cyanobacterium), *Ustilago maydis* (basidiomycete fungus) and *Saccharomyces cerevisiae* (ascomycete yeast).

Co-cultivation is based on the carbon source sucrose, which is produced by the phototrophic cyanobacterium using light and CO<sub>2</sub>. Sucrose secretion into the culture medium is achieved by IPTG-inducible, heterologous expression of the sucrose permease gene *cscB* (H<sup>+</sup>-symporter from *Escherichia coli*) combined with the sucrose phosphate synthase gene *sps* (from *Synechocystis* sp. PCC 6803) in *S. elongatus*.<sup>[1]</sup> This approach can be combined with higher osmolarity and pH to increase sucrose production and secretion.

In this project we investigate the effects of pH and osmolarity on the sucrose production of *S. elongatus* *sps cscB* in axenic cultures and the phototroph-to-heterotroph ratio in co-cultures under controlled conditions. In order to do this, we established turbidostat cultivation with pH control in a photobioreactor set up as well as single-cell flow cytometry analysis for quantification of individual populations within the co-culture.<sup>[2]</sup> We are also exploring fluorescent protein biosensor approaches to track sucrose production and availability in cyanobacteria.

Exploiting these established tools, we created a stable cross-kingdom synthetic microbial community which we further use to characterize the nutrient exchange in microbial consortia with a special focus on carbon economics and logistics.

[1] Abramson, B.W., Kachel, B., Kramer, D.M., and Ducat, D.C. (2016). Increased Photochemical Efficiency in Cyanobacteria via an Engineered Sucrose Sink. *Plant Cell Physiol.* 57, 2451–2460.

[2] Hasenklever, D., Pohlentz, J.C., Berwanger, T., Kokarakis, E.J., Hassan, T., Schipper, K., Matuszyńska, A., Axmann, I.M., and Ducat, D.C. (2024). Assembly and quantification of co-cultures combining heterotrophic yeast with phototrophic sugar-secreting Cyanobacteria. *J. Vis. Exp.*, e67311. <https://doi.org/10.3791/67311>.

## P-MC-017

### Microbial and hepatic metabolism of sucralose and sucralose-6-acetate

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Sucralose is one of the most widely used high-intensity sweeteners, long considered chemically stable and biologically inert. However, recent analytical studies have identified measurable levels of contamination in commercial sucralose of its chlorinated intermediate, sucralose-6-acetate (S6A), at concentrations of 0.7-1.3% (Schiffman *et al.*, 2023). Of particular concern, *in vivo* data suggest that S6A may not only occur as a contaminant but can also be formed endogenously after sucralose ingestion through human-dependent acetylation reactions (Bornemann *et al.*, 2018). Given its reported genotoxic potential and interference with xenobiotic-metabolizing enzymes, the presence and possible endogenous formation of S6A raise additional risks regarding the safety profile of sucralose (Schiffman *et al.*, 2023). This study investigated the endogenous metabolic fate of sucralose, focusing on the potential formation and degradation of toxicologically relevant metabolites across human and rat microbial gut models (ileum and colon), as well as liver preparations. Therefore, *in vitro* bacterial gut models were incubated anaerobically with physiologically relevant concentrations of sucralose (5 mM) or S6A (100-300 µM). Both compounds were non-cytotoxic up to 5 mM sucralose and 0.5 mM S6A, confirming full microbial viability. Quantification of sucralose and S6A was performed using a validated LC-MS/MS method (LOD ≤ 0.113 µM). In a complementary approach, human and rat liver cytosol was analyzed to evaluate potential enzymatic acetylation of sucralose. Across all microbiome models, no microbial acetylation of sucralose to S6A was detected. Sucralose remained chemically and biologically stable in bacterial models representing the microbiome of the small and large-intestine. In contrast, S6A was rapidly degraded in bacterial colon models, with markedly faster kinetics in human compared to rat microbiota (t<sub>1/2</sub> human: 0.7-1.1 h; rat: 1.5-2.2 h). Preliminary experiments with human liver cytosol did not indicate any enzymatic formation of S6A, suggesting that hepatic acetylation is unlikely under physiological conditions. Overall, the data demonstrate that sucralose is chemically and biologically stable in all tested gut models, with no evidence of microbial transformation. In contrast, S6A was rapidly degraded by the colonic microbiota, particularly in human models, highlighting a potential detoxification role of the intestinal community.

## P-MC-018

### Cloud microbiomes reveal complex bacterial defense and viral interactions

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## Introduction

Bacteria and their viruses are engaged in an ongoing arms race, driving the development of sophisticated bacterial defense mechanisms. However, host-virus interactions and the full range of bacterial defense mechanisms remain poorly understood, especially in extreme environments like clouds, which mimic extraterrestrial conditions such as extreme temperatures, limited nutrients, and high ultraviolet radiation.

## Goal

This study focused on the metagenomic profiling of the cloudwater microbiome, aiming to characterize defense and antidefense systems in isolated bacterial strains, metagenome-assembled genomes (MAGs), and mobile genetic elements (MGEs).

## Methods

DNA from cloudwater samples collected on Mount Verde in the tropical Atlantic Ocean, along with bacterial isolates, was subjected to (meta)genomic sequencing and analysis.

## Results

Cold-shock protein-encoding genes were present in both bacterial and viral genomes, potentially aiding survival in freezing cloudwater conditions. In bacteria, the number of cold-shock proteins showed a positive correlation with genome size and the number of coding sequences, suggesting adaptations to cold environments. The presence of 24 prophages and a diverse arsenal of antiviral defense systems, including restriction modification systems and adaptive CRISPR immunity targeting viral operational taxonomic units (vOTUs) of the sample, indicates ongoing bacterial-viral interactions in cloudwater. A prophage from *Curtobacterium nubigenum* MPC39 could be triggered into the lytic cycle and observed in electron microscopy. UV stress tests on this strain revealed resistance of up to 24 h of exposure to 305 nm UV light, with an 18% reduction in live cells. Metagenomic sequencing identified 458 vOTUs, with the major bacterial host genus predicted as *Sphingomonas* spp. The isolate genome of *Sphingomonas panni* MPC37 encoded a significant number of defense systems (10), indicating its potential interactions with viruses and ecological significance.

The presence of (anti) defense systems in MGEs elucidates the complexity of microbial and viral survival strategies in these extreme environments.

## Conclusion

Our findings show that clouds are dynamic systems harboring complex microbial interactions and diverse defense strategies, providing insights into analogous processes in extraterrestrial environments.

## P-MC-019

### **Angomonas deanei - A rising star in endosymbiosis research enables the dissection of molecular mechanisms underlying the host/endosymbiont interaction using advanced genetic tools**

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**Introduction:** The trypanosomatid *Angomonas deanei* contains a vertically transmitted, genomically reduced  $\beta$ -proteobacterial endosymbiont that supplies its host with various co-factors and metabolic intermediates. Interestingly, each *A. deanei* cell contains only a single endosymbiont that grows and divides in synchrony with the host. Proteomic studies as well as the development of protocols to express transgenes and generate targeted gene knock-outs in the host genome enabled the identification of endosymbiont-targeted host proteins that provide the host with nuclear control over endosymbiont division. However, experimental work with this emerging model system for endosymbiosis research is still heavily restricted by the lack of conditional gene expression systems.

**Goal:** In order to facilitate functional studies of essential genes, we aim for the establishment of conditional knock-out systems where expression of the gene of interest is controlled by the application of certain chemical or physical inducers.

**Methods & results:** To this end, we generated an *A. deanei* strain that expresses the T7 RNA polymerase (T7RNAP) from an endogenous expression locus. Sufficient production and functionality of the T7RNAP were confirmed using reporter genes flanked by T7 transcriptional elements and integrated into originally silent loci. We then exchanged the constitutive T7RNAP with a blue light-inducible variant and assessed the light-dependent expression of the reporter gene. In addition, we integrated TetR as transcriptional regulator and optimized its functionality. Finally, we employed Cas9 as an alternative approach to generate conditional knockout mutants.

**Summary:** *A. deanei* is an emerging model system to study endosymbiont integration. The conditional knock-out system, that enables now inducible perturbations of the system, will be key for the in-depth exploration of the host-endosymbiont interaction, and, in particular, to study the cellular functions of essential endosymbiont-targeted proteins.

## P-MC-020

### **Modelling Disease-Associated Microbiome Changes *In Vitro* Using MyBioScope**

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## Introduction

The gut microbiota plays essential roles in nutrient metabolism, immune regulation, and overall host physiology. However, its precise contributions to disease development and progression remain poorly defined. A major limitation in microbiome research is the lack of physiologically relevant and reproducible *in vitro* models that can replicate the complex and dynamic environment of the human gastrointestinal (GI) tract.

## Goals

To address this challenge, we developed MyBioScope, a human stool-based *in vitro* model utilizing the DASbox<sup>®</sup> mini bioreactor system. This platform recreates the anaerobic environment of the GI tract, enabling controlled, scalable, and reproducible studies of microbial dynamics and metabolism under disease-relevant conditions.

## Materials & Methods

In MyBioScope, bioreactors (120–200 mL) are inoculated with human stool slurry, stabilized for 24 hours, and maintained with customizable feeding regimens for multi-day cultivation. The system allows precise modulation of environmental factors such as nutrient supply and microbial perturbations. Microbial composition and metabolic activity are assessed through samples collected every 24 hours and analyzed using 16S rRNA sequencing, qPCR, and metabolomics to capture both community-level and functional responses.

## Results

MyBioScope's versatility was validated through pilot studies modelling distinct disease-relevant microbiome alterations. In a proton pump inhibitor-induced oralization model, daily spiking with oral bacteria reproduced progressive enrichment of *Veillonella parvula* and *Streptococcus salivarius*. Supplementation with a multispecies probiotic could reduce *V. parvula* abundance in the bioreactors, which could be reproduced in a clinical pilot trial. Healthy microbiomes remained significantly distinct from microbiomes derived from patients with sarcopenia throughout cultivation. L-glutamine supplementation in microbiomes from patients with sarcopenia increased formic, lactic, and succinic acids, galactose, and glutamine, while decreasing acetic and fumaric acids; these effects were not observed in healthy controls.

## Summary

In conclusion, MyBioScope enables reproducible, controlled studies of gut microbial dynamics and provides a scalable

tool for investigating disease-specific microbiome changes. This platform supports translational efforts to integrate microbiome insights into clinical research, therapeutic development, and personalized medicine.

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#### P-MC-021

##### **Capture of viable bacteria from liquid samples to enhance sensitivity of classical microbiological methods**

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Culturing of bacteria and fungi is a cost-efficient and well-established method for environmental monitoring, food & water safety testing, as well as clinical diagnostics. However, the methodology is limited by the sample volume that can be applied to the growth medium. This bottleneck is currently addressed by various concentration methods such as filtration and centrifugation protocols. In food testing it is circumvented by pre-culturing in liquid growth media. Each of these methods come with certain disadvantages.

The goal of this project is to develop a fast and user-friendly method to concentrate live bacteria from large volumes. Our aim is to capture bacteria with high cell release and survival rate without the use of filtration or centrifugation.

We implemented a workflow utilizing buffer-optimized magnetic separation. The performance of this workflow was tested using both Gram-positive and Gram-negative bacteria and capture efficiency was determined by comparing colony counts of plated bacterial populations before and after the capture process.

Preliminary results indicate a near-complete recovery of Gram-positive bacteria, showing strong potential for high-efficiency live bacterial capture. Ongoing work focuses on optimization of Gram-negative bacterial capture and maintaining viability across all bacterial types using common buffer conditions.

These findings demonstrate the feasibility of our approach to recover live bacteria from large-volume samples and even if present in very low concentration. With further optimization, this method holds significant potential for broad applications in rapid and accessible pathogen detection, microbiome studies, and various microbiological analyses where maintaining bacterial viability is essential and where classical microbiology's sensitivity is currently hampered by insufficient sensitivity.

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#### P-MC-022

##### **Soil Microaggregates as Microniches in Antarctic Soils: Effects of Aggregate Size and Structure on Microbial Community Composition**

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Soil microaggregates are among the most stable structural building blocks of soils and among the first to form during pedogenesis. They provide protected microhabitats for microorganisms and act as hotspots for early biogeochemical transformations. To explore the mutual

relationship between microorganisms and soil structure, we aim to understand how microbes influence aggregate formation and, in turn, how aggregate size and structure influence microbial community assembly in initial soils.

Bulk soil from Livingston Island, Antarctica, was fractionated through slaking, sieving, ultrasonic dispersion, and centrifugation to separate free and occluded microaggregates of defined size classes (<20 µm, 53–20 µm, and 250–53 µm). DNA was extracted using the Qiagen PowerSoil Pro Kit, and 16S rRNA gene sequencing was conducted for all fractions.

Across all samples, microbial communities were dominated by *Actinomycetota*, *Pseudomonadota*, and *Chloroflexota*, with *Acidobacteriota*, *Gemmatimonadota*, and *Verrucomicrobiota* also consistently present. Alpha diversity (Shannon index) was highest in free aggregates, indicating that enclosure within larger aggregates restricts microbial diversity. Beta diversity (Bray–Curtis dissimilarity) revealed clear clustering by both aggregate size and structural fraction.

Specialist–generalist analysis identified generalists mainly from the dominant phyla, indicating broad ecological tolerance across habitats. Interestingly, only the < 20 µm fraction harbored specialist ASVs (15 in total), including members of *Streptomyces* and *Sphingomonas*, taxa known for exopolysaccharide (EPS) production and involvement in organic matter turnover.

The occurrence of these EPS producers highlights that microorganism may actively contribute to early microaggregate formation and stabilization. Furthermore, differences in alpha and beta diversity across fractions underline the reciprocal relationship between soil structure and microbial communities, where aggregate architecture both shapes and is shaped by microbial activity.

Ongoing analyses of C, N, and S contents, elemental composition, pH, and enzyme activities, as well as assessments of the functional potential based on 16S data and forthcoming 18S rRNA analyses, will further clarify not only microbial specialization but also the functional roles microorganisms play in the formation and stabilization of soil aggregates during early soil development.

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#### P-MC-023

##### **Microbiome richness varies among phytoplankton species of different sizes**

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One of the general laws of ecology is the island-species-area relationship: larger islands harbor more species than smaller ones. In this study, we isolated 13 phytoplankton species of different sizes from Port Hacking, Australia, and analyzed the richness of their associated microbiomes.

Indeed, we found higher microbiome richness associated with larger phytoplankton species, confirming the ubiquity of the island-species-area relationship. However, the slope of this relationship was lower than that observed in other habitats involving aquatic bacteria. To investigate this further, we applied a model simulating phytoplankton-bacteria



interactions, and identified several mechanisms contributing to the lower slope:

i) reduced release of DOM from the phycosphere of larger phytoplankton cells, caused by more bacteria taking up more DOM in the phycosphere, which decreases encounter rates of chemotactic bacteria; and

ii) a lower probability of "naked" large phytoplankton cells (i.e., cells without attached bacteria) after cell division, i.e. less space and substrate for new attachments;

Finally, we discuss factors that may have influenced our findings, such as differences between single-cell and population-level microbiomes, and place the observed patterns within a broader theoretical context.

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## P-MC-024

### Dual-species interaction provides protection of *Campylobacter* against several ribosome-targeting antibiotics

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#### Introduction and Goals

*Campylobacter* is a major food-borne pathogen. In severe cases, treatment is recommended by fluoroquinolone (FQ) and macrolide antibiotics. However, high levels of FQ resistance leave macrolides as the only other first-line treatment option. Within the framework of *Campylobacter* surveillance in Germany, we investigated multidrug resistance (MDR) towards FQ and macrolides of clinical *Campylobacter* from 2010 to 2022.

#### Methods

6950 samples were single-colony purified and analyzed for antimicrobial resistance phenotypes. In 2912 cases, genomes were sequenced and screened for antimicrobial resistance genes. Cultures which showed MDR were analyzed for contaminating DNA and studied using scanning electron microscopy (SEM).

#### Results

451 (6.5 %) *Campylobacter* samples were resistant to FQ (FQR) and macrolides, and two *C. jejuni* samples were even resistant to antibiotics from ten different classes. Genome analysis revealed that the two samples, although derived from single colonies, contained more than 10% of *Enterococcus* DNA reads. SEM confirmed the presence of coccoid bacteria alongside to spiral-shaped *Campylobacter*. In both cases, culture-based purification steps separated FQR but not macrolide resistant *Campylobacter* from *Enterococcus* displaying MDR (including macrolide resistance). This suggests that the macrolide resistance in *Campylobacter* was caused by intimate interaction with an *Enterococcus* strain showing MDR. Overall, genome analysis detected contaminating bacterial species in 19 samples (0.7 %).

#### Conclusion

MDR phenotypes in *Campylobacter* cultures may be caused by dual-species interaction with other highly resistant intestinal bacteria, such as MDR Enterococci. We postulate that protection of *Campylobacter* by *Enterococcus* is provided extrinsically.

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## P-MC-025

### Unwanted guests: Quantification and identification of bacteria on self-ordering touchscreens in fast food restaurants at times of high and low occupancy

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**Introduction:** Surfaces in public spaces can act as fomites (1). Interestingly, the microbial contamination of self-ordering touchscreens in fast food restaurants has not yet been thoroughly investigated, even though food is typically consumed with bare hands shortly after ordering.

**Goals:** To assess the hygienic relevance of bacterial contamination on self-ordering touchscreens, we surface sampled ordering terminals from three different fast food restaurants in the greater area of Villingen-Schwenningen, Germany (2).

**Materials & Methods:** Self-ordering touchscreens were analyzed using RODAC surface sampling, followed by identification of dominant morphotypes via MALDI-TOF MS. Touchscreens from three different fast food restaurants were sampled repeatedly (n = 5) during periods of low ( $\leq 5$  guests) and high occupancy ( $\geq 20$  guests).

**Results:** Sampling with VRBG plates revealed no contamination with enteric bacteria, while sampling with TSA plates showed contamination for 28 out of 30 (93.3 %) investigated touchscreens. Bacterial loads ranged from 0.08 to 6.4 CFU/cm<sup>2</sup>, with median bacterial contamination levels being 2.8 to 7.5 times higher during high occupancy periods compared to low occupancy. Interestingly, the germ counts seem to be lower than those previously reported for conventional (paper) restaurant menus, where levels of up to 100 CFU/cm<sup>2</sup> of aerobic bacteria have been observed (3). Across all samples, 126 isolates were identified at least on genus level. The dominating genera were *Staphylococcus* (60.3 %) and *Micrococcus* (23 %), which indicates human skin microbiota as the primary source of contamination.

**Summary:** These findings highlight the importance of regular touchscreen cleaning and proper hand hygiene before meals, particularly during times of high customer occupancy.

#### References

1. Stephens et al. (2019), Curr Pollut Rep. 2019;5(4):198–213.
2. Schwendenmann et al. (2025), BMC Res. Notes. submitted.
3. Sirsat et al. (2013), J Environ Health. 2013 Mar;75(7):8–14; quiz 54.

## P-MC-026

### Application of DNA-SIP on sulfoquinovose-degrading bacterial communities in forest soil

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Elucidating microbial degradation of sulfoquinovose (SQ) in natural ecosystems is essential to understand its role in the biogeochemical sulfur and carbon cycling. SQ is a plant-derived sulfonated sugar and one of the most abundant organic sulfur compounds [1]. In the recent years, several biochemical pathways for SQ degradation have been revealed in laboratory model bacteria, yet little is known about the microbes catalyzing SQ degradation in the natural environment [2, 3, 4, 5]. In this study, methods such as respirometry, cavity ring-down spectroscopy (CRDS), liquid chromatography-mass spectrometry (LC-MS) and DNA-stable isotope probing (DNA-SIP) were combined, in an attempt to track the mineralization and assimilation of fully <sup>13</sup>C-labeled SQ in forest soil. By incorporation of the <sup>13</sup>C into DNA, the responsible microbes for SQ degradation may be identified. Initial respirometry and CRDS experiments using unlabeled SQ revealed total mineralization of 44% of the SQ-carbon to CO<sub>2</sub> after 50 hours. LC-MS analysis indicated conversion of SQ to known organosulfonate intermediates, and their subsequent turnover, suggesting a complex consortium involving primary and secondary SQ degraders. In order to identify likely SQ-degrading taxa (amplicon sequence variants, ASVs), DNA-SIP and subsequent Illumina sequencing of 16S-rRNA gene amplicons are being performed. In conclusion, this study thus far established a new combination of respirometry and CRDS to "online" track the dynamics of mineralization of <sup>13</sup>C-labeled substrates in complex microbial communities, such as forest soil. This approach will allow for the determination of optimal sampling time point(s) for DNA-SIP analysis. In future, this combination of methods will greatly facilitate linking microbial taxa to biodegradation processes in natural environments.

#### References:

- [1] C. Benning, *Annu. Rev. Plant Physiol. Mol. Biol.*, **1998**, 49, 53-75.
- [2] K. Denger *et al.*, *Nature*, **2014**, 507,7490: 114-7.
- [3] A.K. Felux *et al.*, *Proceedings of the National Academy of Sciences of the United States of America*, **2015**, 112,31: E4298-305.
- [4] Y. Liu *et al.*, *Biochemical and biophysical research communications*, **2021**, 533,4: 1109-1114.
- [5] M. Sharma *et al.*, *Proceedings of the National Academy of Sciences of the United States of America*, **2022**, 119,4: e2116022119

## P-MC-027

### Artificial Leaves: A Novel System for Studying Microbial Interactions on Leaf Surfaces

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Natural leaf surfaces represent complex, heterogeneous habitats for microbial colonization. Their variability in nutrient content, surface chemistry, and microstructure complicates controlled experimentation and the generalization of microbial behavior. This complexity limits current understanding of bacterial aggregation, colonization, and survival on plant leaves. To address this within agriculturally relevant contexts, our work focuses on fruit trees of major economic importance, including apple, plum, peach, pawpaw, cherry, and apricot.

This study aims to develop and validate an artificial leaf system that replicates the physical microtopography of natural leaves while eliminating biological variability. The system offers a controlled platform to study how surface structure alone influences microbial colonization and persistence in the phyllosphere.

Artificial leaves were produced using a two-step casting technique with polydimethylsiloxane (PDMS), based on high-resolution 3D reconstructions of the sampled leaf surfaces. The diffusion properties of PDMS were evaluated using bacterial bioreporters, revealing that water and small polar molecules can permeate the surface, similar to the natural leaf cuticle. Bacterial populations maintained viability for up to 14 days on these artificial surfaces, enabling analysis of the spatial and temporal dynamics of microbial communities over time.

This biomimetic leaf model enables precise and reproducible studies of microbial behavior on plant-relevant surfaces. By isolating topographical effects, it supports mechanistic insights into bacterial adhesion, arrangement, and persistence. The platform offers potential applications for breeding pathogen-resistant crops, improving sanitation strategies, and designing bioinspired antimicrobial materials, thereby advancing research in microbial ecology, food safety, and sustainable agriculture.

## P-MC-028

### Quantitative Insights and Visualization of Antimicrobial Tolerance in Mixed-Species Biofilms

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**Introduction:** Biofilms are a major problem in hard-to-heal wounds. They are composed of different species and are often tolerant to antimicrobial agents. At the same time, interspecific synergy and/or competition occurs when some bacterial species clash.

**Goals:** Regardless of bacterial coexistence in wound biofilm of chronic wounds, the question arises whether bacteria in a biofilm with multiple species become more tolerant to antimicrobial agents and wound dressings containing active ingredients. Under this challenge, dominance of one species

over another could be enhanced, or circumstantial pressure might select species of lesser quantities to gain pathogenic potential.

**Method:** A human-like wound biofilm model was created using fresh frozen plasma and immune cells from buffy coat. It was inoculated with dual species: *P. aeruginosa* and either *S. aureus* or *E. faecium*. Its tolerance against antimicrobials, and antimicrobial dressings were analyzed quantitatively and by confocal laser scanning microscopy (CLSM). The results were compared to findings with planktonic bacteria.

**Results:** Octenidine-dihydrochloride/phenoxyethanol and polyhexamethylene biguanide (PHMB) solutions showed a significant, albeit delayed reduction in biofilm bacteria, while the PHMB dressing failed to induce this effect. The cadexomer-iodine dressing caused a sustained reduction and killed almost all bacteria down to  $10^2$  cfu/mL within 6 days compared to the control ( $10^{10}$  cfu/mL). CLSM of untreated human biofilm models showed *P. aeruginosa* dominating over *E. faecium* and *S. aureus*. *P. aeruginosa* appeared as a vast bottom layer, while *S. aureus* formed cocci clusters. Only few *E. faecium* were visible, contrasting the *P. aeruginosa* layer in the second model. The species appear to avoid each other and occupy respective niches. These mixed-species biofilm models showed that efficacy and tolerance to antimicrobial substances are nearly species-independent. Frequent application appears important. The bacterial wound biofilm remains a challenge in treatment and requires new, combined therapy options.

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#### P-MC-029

##### The effect of community diversity on bacterial spatial patterns in the phyllosphere

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Bacterial communities colonizing leaf surfaces, the phyllosphere, affect host health by enhancing nutrient acquisition, stress tolerance and pathogen proliferation. However, there is a general lack of understanding about the underlying processes of bacterial community assembly in the phyllosphere.

Building upon previous research from our group, my work aims to expand the current knowledge of the ecology of bacterial community diversity in the phyllosphere and its influence on bacterial community spatial structures.

I will inoculate the model plant *Arabidopsis thaliana* Col-0 with synthetic communities consisting of a core community of eight bacterial strains that will be supplemented by additional bacteria mimicking environmental bacterial communities. Two core community strains are genetically modified to constitutively express fluorescent proteins. Using fluorescence microscopy combined with image cytometry and spatial statistics, I will determine the effect of the different synthetic communities on the labelled bacteria strains. This will allow me to understand the relation between spatial distribution and bacterial community complexity. I will perform my measurements in a time-dependent fashion to also unravel the effect of community development on spatial arrangements.

My research will provide deeper insights into bacterial interactions and community dynamics at the single cell

resolution under nature-like conditions, improving our understanding and predictability of phyllosphere microbial ecology.

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#### P-MC-030

##### Potential Impact of Engineered Nanomaterials on Microbial Communities in the Aquatic Environment

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Engineered nanoparticles are increasingly used in industrial and consumer products, raising concerns about their release into the environment. Although nanoparticles are increasingly distributed into the environment, their interactions with living organisms remain poorly understood. Ecotoxicological assessments have primarily focused on higher organisms like fish, zooplankton, or algae, while the responses of microbial communities, which are vital for processes such as nutrient cycling, biodegradation, and maintaining ecosystem health and functioning, are still underexplored.

The aim of this research is to investigate the impact of nanomaterials on aquatic microbial communities using a combination of laboratory experiments and field-sampling along a gradient from low to highly anthropogenically impacted aquatic ecosystems around Berlin, Germany. To comprehensively assess shifts in microbial composition and functional activity following nanoparticle exposure, we will employ a combination of molecular, microbiological, analytical, and OMICs-based approaches.

We hypothesize that nanoparticles significantly impact microbial community structures, potentially leading to alterations in stress responses and other functional effects.

Although nanomaterials offer potential environmental benefits, such as enhancing energy efficiency, pollution remediation, and water treatment, evaluating their ecological safety across environmentally relevant concentrations is essential to ensure sustainable use. Ultimately, this research will improve the accuracy of environmental risk assessments and support the responsible development and deployment of nanotechnology.

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#### P-MC-031

##### From Molecules to Communities: Establishing a Metabolomics Workflow for Microbial Interaction Studies

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Understanding metabolic interactions within and between microorganisms is key to unraveling the mechanisms that drive microbial community formation and function. To enable such investigations, we established a comprehensive metabolomics workflow for the generation of quantitative intra- and extracellular metabolic data from a broad range of microorganisms and microbial consortia.

A critical step in workflow development was the establishment of a sampling and metabolite extraction pipeline compatible with diverse microbial physiologies. To ensure data quality, we implemented energy charge

measurements as a reliable indicator of physiological state and sample integrity.

Because extracellular metabolite exchange plays a central role in the establishment, stability, and function of microbial communities, we further developed a dedicated workflow for the analysis of extracellular metabolite pools in mono- and co-cultures. Given that most conventional growth media are incompatible with mass spectrometry, we introduced chemical tagging and purification strategies to enable the detection and quantification of amino acids and other organic compounds from complex media matrices.

This integrated workflow provides a robust foundation for the systematic investigation of intra- and intercellular metabolic networks, thereby advancing our understanding of metabolic interactions that shape microbial communities.

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## P-MC-032

### Hormetic Response of Otitis Media Pathogens to Formaldehyde Exposure: Toward the Development of an Experimental Biofilm Model for Otitis Media using Optical Coherence Tomography Enabled Label-Free Imaging

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Otitis media (OM) is a prevalent infectious disease characterized by biofilm formation within the middle ear. The resilience of biofilms contributes to treatment failure and recurrence.(1) To enhance the diagnostic accuracy and improve patient outcomes, the development of reliable *in vitro* models is essential. Formaldehyde-fixed tissues represent a promising substrate for OM biofilm studies. However, the impact of residual formaldehyde on microbial physiology and biofilm formation remains insufficiently understood.(2)

The aim of this study was to assess how planktonic and biofilm forms of *Moraxella catarrhalis* and *Haemophilus influenzae* respond to residual formaldehyde in fixed tissue substrates.

Planktonic and biofilm cultures of *M. catarrhalis* and *H. influenzae* were incubated for 48 h and exposed to formaldehyde concentrations ranging from 0.0002% to 0.2%. Planktonic growth kinetics were monitored via optical density at 600 nm. Biofilm viability and thickness was assessed using Syto 9/Propidium Iodide staining and colony-forming unit counts. Optical coherence tomography (OCT) enabled label-free imaging of formaldehyde-fixed biofilms grown on semi-solid agarose. Statistical analysis (ANOVA) was performed to evaluate dose-dependent effects and to identify potential biphasic responses.

At low concentrations, formaldehyde stimulated planktonic growth, while higher concentrations (>0.02%) exerted inhibitory effects, consistent with a hormetic dose-response. In biofilm-associated cells, the stimulatory range was shifted

toward higher concentrations, suggesting increased tolerance or adaptive stress responses in the biofilm state. OCT successfully enabled the visualization of distinct biofilm architectures between fixed biofilms of both bacterial species, supporting its utility for future non-invasive, real-time, label-free biofilm monitoring.

These findings demonstrate that otitis media pathogens exhibit hormetic responses to formaldehyde, with distinct dose-response profiles in planktonic and biofilm states. This work provides a foundation for developing an *in vitro* otitis media biofilm model to facilitate future label-free biophotonic analysis.

(1) Schilder AGM, Chonmaitree T, Cripps AW, Rosenfeld RM, Casselbrant ML, Haggard MP, Venekamp RP. Otitis media. *Nature Reviews Disease Primers*. 2016;2(1):16063.

(2) Chen NH, Djoko KY, Veyrier FJ, McEwan AG. Formaldehyde Stress Responses in Bacterial Pathogens. *Frontiers in Microbiology*. 2016;Volume 7 - 2016.

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## P-MC-033

### Antibiotic tolerance of biofilms emerging from multicellular effects of antibiotic efflux

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Biofilms are multicellular assemblages of bacteria living in a self-produced extracellular matrix. Different mechanisms, like the development of highly tolerant persister cells or increased expression of efflux pumps make them tolerant. Efflux pumps extrude various antimicrobials out of the cells. AcrAB-TolC is the major efflux pump in *E. coli*. It has been shown that efflux pumps can be linked to cell-to-cell-interactions, membrane integrity, and by global regulators to persister and biofilm formation. The aim of this study is to investigate the survival of cells in biofilms upon exposure to different antimicrobials (ampicillin, benzalkonium chloride, kanamycin, and tetracycline) through the interplay of efflux-mediated spatial interactions and efflux-linked persistence. To this end, we generated fluorescently labeled *E. coli* strains with an *acrB* knock-out or with *acrAB* under the control of an inducible promoter, leading to different levels of efflux activity. The strains were characterized in terms of their antimicrobial susceptibility and efflux activity. To investigate the spatial arrangement of strains with different efflux activity in a colony biofilm, strains labeled with different fluorescent proteins and efflux activity were mixed in a ratio 1:1 and grown on agar supplemented with antimicrobials at sub-inhibitory concentrations. Analysis of the colonies with fluorescence microscopy shows that the absence of the AcrAB-TolC efflux pump affects the structure of sector formation and morphology within the colony depending on the supplemented antimicrobial substance. We observed relatively large sectors with similar surface area for high efflux and low efflux cells in the absence of antimicrobials and at low concentrations. Higher ampicillin and benzalkonium chloride concentrations lead to reduced presence of efflux deficient cells. In contrast, sectors are disappearing in the presence of the low efflux strain with increasing antibiotic concentration upon exposure to tetracycline. Even though kanamycin is not a substrate of AcrAB-TolC, a strong effect on the spatial arrangement in the biofilm structure was observed. Taken together, our data suggest that efflux shapes cell-to-cell interactions and these

interactions affect the spatial arrangement of cells with different efflux activity in biofilms. Understanding the dynamics, provides insights into the selection dynamics of resistant bacteria in bacterial biofilm communities in different environments.

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#### P-MC-034

##### Enrichment of sulfate-reducing microorganisms from metal contaminated sites for wastewater treatment applications

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In mining-affected regions such as the Ore Mountains (Germany), acid mine drainage, characterized by low pH and high metal and sulfate loads, continues to pose serious environmental challenges. Sulfate-reducing microorganisms offer promising biotechnological approaches for the treatment of such waters, as they contribute to the removal of sulfate and toxic metals while enabling the recovery of valuable resources.

To gain deeper insights into reductive microbial processes and to identify suitable organisms for water treatment, naturally occurring sulfate-reducing organisms were enriched and identified from contaminated sites. Samples were collected from the "Reiche Zeche" mine and the "Roter Graben" mine drainage in Freiberg to establish enrichment cultures under anaerobic conditions. Cultures were incubated under varying pH, temperature, and substrate conditions. Sulfate-reducing communities were successfully enriched from both sampling sites, as evidenced by growth in Postgate B medium and sulfide formation. DNA was extracted from the enrichment cultures and analyzed by Illumina sequencing of the 16S rRNA gene to characterize the microbial community composition and identify dominant taxa.

Current work includes the determination of sulfate reduction rates to establish parameters for bioreactor applications. These results form the basis for the development of controlled, microbially-driven treatment systems for metal- and sulfate-laden waters, combining environmental protection with resource recovery.

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#### P-MC-035

##### Iron acquisition mechanisms are signatures for ecological adaptation of the human nasal commensal *Corynebacterium accolens*

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The nasal microbiome is considered a gatekeeper of the human respiratory system. Numerous studies have highlighted the contribution of *Corynebacterium* species in this protective function, particularly *Corynebacterium accolens*. Yet the ecological adaptation of *C. accolens* within the nasal habitat remains poorly characterized. To fill this gap, we performed a comparative analysis between *C. accolens* and its closest relative, *Corynebacterium macginleyi*, which is frequently associated with ocular infections. We found that *C. accolens* isolates represent not a homogenous species but can be grouped into four distinct clusters based on Average Nucleotide Identity (ANI), with

cluster I comprising ~90% of the genomes. Comparative genomic analyses revealed functional differences between *C. accolens* clusters and *C. macginleyi*, particularly in categories related to lipid metabolism and inorganic ion transport. Interestingly, all *C. macginleyi* genomes possess an iron siderophore biosynthetic gene cluster (BGC) that is present only in a single *C. accolens* isolate in a similar genomic coordinate, suggesting a potential loss of this BGC in *C. accolens* species. In contrast, *C. accolens* cluster I is enriched in import systems for xenosiderophores and heme, which are largely absent in *C. macginleyi*. Consistent with the concept of siderophore piracy among nasal commensals, all isolates from *C. accolens* cluster I can grow on iron-depleted medium only in the presence of xenosiderophores or human hemoglobin. RNA-sequencing analysis confirmed that under iron-limiting conditions, *C. accolens* cluster I upregulates the expression of nine operons involved in iron acquisition, including those related to heme and xenosiderophore uptake. These findings underscore the role of iron uptake mechanisms in the adaptation of *C. accolens* to the competitive environment of the human nasal cavity.

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#### P-MC-036

##### Ribosome profiling uncovers the "translatome" of minimal gut consortia *in vitro* and *in vivo*

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Ribosome profiling is a powerful tool for detecting the "translatome" by measuring the ribosome coverage of mRNA<sup>1</sup>. This technique is not affected by biases like trypsin digestion, size, or concentration of the final proteins, unlike mass spectrometry<sup>2</sup>. With those advantages in mind, ribosome profiling has been applied in numerous studies on single-organism cultures. In such studies, novel proteins were detected, the coupling of translational speed and sequence was examined, and the differential translational regulation of genes was measured<sup>3-5</sup>. Yet, the application to consortia (i.e., mixed species) has rarely been attempted, since the mRNA-fragments from within the ribosome are short and mapping is challenging. Here, we employ the ribosome profiling protocol, combined with optimized mapping procedures, to quantify bacterial translation within the context of a minimal gut consortium. We used the Oligo-Mouse-Microbiota with 12 species (OMM<sup>12</sup>), both *in vitro* using batch culture and *in vivo* from mouse feces. We evaluated the quality of the signals, considering both mapping precision and the treatment of homologous genes. We were able to draw meaningful conclusions about the translation within minimal gut consortia. Thus, the protocol enables ribosome profiling with samples of some complexity, providing guidelines on how to proceed with analysis and highlights our most important findings.

<sup>1</sup>Ingolia *et al.*, Science, 2009

<sup>2</sup>Prensner *et al.*, Mol Cell Proteomics, 2023

<sup>3</sup>Graf *et al.*, Microbiol Spectr, 2023

<sup>4</sup>Szavits-Nossan and Ciandrini, Nucleic Acids Research, 2020

## P-MC-037

### **MOlecular MONitoring of bacterial BIOdiversity (MOMOBIO) in the water cycle in the city of Hamburg**

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Anthropogenic factors like antibiotics, biocides, and microplastics directly and indirectly impact biological diversity and promote the spread of antimicrobial resistance, representing a serious threat to human and animal health. The inability to quantify these diverse influences hinders a well-founded risk assessment in urban regions. In this project we will generate detailed, predominantly 'omics'-based datasets to continuously monitor and predict the urban microbiota, as well as changes in its biodiversity and resistance profile, along the wastewater streams of the Hanseatic City of Hamburg and its urban surface waters. Our main objective is to use changes in microbial biodiversity as an indirect measure of human and animal health. Over a period of 2.5 years, we will collect samples three times a year at 38 different sites across Hamburg including locations along the River Elbe, parks, canals, lakes and locations within the wastewater system, like the wastewater treatment plant, tertiary care hospitals, residential areas and several industrial sites. The samples were filtered and DNA was extracted from the collected biomass and subjected to high-throughput sequencing. The resulting datasets were analyzed using various bioinformatic tools to assess bacterial community composition and diversity, identify antimicrobial resistance genes, and detect potential plastic-degrading enzymes using Hidden Markov Models. First results show minor differences in bacterial community composition between surface water and wastewater samples. However, the most abundant phyla were largely shared between both sample types. Alpha diversity was higher in surface water samples compared to wastewater samples. In contrast, beta diversity was higher among wastewater samples, indicating greater variability in community composition between different sampling sites. Several potential plastic-degrading enzymes were identified, with a higher number detected in surface water samples. In addition, multiple antimicrobial resistance genes, including potential beta-lactamase genes, were detected in both surface water and wastewater samples, with a higher frequency in wastewater. In summary, no detectable influence of the wastewater treatment plant effluent on the bacterial community composition was observed in the surface water samples collected downstream of the WWTP along the Elbe River, suggesting microbial diversity is an excellent tool to monitor water quality in urban water cycle.

## P-MC-038

### **Unraveling Iron and Siderophore-Driven Interactions in Microbial Communities through Microfluidic Single-Cell Analysis**

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Microbial communities have been observed to thrive in environments with fluctuating nutrient availability and spatial

heterogeneity. These environmental factors profoundly influence cell growth, and interactions between species. Among essential micronutrients, iron plays a pivotal role. However, its low solubility and potential toxicity under aerobic conditions require precise microbial strategies to maintain iron homeostasis. To explore these processes, we are investigating synthetic microbial consortia consisting of *Escherichia coli*, *Pseudomonas putida*, and *Corynebacterium glutamicum*. These bacteria represent producers, cheaters, and exploiters of different siderophores. These model organisms enable the systematic study of competitive and cooperative strategies for iron uptake within specific spatial contexts.

This approach utilizes sophisticated microfluidic cultivation systems that facilitate precise spatial and temporal regulation of environmental parameters. The microfluidic single-cell analysis enables long-term co-cultivation under controllable conditions of nutrient and iron concentrations, offering high-resolution insights into microbial growth dynamics, siderophore-mediated interactions, and resulting community structures at the level of individual cells. The microfluidic cultivation bridges the gap between conventional static assays and the dynamic complexity of natural microbial ecosystems.

To more accurately replicate natural heterogeneity, hydrogels can be integrated into microfluidic growth chambers to serve as localized reservoirs for nutrients and trace elements, such as glucose or iron. This incorporation enables regulated, stimulus-responsive release processes, augmenting the environment's realism without compromising experimental control. Clearly, the microfluidic framework provides a powerful, versatile platform for investigating how spatiotemporal environmental changes influence iron management strategies and microbial community dynamics.

The project is part of the SFB 1535 MibiNet (Microbial networking – from organelles to cross-kingdom communities).

## P-MC-039

### **ESBL displacement: Investigating the acquisition and loss of ESBL *E. coli* in Swiss travellers**

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Extended-Spectrum Beta-Lactamase – ESBL - producing Enterobacterales pose a significant risk to public health, both in healthcare and community settings (1). While not extensively prevalent in Europe and North America, travel to areas where these highly antibiotic-resistant *E. coli* and *Klebsiella* are endemic increases the chance of acquisition and transmission (2). To study the carriage and subsequent loss of ESBL *E. coli* by international travellers, a study was conducted through the Swiss Tropical Institute in which 40 healthy individuals travelling from Switzerland to South/South-East Asia were monitored for ESBL *E. coli* intestinal carriage. 63% of the returning travellers harboured ESBL *E. coli*, a status they lost over the course of the following year. In the scope of the study, more than 4500 ESBL resistant and susceptible (ESBL and nESBL) human gut *E. coli* isolates were sequenced. While microbiota 16s sequencing showed no significant changes on the family level, it was determined through a statistical model outlining the possible interactions between coexisting *E. coli* strains that *E. coli*-*E. coli* competition helps mediate the clearance of ESBL strains from the gut upon return from

travel. We established a mouse model to study this interference and were able to recapitulate specific strain-level competition observed in the participants. Further, we analysed the carbon-source utilization capacities of the individual strain pairs and determined through the targeted generation of mutants that *in-vivo* competition for carbon sources plays a significant role in mediating the loss of ESBL *E. coli*.

1. WHO Bacterial Priority Pathogens List, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. Geneva: World Health Organization; 2024.
2. Arcilla, M. S., van Hattem, J. M., Haverkate, M. R., Bootsma, M. C. J., van Genderen, P. J. J., Goorhuis, A., Grobusch, M. P., Lashof, A. M. O., Molhoek, N., Schultsz, C., Stobberingh, E. E., Verbrugh, H. A., de Jong, M. D., Melles, D. C., & Penders, J. (2017). Import and spread of extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): A prospective, multicentre cohort study. *The Lancet Infectious Diseases*, 17(1), 78–85. [https://doi.org/10.1016/S1473-3099\(16\)30319-X](https://doi.org/10.1016/S1473-3099(16)30319-X)

#### P-MC-040

##### How plant secondary compounds can inhibit the formation of bacterial biofilms by multiple targeting of biofilm control networks – *Acacia nilotica* acting on *E. coli* and *B. subtilis*

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The tropical plant *Acacia nilotica*, which is rich in wattle tannins (oligo-flavonoids), is a medicinal plant widely used from Africa to India and Australia. In West-Africa it is also used in mud dyeing of textiles. In a project that brings together Microbiology, Anthropology and local traditional craft (of the Marka-Dafing community, Burkina Faso) to study the traditional mud dyeing procedure, we have found that the presence of *A. nilotica* actively prevents the formation of biofilms by aerobic soil and enteric bacteria, which would otherwise inhibit textile coloration by FeS generated by anaerobic mud bacteria (1).

Biofilm formation confers protection to bacteria against antibiotics and immune attacks, which leads to chronic infections. In *E. coli*, production of key biofilm matrix components depends on the biofilm master regulator CsgD. We have previously shown that plants use secondary metabolites – incl. catechins (e.g., EGCG) and flavonoids – to control bacterial colonization of their surfaces by targeting biofilm matrix formation (2,3).

Here, we show how plant extracts from *A. nilotica* prevent biofilm formation of *B. subtilis* and *E. coli* (macrocolonies, pellicles and submerged biofilms). For *E. coli*, the extract not only strongly inhibits the polymerization of curli-fiber subunits (CsgA), but also nearly eliminates the master regulator CsgD and therefore expression of the biofilm matrix genes *csgB* and *dgcC*. Our data indicate, that CsgD expression is down-regulated by the RpoE- and RpoH-dependent activation of the cell envelope and heat shock stress responses, respectively, as well as by increased expression of flagellar genes, which include *pdeH* (encoding the master c-di-GMP

phosphodiesterase in *E. coli*). We also show that the individual tannins contained in the plant extracts, ellagic acid and methyl gallate, delay the detection of CsgD levels in cultured cells.

(1) Douny et al. (2023) Acta HorEc. 1361, 67-76

(2) Serra et al. (2016) Mol. Microbiol. 101,1 (2016): 136-51

(3) Pruteanu et al. (2020) Env. Microbiol. 22(12):5280-5299

## Phage biology

#### P-PB-001

##### Diverse bacterial and viral assemblages in ash from Mount Etna

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**Introduction** Volcanoes shape landscapes and influence ecosystems through the release of ash, gases, and heat. Volcanic ash forms a nutrient-rich yet extreme habitat and can be dispersed over hundreds of kilometers from the eruption site. Although viruses, especially bacteriophages, are widespread in soils and other extreme environments, their diversity and ecological roles in volcanic ash remain largely unexplored.

**Goals** This study examined prokaryotic and viral diversity and their interactions on volcanic ash particles.

**Methods** Fresh ash samples from three sites on Mount Etna, Sicily, were collected immediately after the fallout and analyzed by Illumina metagenomic sequencing and bioinformatics. Enrichment cultures were performed in Reasoner's 2A, LB, and marine broth, with isolates identified via 16S rRNA gene sequencing. Virus particles in the 0.2  $\mu$ m flow-through of selected enrichments were concentrated using polyethylene glycol (PEG) precipitation and examined by transmission electron microscopy (TEM) after negative staining.

**Results** Dominant bacterial genera in ash metagenomes included *Pseudomonas*, *Sphingomonas*, *Massilia*, and *Delftia*. A total of 20 high-quality, dereplicated metagenome-assembled genomes (MAGs) were recovered. Viromics analysis identified 1139 viral operational taxonomic units (vOTUs), with approximately one-third assigned to predicted hosts, including *Telluria* and *Pseudomonas*. CRISPR spacer matches linked the MAGs of *Candidatus Humibacillus vulcanicus* and *Delftia acidovorans* to multiple vOTUs detected in the same sample. A complete 336-kb jumbo phage genome encoding 539 ORFs was recovered, revealing a largely autonomous genetic toolkit, including its own transcriptional and DNA replication machinery, T4-like structural proteins, and genes for host manipulation and defense evasion. A lytic phage infecting the isolate *Bacillus*

sp. Vol12 exhibited an unusual morphology, with a small capsid and disproportionately elongated tail. Additional phages particles were observed by TEM following PEG precipitation.

**Conclusion** Our findings provide novel insights into the ecology of microbes and their viruses associated with volcanic ash, highlighting complex virus-host interactions and a diverse viral community. The results have implications for understanding microbial survival strategies at the biosphere-atmosphere interface in extreme volcanic environments.

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#### P-PB-003

##### Genetic studies of prophage SP $\beta$ on *Bacillus subtilis*

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Temperate bacteriophages profoundly influence bacterial evolution by integrating into host genomes and reshaping genetic networks through horizontal gene transfer and lysogenic conversion. Despite their significance, the molecular mechanisms underlying this transition remain poorly understood. Understanding how prophages establish, maintain, and exit lysogeny is critical for explaining bacterial adaptation and genome plasticity, yet systematic genetic tools to study these processes in *Bacillus subtilis* have been limited.

To address this gap, we are developing a phage-driven mutant library designed to enable high-resolution functional analyses of phage-host interactions. Gene deletions from the well-characterized *B. subtilis* collection (1) were combined with strain carrying the prophage SP $\beta$  (2). The lytic cycle of these mutants can be readily activated, creating a defined platform to systematically investigate the molecular determinants of lysogeny, prophage induction, and horizontal gene transfer in *B. subtilis*. This new mutant library enables detailed studies of how individual host genes influence the switch between lysogenic and lytic states, offering insights into SP $\beta$ -like prophage biology.

(1) Koo *et al.* (2017) *Cell Systems*, 4(3), 291–305.e7

(2) Kohm, *et al.* (2023) *Nucleic Acids Research*, 51(3), 1312–1330.

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#### P-PB-004

##### Deciphering phage-host Interactions in the *Drosophila melanogaster* Gut Ecosystem

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**Introduction:** Bacteriophages, or phages, are viruses that infect bacteria and are among the most abundant biological entities in animal guts. Despite their ubiquity and potential to shape bacterial communities, little is known about their composition, diversity, and physiological significance within the gut. The fruit fly *Drosophila melanogaster* provides an ideal model to address these gaps due to its genetic tractability and its simple, well-characterized, and cultivable gut microbiota. While the bacterial members of the *Drosophila* gut have been extensively studied, the viral component remains largely unexplored.

**Goals:** This study aimed to investigate how bacteriophages modulate the composition and function of the *Drosophila* gut microbiota, with a particular focus on the dominant commensal *Lactiplantibacillus plantarum* and its physiological impact on the host.

**Methods:** A total of 950 wild-type flies were collected from multiple geographic locations across Germany using standardized trapping methods. Gut bacterial isolates were obtained through culture-dependent techniques and characterized by whole-genome sequencing. The analysis revealed a microbial community dominated by lactic acid bacteria, including *Lactiplantibacillus plantarum* (Lactobacillaceae) and *Leuconostoc mesenteroides* (Leuconostocaceae). Using isolated *L. plantarum* strains, we enriched gut samples and isolated a novel bacteriophage infecting this species, which we named **Drosophage**. The phage was subsequently characterized genomically and morphologically to assess its role in regulating gut microbial dynamics and host physiology.

**Results:** Characterization of Drosophage revealed that it belongs to the order *Caudovirales* and is capable of infecting and modulating *L. plantarum* populations, leading to measurable changes in gut microbial community structure. These alterations were associated with shifts in host physiological parameters, indicating that phage-bacteria interactions can indirectly influence host biology.

**Conclusion:** Our findings demonstrate a complex and dynamic interplay between bacteriophages, gut bacteria, and the *Drosophila* host. Phages act as regulatory agents that shape microbial community composition and, consequently, host physiological responses. This work provides new insights into the ecological and functional significance of phage-microbiota interactions and establishes *D. melanogaster* as a valuable model for studying tripartite host-bacteria-phage relationships.

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#### P-PB-005

##### The co-localizing Zorya II, Druantia III, and ARMADA II defense systems on O-island 172 confer synergistic anti-phage defense in enterohemorrhagic *Escherichia coli*

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**Introduction:** Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 strain EDL933 is a foodborne pathogen that causes life-threatening diarrheic infections. We found that EDL933 is highly resistant to many phages, that efficiently lyse *E. coli* K12 strains. The genes encoding the three phage defense systems Zorya II, Druantia III, and ARMADA II were shown to co-localize on O-island 172 in EDL933. Bioinformatics analysis revealed that the three phage defense systems co-occur in 426 *E. coli* strains, comprising 66 distinct serotypes, including 277 O157:H7 isolates and 117 diverse non-pathogenic strains [1]. **Goals:** We hypothesized that the three defense systems act synergistically in the phage defense, together contributing to the strong phage resistance



of EHEC. **Materials & Methods:** Efficiency of plating (EOP) assays of single and combined defense system were used to explore the phage spectrum and synergy of the three defense systems. **Results:** Using DzorABE, DdruHE, and DarmABCD single system deletion mutants, we showed that Druantia III and ARMADA II protect to different extents against a broad spectrum of phages, including the *Drexlerviridae*, *Siphoviridae*, *Demereciviridae*, *Vequentavirinae* and *Autographiviridae*, but not against hypermodified *Tevenvirinae*. Additionally, Zorya II caused strong protection only against *Autographiviridae*. EOP assays of combined system mutants revealed strong synergistic interactions of Druantia III and ARMADA II to provide more robust immunity against a similar phage spectrum than the additive protection of the individual systems. In contrast, Druantia III and Zorya II act only weakly synergistically against a few phages [1]. **Conclusions:** Our results revealed that Druantia III and ARMADA II are responsible for most of the phage resistance of EDL933, whereas Zorya II provides additional immunity against podoviruses. Thus, the design of novel inhibitors against the Druantia III and ARMADA II systems could be combined with phage therapies to efficiently eradicate EHEC.

#### P-PB-006

##### Dual time-resolved tRNA modification profiles and their influence on T5 replication in *Escherichia coli*

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The epitranscriptome is the sum of all RNA modifications in an organism. It represents an additional layer of information beyond the base RNA sequence. Over 160 modified RNA bases are reported across all RNA types<sup>1</sup>. In bacteria, RNA modifications contribute to translational regulation, decoding, tRNA stability, and metabolic regulation under stress<sup>2</sup>. Recently, our group showed that m<sup>6</sup>A depletion in *Escherichia coli* RNA reduces the infectious potential of the lytic *E. coli* phage T5<sup>3</sup>. However, data regarding the presence and dynamics of modifications in host and phage RNAs during infection remain scarce.

To fill this gap, we focused on *E. coli* infected with T5, a member of the *Demereciviridae* family that encodes 25 tRNAs, using direct RNA sequencing (DRS) and mass spectrometry (MS). Using multiplexed tRNA sequencing, we detected T5 tRNA expression in the late infection stage. Most host tRNA modifications remained stable during infection. The modification patterns of T5 tRNA differed from those of the host, particularly in the D-loop. This pattern was also conserved in another T5-like phage, Bas29. Analysis of the DRS data suggested that mnm<sup>5</sup>s<sup>2</sup>U34 was present in GluT5, which was important for the phage replication cycle.

This study provides the overview of the time-resolved dynamics of expression and modification of both host- and phage-encoded tRNAs during T5-infection. Some tRNAs modifications directly affect phage replication, providing new insights into the role of phage tRNAs in translation.

1. Cappannini, A. et al. MODOMICS: a database of RNA modifications and related information. 2023 update. *Nucleic Acids Res.* 52, D239–D244 (2024).
2. Riquelme-Barrios, S. et al. Epitranscriptomic control of stress adaptations in *Escherichia coli*. (preprint) *bioRxiv* (2025).
3. Saikia, B., et al. Depletion of m<sup>6</sup>A-RNA in *Escherichia coli* reduces the infectious potential of T5 bacteriophage. *Microbiol. Spectr.* 12, e0112424 (2024).

#### P-PB-007

##### The role of phage Thanatos ADP-ribosyl-transferases Alt1 and Alt2 in disarming and preparing the host

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ADP-ribosyltransferases (ARTs) are versatile enzymes present in prokaryotes, eukaryotes, and viruses. Their roles in phages, however, remain poorly understood. To date, only the ARTs of the model *Escherichia* phage T4 (Alt, ModA, and ModB) have been characterized in more detail. These enzymes modify host proteins through NADylation or RNAlation, primarily targeting components of the transcription and translation machinery, thereby contributing to host cell reprogramming. Here we investigate the functions of two ARTs - Alt1 and Alt2 - from the *Shewanella* phage Thanatos. Both enzymes are incorporated into the phage particle and actively NADylate host proteins immediately upon infection, enabling rapid host takeover. The Alt proteins can undergo self-modification and a loss of functional ARTs markedly reduces phage fitness, as mainly reflected by a decreased burst size. Several host proteins targeted and modified by Thanatos Alt1 and Alt2 were identified either through comparison with T4 Alt or directly via co-immunoprecipitation. These include the host RNA polymerase and components of bacterial defense systems such as toxin-antitoxin modules and a dNTPase. Consistently, in a heterologous *Escherichia coli* host, expression of Alt1 and Alt2 can restore infectivity of phage T5 in the presence of a *Shewanella* dGTPase. Our results support the hypothesis that Alt1 and Alt2 act as early infection effectors that disrupt host defense mechanisms and prepare the host cell for phage replication.

#### P-PB-008

##### Dissecting antiviral defense systems in Actinobacteria – from single cells to the community level

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Viruses infecting bacteria, so-called bacteriophages (phages for short) account for the most abundant biological entities on the planet and pose a constant threat for bacteria. Due to that omnipresent evolutionary pressure, bacteria evolved complex "immune systems" with a great diversity of antiviral defense mechanisms, such as abortive infection (Abi), restriction modification (RM) systems or CRISPR-Cas. In recent years, chemical antiviral strategies on a community level, mediated by small molecules and secondary metabolites of Actinobacteria, came into the focus of attention.<sup>1</sup> There are indications that the antiviral immune

defense of bacteria is not limited to single cells, but rather plays a communal role in the so-termed "pan-immune system"<sup>2</sup>.

Previous studies revealed that defense systems often cluster within "defense islands". Based on these findings, bioinformatic prediction tools have been developed for the identification of new, yet uncharacterized defense systems.<sup>3</sup> These tools show a particular bias toward Proteobacteria, and analysis across other bacterial phyla have revealed a notable "depletion" of so far known defense systems in other large phyla, such as the diverse phylum of Actinobacteria.<sup>4</sup> Here, we leveraged existing bioinformatics prediction tools to identify potential antiviral defense systems in the actinobacterial model strain *Corynebacterium glutamicum*. Using DefenseFinder and PADLOC, we identified overall 12 different systems with a putative role in antiviral defense.

Here, we investigate the role and potential co-regulation of selected defense systems during phage infection. Our initial screenings against a collection of 5 phages indicated that, in particular genomically-integrated (cryptic) prophages represent a hotspot for systems involved in antiviral defense.

## References

1. Georjon, H. & Bernheim, A. The highly diverse antiphage defence systems of bacteria. *Nat. Rev. Microbiol.* 21, 686–700 (2023).
2. Bernheim, A. & Sorek, R. The pan-immune system of bacteria: antiviral defence as a community resource. *Nat. Rev. Microbiol.* 18, 113–119 (2020).
3. Makarova, K. S., Wolf, Y. I., Snir, S. & Koonin, E. V. Defense Islands in Bacterial and Archaeal Genomes and Prediction of Novel Defense Systems. *J. Bacteriol.* 193, 6039–6056 (2011).
4. Georjon, H., Tesson, F., Shomar, H. & Bernheim, A. Genomic characterization of the antiviral arsenal of Actinobacteria: This article is part of the Microbial Evolution collection. *Microbiology* 169, (2023).

effective antibiotic, an expensive reserve antibiotic with serious side effects<sup>8</sup>.

Therefore, alternative treatment strategies for VRE are urgently needed. We evaluated the efficacy of a four-bacteriophage cocktail designed for preventive VRE decolonization. Using anoxic growth kinetic assays and a bioreactor-based *in vitro* gut community model (ivGCM), we evaluated its activity under colon-like conditions, which can alter phage performance through metabolic and receptor-related changes<sup>9</sup>. We could demonstrate that under these conditions, the phages strictly required divalent cations (Mg<sup>2+</sup> and Ca<sup>2+</sup>) for effective VRE lysis in monoculture and within a representative gut microbiota. Overall, we were able to reduce VRE colonization inside the ivGCM by 2.5-log steps with a single dosage of the phage cocktail and the supplementation of divalent ions. The results deliver important preclinical data relevant for future formulations of a suitable phage product based on the selected bacteriophages. By demonstrating that the efficacy of the selected phages depends critically on the availability of divalent ions, we identified both a promising therapeutic candidate and an important ecological factor that influences the outcome of the treatment.

<sup>5</sup> Miller, W. R. & Arias, C. A (2024) in: *Nat Rev Microbiol* 22, 598–616

<sup>6</sup> Sati, H. et al. (2025) in: *The Lancet Infectious Diseases* 25, 1033–1043

<sup>7</sup> Zhang, M. & Guo, H. (2024) in: *Cell Host & Microbe* 32, 1455–1457

<sup>8</sup> Di Paolo, A. et al. (2010) in: *Clin Pharmacokinet* 49, 439–447

<sup>9</sup> Hernández Villamizar, S. et al. (2023) in: *Applied and environmental microbiology* 89, e0149123

## P-PB-009

### Ion-dependent phage decolonization of vancomycin-resistant *Enterococcus faecium* (VRE) in an *in vitro* gut model

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*Enterococcus faecium* is a natural member of the human gut microbiome but also part of the infamous group of ESKAPE pathogens. Under healthy conditions, this pathobiont exhibits low virulence and is usually harmless. However, diseases that alter immune function or trigger dysbiosis can promote *E. faecium* overgrowth, leading to severe infections such as sepsis and endocarditis<sup>5</sup>. In particular, vancomycin-resistant *Enterococcus* (VRE) strains, classified by the WHO as "high-priority" pathogens<sup>6</sup>, represent a major cause of nosocomial infections, especially among immunocompromised patients. Oncology patients are particularly affected due to chemotherapy-induced gut damage and microbial dysbiosis, which facilitates VRE colonization<sup>7</sup>. In general, VRE infections are difficult to treat, and linezolid remains the only

## P-PB-010

### Best enemies: How an antibiotic can save bacteria from being killed by phages

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Bacteriophage-antibiotic combinational treatment is a promising strategy to counter the surge of non-treatable antimicrobial resistant pathogens, and it has been successfully employed in a few critical cases. However, rational design of phage-antibiotic combinations is hindered by the limited knowledge of their underlying mechanisms. The cellular impact of antibiotics and phages alone is highly complex, and often not well understood. This is even more so for phage-antibiotic combinations, which are largely uncharacterized, and might involve molecular mechanisms beyond the regular drug and phage targeted processes. This project deploys an innovative systems biology approach to uncover the molecular underpinnings of phage-antibiotic interactions. In a pioneer study, we recently discovered that CBASS drastically enhances the activity of antifolates in a synergistic manner, in the absence of phages. However, now we observe that the antifolate rescues the bacterial cell from being killed by phages, without the presence of CBASS. We hypothesize an antagonistic interaction between the antifolate sulfamethoxazole (SMX) and the T4 phage. The

aim now is to reveal the mechanism underlying this interaction as well as systematically evaluate phage-antibiotic interactions between antifolate antibiotics and the BASEL collection of phages in *Escherichia coli*.

1 Theuretzbacher, U et al. (2020). Nat Rev Microbiol 18, 275–285

2 Georjon, H & Bernheim, A. (2023). Nat Rev Microbiol 21, 686–700

3 Brenzinger, S et al. (2024). Nat Microbiol 9, 251–262

4 Maffei, E et al (2021). PLoS Biol 19(11): e3001424

## P-PB-011

### Regulation of lysis-lysogeny decision in *Corynebacterium glutamicum* ATCC 13032 harboring the CyranPS prophage

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Prophages are found in more than half of all bacteria and are ubiquitous elements of bacterial life (1). As major contributors to bacterial genetic diversity, prophages can impact host fitness and confer new phenotypes (lysogenic conversion) as they often encode defense systems or virulence factors (2,3). Intriguingly, many bacteria harbor multiple prophages – so-called polylysogens – making them valuable models to study phage-host and phage-phage interactions (4).

The actinobacterium *Corynebacterium glutamicum* ATCC 13032 harbors three cryptic prophages. Among them, the cryptic prophage CGP3 comprises nearly 6% of the genome and provides immunity against phage infection (5).

Here, we investigated the new temperate phage CyranPS infecting *C. glutamicum* ATCC 13032. Interestingly, the attachment site of CyranPS was found to be located within the phage repressor gene, resulting in repressor truncation upon chromosomal integration. Expression of the full-length versus truncated variant of the repressor revealed that the truncated variant is indeed the active form of the protein.

We further analyzed the impact of factors encoded by the cryptic prophage CGP3 on the lysis-lysogeny decision of the newly introduced prophage CyranPS. Our studies provide evidence that interprophage crosstalk occurs in *C. glutamicum*, with the presence of CGP3 significantly reducing spontaneous induction of CyranPS. Transcriptomic analyses revealed increased expression of CyranPS lytic genes in the absence of CGP3. A forward genetics screen identified two adjacent genes with a previously unrecognized role in interprophage competition. Together, these findings demonstrate that interactions between prophages and other mobile elements play a pivotal role in shaping infection dynamics.

(1) Touchon et al. (2016) ISME J, doi.org/10.1038/ismej.2016.47

(2) Pfeifer et al. (2022) Curr Opin Microbiol, doi.org/10.1016/j.mib.2022.102230

(3) Harrison et al. (2017) Bioessays, doi.org/10.1002/bies.201700112

(4) Silpe et al. (2023) Nature, doi.org/10.1038/s41586-023-06376-y

(5) Pfeifer et al. (2016) Nucleic Acids Res, doi.org/10.1093/nar/gkw692

## P-PB-012

### Systematic studies of bacteriophage confinement in glycan-based biofilms of *Erwinia amylovora*

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Biofilms have served as a protective microbial lifestyle in harsh environments for millions of years. Many bacteria embed themselves in a viscous extracellular polymeric substance (EPS) composed of DNA, proteins, and glycans. In plant pathogens such as *Erwinia* spp. highly viscous, anionic polysaccharides are the main EPS component, regulating molecular transport and serving as major diffusion barrier to bacteriophages.

We investigated phage diffusion in glycan-based biofilms using *Erwinia amylovora* podovirus ΦEa1h and myovirus ΦEaJ08C. Polysaccharide matrices from *Pantoea stewartii* and *Erwinia amylovora* served as model systems. Exopolysaccharide was isolated from eight bacterial strains (two *P. stewartii*, six *E. amylovora*) and subjected to extensive structural characterization, including analysis of monosaccharide composition, polymer chain length, and production yield. These analyses provided important insights into the biochemical diversity and physical properties of the EPS between different strains.

To date, there has been no quantitative analysis of diffusion parameters in biofilms. We therefore need data that quantifies how phages move in a viscous matrix and reach their host. We tracked fluorescently labeled phages and microspheres of comparable size with fluorescence microscopy using single particle tracking. From particle trajectories, we developed models to identify populations with distinct diffusion behaviors. These models also enabled the establishment of quantitative methods for determining phage concentrations under diffusion-limited conditions and varying physicochemical environments.

Our results show that phage diffusion is strongly influenced by EPS concentration, pH, ionic strength, and tracer properties. Phage-encoded depolymerase-mediated degradation of stewartan proved to be a rapid and effective mechanism for modulating particle dynamics within the matrix [1]. Finally, we link phage diffusion to host infection dynamics, providing insights into how bacteriophages navigate, penetrate, and infect their bacterial hosts in the context of dense, viscous biofilm environments.

1. Dunsing V, Irmscher T, Barbirz S, Chiantia S. Purely Polysaccharide-Based Biofilm Matrix Provides Size-Selective Diffusion Barriers for

### P-PB-013

#### Potential of bacteriophages as alternative agent for the precision decolonization of nasal *Staphylococcus aureus*

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#### Introduction:

*Staphylococcus aureus* is a major human pathogen which causes a wide variety of infections. *S. aureus* colonizes approximately the nares of 20%-30% of the human population, which is a risk factor for subsequent infections. The proven method for decolonization of *S. aureus* is a treatment with the antibiotic mupirocin, which leads to problems on the long run. Firstly *S. aureus* recolonizes usually after the treatment has stopped. Secondly antibiotic resistance to mupirocin in *S. aureus* is emerging.

#### Goals:

*S. aureus* is part of a complex microbiome in the human nares. Other bacterial commensals have promoting or suppressive effects on *aureus* nasal colonization. The effect of mupirocin treatment on the nasal microbiome is poorly understood on the species and strain level. Contrastingly bacteriophages, microbial viruses, have the potential to eradicate bacteria in a highly selective manner. In this study we determine the effect of mupirocin- and phage-based treatment strategies on nasal commensal with the aim to develop long-term as wells as species- or strain-specific nasal decolonization strategies of *S. aureus*.

#### Methods:

We measured the effect of mupirocin and bacteriophages on nasal commensals on the individual and in binary co-cultures of commensals. Additionally, we performed in silico comparative genomics of nasal commensals to identify genetic markers for antimicrobial susceptibility.

#### Results and Conclusion:

We show that certain genetic features explain antimicrobial susceptibility across a wide variety of nasal commensals from very diverse phylogenetic clades. Presence of identified antibiotic or phage resistance determinants also governs growth in a competitive *in vitro* system. Observed microbiome disturbances caused by mupirocin treatment correlate well with *in vivo* colonization data. Side effects are especially pronounced on other- often harmless - commensal staphylococci, which are frequently antagonizing *S. aureus* colonization.

Hence, we propose alternative antimicrobial approaches targeting molecular structures which are unique to pathogenic commensals. We demonstrate the potential of phages as promising alternative therapeutic. In contrast to frequently used antibiotics bacteriophages target distinct surface structures that are unique to *S. aureus* allowing potentially selective and controlled eradication of this pathogen from the human nose.

### P-PB-014

#### Isolation and Characterization of Bacteriophages Targeting *Enterobacter* and *Citrobacter* Species

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*Enterobacter* and *Citrobacter* are both Gram-negative bacteria that can be found in a variety of environments, including soil, water, plants and the gastrointestinal tracts of animals and humans. In the human environment, especially in hospitals, they colonise various habitats, such as medical equipment, wastewater systems, sinks and drains. In healthcare settings, *Enterobacter* (particularly the *E. cloacae* complex) and *Citrobacter* (especially *C. freundii* and *C. koseri*) are well-known opportunistic pathogens that cause nosocomial infections. *Enterobacter* is frequently implicated in pneumonia, urinary tract infections (UTIs), sepsis and wound or device-related infections. Likewise, *Citrobacter* spp. cause UTIs, bloodstream infections and pneumonia in vulnerable patients, such as neonates, ICU patients and the immunocompromised. More concerning, *C. koseri* and *C. freundii* can invade the central nervous system of neonates, causing meningitis and brain abscesses with high mortality rates. Both *Citrobacter* and *Enterobacter* spp. pose a growing global threat of multi-drug resistance (MDR). *Citrobacter* species are now considered important reservoirs of extended-spectrum beta-lactamase (ESBL) and carbapenemase genes, with high MDR prevalence limiting treatment options. More critically, *Enterobacter* ranks among the top carbapenem-resistant *Enterobacterales*. Current data emphasize the need for new therapeutics such as  $\beta$ -lactamase inhibitors and combination therapies, to manage these infections. A potential approach to controlling these pathogens might be to use bacteriophages. In this regard, we aimed to isolate new phages against clinically relevant *Citrobacter* and *Enterobacter* strains. Five *Citrobacter* and five *Enterobacter* strains, all of which were isolated from patients or hospital settings, were selected as hosts. We have isolated a set of 44 phages and further characterization has led us to focus initially on six of them. This selection was made based on host range analysis, which revealed cross-species infection with *Citrobacter* phage Cfr-4 and *Enterobacter* phage Esa-44. The other four phages, *Citrobacter* phages Cfr-14 and Cko-28, and *Enterobacter* phages Ero-32 and Ecl-38, were selected for their broad host range within their respective genera. Further experiments are in progress to fully characterize these selected phages, such as measuring their lysis and adsorption kinetics or analyzing their genomes.

### P-PB-015

#### Analyse of phage related response of *S. maltophilia* in single and mixed species biofilms

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**Introduciton:** *Stenotrophomonas maltophilia* is a multidrug-resistant opportunistic pathogen that causes various nosocomial infections, including pneumonia, bloodstream infections, and urinary tract infections. Over the past decade, *S. maltophilia* has become an important clinical concern due to its intrinsic resistance to multiple antibiotics, low pathogenicity, and strong ability to form biofilms. Because of the increasing incidence of *S. maltophilia* related infections, a better understanding of its treatment strategies is urgently

needed. Multidrug resistant pathogens, particularly in biofilms, are difficult to treat and pose a major clinical threat. As antibiotic efficacy declines, phage therapy is emerging as a promising alternative, though phage biofilm interactions remain poorly understood.

**Goal:** The main objective of this study is to gain a deeper understanding of the interactions between bacteriophages and biofilms, with a particular focus on single and mixed-species biofilms using the following pathogens: *S. maltophilia*, *P. aeruginosa*. We aim to determine how phages influence biofilm formation in both single and mixed-species biofilms and to identify genes that are differentially expressed in response to phage predation. By elucidating these mechanisms, we seek to contribute to a better understanding of phage biofilm dynamics as a potential foundation for phage-based biofilm control strategies.

**Methods:** Phages were isolated from wastewater samples collected in a clinical environment. Biofilms were cultivated under continuous flow and static conditions until maturation, followed by phage treatment. Biofilm structure and integrity were analyzed using laser scanning confocal microscopy and quantified with dedicated image analysis software.

**Results/Conclusion:** Our preliminary results indicate that bacteriophages have a significant impact on biofilm structure and viability. Both biofilm formation assays and live/dead staining revealed partial biofilm disruption and cell death after phage treatment. Time series analyses revealed that phage infection initially degraded *Pseudomonas aeruginosa* biofilms but, after about 20 hours, partial regrowth occurred. These findings confirm that phages affect biofilm integrity and dynamics. Further experiments, including transcriptomic analyses, will be conducted to elucidate the molecular mechanisms underlying phage biofilm interactions.

#### P-PB-016

##### The holin of phage T4 forms a defined pore that can be closed by fused mEGFP

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Most DNA phages release their progeny from the host by inducing host cell lysis [1]. To achieve this, hole-forming membrane proteins are produced, so-called holins, that enable the passage of cell wall-degrading endolysins through the cytoplasmic membrane. We recently showed evidence for the formation of rings by the holin of phage T4 [2]. Such rings can in principle generate a hydrophilic membrane pore by flipping an N-terminal amphipathic helix from the inner membrane surface to a transmembrane orientation. In initial studies with N-terminal mEGFP fusions, we unexpectedly found that these did not interfere with holin function. As an unfolded region of the N-terminus could have functioned as flexible linker that prevented holin pore closure by the fused mEGFP, we removed this linker and found that indeed the resulting constructs were not functional. Moreover, we could show that the holin-induced membrane permeability for the dye propidium iodide was abolished. In contrast, when the unfolded region was removed without a fused mEGFP, the holin was highly active, indicating that indeed the fused mEGFP blocks hole formation, likely by preventing the flipping of the amphipathic helix of the holin.

[1] Fernandes S. and Carlos São-José C. (2018) Viruses 10:396. doi: 10.3390/v10080396

[2] Schwarzkopf J.M.F., Viveros R.P., Burdur A.N., Mehner-Breitfeld D., Tschowri N., Brüser T. (2025) Front Microbiol. 16:1579756. doi: 10.3389/fmicb.2025.1579756

#### P-PB-017

##### DNA loaded outer membrane vesicles after bacteriophage treatment

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Bacteria use a variety of defence systems to control bacteriophage infection, including extracellular vesicles (EVs) [1, 2, 3]. In particular, bacteriophage infection or antibiotic treatment triggers the production of EVs [3]. These EVs have an effective capacity to bind and reduce the number of infectious bacteriophage particles within a bacterial population [2]. However, the precise mechanism underlying the interaction between EVs and bacteriophages as well as the fate of these phage-loaded EVs are not well studied so far.

Gram-negative bacteria release extracellular vesicles that consist only of the outer layer of the membrane. These vesicles are called outer membrane vesicles (OMVs). OMVs contain the surface receptors that can be involved in bacteriophage infection. We work on tailed bacteriophages that are specific for lipopolysaccharide of Gram-negative bacteria as the receptor molecule. In this work we use podovirus P22 as a model phage specific for *S. Typhimurium*. We have shown that phage P22 binds to OMVs of *S. Typhimurium* and injects its DNA into the vesicle lumen [2]. The aim of this study is to characterise these "DNA-loaded" OMVs and analyse their fate in a bacterial population. We will study the change in the surface composition of the OMVs upon phage challenge, with a particular focus on their LPS composition. To some OMVs phages are only bound and their DNA is not injected into the OMVs. These phages remain infectious and can effectively use the OMV as a 'hop-on, hop-off' vehicle. We will also investigate the fate of bacteriophage particles bound to the OMV surface. For our studies we compare OMVs obtained from either explosive cell lysis or from natural budding into culture supernatants.

[1] Adam Kulp and Meta J Kuehn. Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annual review of microbiology, 64:163–184, 2010.

[2] Mareike S Stephan, Nina K Broeker, Athanasios Saragliadis, Norbert Roos, Dirk Linke, and Ste-fanie Barbirz. In vitro analysis of O-antigen-specific bacteriophage P22 inactivation by Salmonella outer membrane vesicles. Frontiers in Microbiology, 11:510638, 2020.

[3] Ethan Hicks, Nicholas MK Rogers, Christine Ogilvie Hendren, Meta J Kuehn, and Mark R Wiesner. Extracellular vesicles and bacteriophages: New directions in environmental biocolloid research. Environmental Science & Technology, 57(44):16728–16742, 2023

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## P-PB-018

### Investigation of the dynamics between the Inovirus Dolos and its host *Shewanella oneidensis* MR-1

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Inoviruses are widely occurring filamentous phages; the best-known members of this family are Ff phages, including F1, Fd, and M13 that have been studied extensively in recent decades to investigate fundamental aspects of molecular biology. However, outside of the Ff group, knowledge about the fundamental mechanisms of Inovirus infection and egress is rather scarce.

We recently isolated a novel phage, *Shewanella* phage Dolos, which belongs to the family of Inoviridae. We have found that the infection and extrusion process of the phage Dolos does not follow the dogmatic regimen of Ff phages: Dolos most likely binds to the side of the type IV MSHA pili as its primary recognition structure in order to attach and invade the cell. After inoviruses reach the cell surface by retraction of the pili, all characterized phages of this type strictly require the TolA protein of the TolQRA system in order to enter the cytoplasm of their host. We found normal Dolos infection in the absence of TolA, the first example for an Inovirus that infects its host independently of the TolQRA system. For egress of the Inovirus, which occurs as an extrusion across the host cell envelope, usually requires adhesion zones between the inner and outer membranes. While Ff phages provide all the components necessary for their egress, phage Dolos does not encode an outer membrane export protein. We were able to show that the *Shewanella* phage uses the same structure for exit as for entry by utilizing the host's MSHQ secretin for virion export. Furthermore, we found that Dolos suppresses the *Shewanella* prophage LambdaSo after infection to ensure its own establishment, likely by encoding an accessory protein predicted as an RstR-like protein that controls phage replication regulation and prevents superinfection. Thus, Dolos provides a great model system to challenge the paradigm models of Inoviruses.

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## P-PB-019

### Genetic modification of intractable staphylococcal clones by heat-shock facilitated phage transduction

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Increasing recognition of commensal bacteria as a requirement for microbiome integrity and pathogen exclusion puts urgency to the molecular characterization of commensal interactions. However, many commensals cannot be transformed with available methodology because of potent restriction barriers. We developed a new method for the introduction of plasmid DNA into otherwise intractable non-*Staphylococcus aureus* (NAS) staphylococci, important commensal members of human nose and skin microbiomes, by phage transduction. We demonstrate that a precise pulse of recipient bacteria with elevated temperatures prior to exposure to transducing phages renders NAS isolates

effectively and transiently amenable to transduction. Transduction of NAS mutants lacking restriction-modification (RM) systems did not profit from a heat shock indicating that it is the transient deactivation of RM enzymes that permit transduction. Our method also facilitated the transduction of other Bacillota from the genera *Bacillus* and *Listeria* indicating that it will support research on different bacterial groups from diverse ecosystems.

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## P-PB-020

### An old idea revisited: Bacteriophages as a therapy against antibiotic-resistant ESKAPE-Bacteria in a translational biofilm model

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**Introduction** The use of bacteriophages to combat multidrug-resistant bacteria represents a promising alternative to established antibiotic therapies due to their high specificity and efficacy. Particularly in chronic wound infections caused by antibiotic-resistant ESKAPE-pathogens such as *Pseudomonas aeruginosa*, phages could offer competitive therapeutic options. Clinical case reports have demonstrated successful outcomes, and numerous in-vitro studies show encouraging results. Translational settings are essential to bridge the preclinical gap and to provide factual evidence for the efficacy and safety of phage therapy.

**Methods** To analyze the effects of phages, a biofilm model composed of human plasma, buffy coat (immune cells), and *P. aeruginosa* PA14 was established. Phage lysates were isolated and enriched using adapted environmental isolation protocols. The infected model was treated daily with two different lysates as well as their combination, and analyzed after 24, 48, and 72 hours. Treatment effects were visualized using confocal laser scanning microscopy and quantified through suspension-based assays and image cytometry.

**Results** All treatments with *P. aeruginosa*-specific phages resulted in a significant reduction of bacterial load and biofilm volume. The combined application of multiple phages ("phage cocktails") did not yield a higher level of bacterial eradication in this case, suggesting that the total phage concentration is a key determinant of efficacy. Nevertheless, phage cocktails remain highly relevant in clinical practice to ensure effectiveness against the complex microbiome of wound biofilms.

**Conclusion** Specific phages significantly reduce *P. aeruginosa* in a single-species biofilm model, corroborating clinical case reports. Further research into infection mechanisms and possible interactions with human cells is required. In the long term, this approach may enable the development of individualized phage therapies for difficult-to-treat, antibiotic-resistant wound infections.

**Take-Home Message** In view of the increasing spread of antimicrobial resistance, bacteriophages should once again be considered as a valuable therapeutic option.

## P-PB-021

### The permanent lifestyle potential in *Chimalliviridae*

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Until recently, phages were classified according to a binary system that distinguished clearly between lytic and lysogenic lifecycles, either actively lysing the host cell or with the potential to integrate as prophages into the bacterial genome. However, recent studies challenge this dichotomy, suggesting that virulent phages with a strictly lytic lifecycle may maintain a permanent infection state within their host cells. Bacterial assemblies sequenced from single clones contained in about 0.1% of the cases virulent *Chimalliviridae* phages suggesting a permanent presence in bacteria<sup>1,2</sup>. These phages form nucleus-like compartments, thereby enabling them to operate largely independently of the host cellular machinery<sup>3</sup>. We hypothesize that nucleus-forming phages enter a state of permanent coexistence with the host, which is induced under adverse conditions and provides this phage family with an advantage over other phages that could impact also their therapeutic potential.

## References

1. Dougherty, P. E. et al. Persistent virulent phages exist in bacterial isolates. *bioRxiv*, doi: 10.1101/2024.12.31.630880 (2025).
2. Perfilyev, A., Gæde, A., Hooton, S. et al. Hidden in plain sight: Bacterial genomes reveal thousands of lytic phages with therapeutic potential. *bioRxiv*, doi: 10.1101/2025.05.06.652010 (2025).
3. Prichard, A. & Pogliano, J. The intricate organizational strategy of nucleus-forming phages. *Curr. Opin. Microbiol.* **79**, 102457 (2024).

## P-PB-022

### Phage-encoded ribosome-targeting factors in bacterial takeover

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Bacteria employ a variety of strategies to defend themselves against bacteriophage infections. Bypassing these strategies is crucial for determining the fate of the bacteriophage replication and efficiency in therapeutic setups. Through co-fractionation in gradients, we identified the  $\Phi$ KZ-encoded factor  $\Phi$ KZ014 as a novel interaction partner with the protein production machinery<sup>1</sup>. This factor is unique in that it binds to the 5S rRNA of the large ribosomal subunit, and it is one of the earliest phage proteins expressed upon infection. In a clinical *Pseudomonas aeruginosa* isolate,  $\Phi$ KZ014 is essential for phage replication. The relationship between translation and host takeover remains largely unexplored in general. We hypothesize that the role of  $\Phi$ KZ014 in the host's translation machinery may be connected to the circumvention of bacterial defence or stress response and surveillance mechanisms. Elucidating the relevant host factors and determining how they are affected by  $\Phi$ KZ014 is essential to understanding its broader role in orchestrating host takeover.

## References

1. Gerovac, M. et al. Phage proteins target and co-opt host ribosomes immediately upon infection. *Nat. A Microbiol.* **9**, 787–800 (2024).

## P-PB-023

### High-throughput analysis of bacteriophage sensitivity with focus on bacteriophage resistance determinants of clinical *Staphylococcus aureus*

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Antibiotic-resistant bacteria continue to pose major public health threats globally, prompting the urgent need for novel and innovative therapeutic strategies. Our project aims to identify and optimize bacteriophages which target the antibiotic-resistant pathogen *Staphylococcus aureus* (MRSA), while additionally describing bacteriophage resistance determinants of clinical strains that could impede phage therapy.

By combining next-generation sequencing techniques and high-throughput sensitivity testing, we want to use our extensive collection of clinical *S. aureus* isolates in order to determine the most suitable therapeutic phages and to describe bacteriophage resistance patterns for hundreds of clinical *S. aureus* isolates. Furthermore, we want to investigate how phage genomes and phylogeny affect host range, immune system activation and serum inactivation, in order to suggest the most promising genetic traits of phage therapy candidates. This will offer insights into the therapeutic capacity of different phage groups and predict problems arising upon treatment of bloodstream infections.

Up to now, we have isolated several bacteriophages from waste water, from river water, or induced from clinical isolates. All phage genomes and *S. aureus* isolates will be sequenced and analyzed, using a dedicated bioinformatics pipeline to identify the most promising phage specimen and the genetic determinants conferring resistance to phages.

On the long term, the most potent phage candidates with the best serum activity will be further optimized through genetic engineering to expand host range, enhance therapeutic efficacy and to help promote next-generation phage-based therapeutics.

## Prokaryotic cell biology

### P-PCB-001

#### Mining of *Vibrionaceae* genomes for single-copy plasmid origins and investigation of their replication and segregation machineries

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Plasmids drive bacterial adaptation and pathogenicity and carry genes for niche-specific traits, yet the molecular mechanisms governing their maintenance remain poorly understood in many bacterial families. In *Vibrionaceae* a family including the human pathogens *Vibrio cholerae* and



*Vibrio parahaemolyticus*, approximately one-third of isolates harbor plasmids often encoding genes critical for virulence and niche adaptation. Despite extensive characterization of plasmid cargo genes, the systems for the maintenance of these plasmids remain largely unexplored.

We are interested in single-copy plasmids carrying dedicated systems for plasmid replication and segregation. We applied a Hidden-Markov Model to identify genes encoding replication (*rep*) and segregation (*parA*, *parB*) proteins in thousands of *Vibrionaceae* genomes. We then combined computational and experimental approaches to explore the diversity of these systems. We revealed typical iteron sequences, which are known to be binding sites of the replication protein and therefore essential for replication initiation. The identified iterons differed significantly in the number of repeats and in the nucleotide sequence. For three selected candidates, we applied error prone PCR, followed by deep sequencing to map essential positions at single nucleotide resolution and to confirm the computational predictions.

Single-copy plasmids often encode the tripartite ParABS segregation system, comprising of the centromere-like *parS* sequence, the CTPase ParB that binds *parS*, and the ATPase ParA that segregates the ParB-*parS* complex to opposite cell poles. While ParA and ParB could be identified bioinformatically, the *parS* sequences are too diverse and have no conserved sequence or structure. We again employed a sequencing-based approach to define the functional boundaries of *parS* for some selected candidates.

Lastly, we characterized the performance of these single-copy origins in the fast-growing bacterium *Vibrio natriegens*. We observed a temperature dependent plasmid stability with almost perfect stability at lower temperatures and a very homogenous distribution in the population.

This work establishes an integrative computational-experimental workflow for mapping essential sequence features of single-copy plasmids. Because similar replication and segregation systems are widespread across bacterial plasmids, our approach provides a blueprint for revealing the unexplored diversity of plasmid maintenance mechanisms.

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## P-PCB-002

### Breaking barriers in evolutionary biology: Structure based homology discovery across the tree of life

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The advent of highly accurate structure prediction tools has transformed structural biology, enabling fold-based homology searches that go beyond the limits of classical sequence-based methods. Leveraging local cluster nodes equipped with the AlphaFold database and FoldSeek, our group wants to develop a high-throughput platform for structural homology analysis across prokaryotic and eukaryotic proteomes. We have utilised this strategy to discover protein complex conservation across taxa and novel protein architectures containings domains of interest. This approach has been applied to investigate the conservation of multi-protein systems involved in cell curvature control, building on the work of Pöhl et al., 2024. Compared to sequence homology, fold-based searches offer improved sensitivity and structural insight, allowing the refined classification of bactofilins into subfamilies distinguished by domain size. Moreover, our

approach has facilitated the discovery and characterization of previously unannotated proteins carrying domains of interest such as the ParB-like nucleotide switch, expanding our understanding of domain architecture and functional diversity across taxa. Highlighting new connections between function and structure across diverse lineages. We aim to demonstrate the potential of this approach for large-scale discovery and invite collaboration to extend these analyses across the tree of life.

Jumper, J., Evans, R., Pritzel, A. et al. Highly accurate protein structure prediction with AlphaFold. Nature 596, 583–589 (2021). <https://doi.org/10.1038/s41586-021-03819-2>

van Kempen, M., Kim, S.S., Tumescheit, C. et al. Fast and accurate protein structure search with Foldseek. Nat Biotechnol 42, 243–246 (2024). <https://doi.org/10.1038/s41587-023-01773-0>

Pöhl, S., Giacomelli, G., Meyer, F.M. et al. An outer membrane porin-lipoprotein complex modulates elongasome movement to establish cell curvature in *Rhodospirillum rubrum*. Nat Commun 15, 7616 (2024). <https://doi.org/10.1038/s41467-024-51790-z>

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## P-PCB-003

### Disentangling Qualitative and Quantitative Effects of Cellular CurT Protein on Cell Division and Photosynthetic Efficiency in *Synechocystis* sp. PCC 6803

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**Introduction:** All but the most early-branching oxygenic photoautotrophs perform their photosynthetic light reactions within a specialized intracellular membrane system called thylakoids. The Curvature Thylakoid (CurT) protein is an integral thylakoid membrane protein acting as both key determinant of thylakoid architecture [1, 2] and facilitator of symmetrical cell division in the model cyanobacteria *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 [3]. While CurT-dependent formation of thylapase structures (*i.e.*, zones of thylakoid membrane convergence towards the plasma membrane) and their functional involvement in photosystem II (PSII) biogenesis have been studied in some detail [2, 4, 5], dual involvement of CurT in structure-mediated differential thylakoid functionalization and divisome orchestration results in overlapping and pleiotropic phenotypes, obscuring precise analysis of its separate molecular functionalities.

**Goals:** Clear distinction between molecular functions of cyanobacterial CurT. Quantitative elucidation of CurT-dosage effects on cell division, cellular ultrastructure, and photosynthesis.

**Methods:** Genetic titration and biochemical quantification of cellular CurT levels, TEM-resolution of cellular ultrastructure, and physiological assessment of photosynthetic performance by chlorophyll fluorometry and oxygen evolution.

**Results and Conclusion:** Minimal cellular CurT levels enable efficient and symmetrical cell division, while PSII quantum yield and cellular oxygen evolution are compromised despite restored formation of thylakoid convergence zones. Meanwhile, enhanced cellular CurT levels result in an increased number of thylakoid membrane



facies and higher PSII quantum yield despite overall reduction in cellular PSII. This indicates a functional divide between qualitative membrane-restructuring properties of CurT affecting cell division and thylakoid membrane architecture, and quantitative properties of CurT facilitating PSII *de-novo* biogenesis and/or repair.

[1] Armbruster, U., *et al.*, *The Plant Cell* 25.7 (2013): 2661-2678.

[2] Heinz, S., *et al.*, *The Plant Cell* 28.9 (2016): 2238-2260.

[3] Dann, M., *et al.*, *Nature Communications* 16.1 (2025): 8424.

[4] Ostermeier, M., *et al.*, *Physiologia Plantarum* 177.2 (2025): e70234.

[5] Zhang, Z., *et al.*, *mLife* 4.5 (2025): 567-571.

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#### P-PCB-004

##### **A structurally coordinated maturation process for pyoverdines in the periplasm of pseudomonads**

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Most *Pseudomonas* species synthesize pyoverdines, which are fluorescent siderophores that are derived from ferriobactin, a non-ribosomal peptide [1]. After its cytoplasmic synthesis, ferriobactin needs to be transported into the periplasm, where a dihydroxyquinoline fluorophore is formed in a series of maturation steps. Several periplasmic enzymes are involved in this process, including the tyrosinase PvdP and the formylglycine-generating enzyme-related PvdO, which together catalyze the transformation of a strictly conserved D-Tyr/L-Dab sequence to the fluorophore that is characteristic for all pyoverdines. After having clarified the functions of PvdP and PvdO [2], we discovered that PvdM, a membrane-anchored periplasmic protein, is essential for the function of PvdP [3]. However, the exact role in the process was unresolved so far. Here we show that PvdM forms a complex with PvdP, which likely serves to channel the ferriobactin to PvdP. The unusual structural organization of this complex is unprecedented in membrane-associated protein systems and appears to be designed to ensure complete turnover of ferriobactin to pyoverdine, thereby suppressing the wasteful loss of biosynthetic intermediates from the periplasm into the environment.

[1] Ringel M.T. and Brüser T. (2018) *Microb Cell*. 5:424-437. doi: 10.15698/mic2018.10.649

[2] Ringel M.T., Dräger G. and Brüser T. (2018) *J Biol Chem*. 293:2330-2341. doi: 10.1074/jbc.RA117.000121

[3] Sugue M.F., Burdur A.N., Ringel M.T., Dräger G. and Brüser, T. (2022) *J Biol Chem*. 298:102201. doi: 10.1016/j.jbc.2022.102201

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#### P-PCB-005

##### **Polar Identity in *Shewanella putrefaciens***

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#### **Background:**

The polar identity of rod-shaped bacteria, like *Shewanella putrefaciens*, is essential for many processes and organization, like flagellar assembly or a working chemotaxis system. For this, bacteria have a complex, spatiotemporal organization that maintains this polar identity through every cell cycle. Every cell division results in two new existing cell poles arising from the divisome. This results in a switch of polar identity from the former "new" cell pole, which now becomes an old cell pole that has to be restructured accordingly. How this process is mediated is still unknown. Many of the polar processes rely on landmark proteins like the conserved protein HubP (Hub of pole), which recruits other proteins to the cell pole involved in many different processes from type IV pili-mediated twitching-motility via flagellar assembly to chromosome segregation. We want to investigate the identity of the cell poles and the specific switch throughout the cell cycle.

#### **Methods and Results:**

Mini cell approach: To understand the conditions at the cell poles, specifically the differences between the old and new cell pole, we produced modified *S. putrefaciens* mini cells. To reach this goal, we deleted two genes, *minD* and *parA*, to enable *ftsZ*-ring assembly at the poles. Septum formation at the cell poles results in the production of mini cells, which contain pole-specific RNAs and proteins. To investigate the differences between the old and new cell poles, we tagged the hook of the flagellum with an amino acid exchange to separate the two types of mini cells for downstream analyses in regard of protein and mRNA contents.

BioID approach:

To find novel transient interaction partners of FimV/HubP, we fused the protein to UltraID, a hyper-active biotin ligase, which biotinylates all proteins in immediate vicinity. These are subsequently enriched and identified by mass spectrometry.

#### **Discussion and outlook**

Both methods will lead to the discovery of novel actors in polar identity of *S. putrefaciens*. This will help to better understand the identity switch from a new to old cell pole. Follow-up research will show if the mechanisms identified are conserved in other rod-shaped Gram-negative bacteria and lead to a better understanding the cellular tempo-spatial organization in bacteria.

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#### P-PCB-006

##### **The last resort antibiotic daptomycin exhibits two independent antibacterial mechanisms of action**

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Daptomycin is a lipopeptide antibiotic that exhibits excellent activity against Gram-positive organisms and is often used as a last-resort treatment for multidrug-resistant bacterial infections. Despite its vital clinical role, the exact mechanism of action of daptomycin remains a topic of debate. In particular, considerable controversy surrounds whether daptomycin permeabilises the cytoplasmic membrane of target cells by forming large pores. Using a series of fluorescence microscopy-based techniques on the Gram-positive model organisms *Bacillus subtilis* and *Staphylococcus aureus*, we found that daptomycin triggers extensive autolysis, which has likely been misinterpreted as membrane pore formation in previous *in vivo* studies. In cells lacking key cell wall hydrolytic enzymes, even prolonged incubation with daptomycin is unable to trigger membrane pore formation. In contrast, daptomycin causes a partial depolarisation of the cytoplasmic membrane which, in turn, is responsible for inducing cellular autolysis.

Using *B. subtilis* L-forms deficient for the *uppS* gene, we further demonstrate that, in the absence of daptomycin's recently identified interaction with the undecaprenyl-coupled cell wall precursors, fluid membrane microdomains associated with daptomycin's antibacterial activity do not form. Moreover, we show that these microdomains characteristic of daptomycin's mode of action depend on *B. subtilis* IM30/phage shock protein A homologue LiaH, which is strongly induced in response to daptomycin.

In summary, daptomycin exhibits two independent antibacterial mechanisms of action: (i) targeting of cell wall precursor lipids that leads to the inhibition of cell wall synthesis, and (ii) membrane depolarisation that does not rely on interactions with cell wall precursor lipids. These findings demonstrate that antibiotics with dual mechanisms can be clinically successful and show favourable characteristics regarding resistance development.

Buttress et al. (2024) The last resort antibiotic daptomycin exhibits two independent antibacterial mechanisms of action. *bioRxiv*, doi: 10.1101/2024.12.20.629673

Gray & Wenzel (2020) More than a pore: A current perspective on the *in vivo* mode of action of the lipopeptide antibiotic daptomycin. *Antibiotics*, 9(1):17. doi: 10.3390/antibiotics9010017

## P-PCB-007

### Molecular basis for the targeting process of injectisome substrate proteins in *Salmonella enterica*

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A major virulence factor of many pathogenic gram-negative bacteria, including *Salmonella enterica* Typhimurium, is the injectisome, a needle-like structure that injects toxins into host cells during infection. The injectisome employs a type-III secretion system (T3SS) for the export of toxin proteins.

Before initiating toxin secretion, it secretes its own structural components through a highly controlled self-assembly process. However, because T3SS substrates do not contain a typical cleavable secretion signal in the amino-terminal region of the peptide sequence [1], it remains unknown how these substrates are selected from thousands of cytoplasmic proteins and specifically targeted to the T3SS. This project explores the hypothesis that substrate gene transcription and translation occur in close proximity to the T3SS, enabling efficient recognition and secretion. This concept is inspired a process known as transertion in which transcription, translation, and membrane insertion of proteins are spatially coordinated. Recent findings suggest that *Vibrio parahaemolyticus* utilizes transertion for T3SS substrate secretion [2], but its role in *Salmonella* remains unclear. To test this hypothesis, co-localization analyses of chromosomal substrate gene loci and the T3SS, as well as spatial studies on membrane proximity will be conducted. In summary, this project will provide critical insights into the type III secretion process, shedding light on the assembly mechanism of the injectisome and the secretion of toxins during infection. Ultimately, it will elucidate the potential role of local transcription and translation of substrate genes in these processes.

[1] Michiels T, Wattiau P, Brasseur R, Ruyschaert JM, Cornelis G (1990). Secretion of Yop proteins by *Yersinia*. *Infect Immun* 58(9), 2840–2849

[2] Kaval KG, Chimalapati S, Siegel SD, Nalleli G, Jananee J, Ankur BD, Kim O (2023). Membrane-localized expression, production and assembly of *Vibrio parahaemolyticus* T3SS2 provides evidence for transertion. *Nat Commun* 14, 1178

## P-PCB-008

### A novel putative DNA damage-repair system Dip in *Corynebacterium glutamicum*

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The bacterial SOS response is a generalized mechanism that upon stress allows efficient DNA damage repair and pauses cell division to prevent the segregation of damaged chromosomes. In *Corynebacterium glutamicum*, a biotechnological workhorse and a model organism for the cell biology of Mycobacteriales, the SOS regulon comprises 48 genes, nearly half of which with unknown functions. We identified a new SOS-regulated operon *dipABCD* (DNA damage-induced proteins) and explored its role in DNA repair. CRISPRi-seq data and phenotypical assays confirmed that *dip* deletions affect other DNA repair and oxidative stress pathways. Through *in vitro* and *in vivo* approaches, we showed that the Dip system includes the GTP-dependent DNA nickase DipA and the ATP-dependent helicase DipD that binds near DNA damage sites. Additionally, DipD was found to form oligomers under oxidizing conditions *in vitro*, suggesting that the Dip system could play a specialized role in DNA repair under oxidizing conditions. Single-molecule localization microscopy revealed that DipA influences the dynamics of DipC and DipB. Thus, the DNA nicking activity of DipA may be necessary for the DNA binding of other Dip proteins. In summary, our results suggest a potential role of the Dip system in oxidative DNA damage repair.

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## P-PCB-009

### Dam it! Tracking DNA methylation-driven regulation in *E. coli*

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Dam-dependent methylation is known to regulate the initiation of DNA replication in *Escherichia coli* by modulating the replication origin binding of the sequestration protein SeqA. Evidence exists for methylation-dependent gene regulation, yet the exact molecular mechanisms involved remain incompletely characterized. Notably, no previous studies have specifically analyzed the effects of Dam or SeqA on gene expression apart from their functions in replication control. To address this gap, a strain was engineered in which the native origin of replication was replaced by the F plasmid origin. Since replication initiated from *oriF* is independent of SeqA regulation, this model enables targeted investigation of SeqA and Dam as transcriptional regulators. Global transcriptomic analysis using RNA-seq was performed on exponentially growing *seqA* and *dam* deletion mutants carrying *oriF*. Notably, both mutants exhibited activation of the SOS response, with *dam* deletion displaying a more pronounced effect, indicating that methylation-dependent repression is involved in regulation of the process. Subsequent experiments sought to elucidate the mechanisms underlying the observed gene expression changes. To assess the potential for SeqA to function directly as a transcriptional regulator, a series of synthetic promoters containing multiple GATC motifs as binding sites for SeqA were designed and constructed. The characterization of respective reporter fusions in synchronized populations has the potential to uncover a direct effect of SeqA on transcription initiation. DNA methylation has been extensively studied in *E. coli*, however the molecular basis of its regulatory functions remains complex and not fully resolved. This project advanced the investigation of global effects of methylation-dependent regulation in the cell and brought potential strategies that can be used to unravel the principles behind its mechanism.

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## P-PCB-010

### Investigations on cell cycle and shape determination in star-shaped bacteria from the genus *Stella*

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The physiological and morphological diversity of bacteria is enormous, ranging from simple rods or cocci to extraordinary asymmetrical cell shapes. In the second half of the last century, Heinz Schlesner (Kiel University) isolated over 500 strains based on their unusual morphology from various habitats and deposited them in his personal bacterial strain collection. From this collection, we investigated 17 strains, which are members of the genus *Stella* within the Alphaproteobacteria. Members of this genus are described to form unusually shaped cells, which resemble flat, six-pointed stars (Vasilyeva, 1970). The cells were proposed to divide by constricting at midcell, leaving three-pronged, crown-like daughter cells that would regrow the missing three prongs until their next division (Vasilyeva, 1972). However, our microscopic observations indicate otherwise and suggest a much more complex shape and cell cycle for these cells. Using genetically modified strains, we started to address the

differences between our and previous observations by moving from 2D observations into a 3D environment.

## References:

Vasilyeva, L. V. 1970. 'Звездообразный почвенный микроорганизм', *Известия Академии Наук СССР Серия биологическая*: 308-10.

Vasilyeva, L. V. 1972. 'Особенности ультраструктуры и цикл развития *Stella humosa*', *Известия Академии Наук СССР Серия биологическая*: 782-88.

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## P-PCB-011

### Bacteria of Prey: Fantastic Microorganisms and Where to Find Them

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Since their discovery, members of the phylum *Planctomycetota* have surprised scientists with their unique features. Although the molecular mechanism is yet to be elucidated, bacteria from the class *Planctomycetia* are well known for their budding cell division. Recently, several environmental isolates belonging to the phylum *Planctomycetota* were discovered, which challenge commonly accepted principles of bacterial cell biology yet again. One of these exceptional strains was reported by Shiratori et al. (2019): In addition to its eukaryotic-like locomotion and division, it is capable of engulfing prey bacteria in a phagocytosis-like process. We now isolated nine similarly behaving bacteria, which are phylogenetically related to the strain reported by Shiratori et al. (2019), from environmental samples and studied their various peculiar features in more detail.

## References:

Shiratori, T., Suzuki, S., Kakizawa, Y., and Ishida, K. I. 2019. 'Phagocytosis-like cell engulfment by a planctomycete bacterium', *Nature Communications*, 10: 5529.

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## P-PCB-012

### The dynamin-like protein DynA confers resistance against bacteriophages through membrane protection in *Bacillus subtilis*

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In bacteria, membrane remodeling is essential for fundamental molecular processes such as growth, cell differentiation, and pathogenesis. Membrane fusion, fission, and reshaping of membranes can be mediated by members of the dynamin-like protein family (DLPs). These large GTPases possess a modular architecture that includes a GTPase domain, a four-helix bundle domain responsible for oligomerization and trunk domains that may incorporate a paddle domain required for membrane tethering [1]. While genes encoding DLPs are often found in tandem in bacteria, in *Bacillus subtilis* we identified a two-headed DLP, termed DynA, composed of two fused dynamin-like subunits. Although many DLPs have been found in bacteria, only a few have been functionally characterized. In this study, we revealed that DynA membrane binding is mediated by a paddle domain with a hydrophobic tip that inserts into the membrane. This interaction is stabilized by positive charges, provided by two flanking lysine residues [2]. DynA is highly dynamic in the cell, but forms stable clusters in response to membrane stress induced by bacteriophages or nisin [3]. Consequently, DynA protects membranes at sites of pore formation, thereby delaying cell lysis and conferring resistance to bacteriophage infection. In this process, membrane binding of DynA is required for its protective effect, while GTP hydrolysis is dispensable for liposome fusion in vitro but essential for anti-phage defense in vivo. In summary, our results demonstrate that DynA functions as a membrane-protective factor, providing resistance against both nisin and bacteriophages  $\Phi$ 29 and SP $\beta$  in *B. subtilis*.

[1] Bohuszewicz, O., Liu, J. & Low, H. H. (2016). Membrane remodelling in bacteria. *Journal Of Structural Biology*, 196(1), 3–14. <https://doi.org/10.1016/j.jsb.2016.05.010>

[2] Shafqat, S., Repnik, U. & Bramkamp, M. (2025). Binding of *Bacillus subtilis* dynamin-like protein DynA to the bacterial membrane is essential for effective phage defense. *FEBS Journal*. <https://doi.org/10.1111/febs.70282>

[3] Guo, L., Sattler, L., Shafqat, S., Graumann, P. L. & Bramkamp, M. (2022). A Bacterial Dynamin-Like Protein Confers a Novel Phage Resistance Strategy on the Population Level in *Bacillus subtilis*. *mBio*, 13(1), e0375321. <https://doi.org/10.1128/mbio.03753-21>

## P-PCB-013

### The periplasmic PtaA of *Pseudomonas fluorescens* is an unusual transaminase

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The fluorescent siderophore pyoverdine, which is produced by most pseudomonads in response to iron limitation, is always non-ribosomally synthesized with an N-terminal glutamic acid residue that is bound to the dihydroxyquinoline fluorophore via an isopeptide bond [1]. In many *Pseudomonas* species, the  $\alpha$ -amino group of the glutamic acid is exchanged by a keto group at a late stage of biosynthesis, and this conversion depends on PtaA, a predicted transaminase [2]. To examine whether this potential transaminase indeed catalyzes the reaction, we purified recombinant PtaA of *P. fluorescens* A506 from the cytoplasm of *Escherichia coli* and examined its biochemical and enzymatic characteristics. The purified enzyme was

homodimeric and contained two pyridoxamine phosphate cofactors per dimer, indicating that an accepted amino group donor was present in the cytoplasm of *E. coli*, whereas an efficient amino group acceptor was lacking in that compartment. We were able to achieve a turnover with artificial amino group acceptors.

[1] Ringel M.T. and Brüser T. (2018) Microb Cell. 5:424-437. doi: 10.15698/mic2018.10.649

[2] Ringel M.T., Dräger G. and Brüser T. (2017) J Biol Chem. 292:18660-18671. doi: 10.1074/jbc.M117.812545

## P-PCB-014

### Purification and Proteomic Analysis of Premagnetic Magnetosome Vesicles

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Magnetosomes are sensory organelles which are formed by magnetotactic bacteria and consist of magnetite crystals (~40 nm) enveloped by a proteinaceous lipid-bilayer membrane. In the alphaproteobacterium *Magnetospirillum gryphiswaldense* magnetosomes are formed by a genetically encoded pathway, which starts with the invagination of empty magnetosome membrane vesicles (eMMs) from the cytoplasmic membrane, simultaneous with the targeting of ca. 30 MM-specific proteins and followed by iron uptake and biomineralization of the magnetite core [1, 2]. While purification and analysis of mature magnetite-containing magnetosomes (magMM) by sedimentation and magnetic separation is relatively straightforward, isolation of pre-magnetic eMMs has proven to be challenging due to their low abundance and the lack of magnetic and density tags. Thus, little is known about the early stages of magnetosome biosynthesis represented by eMMs.

In this study, we employed different synthetic tags, which were specifically anchored to the eMMs by genetic fusion to abundant magnetosome proteins. Affinity chromatography from disrupted iron-starved and non-magnetic mutant cells resulted in the selective purification of "empty" membrane vesicles, but with the characteristic size and appearance of magnetosome membranes as revealed by electron microscopy. For in depth biochemical analysis, the proteome of these eMMs was compared with all other cellular compartments. The about 30 known MM-specific proteins were most abundant in the magMM, and also significantly enriched in the eMMs. However, the latter also contained a larger fraction of proteins typical for the cytoplasmic membrane, consistent with the origin of early stage eMMs from this compartment.

In summary, we demonstrate that engineered pre-magnetic eMM vesicles can be purified to homogeneity via affinity chromatography. Moreover, electron microscopy and comparative proteomics seem to indicate that eMMs represent compartments with a distinct biochemical composition.

[1] Schüler D. et al, Biosynthesis and function of magnetic organelles in magnetotactic bacteria., *Nat. Rev. Microbiol.* 2015, 1-15

[2] Raschdorf O. et al., Genetic and ultrastructural analysis reveals the key players and initial steps of bacterial magnetosome membrane biogenesis, *PLoS Genet.* **2016**, *12*, e1006101

## P-PCB-015

### Untangling Cell Division Mechanisms in the Phylum *Planctomycetota*

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Members of the phylum *Planctomycetota* exhibit remarkable deviations from the cell biology of model bacteria<sup>1</sup>. One of their most distinctive features is their unusual asymmetric cell division. During this process, a small daughter cell is formed on one side of the mother cell, grows over time, and eventually pinches off. Although members of the phylum possess a peptidoglycan (PG) sacculus<sup>2</sup>, several genes encoding PG biosynthetic enzymes appear to be non-essential<sup>3</sup>. Moreover, planctomycetes lack genes encoding FtsZ and most of the otherwise canonical cell division proteins. Studying cell division in this phylum, however, is constrained by the lack of advanced genetic tools. Thus, molecular mechanisms driving PG synthesis, PG insertion and therefore planctomycetal cell division remain enigmatic.

Here, we present an updated molecular toolbox for members of the phylum *Planctomycetota* featuring streamlined plasmids for the construction of sequential gene deletions, a diverse set of fluorescent proteins suitable for protein fusions as well as a naturally-occurring sugar-dependent regulator/regulated promoter pair which was turned into an inducible gene expression system. Using these molecular tools and microscopic investigations, we analyzed cell division across phylum members and identified distinct clades with variations in the cell division modes. Based on these analyses, we mapped the morphological dynamics of planctomycetal cell division over time. Staining with fluorescent dyes enabled the visualization and characterization of important processes involved in cell division and immunostaining revealed protein candidates potentially involved in the process.

Together, these results reveal some of the exceptional nature of planctomycetal cell division and establish a foundation for future molecular and mechanistic exploration of this phylum.

#### References:

<sup>1</sup>Boedeker, C. et al. Determining the bacterial cell biology of Planctomycetes. *Nature Communications* **8**, 14853, doi:10.1038/ncomms14853 (2017).

<sup>2</sup>Jeske, O. et al. Planctomycetes do possess a peptidoglycan cell wall. *Nature Communications* **6**, 7116, doi:10.1038/ncomms8116 (2015).

<sup>3</sup>Rivas-Marin, E. et al. Essential gene complement of Planctopirrus limnophila from the bacterial phylum Planctomycetes. *Nature Communications* **14**, 7224, doi:10.1038/s41467-023-43096-3 (2023).

## P-PCB-016

### Does population heterogeneity facilitate survival upon exposure to quaternary ammonium compounds in *Mycobacterium*?

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Mycobacteria differ from other rod-shaped bacteria in that they elongate at the poles and divide asymmetrically, resulting in emergence of two uneven daughter cells, and in consequence enhances population heterogeneity. In addition, mycobacteria possess a thick, hydrophobic cell envelope, the composition of which can be modified in response to stress factors. These features contribute to resistance of mycobacteria to unfavorable environmental or host-derived conditions, as well as to many inhibitors, including anti-mycobacterial drugs. Given the rising incidence of mycobacterial infections—caused by both pathogenic and environmental species—and the excessive use of disinfectants during the recent COVID-19 pandemic, studying interactions between biocides and anti-mycobacterial drugs is of growing importance.

The aim of the presented studies is to elucidate the mode-of-action of a widely used class of disinfectants, quaternary ammonium compounds (QACs). Utilizing a fluorescently labeled analog of the commonly used dodecyl dimethyl ammonium chloride (BAC12), NBD-DDA, we were able to perform real-time analysis of compound incorporation into *Mycobacterium smegmatis* cells. Our results suggest that NBD-DDA inhibits growth by disruption of cell membrane integrity. Furthermore, excess compound is accumulated within the cells in septum area, off-center, and on the cell poles, forming bright fluorescent foci. Interestingly, the chromosome is excluded from the areas of NBD-DDA accumulation. Quantification of the NBD-DDA signal intensity indicates that the compound is incorporated more efficiently into actively dividing cells than into stationary, metabolically inactive ones. To investigate whether daughter cell heterogeneity contributes to survival of NBD-DDA exposure, we compared the wild-type asymmetric *M. smegmatis* population with a deletion mutant, a  $\Delta lamA$  mutant lacking the LamA protein, which is crucial for mediating asymmetric daughter cell growth. Real-time microscopic analysis suggests that asymmetric cell division might facilitate survival during biocide exposure. Moreover, NBD-DDA appears to penetrate more efficiently into daughter cells inheriting a new cell pole, as opposed to those inheriting the old pole, where the elongation machinery is already assembled.

Taken together, these results provide important insights into the mechanism of QAC-mediated inactivation of Mycobacteria, which could inform and improve future disinfection and treatment strategies.

## P-PCB-017

### Non-resistance genes on native *E. coli* resistance plasmids do not commonly convey fitness benefits

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#### Introduction

Bacteria carrying antimicrobial resistance (AMR) plasmids often persist in farm environments even when antibiotics are no longer given. Although antimicrobial stewardship has improved, resistances even against reserve antibiotics remain present in the population. The plasmids conveying these resistances commonly carry both resistance genes and a variety of accessory genes, some of which have no known function. Past studies have shown altered virulence phenotypes in some *E. coli* strains when their native resistance plasmids were removed ("plasmid curing"). However, it is unclear how common such virulence changes are. We thus employ a plasmid-based CRISPR-Cas9 system to cure chicken-associated *E. coli* of their native  $\beta$ -lactamase plasmids and assess physiological changes in the context of the native host. We look to establish whether physiological benefits contribute to the persistence of AMR plasmids in the absence of antibiotics.

#### Materials & Methods

We generated a plasmid carrying an inducible CRISPR-Cas9 system targeting the *bla*CTX-M/*bla*TEM, and *bla*SHV/*bla*CMY gene families ("pCBL"), allowing for targeted removal of ESBL/pAmpC-plasmids. We chose *E. coli* strains isolated from the broiler chicken context for this. Transformants with pCBL were cured by growth in inducing conditions. Plasmid-cured variants (PCV) were selected by loss of resistance and used in physiological assays.

#### Results

We successfully cured 19 *E. coli* strains of their native resistance plasmids and compared their growth and biofilm formation to the respective wild types. We find that most strains show only minor growth differences in liquid medium. Furthermore, biofilm formation shows few differences at 28 and 37°C. However, many strains show significant growth inhibition during the curing process, which increases with inducer concentration. This is often accompanied by large-scale rearrangements of the *cas9* gene, inactivating pCBL. This toxicity only occurs when the  $\beta$ -lactamase gene present in the strain matches the target of the pCBL version.

#### Summary

Our findings show no general fitness benefit to ESBL/pAmpC-plasmid carriage. Instead, the high toxicity of plasmid loss, which is likely due to the action of plasmid stability systems present on AMR plasmids, is likely to stabilise plasmid carriage. We thus propose that the maintenance of AMR plasmids in *E. coli* is more strongly linked to plasmid stability systems than to fitness benefits derived from the plasmid backbone.

## P-PCB-018

### Super-resolution microscopic analysis of the spatial organization of the polar elongasome in *Corynebacterium glutamicum*

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The complex cell envelope of *Mycobacterials* represents one of the most important targets for antimicrobial agents. Consequently, the biosynthesis of their unique diderm envelope has been intensively studied. This multilayered structure consists of an initial peptidoglycan (PG) layer covalently linked to an arabinogalactan (AG) polysaccharide, which is further esterified with long-chain mycolic acids (MA). Together, these components form the mycolyl–arabinogalactan–peptidoglycan (mAGP) complex, a hallmark of the genus [1]. The enzymes and protein machineries involved in the synthesis of all three layers have been largely identified, including the apically localized elongasome responsible for polar growth and the divisome required for cell division [1].

Despite this progress, the spatial and temporal coordination of these biosynthetic systems remains poorly understood. Using the model organism *Corynebacterium glutamicum*, this study aims to elucidate the spatial regulation of mAGP synthesis at the septum and cell poles through high-resolution fluorescence microscopy and analysis of molecular dynamics. In particular, the localization and dynamic behavior of key enzymes representing each layer, RodA (*cg0061*) for PG synthesis, Emb (*cg0235*) for AG synthesis, and Cmt2 (*cg3186*) for MA synthesis, will be investigated [2]. These experiments will provide new insights into how the individual layers of the mycobacterial cell envelope are synthesized and integrated in space and time, ultimately contributing to a deeper understanding of envelope biogenesis and its potential as a drug target.

[1] Jankute, Monika, Jonathan A. G. Cox, James Harrison, and Gurdyal S. Besra. (2015). Assembly of the *Mycobacterial* Cell Wall. *Annual Review of Microbiology* 69: 405-23. <https://doi.org/10.1146/annurev-micro-091014-104121>.

[2] Meyer, Fabian M, and Marc Bramkamp. (2024). Cell Wall Synthesizing Complexes in *Mycobacterials*. *Current Opinion in Microbiology* 79: 102478. <https://doi.org/10.1016/j.mib.2024.102478>.

## P-PCB-019

### Bacterial motility and chemotaxis in porous media: lophotrichously flagellated *Pseudomonas putida* exhibits run motility with mechanical trapping and active turning events that enable chemotaxis based on a turn-angle bias

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Bacterial motility and chemotaxis have been extensively studied in bulk liquid environments, particularly for the peritrichously flagellated model organism *Escherichia coli*, which shows a run and tumble/turn motility. In such settings, navigation toward a source of chemoattractant relies on run-time bias, where run phases are extended when bacteria swim toward higher nutrient concentrations.

Much less is known about how bacteria perform chemotaxis in complex, structured environments, where physical confinement restricts free swimming paths and a run-time bias is less feasible. Previous studies have suggested that *E. coli* also bias their turning angle during tumbles, adjusting reorientations to favor subsequent runs toward the chemoattractant, a strategy that could be advantageous under confinement.

In a recent study, we analyzed the motility of the lophotrichously flagellated soil bacterium *Pseudomonas putida* in porous media and identified run phases and active turning events -known from bulk liquid- and additional mechanical trappings caused by the porous environment [1].

We provide experimental evidence that *P. putida* also performs chemotaxis in porous media by employing a turn-angle bias strategy [2]. We show that the resulting directional preference of runs arises from the active, motor-induced turning events, while passive mechanical trapping in the porous matrix weakens this bias [2]. Agent-based simulations, parameterized with experimentally observed swimming statistics, indicate that the turn-angle bias represents the predominant chemotactic strategy of bacteria navigating porous environments [2].

Our results suggest that the turn-angle bias may represent a widespread, common strategy employed by different bacteria to navigate different environments.

[1] Datta A. et al.: "Bacterial swimming in porous gels exhibits intermittent run motility with active turns and mechanical trapping", Sci Rep 15, 20320 (2025)

[2] Beier S. et al.: "Dual Chemotaxis Strategy of Bacteria in Porous Media: Run Time and Turn Angle Bias", arXiv:2503.05286 (2025)

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## P-PCB-020

### Profile of targeted penicillin binding proteins drives synergy between $\beta$ -lactams and aminoglycosides

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Antibiotic combinations have been previously shown to be highly species-specific. While a mechanistic basis for such specificity is yet to be understood, the potential to use combinations for narrow-spectra treatment, where the pathogen is primarily targeted and the commensal bacterial population is protected, is there. Here, we investigate species-specific synergistic combinations against *Pseudomonas aeruginosa*, a particularly problematic Gram-negative pathogen, given its intrinsic resistance to major classes of antibiotics. We focus on understanding the mechanism of action underlying synergy between aminoglycosides and  $\beta$ -lactams, because of its clinical significance. We started by assessing synergy across different bacterial strains beyond *P. aeruginosa*, including *Escherichia coli*, *Salmonella Typhimurium*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Burkholderia pseudomallei*. Our data shows that synergy potency widely varies across all species. It is particularly potent against *P. aeruginosa*, while the weakest effect is observed for *E. coli*

strains. Current evidence suggests possible cross-permeabilization between  $\beta$ -lactams and aminoglycosides as the mechanism underlying synergy, but they do not determine the species-specificity of the combination.

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## P-PCB-021

### Role of chaperons and the Ap4A second messenger in the *Bacillus subtilis* stress response

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**Introduction:** The heat shock response in *Bacillus subtilis* involves a complex network of signaling pathways and protein quality control mechanisms that ensure cellular survival under stress. Diadenosine tetraphosphate (Ap4A) is a nucleotide found in many organisms, but its physiological role is unclear. It is synthesized by aminoacyl-tRNA synthetases and degraded by specific hydrolases. Ap4A levels increase during heat or oxidative stress, suggesting it may play a role in stress adaptation, although its specific targets and pathways remain unknown. Chaperons ClpC and HtpG contribute to the protein quality control, ClpC by disaggregating, unfolding, refolding or degrading misfolded proteins, and HtpG by assisting in the late stages of folding and stabilizing near-native substrates.

**Goals:** The aim of this work is to study the role of Ap4A and chaperones ClpC and HtpG in maintaining protein homeostasis under stress.

**Materials and methods:** A genetic approach was used to control Ap4A levels. The resulting strains were analyzed for growth under heat shock and oxidative stress. In parallel, *htpG* and *clpC* deletion mutants were created to evaluate their roles in stress response and protein quality control.

**Results:** Ap4A levels were manipulated by deleting or overexpressing the *yqeK* gene, which encodes an Ap4A hydrolase. The  $\Delta yqeK$  mutant showed increased sensitivity to heat shock during exponential growth; however, overexpression of *yqeK* did not noticeably affect growth or stress response. Additionally, the roles of ClpC and HtpG in stress adaptation were studied. Deleting either *clpC* or *htpG* reduced growth rate, and removing both led to a severe growth defect and increased oxidative stress sensitivity.

**Summary:** These results suggest that decreased intracellular Ap4A levels do not significantly impact bacterial growth. In contrast, elevated Ap4A levels result in a growth defect under heat stress, suggesting Ap4A may have an inhibitory function during the stress response. Additionally, it was found that ClpC and HtpG play compensatory roles in maintaining cellular homeostasis, suggesting that HtpG may stabilize key proteins or contribute to protein degradation or regulatory pathways during stress adaptation.

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## P-PCB-022

### MurA interacts with proteins involved in phospholipid and lipopolysaccharide synthesis in *Escherichia coli*

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The envelope of Gram-negative bacteria contains an inner membrane consisting of a phospholipid bilayer and an outer

membrane which is made of phospholipids (PL) in the inner leaflet and lipopolysaccharides (LPS) in the outer leaflet. The periplasm is located between the two membranes and contains a thin peptidoglycan (PG) layer. The balance of LPS and PL in the outer membrane is crucial for the survival of bacteria, therefore, the biosynthesis pathways of both components need to be tightly regulated. The levels of LPS and PL are monitored by a complex machinery including the FtsH protease and the inner membrane protein LapB. LapB serves as an anchor for the cytosolic proteins of both pathways (LpxA, LpxC, LpxD, FabZ) and facilitates the post-translational degradation of the key enzyme of LPS biosynthesis LpxC. Moreover, PL, LPS and PG biosynthesis share same substrate pools, but how PG synthesis is synchronized with PL and LPS biogenesis is poorly understood.

To investigate the coordination between PG and LPS/PL synthesis, we employed complementary studies using bacterial two-hybrid assay, pulldown and microscale thermophoresis. We discovered protein-protein interactions of the initial PG biosynthesis enzyme MurA with LapB and the cytosolic proteins LpxA, LpxC, LpxD and FabZ. Furthermore, we analyzed whether MurA is degraded by the LapB/FtsH protein network like LpxC. However, our results show that MurA is not post-translationally controlled by this machinery.

Overall, our studies indicate that PG synthesis might be synchronized with LPS and PL production via direct protein-protein interactions of MurA and emphasize LapB as a central scaffold for cell envelope biogenesis in *E. coli*.

#### P-PCB-023

##### Protein-protein interactions of the membrane anchor LapB coordinate cell envelope synthesis in *Escherichia coli*

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The cell envelope of Gram-negative bacteria is composed of phospholipids (PL), peptidoglycan (PG) and lipopolysaccharides (LPS). The biosynthesis pathways of these compounds are connected by the shared precursor molecules (R)-3-hydroxy-myristoyl-ACP (acyl-carrier protein) and UDP-GlcNAc (UDP-N-acetylglucosamine). In *Escherichia coli*, LPS synthesis is further regulated by post-translational degradation of the key enzyme LpxC. For this process, the adaptor protein LapB (YciM) binds LpxC and directs it to the membrane-bound protease FtsH. While LapB is integrated into the inner membrane via its transmembrane domain, the cytosolic part of the protein harbors nine tetratricopeptide repeat motifs (TPRs) and a C-terminal rubredoxin domain. TPRs are known to mediate protein-protein interactions and are often associated with multiprotein complexes. Previous studies demonstrated that LapB not only interacts with LpxC, but also with LpxA and LpxD, as well as with FabZ, the first enzyme in the PL synthesis pathway (Möller *et al.*, 2023). Thus, we propose that LapB serves as an anchor for the cytosolic enzymes required for cell envelope synthesis by enabling the formation of a large multiprotein complex.

We aim to investigate how LapB coordinates these presumably dynamic interactions. To identify which TPRs are required for specific binding partners, we used targeted mutagenesis for the deletion of single TPRs of LapB. With these mutants we performed *in vivo* (bacterial two-hybrid)

and different *in vitro* protein-interaction studies. The results will provide insights into how LapB navigates its protein interactions and how this affects the coordination of cell envelope biosynthesis pathways.

#### P-PCB-024

##### Insights into unique flagellar motor dynamics in an amphitrichously flagellated magnetotactic spirillum

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Efficient flagellar motility is crucial for environmental colonization and survival for many bacterial species. Flagellar arrangements vary widely, and while research is often focused on traditional model species, alternative flagellation and motility patterns remain incompletely understood.

Here, we established live-cell visualization of flagella in *Magnetospirillum gryphiswaldense*, a model organism for magneto-aerotaxis. Through intracellular magnetic organelles called magnetosomes, *M. gryphiswaldense* aligns within Earth's magnetic field. Passive magnetic alignment, combined with aerotactic sensing and flagellar motility, is thought to facilitate navigation toward favored, low-oxygen environments.

Despite initially reported bipolar flagellation (one flagellum per pole), we show that a single flagellum is sufficient for motility including directional reversals, with the flagellar filament alternating between wrapping around the cell body from the leading pole or adopting a pushing configuration when at the lagging pole. However, monoflagellated cells swam significantly slower when their flagellum was wrapped, showing that bipolar flagellation allows cells to maintain higher swimming speeds throughout directional reversals.

Using high-temporal resolution TIRF imaging, we reveal that both flagellar conformations rotate counterclockwise with respect to the direction of movement, driving clockwise rotation of the cell body. By analyzing tethered cells, we discovered that individual motors are locked in either counterclockwise or clockwise direction in the absence of the major chemotactic signaling pathway – a deviation from well-studied model organisms.

Building on our experimental observations, we discuss how opposing flagellar motors are coordinated, how motor rotation influences distinct flagellar configurations, and how flagellar asymmetry might enable directionally biased North- or South-seeking motility within Earth's magnetic field. In summary, our work provides detailed insights into a motility system deviating greatly from traditional models, along with first direct evidence of flagellar motors defaulting to both counterclockwise and clockwise rotation in an amphitrichously flagellated spirillum.

#### P-PCB-025

##### Reporter-free observation of the circadian rhythm in individual *Synechocystis* cells using single-cell resolution time-lapse imaging

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All life on Earth is subject to rhythmic environmental changes with a 24-hour period, resulting from the Earth's rotation around its axis. Most organisms have evolved mechanisms, known as circadian clocks, that allow them to predict these oscillations and prepare accordingly. In cyanobacteria these oscillations have often been detected by expressing fluorescence reporters under the control of circadian-regulated promoters. This however, requires knowledge of suitable promoters and the availability of genetic toolboxes. Here, we report the use of microfluidic single-cell cultivation technology to detect circadian oscillations in individual, reporter-free *Synechocystis* cells.

We used a microfluidic cultivation technology adapted for phototrophic growth in combination with single-cell time-lapse imaging (Witting et al., *Lab on a Chip*, 2025.) to observe individual *Synechocystis* cells for up to ten days. Cultivations were performed at 30 °C under 4 µmol·m<sup>-2</sup>·s<sup>-1</sup> constant cool white illumination. We processed terabytes of phase-contrast image data using our deep learning cell segmentation workflow. Using python-based analysis workflows data on cell size and average phase-contrast intensity were collected for each cell over time. Further, we analyzed hundreds of division events and mapped these based on the time of day they occurred. Wildtype *Synechocystis* cells exhibited a roughly 24-hour oscillation in the average brightness of phase-contrast images. These oscillations were strikingly similar to those previously reported during backscatter measurements (Berwanger et al., *PNAS Nexus*, 2025). The increase in cell size was exponential without any superimposed oscillations and cell division events occurred equally distributed throughout the day. As previously shown by other researchers, two *kaiABC* protein oscillators are required for stable oscillations in *Synechocystis* (Köbler et al., *Nature Communications*, 2024). Consequently, knocking out the entire *kaiA1B1C1* or *kaiA3B3BC3* gene clusters, or parts of them (*kaiC3* and *kaiA3*), lead to a loss of the phase-contrast oscillations. Throughout the project, a comprehensive dataset on *Synechocystis* clock mutants was obtained. It shows that the circadian clock causes the refractive index of *Synechocystis* cells to oscillate with a period of 24 hours. We expect our method to increase the number of available model strains for circadian research by allowing circadian rhythms to be analyzed without expressing reporter molecules.

#### P-PCB-026

##### **Structural and functional characterization of RlmF, m<sup>6</sup>A writer in *Escherichia coli***

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The diverse chemical modifications on RNA that control the flow of genetic information in a noncanonical manner are referred to as epitranscriptomics. Epitranscriptomic modifications are dynamic and controlled by a set of writers and erasers that add and remove these modifications in response to changing environments. Methyltransferases catalyze the addition of methyl groups to nucleotides and proteins. RlmF is a prokaryotic methyltransferase responsible for the N6-methyladenosine modification (m<sup>6</sup>A), one of the most widely distributed modification in biological

systems. Although RlmF is known as an S-adenosylmethionine (SAM)-binding methyltransferase, the enzymatic mechanism has remained elusive. In this study, we aimed to elucidate the structure of the m<sup>6</sup>A writer RlmF. The enzyme is composed of two domains. Our study identified the amino acids that form the SAM-binding site in the methyltransferase domain. We also identified key amino acids in the N-terminal domain that recognize RNA. Based on our biochemical data, we propose a mechanistic order for the methyltransferase reaction. Furthermore, our study revealed that methylation by RlmF is restricted to a specific motif. RlmF is similar to the eukaryotic ortholog METTL16, a methyltransferase that also acts as a reader. The comprehensive structural and biochemical characterization of RlmF provides the ground to analyze its putative multifunctional role in bacteria.

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## **Prokaryotic physiology and metabolism**

#### **P-PPM-001**

##### **Itaconic acid: Logistics and cooperativity of mitochondrial metabolism**

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The smut fungus *Ustilago maydis* is a natural producer of itaconic acid. Due to the acid's industrial relevance as a platform chemical, itaconate production in *U. maydis* has been engineered to achieve high yields and titers, with by-product formation effectively eliminated [1]. The resulting production strain enables direct conversion of the tricarboxylic acid (TCA) cycle intermediate *cis*-aconitate to itaconate under nitrogen-depleted pseudosteady-state conditions. Since *cis*-aconitate originates in mitochondria, while itaconate synthesis occurs in the cytosol, this system provides a direct readout to analyze the linkage between mitochondrial and cytosolic metabolism. Despite being the central hub of the itaconate pathway, mitochondrial involvement in itaconate formation has remained largely unexplored.

To investigate how mitochondrial structure and available membrane space affect fungal carbon and energy metabolism within the itaconate production strain, we deleted a core component of the mitochondrial contact site and cristae organizing system (MICOS), thereby manipulating the organization of the inner mitochondrial membrane. The resulting deletion mutant was characterized with respect to respiratory activity, substrate uptake, and itaconate production, providing new insights into the complex interplay between *U. maydis* and its mitochondrial endosymbiont.

#### References

[1] Becker, J.; Hosseinpour Tehrani, H.; Gauert, M.; Mampel, J.; Blank, L.M.; Wierckx, N. An *Ustilago maydis* chassis for itaconic acid production without by-products. *Microb. Biotechnol.* 2019, 2, 13.

## P-PPM-002

### Functional analysis of a conserved gene cluster indicates a so-far unknown pathway for cleavage of the carboxylic side chain from the steroid skeleton in the bile-acid-degrading *Sphingobium* sp. strain Chol11

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Bile acids, such as cholate, are steroids produced by vertebrates in their digestive systems. After excretion, environmental bacteria can use them as carbon and energy sources via two pathway variants that differ in their side-chain degradation mechanism. The  $\Delta^{1,4}$ -variant proceeds via stepwise aldolytic cleavage. In contrast, the  $\Delta^{4,6}$ -variant, which seems to be unique to the *Sphingomonadaceae* family, does not produce intermediates with shorter side-chain. In this variant, side-chain degradation begins with a desaturation of the side chain involving a CoA ligase (*ScIA*), and an acyl-CoA dehydrogenase (*Scd4AB*). A proteomic analysis of *Sphingobium* sp. strain Chol11 revealed a gene cluster that is specifically upregulated during side-chain degradation containing genes for a putative amidase (*nov2c229*), and a putative Rieske monooxygenase (*nov2c228*) in addition to *scIA* and *scd4AB*.

Attempts to delete *nov2c228* resulted in the mutant strain m25, which was unable to grow with cholate but transformed it to intermediates with an intact carboxylic side chain. Chol11 m25 was able to degrade cholate degradation intermediates without side chain. Genome sequencing revealed that it has a 64-kb deletion encompassing all genes of the aforementioned cluster except *scIA*.

Complementation of Chol11 m25 with *nov2c228*, *nov2c229* and *scd4AB* completely restored growth with cholate albeit with a slower rate compared to the wild type (WT). Complementation with only *nov2c228* +/- *nov2c229* led to the accumulation of intermediates with an additional hydroxylation as dead-end products. Upon complementation with *nov2c228* and *scd4AB*, a compound with a  $\Delta^{4,6}$ -structure was converted into a  $\Delta^{1,4,6}$ -derivative with an unsaturated side chain, which was subsequently hydroxylated at an unidentified position. This compound, which is currently being analyzed by NMR, was degraded by Chol11 WT, suggesting that it is a true intermediate of the  $\Delta^{4,6}$  variant, and may be the substrate for the enzyme encoded by *nov2c229*.

These results show that the enzymes encoded by *nov2c228* and *nov2c229* in combination with *ScIA* and *Scd4AB* are necessary and sufficient for the cleavage of the carboxylic side chain from the steroid skeleton. The incremental complementation approach suggests that the unsaturation of the side-chain precedes the *Nov2c228*-mediated hydroxylation, and that *Nov2c229* is then catalyzing the ultimate cleavage via a so-far unknown reaction.

## P-PPM-003

### Exploring Transit Peptides through Photosynthetic Restoration in *Chlamydomonas*

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In plants and algae most of the proteins necessary for chloroplast physiological activities, including photosynthesis,

are encoded in the nucleus and afterwards imported. Chloroplast transit peptides (cTPs) are essential molecular components that direct thousands of nuclear-encoded proteins to chloroplasts, having a central role in photosynthesis and other metabolic processes. Despite decades of study, cTPs remain enigmatic: they lack a conserved sequence, yet reliably mediate specific and efficient protein import. Using *Chlamydomonas* as a model organism, the aim of the research is to better understand the importance of the amino acid residues in the cTPs during protein import, this would be evaluated by the restoration of photosynthetic activity in *Chlamydomonas* mutants. To achieve this goal, a mixed arrays of wet laboratory and *in-silico* predictions are used. The project is divided into five major segments: cloning, qualitative and quantitative cTPs evaluation, generative cTPs design and, testing for protein and membrane interactions. Cloning will be performed by Golden Gate and Modular Cloning, allowing us to test many transit peptides. The quantitative evaluation relies on peptides identified in complemented transformants are certain to direct sufficient protein import into the chloroplast stroma to restore photo-autotrophic growth, the positive subjects from the quantitative analysis will generate variants that test cTPs features in a hypothesis-driven fashion and fed into a quantitative version of the screen. Based on the insights gained, a simple feature-based generative model will be proposed that captures the principles underlying chloroplast targeting efficacy. The protein interaction test will be centred mostly around the Toc159-family proteins while the membrane interaction will be performed using synthetic vesicles to simulate the lipid composition of the chloroplast membrane. The research aims to establish a clearer panorama of the importance of the amino acid sequence and features constraints of amphipathic helices, while shading light into the different interactions with different import mechanism.

## P-PPM-004

### Redirecting carbon flux towards products in cyanobacteria by engineering the regulatory mechanism of the 2,3-bisphosphoglycerate-independent phosphoglycerate mutase reaction

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## Introduction

As cyanobacteria receive growing interest as biocatalysts in photo-biotechnology it is important to understand native molecular processes that control metabolism. Recently, the 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (PGAM) has been shown to serve as a central control point determining the carbon flux from the Calvin-Benson-Bassham cycle to lower glycolysis in the model organism *Synechocystis* sp. PCC6803. In particular, PGAM activity is controlled by the small protein PirC that also binds to the central regulatory protein PII. Upon PirC-binding, PGAM is inactivated leading to glycogen accumulation within the cell.<sup>1</sup>

## Material and Methods

Expression of the genes encoding PGAM and PirC was manipulated by using controllable promoters that respond to copper ions. To reduce PGAM activity we aimed for an

inducible downregulation of *pgam* and simultaneous upregulation of *pirC*. Similar cloning strategies were used to rationally design sucrose production strains, achieved by overexpression of the *sps* gene and by installing an *invertase* gene knockout (similar to Du et al.2). Glycogen and sucrose formation were analyzed in the obtained strains.

## Results

Recombinant *Synechocystis* strains showing Cu<sup>2+</sup>-inducible downregulation of *pgam* and overexpression of *pirC* as well as a combination of both were engineered. In all three mutant strains an accumulation of glycogen after addition of copper ions was determinable. These data confirm that the overall flux towards glycogen can be tuned by modulating PGAM abundance and direct tuning of PGAM activity. To confirm an effect on other products of gluconeogenesis we demonstrated that salt-dependent sucrose formation is also elevated in all strains. This could be further enhanced by additional overexpression of the *sps* gene, encoding sucrose phosphate synthase and deletion of the intrinsic *invertase* gene to avoid sucrose degradation.

Altogether, with this study we demonstrate the feasibility of engineering the PGAM valve to boost C-based product formation in cyanobacteria.

1 T. Orthwein, J. Scholl, P. Spät, S. Lucius, M. Koch, B. Macek, M. Hagemann, K. Forchhammer. PNAS, 2021, 118(6): e2019988118

2 Du, W.; Liang, F.; Duan, Y.; Tan, X.; Lu, X. Exploring the photosynthetic production capacity of sucrose by cyanobacteria. Metabolic engineering 2013, 19, 17–25

## P-PPM-005

### Transcriptional activity of *Methylocystis sumavensis* under pH stress

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Methanotrophic bacteria in peatlands mitigate emissions of methane (CH<sub>4</sub>), a potent greenhouse gas. Yet, the identity, physiology, and adaptive traits of methanotrophs inhabiting acidic peatlands are still not fully characterised. Using classical enrichment methods and single-cell sorting, we isolated a novel bacterial methanotroph species from Czech peatland soil: *Methylocystis sumavensis*. *M. sumavensis* is moderately acidotolerant, growing optimally at pH 6.8, but at a wide range from pH 5 to 9. To better understand the adaptive mechanisms employed by the new methanotroph to survive and remain active during acidic and alkaline conditions, we combined controlled incubations of the organism at pH 5 and 9 with transcriptome sequencing. Under both extreme pH conditions methane oxidation was continued and we observed a significant down regulation of one of the two present particulate methane monooxygenase isoforms and an up regulation of the lanthanide-dependent methanol dehydrogenase compared to the control incubation at pH 6.8. Moreover, transcripts involved in cell division and protein synthesis were significantly down regulated at both extreme pH levels, however genes involved in carbon fixation were only significantly down regulated under pH 9. Even though a diverse set of genes for antiporters likely involved in ion homeostasis were over transcribed at pH 5 and 9, the multiple subunit *mnh* sodium/proton antiporter,

often discussed for balancing pH stress, was not differentially expressed. Finally, we observed a significantly higher transcription of multiple genes involved in the maturation and function of a type 4f membrane-bound [NiFe]-hydrogenase at pH 5, but not pH 9, implying its role in the balancing of acidic stress. We are tempted to speculate that this could possibly take place through cytoplasmatic proton consumption and hydrogen evolution coupled with the oxidation of formate, but future work is needed to verify this assumption. Together, our data provide crucial first insights into the diverse mechanisms of peat-inhabiting methanotrophs for retaining activity under fluctuating pH.

## P-PPM-007

### Epistatic interactions drive evolution of divergent antibiotic survival phenotypes in *Staphylococcus epidermidis*

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Effective treatment of infections is complicated by bacteria's capacity to endure antibiotics. This is often not driven by single genes in isolation, but by a complex web of interacting genetic factors that shape survival under drug pressure. To investigate the genomics of antibiotic survival, we analysed *Staphylococcus epidermidis* isolates from clinical infections and asymptomatic carriage using high-throughput phenotyping, population genomics, and directed evolution. We observed wide spread multidrug resistance, with strong links between specific genomic elements and resistance. All isolates harbouring *mecA* were resistant to oxacillin, though MICs varied significantly, suggesting the modulation by additional genetic factors. Directed evolution revealed potentiating mutations that enhanced oxacillin resistance in *mecA*<sup>+</sup> strains. In *mecA*<sup>-</sup> isolates, however, evolution of mutations in the same genes conferred improved survival to oxacillin without significant increased MICs (tolerance). Revealing this complex genetic landscape of epistasis provides new insight into the different antibiotic survival phenotypes in *Staphylococcus epidermidis*.

## P-PPM-008

### The Cobalamin-Dependent Radical SAM Methyltransferase that Methylates SAM – Characterization and Exploration of the Alkyl-Transfer Potential of Orf29

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The unusual cyclopropane-containing amino acid (1*S*,2*S*)-2-methyl-1-aminocyclopropane-1-carboxylic acid ((1*S*,2*S*)-MeACC) is biosynthesized in a two-step enzymatic process that utilizes *S*-adenosyl-L-methionine (SAM) as initial substrate. In the first step, a cobalamin-dependent radical SAM methyltransferase (B12-RSMT), termed Orf29, catalyzes the methylation of the methionine moiety of SAM yielding (4''*R*)-4''-methyl-SAM (1). Second, a cyclization reaction leads to formation of (1*S*,2*S*)-MeACC (1). This product serves as a building block for natural products exhibiting antibiotic and/or antitumor activities (2,3).

The putative Orf29 from *Streptomyces coffeae* (Sc) was characterized as a homo-dimer which contains one [4Fe-4S]-cluster and one cobalamin (Cbl) in base-off conformation as described by spectroscopic methods. LC-MS analysis of *in vitro* activity assays of ScOrf29 revealed a +14 Da shift consistent with the methylation of SAM to 4"-methyl-SAM. The formation of 5'-deoxyadenosine (5'-dA) and S-adenosyl-L-homocysteine (SAH) was observed by HPLC analysis in an approx. 1:1 ratio and the enzyme followed a typical B12-RSMT mechanism (4). Next, we elucidated binding promiscuity, affinities and the alkyl-transfer potential of ScOrf29 using different alkyl-cobalamins prepared as described by literature methods (5). The respective alkyl-cobalamins were bound in the enzyme and the transfer of an ethyl group was catalyzed by Orf29. Additionally, SAM nucleobase analogues were employed to explore how SAM is used as substrate, co-factor and methyl-group donor during catalysis.

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(1) Maruyama et al., *Biomolecules* 2020, 10 (5), 775. DOI: 10.3390/biom10050775.

(2) Kurosawa et al., *J Antibiot* 2001, 54 (8), 615–621. DOI: 10.7164/antibiotics.54.615.

(3) Nakaya et al., *Biosci Biotechnol Biochem* 2007, 71 (12), 2969–2976. DOI: 10.1271/bbb.70371.

(4) Gagsteiger et al., *Angew. Chemie Int. Ed.* 2022, 61 (32). DOI: 10.1002/anie.202204198.

(5) Hannibal et al., *Inorg. Chem.* 2009, 48 (14), 6615–6622. DOI:10.1021/ic900590p.

## P-PPM-009

### Mind the Gap(N): GapN as a Potential Novel Antimicrobial Target in *Streptococcus pyogenes*

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In 2024, macrolide-resistant *S. pyogenes* were added to the WHO list of priority pathogens, highlighting the urgent need for new antimicrobial strategies against this important pathogen [1]. The non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase GapN is a promising antimicrobial target in *S. pyogenes* [2]. As these bacteria lack the oxidative branch of the pentose phosphate pathway, GapN serves as the main NADPH-producing enzyme [3].

In this study, potential competitive inhibitors were identified by *in silico* docking of compound libraries against the substrate-binding pocket of GapN using a homology model of the tetrameric holoenzyme as the structural basis.

Among the screened compounds, glyoxal bisulfite exhibited the strongest inhibition of GapN activity *in vitro*. It also showed dose-dependent impairment of GapN and markedly

increased the Km for its natural substrate glyceraldehyde 3-phosphate, indicating a competitive inhibition.

Complementary to this, fragment-based crystallographic soaking experiments revealed that the low molecular weight compounds pyrimidine-5-amine and 4-hydroxypyridazine bind in the NADP(H)-binding pocket of GapN. When applied to the purified GapN enzyme *in vitro*, both fragments caused an impairment and revealed additive or slight synergistic effects when combined with glyoxal bisulfite. When assessed in *S. pyogenes* survival assays, the combination of glyoxal bisulfite and 4-hydroxypyridazine led to accelerated bacterial killing, suggesting a potentiated antimicrobial effect.

Taken together, our results demonstrate glyoxal bisulfite - alone or in combination with cofactor-binding fragments - as a promising starting point for the development of specific GapN inhibitors with antimicrobial potential.

[1] WHO Bacterial Priority Pathogens List, 2024.

[2] Eisenberg et al., *Front Microbiol*, 2022, 10.3389/fmicb.2022.802427.

[3] J. Levering et al., *J Biotechnol* 2016, 10.1016/j.jbiotec.2016.01.035.

## P-PPM-011

### Bacterial membrane toxins: a systematic characterization of type I toxin-antitoxin systems

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Bacteria produce a wide variety of toxic proteins. These toxins can either be secreted to inhibit the growth of competing microbes, or can mediate intracellular effects in the producing bacterium. To control unwanted intracellular activity, toxin-producing bacteria have evolved regulatory toxin-antitoxin systems (TA systems). In these systems, the activity of the toxin protein is controlled via an antitoxin. In type I TA systems, the antitoxin is an antisense RNA that inhibits the translation of the toxin protein by binding to the toxin mRNA. Under stress conditions, inhibition by the antitoxin is relieved, resulting in toxin production and cellular effects. Most type I toxins are small hydrophobic proteins that integrate into the cytoplasmic membrane, disrupting the membrane potential and leading to growth arrest. Here, we systematically investigate the toxicity of diverse type I toxins. To this end, a spectrum of type I toxins from both Gram-negative and Gram-positive bacteria were cloned into a plasmid system under the control of an L-arabinose-inducible promoter and transformed into *Escherichia coli* MG1655. We then measured the effects of toxin expression on growth inhibition, ATP depletion, and toxicity in this Gram-negative model organism. While most toxins inhibited *E. coli* growth and depleted intracellular ATP levels, we found that toxins from Gram-negative bacteria exhibited a stronger killing effect on *E. coli* than those from Gram-positive bacteria. Our study suggests that bacterial membrane toxins from type I TA systems are universally active, however, the strength of their effects can vary depending on the target bacterium.

## P-PPM-012

### Dormancy of the probiotic strain *Escherichia coli* Nissle 1917 after long-term storage: a physiological and molecular characterization

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Mutaflor is a pharmaceutical product containing the probiotic strain *Escherichia coli* Nissle 1917. It is used to restore the human intestinal flora after an infection and antibiotic treatment. After long-term storage (LTS) of Mutaflor suspensions at 4°C, Nissle cells are impeded in their ability to form colonies on agar plates, while short-term storage (STS) does not affect culturability. The observed plating defect after LTS can be overcome by a regeneration of suspensions at 37°C. Here, we use the different Mutaflor suspensions as a model system for bacterial dormancy and investigate the physiological and molecular differences. STS and LTS suspensions were either tested directly or after regeneration at 37°C using cultivation-based approaches in solid and liquid media. Furthermore, experiments with antibiotics, microscopic analysis, and ATP assays were performed and compared to exponential and stationary phase of laboratory cultures. To elucidate the molecular response, total protein and RNA were isolated and analyzed by gel-based approaches. In addition, RNA-sequencing was performed to identify global molecular patterns. We found that Mutaflor suspensions showed an increased antibiotic tolerance as compared to regular Nissle cultures, clearly indicating the expected stress tolerance of dormant cells. The cultivation-based approaches further demonstrated that Nissle cells in LTS suspensions regenerate in dependence of their cell density. Finally, the RNA-sequencing analysis revealed that LTS cells are characterized by a global mRNA depletion, and that regeneration leads to the replenishment of the mRNA pool. Our study provides new insights into the dormancy and regeneration of bacteria and represents an important basis for future research.

## P-PPM-013

### Elucidating the role of a cryptic small protein in the assimilation of inorganic carbon in cyanobacteria

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Cyanobacteria have evolved a sophisticated carbon concentrating mechanism (CCM) to significantly enhance the CO<sub>2</sub> concentration in close proximity to the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase and thereby diminish the frequency of its detrimental oxygenation reaction<sup>1</sup>. While previous research has extensively studied the sodium dependent bicarbonate transporter SbtA and its regulator SbtB<sup>2</sup>, both crucial for the CCM, a so far non-annotated gene encoding for a small protein of 80 amino acids has been identified upstream of the *sbtAB* operon in many cyanobacterial genomes. We hypothesize a function linked to the CCM and SbtA or B in particular and hence termed the protein SbtC. The 3D structure of SbtC, conserved regions as well as potential binding sites for interactions with other proteins were identified *in silico*. Structural analysis of SbtC identified a central

transmembrane helix and a downstream region displaying a protein-binding motif. Moreover,  $\Delta$ *sbtC* mutants have been generated using the cyanobacterial model *Synechocystis* sp. PCC 6803 and comparative growth analyses have been conducted compared to wild type and other mutants deficient in other inorganic carbon transporters such as NDH-13, NDH-14, and CmpA. We also performed immunoblot-based analyses to identify interaction partners of the novel protein. Indeed, an interaction with both the bicarbonate transporter SbtA and the small regulator protein SbtB were identified. In combination with the reduced growth exhibited by the  $\Delta$ *sbtC* strain under diurnal illumination this may point towards an involvement of SbtC in the adaptation of SbtA-mediated bicarbonate uptake to changing light and bicarbonate availabilities.

1 Price, G. D.; Badger, M. R.; Woodger, F. J.; Long, B. M. (2008): Advances in understanding the cyanobacterial CO<sub>2</sub>-concentrating-mechanism (CCM): functional components, Ci transporters, diversity, genetic regulation and prospects for engineering into plants. In *Journal of experimental botany* 59 (7), pp. 1441–1461.

2 Selim, Khaled A.; Haase, F.; Hartmann, M. D.; Hagemann, M.; Forchhammer, K. (2018): PII-like signaling protein SbtB links cAMP sensing with cyanobacterial inorganic carbon response. In *Proceedings of the National Academy of Sciences of the United States of America* 115 (21), E4861-E4869.

## P-PPM-014

### Markerless Gene Deletion and Selection Strategies in *Hyphomicrobium denitrificans* for Investigating Dissimilatory Sulfur Oxidation

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The budding Alphaproteobacterium *Hyphomicrobium denitrificans* XT exhibits remarkable metabolic versatility. It utilizes C1 and C2 compounds such as methanol, methylamines, and acetate as sole sources of carbon and energy, and can oxidize both organic and inorganic sulfur compounds via the sulfur-oxidizing heterodisulfide reductase-like (sHdr) system [1]. The complete sulfur oxidation pathway and the function of each protein encoded in the vicinity of the *shdr* genes remain to be fully elucidated. A particular focus lies on a lipocate-binding protein (LbpA), which is encoded next to the *shdr* genes, as well as a cluster of genes for enzymes that are likely involved in the assembly of the lipoyl moiety. Targeted gene deletions and phenotypic characterization of mutant strains are valuable tools for analyzing the *in vivo* function of uncharacterized proteins. A two-step homologous recombination strategy based on the pk18mobsacB vector [2] introduced via electroporation has successfully generated markerless gene deletions in *H. denitrificans* [1]. However, the technique produces an unsatisfactory number of transformants. Therefore, we set out to replace electroporation with conjugation, which proved to be much more efficient. Several defined deletion mutants were generated in different genetic backgrounds of *H. denitrificans*, including strains lacking genes that are potentially involved in sulfur oxidation and in the maturation of LbpA. These strains provide a solid basis for systematically analyzing the sulfur metabolism of our model organism. To further expand the available genetic tools for *H. denitrificans*, triclosan was explored as an alternative selection marker. This compound interferes with bacterial

fatty acid biosynthesis by blocking substrate access to enoyl-ACP reductase (FabI) and was found to inhibit the growth of *H. denitrificans* in a concentration-dependent manner. A triclosan resistance cassette was engineered based on the gene for the alternative enoyl reductase FabL from *Bacillus subtilis* [3], under the control of a strong native promoter from *H. denitrificans* to ensure robust expression in the host background. Thus, a foundation was established for future vector development and selection strategies tailored for our model organism.

[1] Koch & Dahl, 2018 ISME J, 12, 2479

[2] Schäfer et al. 1994 Gene, 145, 69

[3] Heath et al. 2000 J Biol Chem, 275, 40128

## P-PPM-015

### Functional expansion of the *Staphylococcus aureus* ClpX interactome using the TIE-UP-SIN workflow

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Maintaining protein homeostasis is essential for sustaining life. In *Staphylococcus aureus* precise control of protein degradation is critical not only for adaptation to and survival of changing environmental and cell status conditions, but also for virulence regulation. Thus, proteins involved in proteostasis and proteolysis, particularly the Clp protease system, inherit great potential for therapeutic targeting. The Clp degradation machinery comprises the proteolytic core ClpP and associated ATPases, ClpX and ClpC, which also function independently as chaperones facilitating protein folding. Within this network, the ClpXP complex is of particular interest due to its involvement in essential cellular processes and pathogenicity. Although several ClpXP substrates have been identified using trapping mutants of ClpP, its interaction network remains incompletely defined.

In this study we aimed to expand the understanding of Clp-ATPase function by detailed functional characterization of the AAA+ ATPase ClpX.

To identify putative interaction partners, we employed a novel quantitative mass spectrometry approach, **TIE-UP-SIN** (Targeted Interactome Experiments for Unknown Proteins by Staple Isotope Normalization) (Schedlowski *et al.*, 2025). This method combines *in vivo* crosslinking of adjacent proteins *via* formaldehyde followed by affinity purification of Twin-Strep-tagged ClpX complexes. A triple-sample isotopic design using unlabeled and <sup>15</sup>N-labeled media enables robust normalization and discrimination of specific versus nonspecific interactions.

Using this workflow, we captured a reproducible and high-confidence set of *S. aureus* ClpX-associated proteins. The resulting interactome complements known ClpXP substrates and provides a pioneering experimental framework for dissecting Clp-mediated proteostasis and its role in bacterial adaptation and virulence.

Our approach opens new avenues for exploring the regulatory complexity of bacterial proteolytic systems and for

uncovering novel targets in the fight against antibiotic resistance.

Schedlowski, M., Michalik, S., Hoffmüller, T., Harms, M., Steil, L., Surmann, K., Hentschker, C., Salazar, M.G., Völker, U., Reder, A., 2025. TIE-UP-SIN: a novel method for enhanced identification of protein–protein interactions. Front. Microbiol. 16. <https://doi.org/10.3389/fmicb.2025.1657647>

## P-PPM-016

### Anaerobic allantoin degradation in *Escherichia coli*: discovery, characterization, and regulation of the oxamate branch enzymes

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*Escherichia coli* can utilize allantoin as its sole nitrogen source under anaerobic conditions. Allantoin degradation releases two molecules of ammonia (NH<sub>3</sub>) and produces ureidoglycolate, which can be further metabolized through two alternative routes: the glycerate branch via glyoxylate, or the oxamate branch, leading to release one additional molecule of NH<sub>3</sub> and substrate-level phosphorylation. While the glycerate branch has been well characterized, the genes and enzymes responsible for the oxamate branch remained unidentified. Our studies identified a gene cluster *fdrA–ylbE–ylbF–ybcF*, located immediately downstream of the *allDCE* operon, that encodes the enzymes of the oxamate branch. Biochemical assays demonstrated that *fdrA*, *ylbE*, and *ylbF* together encode a novel oxamic transcarbamylase (OXTCase), which catalyzes the conversion of oxalurate to carbamoyl phosphate (CP) and oxamate. This OXTCase shares no homology with known transcarbamylases, such as aspartate or ornithine transcarbamylase, suggesting a new enzyme family. Furthermore, the downstream gene *ybcF* encodes a catabolic carbamate kinase (CK) that catalyzes the ATP-generating dephosphorylation of CP. We therefore renamed these genes (*fdrA*, *ylbE*, *ylbF*, and *ybcF*) to *allF*, *allG*, *allH*, and *allK*, respectively, as part of the allantoin gene cluster. Subsequently, we found that the four genes form an operon (*allFGHK* operon), and their expression requires both the local regulator AllS and the global anaerobic regulator ArcA. Electrophoretic mobility shift assays (EMSA) confirmed that AllS and phosphorylated ArcA directly bind to the *allF* promoter region, activating transcription in the presence of allantoin under anaerobic condition. Collectively, these findings define the complete oxamate branch of the anaerobic allantoin degradation pathway in *E. coli* and uncover its regulatory control by AllS and ArcA.

## P-PPM-017

### Investigating consequences of a point mutation in the *spx* gene of *Staphylococcus aureus* HG001

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**Introduction:** Bacterial fitness is determined by the genetic background. The opportunistic pathogen *Staphylococcus aureus* displays high strain diversity, which

is achieved primarily through mobile genetic elements or mutations. Strains, such as HG001 and SH1000, belonging to the NCTC 8325 lineage are commonly used in basic infection and physiology research. The two aforementioned strains are known to mainly differ in the presence of prophages (HG001: prophage-proficient; SH1000: prophage-deficient). However, comparative whole genome sequencing of the strains additionally revealed several point mutations, including a particularly interesting mutation in the *spx* gene of HG001, located in the region encoding the CXXC motif of the regulator. This redox-sensitive motif is critically involved in controlling Spx's activity. The complex regulatory role of Spx is involved in various aspects of bacterial survival such as the response to oxidative and salt stress and in cell wall homeostasis.

**Objectives:** Here, we aimed to investigate the impact of a point mutation affecting the CXXC motif in *S. aureus* under salt and oxidative stress conditions.

**Methods:** The *spx* gene of HG001 was replaced by the *spx* gene of SH1000 using the pSauSE allele exchange plasmid. Afterwards, drop dilution assays on NaCl-supplemented TSB agar plates and diamide growth inhibition assays were performed. In addition, mass spectrometry-based proteome profiles were recorded for SH1000, HG001 and HG001 *spx::spxSH1000* under control conditions and after imposition of salt and oxidative stress.

**Results:** Physiological assays revealed that repair of the *spx* gene in *S. aureus* HG001 led to an increase in salt and diamide stress tolerance, closely resembling that of SH1000. The proteome data support the finding that the point mutation in the encoded CXXC motif of Spx affects the stress tolerance of *S. aureus*. Overall, our results indicate that impairment of the *spx* gene impacts bacterial fitness during different stress conditions.

**Conclusion:** By comparing proteome profiles of isogenic strains carrying different Spx variants, we provide insights into the role of the CXXC motif of Spx, specifically in the response of *S. aureus* to salt and oxidative stress.

## P-PPM-018

### Characterization of *Bacillus subtilis* L-asparaginases AnsA and AnsZ

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Asparaginases (ASNase) hydrolyze asparagine (Asn) into aspartate (Asp) and ammonium. Many industries use ASNases to their advantage. For example, in the food industry, Asn is a precursor to the carcinogenic byproduct acrylamide (AA), which forms during the heat treatment of carbohydrate-rich products. By reducing Asn content beforehand, the formation of AA is reduced. Another example is the treatment of specific cancers where bacterial ASNases are injected into the patients blood to reduce the Asn concentration. This slows or stops the proliferation of cancer cells, as they need Asn to grow [1]. Because of the wide area of application, a detailed characterization and understanding of ASNases is of great interest. *Bacillus subtilis* is known to have two ASNases, AnsA and AnsZ. Although the synthesis of ASNases depends on SigA, their genes are differentially regulated. *ansA* is induced by Asn that inactivates the repressor AnsR; *ansZ* is induced by TnrA

and CodY under nitrogen-limiting conditions [2]. *ansA* forms an operon with AnsB. The encoded ASNase and aspartase contribute to glutamate homeostasis in *B. subtilis* strains with defects in glutamate synthesis and degradation because both enzymes catalyze reversible reactions [3]. Recently, it was shown that AnsZ possesses an N-terminal signal peptide. Only after removing it does AnsZ become active [4]. Even though *B. subtilis* is one of the best studied microorganisms, the function of AnsZ is unknown. We became interested in the ASNases of *B. subtilis* because it has never been demonstrated how each of the enzymes contributes to Asn degradation when the amino acid serves as a single source of carbon and nitrogen. *In vivo* experiments revealed that AnsA seems to be the major ASNase allowing *B. subtilis* to utilize Asn. In contrast, it is unclear whether AnsZ contributes to Asn degradation *in vivo*. Moreover, an *ansA* mutant is genetically stable, and it does not unmask AnsZ activity by mutations that increase *ansZ* expression. Thus, the *B. subtilis ansA* mutant is a perfect platform to screen for ASNase that could be used for medical applications. Currently, purification of AnsA and AnsZ for biochemical characterization of the ASNases is underway.

[1] Wang et al. (2021) Int. J. Biol. Macromol. 186, 975–983.

[2] Fisher et al. (2002) J. Bacteriol. 184(8), 2148–2154.

[3] Mardoukhi et al. (2024) Microb. Biotechnol. 17(3), e14429.

[4] Arredondo-Núñez et al. (2023). Life (Basel, Switzerland) 13(11), 2145.

## P-PPM-019

### Investigating the regulation of the carbon switch in central metabolism of *Synechocystis* sp. PCC 6803

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*Synechocystis* sp. PCC 6803 grows auto-, hetero-, and mixotrophically depending on light and nutrient availability. This metabolic flexibility depends on the reversibility of sugar metabolism, particularly the Embden–Meyerhof–Parnas (EMP) pathway, which must switch between the anabolic and catabolic direction. Such reversibility requires tight regulation and a coordinated interaction between the EMP pathway and the Calvin–Benson–Bassham (CBB) cycle to balance CO<sub>2</sub> fixation and energy metabolism.<sup>1</sup> Within the upper EMP pathway, *Synechocystis* possesses two paralogous copies of glucokinases (GKs), phosphofructokinases (PFKs) and fructose-1,6-bisphosphatase (FBPase) enzymes, whose functions and regulation were previously unknown. Furthermore, the reversible phosphoglucose isomerase (PGI) shunt, directing flux through the non-oxidative pentose phosphate pathway (NOPP) to the CBB cycle, further emphasizes the need for precise control.

Enzyme characterization revealed that both GKs are specific for glucose as substrate and ATP as phosphate donor. GK1 is activated by dihydroxyacetone phosphate ( $K_a = 0.6$  mM), while GK2 is inhibited by ADP ( $K_i = 0.7$  mM). Both PFKs are ADP-dependent, defining a new subclass of the PFK-A superfamily. PFK-A1 is inhibited by 3-phosphoglycerate (3PG), and PFK-A2 by ATP, linking regulation to the cellular

energy state.<sup>2</sup> FBPase is not affected by tested effectors, suggesting a housekeeping role, whereas the bifunctional F/SBPase is inhibited by AMP and activated by DTT.<sup>3</sup> PGI favors the gluconeogenic direction and is inhibited by erythrose 4-phosphate (E4P) and 6-phosphogluconate (6PG), thus representing a control point between the EMP, CBB, and NOPP pathways.

Our data indicate that both ATP-dependent GKs are active under mixotrophic conditions, boosting fructose 6-phosphate (F6P) supply to enhance CO<sub>2</sub> fixation. Under low energy charge, ADP-dependent PFKs drive F6P catabolism, while inhibition by 3PG and ATP prevents futile cycling and supports rapid regeneration of CO<sub>2</sub> acceptors under light conditions. The sustained FBPase activity ensures EMP reversibility, and PGI inhibition by E4P and 6PG acts as a metabolic valve balancing carbon flow between the PGI shunt and NOPP. Together, these results uncover a coordinated regulatory network enabling *Synechocystis* to adjust carbon flux and energy balance across trophic states.

1. Schulze *et al.*, Microb Cell Fact (2022); 21:699.
2. Shen *et al.*, JBC (2024); 107868
3. Caliebe *et al.*, JXB (2025); 10.1093.

## P-PPM-020

### All roads lead to glutamate: versatile genomic adaptations compensate for defects in the glutamate metabolism of *Bacillus subtilis*

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The amino acid glutamate plays important roles in cellular processes in the Gram-positive model bacterium *Bacillus subtilis*. It is the major amino group donor in anabolic reactions and a key molecule connecting carbon and nitrogen metabolism (1). *B. subtilis* synthesizes glutamate via the glutamate synthase (GOGAT)/glutamine synthetase (GS) pathway (1) and degrades it by the glutamate dehydrogenases (GDHs) RocG and GudB. The *B. subtilis* GDHs are strictly catabolic active, due to their low ammonium affinity (1). The GDHs control the conversion of glutamate to 2-oxoglutarate, thus linking nitrogen and carbon metabolism. In the *B. subtilis* SP1 wt, *rocG*, encoding the RocG GDH, is only sufficiently expressed in the presence of arginine, while the major GDH-encoding gene *gudB* is cryptic and does not encode a functional GDH without additional mutations (1). Suppressor mutant screens that aimed to elucidate alternative metabolic pathways for arginine and glutamate degradation, as well as glutamate biosynthesis, corroborated earlier findings of alternative pathways for the degradation (2) and biosynthesis of glutamate (3). Both pathways involve the constitutively expressed aspartate transaminase AspB and the de-repression of the L-aspartase encoding gene *ansB*, as well as secondary mutations facilitating enzymatic function by redirecting the flux of metabolites (3). Interestingly, in a second group of mutants, a putative role of the alternative sigma factor SigL, in restoring catabolism of glutamate and arginine, was revealed. SigL, in concert with specific transcriptional activators, controls many other genes involved in amino acid metabolism, including the BkdR regulon, involved in branched-chain amino acid utilization and the RocR regulon, involved in arginine degradation (4). *bcd* is part of the BkdR regulon and encodes for the branched-chain amino acid dehydrogenase Bcd that has recently been described to also have GDH activity (5). Here, we further characterize and

compare amino acid dehydrogenase activities of RocG and Bcd *in vitro*, as well as the role of *sigL* and *bcd* in an alternative pathway for glutamate degradation *in vivo*.

- (1) Gunka K & Commichau FM. (2012) doi: 10.1111/j.1365-2958.2012.08105.x.
- (2) Flórez LA, *et al.* (2011) doi: 10.1186/1752-0509-5-5.
- (3) Mardoukhi MSY, *et al.* (2024) doi: 10.1111/1751-7915.14429.
- (4) Débarbouillé M, *et al.* (1991) doi: 10.1073/pnas.88.20.9092.
- (6) Ranava D, *et al.* (2025) doi: 10.1038/s41522-025-00750-6.

## P-PPM-021

### The putative copper chaperone NosM is involved in nitrous oxide respiration of *Neobacillus vireti*

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As nitrous oxide (N<sub>2</sub>O) is a highly potent greenhouse gas, the biological catalysis of its reduction to dinitrogen gas is of crucial importance. A diverse group of bacterial species produce the copper-containing enzyme N<sub>2</sub>O reductase (NosZ) as the key enzyme of anaerobic N<sub>2</sub>O respiration. Phylogenetic analyses have classified NosZ enzymes as either clade I or clade II, and it has been predicted that the corresponding organisms will differ significantly in their electron transfer routes to NosZ, as well as in the assembly of the NosZ active site, which contains six copper atoms per monomer.

The Gram-positive, nitrate-ammonifying bacterium *Neobacillus vireti* grows by N<sub>2</sub>O respiration and contains a clade II NosZ, which is encoded by the second gene of the *nosC-Z-B-D-Y-F-M* gene cluster. The *nosM* gene is frequently found within *nos* gene clusters in the phylum Bacillota. The membrane-bound NosM protein is predicted to consist of two domains located at the outside of the membrane, and the N-terminal domain contains a conserved putative metal-binding site (MX<sub>2</sub>MXHX<sub>22</sub>M) that may interact with NosD of the NosDYF complex involved in assembling the copper active site of NosZ.

An *N. vireti* markerless deletion mutant lacking *nosM* was unable to grow by N<sub>2</sub>O respiration and exhibited only a negligible N<sub>2</sub>O reduction rate, as determined by microrespirometry. Copper supplementation in the growth medium partially rescued this phenotype, suggesting that NosM functions as a copper chaperone, similar to NosL in Gram-negative bacteria.

## P-PPM-022

### Exploring novel enzymes for lipoic acid biosynthesis and protein lipoylation

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Lipoic acid is a pivotal organosulfur cofactor that facilitates critical reactions within multienzyme complexes involved in energy metabolism. Present in both prokaryotic and eukaryotic organisms, its biosynthesis and subsequent attachment to specific proteins - termed protein lipoylation - are essential for enzymatic activities including the sulfur-oxidizing heterodisulfide reductase-like (sHdr) system [1,2]. sHdr driven dissimilatory sulfur oxidation is widespread among archaea and bacteria and includes lipoate-binding proteins (LbpA) as essential elements as has been shown by phenotypic characterization of a *Hyphomicrobium denitrificans* mutant carrying an *in frame* deletion of the *lbpA* gene. *H. denitrificans* is a chemolithoautotrophic Alphaproteobacterium capable of using thiosulfate as a supplemental electron donor during growth on C1 or C2 compounds.

In lipoylated proteins, the active lipoyl moiety is generated by combined action of octanoate/lipoate-protein ligases and radical S-adenosylmethionine (SAM) lipoyl synthases, that orchestrate the transfer of octanoate precursors followed by the insertion of sulfur. In 2023, we demonstrated that LbpA proteins are not assembled by the well-described protein lipoylation machinery established in *E. coli* [3]. Instead, *shdr-lbpA* gene clusters come with a set of genes encoding a novel lipoylation pathway, including a specific lipoate:protein ligase sLpl(AB) and two radical SAM proteins, designated LipS1 and LipS2 [3]. The roles of even further gene products such as the FAD-binding oxidoreductase LipT or the putative glutamine amidotransferase LipX are completely unresolved. To answer these remaining questions, we constructed *H. denitrificans* mutants carrying individual markerless in-frame deletions of *lipS1*, *lipS2*, *lipT*, and *lipX*. Growth experiments with thiosulfate as the electron source were conducted and showed that *lip* gene products are indeed crucial for thiosulfate oxidation and consequently for the maturation of the hyphomicrobial lipoate-binding protein. Attempts were also made to purify the LbpA proteins from *H. denitrificans* mutants to gain insight into the identity of lipoyl precursors bound to apoproteins, as well as the function and sequence of various lip enzymes in stepwise lipoyl moiety assembly.

[1] Koch, Dahl 2018 ISME J 12(10), 2479-2491

[2] Cao et al. 2018 Elife 7, e37439

[3] Tanabe et al. 2023 PLoS Biology 21(6), e3002177

## P-PPM-023

### Ca<sup>2+</sup> signaling in filamentous heterocyst forming cyanobacteria

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Free calcium (Ca<sup>2+</sup>) ions can be highly toxic as they can precipitate phosphate ions, which are important for various metabolic pathways. Therefore, cyanobacteria regulate their intracellular Ca<sup>2+</sup>-concentration [Ca<sup>2+</sup>]<sub>i</sub> via pumps, channels and calcium binding proteins.<sup>1,2</sup> In multicellular cyanobacteria, Ca<sup>2+</sup> signaling also plays an important role in heterocyst differentiation.<sup>3</sup> Shortly after nitrogen depletion, a condition that triggers heterocyst differentiation, a transient increase of [Ca<sup>2+</sup>]<sub>i</sub><sup>4</sup> in pro-heterocysts have been reported.<sup>3</sup> In the multicellular heterocyst-forming cyanobacterium *Nostoc* sp. PCC 7120, two calcium binding proteins have

been identified: CcbP (cyanobacterial calcium binding protein)<sup>3</sup> and CSE (Ca<sup>2+</sup> Sensor EF-hand).<sup>5</sup> CcbP is mainly known for its buffer property in capturing free Ca<sup>2+</sup> ions. CSE binds Ca<sup>2+</sup> via two characteristic Ca<sup>2+</sup> sensor EF hand domains.<sup>5</sup> We show that CSE is completely unfolded in the absence of calcium and undergoes a strong conformation change upon Ca<sup>2+</sup>-binding.

Although there is a strong connection between Ca<sup>2+</sup> signaling and heterocyst differentiation<sup>5,6</sup>, the specific functions of Ca<sup>2+</sup>-binding protein, CSE, remain elusive. The knockout of *cse* causes a strong fragmentation phenotype, a characteristic of several mutants involved in cell-cell communication and nanopores array formation. Additionally, strains altered in CSE content exhibit abnormal cell shapes and altered growth under specific conditions compared to the wildtype. Overall, our data suggest that CSE is a crucial buffer protein, regulating various cell processes, including C/N-balance and cell morphology as reaction to changing environments, by maintaining the intracellular Ca<sup>2+</sup> balance which influences many cellular processes.

1. D.E. Clapham, *Cell*, **2007**, 131, 1047–1058.
2. V. Norris *et al.*, *J. Bacteriol.*, **1996**, 178 (13), 3677–3682.
3. Y. Zhao *et al.*, *Proc. Natl. Acad. Sci. U. S. A.*, **2005**, 102 (16), 5744–5748.
4. I. Torrecilla *et al.*, *Microbiol.*, **2004**, 150, 3731-3739.
5. J. Walter *et al.*, *BBA Bioenerg.*, **2019**, 1860 (6), 519-532.
6. J. Walter *et al.*, *BMC Microbiol.*, **2020**, 20:57.

## P-PPM-024

### Genetic Insights into Sulfobacin Biosynthesis in *Flavobacterium johnsoniae*

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Sulfobacins are bacteria-derived bioactive sulfonolipids that suppress inflammation and macrophage polarization in the gut, indicating an immunomodulatory function. Moreover, they are essential for gliding motility in *Flavobacterium johnsoniae* and induce multicellularity in choanoflagellates. Sulfobacins are structurally related to ceramides and formed by N-acylation of a capnine base, the sulfonated analog of sphinganine. Distinct bioactivity is defined by further acylation with isobranched chains, hydroxylation, and desaturation. The initial steps of this pathway involve the condensation of cysteate with an acyl-carrier protein catalyzed by cysteate acyl-ACP transferase (SulA), and reduction of 3-ketocapnine to capnine by dehydrocapnine reductase. However, the enzymes required for the final synthesis of sulfobacins from capnine remain unknown.

This project aimed to characterize the yet unknown genes involved in sulfobacin biosynthesis using *Flavobacterium johnsoniae*, a genetically accessible model organism.

Comparative genomics of bacterial ceramide pathways suggested the presence of a potential sulfobacin synthase gene that catalyzes the N-acylation of the capnine base. In addition, two putative monooxygenases were identified as candidate genes involved in the biosynthesis of the hydroxylated sulfonolipid (sulfobacin). To test the function of

the proposed genes, markerless deletion mutants of *F. johnsoniae* were generated. A *sulA*-deficient strain served as a control. Lipid extracts from deletion mutants were analyzed by lipidomics. As sulfobacins are required for motility of *F. johnsoniae*, the phenotypes were microscopically evaluated.

Gliding motility was completely lost in the putative sulfobacin synthase mutant but was only impaired in the potential monooxygenase mutants. Lipidomic profiling confirmed the absence of sulfobacins and accumulation of the precursor capnine in the sulfobacin synthase mutant, supporting its role in sulfobacin biosynthesis. Sequence comparison further revealed a homologous gene in *Alistipes putredinis*, a sulfobacin-producing species in the human and animal gut.

We identified a previously unknown key enzyme of sulfobacin biosynthesis, a putative sulfobacin synthase, which is essential for gliding motility in *F. johnsoniae*.

#### P-PPM-025

##### **Growth of *Corynebacterium glutamicum* on 5-oxo-L-proline (pyroglutamate) as carbon and nitrogen source requires the PxpR-controlled *pxpTABC* genes**

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**Introduction:** 5-Oxo-L-proline (5-OP) is the cyclic lactam of glutamate. It is formed by the spontaneous cyclization of L-glutamine, L-glutamate, or  $\gamma$ -glutamyl phosphate. Hence, 5-OP is an unavoidable metabolite in all cells. 5-oxoprolinases convert 5-OP to L-glutamate allowing further metabolism. The *pxpABC* genes encode a prokaryotic ATP-dependent 5-oxoprolinase that is widespread amongst bacteria. The utilization of 5-OP as a carbon and nitrogen source for bacteria has rarely been analyzed. *Corynebacterium glutamicum* is of special interest with respect to 5-OP, as it is used for the industrial production of the flavor enhancer L-glutamate at a scale of more than 3 million tons per year and has a high cytoplasmic L-glutamate concentration in the range of 100-200 mM.

**Objective:** The goal of this study was to test if 5-OP can be utilized by *C. glutamicum* as sole carbon and nitrogen source and to identify and characterize genes and proteins involved in 5-OP metabolism.

**Methods:** Construction of *C. glutamicum* deletion mutants via homologous recombination. Growth experiments in BioLector microcultivation systems. Complementation of deletion mutants via plasmid-based expression. Protein overproduction and purification with subsequent isothermal titration calorimetry (ITC) to test for ligand binding.

**Results and Conclusions:** It was shown that *C. glutamicum* can grow well in minimal medium with 5-OP as the sole carbon and nitrogen source. The *pxpTABC* gene cluster was identified to be essential for growth on 5-OP. The *pxpABC* genes encode for an ATP-dependent 5-oxoprolinase and the *pxpT* gene encodes a secondary transporter of the APC superfamily, which most likely catalyzes 5-OP import. Furthermore, it was demonstrated that the *pxpTABC* genes are repressed by the GntR-type transcriptional regulator PxpR. Purified PxpR was shown by ITC to specifically bind 5-OP with a KD of 726 nM, but not L-glutamate, L-glutamine,

or L-proline, suggesting that it is a specific 5-OP biosensor. Overall, the results of this study [1] together with the widespread occurrence of the *pxpABC* genes and the ubiquitous presence of 5-OP in cells indicate that many bacteria may have the ability to use 5-OP as a source of carbon and nitrogen.

[1] Sundermeyer et al. (2025) bioRxiv  
<https://doi.org/10.1101/2025.10.30.685629>

#### P-PPM-026

##### **What is the function of the *Thermoanaerobacter kivui* S-layer? Searching for phenotypes in a knockout strain**

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Many prokaryotes have a crystalline protein or glycoprotein-based component of their cell surface (S-layer). In some archaea this is the only structural component providing rigidity to the cell membrane (1), and it may also provide structural support for gram-negative bacteria, where the peptidoglycan cell-wall is relatively thin (2). Gram-positive bacteria already have a thick and structurally robust layer of peptidoglycan for rigidity, but S-layers have also been found to contribute to virulence in pathogenic bacteria (3). The thermophilic acetogen *Thermoanaerobacter kivui* is gram-positive and non-pathogenic, but still has an S-layer (4). While there are many hypothesized functions for the S-layer aside from structural integrity and pathogenicity, experimental evidence for many of these is lacking.

The S-layer represents a significant investment of energy and carbon, both of which are in short supply during autotrophic acetogenesis, suggesting that the functions provided by *T. kivui*'s S-layer are important enough to be worth the costs. Spontaneous loss of S-layers is sometimes observed in laboratory strains, but we have never observed loss of S-layer in *T. kivui* cultures even in strains passaged hundreds of times under laboratory conditions. Therefore, we generated a knockout of the primary S-layer gene of *T. kivui*. Characterization of this knockout strain should help to better understand the function of the S-layer in this non-pathogenic gram-positive bacterium.

Electron microscopy confirmed the absence of an S-layer in the knockout strain, and knockout cells were longer and more likely to be found in chains than the parent strain. Cells lacking the S-layer also appeared to aggregate more easily, causing flocculation and sedimentation in cultures incubated without shaking. Minimal differences in growth rate were observed.

Evidence for a key function of the S-layer in *T. kivui* is still lacking. Loss of the S-layer appears to disrupt cell division to some extent, since cells are longer and form chains, but the effect is not strong enough to cause a significant growth defect. Efforts to further characterize phenotypic changes caused by deletion of the S-layer, as well as detailed structural analyses, are ongoing.

1) Sivabalasarma S, et al. 2025. *Curr Opin Cell Biol* 95:102534.

2) Trautner C and Vermaas W. 2013. *J Bacteriol* 195:5370–5380.

- 3) Kirk J, *et al.* 2017. *Microb Biotechnol* 10:76–90.
- 4) Lupas A, *et al.* 1994. *J Bacteriol* 176:1224–1233.

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#### P-PPM-027

##### Elucidating the metabolon formed by tricarboxylic acid cycle enzymes in *Bacillus subtilis*

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The tricarboxylic acid (TCA) cycle is a central hub of cellular metabolism, but increasing evidence suggests that its enzymes do not operate as isolated entities. Instead, transient or stable multienzyme assemblies may facilitate substrate channeling and enhance metabolic efficiency. In *Bacillus subtilis*, the structural and functional organization of such a putative TCA-cycle metabolon remains largely unresolved [1].

This project aims to systematically elucidate protein–protein interactions among TCA-cycle enzymes using chemical crosslinking coupled to mass spectrometry [2]. Malate dehydrogenase (MDH) is employed as the first key protein for pull-down experiments, as its structural similarity to the human enzyme allows insights from the bacterial system to be extrapolated to human mitochondria. Comparative analyses between wild-type and enzyme-deletion strains will help to identify core interaction nodes that stabilize complex formation. The resulting interaction network will be complemented by structural modelling to visualize potential spatial arrangements of associated enzymes.

This approach provides a conceptual framework for understanding how higher-order enzyme assemblies may dynamically regulate metabolic flux and redox homeostasis. Moreover, insights gained from MDH-centered interactions in *B. subtilis* can inform our understanding of mitochondrial function and dysfunction in human disease, including metabolic disorders linked to TCA-cycle impairments.

[1] Meyer *et al.* (2011) *Metabolic engineering* vol. 13,1: 18-27

[2] O'Reilly *et al.* (2023) *Molecular systems biology* vol. 19,4: e11544.

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#### P-PPM-028

##### Differential regulation of the three type VI secretion systems of *Pseudomonas aeruginosa*

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*Pseudomonas aeruginosa* is an opportunistic pathogen capable of thriving in diverse ecological niches across a wide temperature range. It encodes three distinct type VI secretion systems (H1-, H2-, and H3-T6SS), each associated with specific effector proteins. These effectors have diverse functions, including anti-eukaryotic activity contributing to virulence, antibacterial activity mediating interbacterial competition, and roles in nutrient acquisition supporting survival in nutrient-limited environments. Despite their

importance, the environmental conditions that trigger activity of the three T6SSs remain poorly understood.

This study investigates how growth under six conditions representing common *P. aeruginosa* habitats influences the expression and activity of its three Type VI secretion systems (T6SSs). RNA-seq analysis was performed for the H1-, H2-, and H3-T6SS gene clusters in static and shaking liquid cultures as well as on solid surfaces at two temperatures, 22°C and 37°C. Secretion activity was evaluated by detecting Hcp, the hallmark T6SS protein. The results showed that the H1-T6SS was highly expressed and active at 37°C in static cultures. The H2-T6SS was strongly expressed and active at 22°C in static culture. The H3-T6SS was highly expressed at 22°C in stationary-phase liquid cultures, both static and shaking, when nutrients were limited.

Together, these results reveal that the three T6SSs are differentially regulated by temperature and growth condition, enabling *P. aeruginosa* to fine-tune its secretion activity to specific environmental and host-associated conditions. Identifying the conditions that activate T6SS is important for understanding *P. aeruginosa* infection dynamics and its persistence in hospital environments.

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#### P-PPM-029

##### Growth Characteristics and Drug Resistance Mechanisms of *Acinetobacter baumannii* in Human Urine

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#### 1. Introduction:

*Acinetobacter baumannii* is a clinically significant, multidrug-resistant Gram-negative bacterium that causes infections, including pneumonia, sepsis, and urinary tract infections (UTIs). However, specialized research on the growth behaviour and resistance mechanisms of *A. baumannii* in the urinary environment remains limited.

#### 2. Goals:

This study aims (1) to elucidate the survival and drug resistance regulatory mechanisms of *A. baumannii* in the UTI environment, and (2) to assess the relevance of two artificial culture systems, Artificial Urine Medium (AUM) and Mueller-Hinton (MH) medium, compared with human urine in antimicrobial susceptibility testing (AST), thereby guiding the selection of appropriate experimental systems.

#### 3. Materials & Methods:

The standard strain *A. baumannii* ATCC 19606 was cultured *in vitro* under three conditions: MH, AUM, and human urine. Growth kinetics and AST were compared across these systems. Transcriptomic profiling (RNA-seq) identified genes with differential expression among the three environments. Selected genes were validated using RT-qPCR. Key

siderophore synthesis genes were inactivated by gene knockout, and the growth of mutant strains in human urine was examined to verify their functional roles.

#### 4. Results:

RNA-seq analysis revealed that siderophore-related genes were significantly up-regulated in human urine compared to MH and AUM. Analysis of iron content confirmed that human urine contained lower iron levels. Supplementation with exogenous iron ions alleviated the growth restriction of *A. baumannii* in human urine. Knockout of key siderophore synthesis genes, such as those involved in acinetobactin production, led to markedly reduced bacterial growth in human urine. AST demonstrated that neither MH medium nor AUM was capable of effectively simulating human urine environment.

#### 5. Summary:

This study elucidate the molecular basis of *A. baumannii* survival and persistence under urinary conditions and highlight the influence of environmental composition and antibiotic susceptibility. Importantly, our results indicate neither MH medium nor AUM fully recapitulates the urinary milieu for AST of *A. baumannii* isolates, underscoring the need for physiologically relevant assay conditions. Collectively, this work provides a framework for improving AST strategies and for investigating host-specific adaptations in *A. baumannii* pathogenesis.

#### P-PPM-030

##### Regulation of the Carbon Flux in *Synechocystis* Using the PGAM-PirC Switch

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Cyanobacteria, such as the unicellular model organism *Synechocystis* sp. PCC 6803, perform oxygenic photosynthesis and convert CO<sub>2</sub> and light into organic compounds, making them promising organisms for sustainable biotechnological use. An important control point of the carbon flux is the phosphoglycerate-mutase (PGAM), which converts 3-phosphoglycerate to 2-phosphoglycerate. This reaction directs carbon flow towards anabolic pathways. Under nitrogen starvation, *Synechocystis* adapts with a reversible process called chlorosis. Then, the PII protein, the sensor protein for the carbon/nitrogen status, releases the small protein PirC. Consequently, PirC binds and thereby inhibits PGAM. As a result, glycogen is formed as carbon storage as well as the biopolymer PHB. Moreover, mutants with impaired carbon flux were shown to excrete organic acids under these stress conditions.

This study aims to exploit the regulatory interaction between PGAM and its inhibitor PirC, to redirect carbon flux toward lower glycolysis. Through metabolomic engineering, value goods should be excreted, such as succinate, which is a valuable product for industrial production.

Different *Synechocystis* strains constitutively overexpressing *pgam* were generated using synthetic promoters. The deletion mutant  $\Delta$ *phaEC*, unable to synthesize PHB, was also used as a background as well as a C-terminus truncated

PGAM variant not inhibited by PirC [2]. Moreover, overexpression of the phosphoenolpyruvate carboxylase and introducing a glyoxylate shunt should boost the carbon flow further into the TCA cycle. Levels of glycogen, PHB and excreted metabolites were analyzed under N-depleted conditions.

PGAM overexpression decreased glycogen accumulation and increased PHB levels under nitrogen starvation, indicating a redirection of carbon flow to lower glycolysis. Deletion of *phaEC* increased metabolite excretion further. The results confirm the central role of the PGAM–PirC regulatory switch in controlling carbon flux in *Synechocystis*. Modulating this control point allows redirection of carbon flux toward PHB formation and organic acid secretion. This work establishes a foundation for metabolic engineering strategies to optimize cyanobacterial production of feedstock chemicals and sustainable biopolymers.

[1] Orthwein et al., *PNAS U.S.A.* (2021), 10.1073/pnas.2019988118

[2] Orthwein et al., *mBio* (2025), 10.1128/mbio.03378-24

#### P-PPM-031

##### Exploration of Jep expression under (patho-)physiologically relevant conditions in the mouse adapted *Staphylococcus aureus* strain JSNZ

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*Staphylococcus aureus* is a pathogen that causes various diseases. Mice are the conventional *in vivo* model for *S. aureus* infections. However, human-adapted strains are less virulent in murine models, a phenomenon attributable to their strong host specificity<sup>1</sup>. Consequently, mouse-adapted strains have been increasingly used to overcome this limitation. One such strain is JSNZ, which secretes the JSNZ extracellular protease (Jep) at extremely high levels<sup>2</sup>. Therefore, we explore how Jep is expressed under different infection-relevant conditions and the co-regulation of other proteins to identify regulators of Jep and to clarify why Jep is the dominating secreted protein of *S. aureus* JSNZ. JSNZ was cultivated at control conditions (37 °C), constant high temperature (42 °C), constant low temperature (32 °C), iron-limiting (with bipyridine) and oxygen-limiting conditions. The expression of *jep* was analyzed at seven time points on transcriptomic level and on proteomic level (cellular proteome and secretome). Secreted Jep makes up about 75% of the supernatant protein fraction when grown at 42 °C, at 37 °C or under iron-limitation. This finding was confirmed at transcript level, where it was shown that *jep* expression increases over time, reaching a plateau in the stationary growth phase. Interestingly, iron limitation caused the highest expression in exponentially growing cells, which then declined, in contrast, the secreted Jep levels accumulated in the supernatant, seemingly without degradation. At 32 °C, both JSNZ growth and Jep secretion showed a delay. Under oxygen limitation, there was barely any Jep detectable. As it is highly similar to the serine-protease-like proteins (*sp*s), it was hypothesized that *jep* could have a similar regulation pattern. However, the most positively correlated protein was another protease, ScpA. *In silico* prediction of possible

regulators of *jep* indicated a high potential for PurR and SaeR regulation. These findings demonstrate that the regulation of *jep* is complex and possibly due to an interaction between various regulators, which requires further investigation.

1. Trübe, P. *et al.* Bringing together what belongs together: Optimizing murine infection models by using mouse-adapted *Staphylococcus aureus* strains. *Int. J. Med. Microbiol.* 309, 26–38 (2019).

2. Wolfgramm, H. *et al.* Integrated genomic and proteomic analysis of the mouse-adapted *Staphylococcus aureus* strain JSNZ. *Curr. Res. Microb. Sci.* 9, 100489 (2025).

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### P-PPM-032

#### Linking Carbon Metabolism to Copper Surface Toxicity in *Escherichia coli*

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Copper is a widely used antimicrobial material due to its ability to disrupt bacterial membranes and redox balance. However, the bacterial metabolic state can strongly influence susceptibility to copper-induced stress on copper surfaces. This study investigated how different carbon sources modulate *Escherichia coli* survival upon exposure to copper surfaces. Using the *E. coli* K-12 BW25113 strain, we compared survival after contact with copper when cells were grown on glucose, glycerol, acetate, gluconate, galactose, fructose, succinate, and pyruvate. These carbon sources differentially affected central metabolism, redox homeostasis, and energy generation, which in turn altered surface-related copper stress responses. The results revealed metabolic determinants of copper tolerance and highlighted physiological vulnerabilities that could be targeted in antimicrobial surface design and bacterial control strategies.

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### P-PPM-033

#### Genome and differential proteome of *Desulfosarcina variabilis* 3be13 – a marine sulfate reducer of many talents

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Members of the deltaproteobacterial family *Desulfobacteraceae* are key players in the remineralization of organic compounds in the marine environment. This prominent role is based to the broad catabolic spectrum ranging from autotrophy via (simple to complex) aliphatic and aromatic compounds to recalcitrant aliphatic and aromatic hydrocarbons. While other family members are either degradation specialists focused on distinct aliphatic (e.g. *Desulfobacterium autotrophicum* HRM2) or aromatic compounds (e.g. *Desulfobacula toluolica* Tol2), or a limited number of both (e.g. *Desulfococcus multivorans* 1be1), *Desulfosarcina variabilis* 3be13 stands out for its very broad spectrum of utilizable substrates.

To understand the molecular basis of this versatility and, hence, the environmental success of *Desulfosarcina* sp., the

genome of *Ds. variabilis* 3be13 was determined. In addition, the soluble and membrane protein fractions of cells adapted to 28 different substrate conditions were differentially analyzed to gain insights into the adaptability to changing environmental conditions.

The 9,639,276 bp circular chromosome (no plasmids) contains a total of 8,578 open reading frames, representing one of the largest genomes of sulfate-reducing bacteria at present. A high genomic plasticity is evident from a large number of mobile genetic elements, 3 CRISPR-Cas loci as well as the presence of 55 predicted genomic islands and a prophage. Notably, proteins of peripheral degradation routes are mostly encoded in operon-like gene clusters that are referred to as pathway modules. Besides catabolic proteins, these pathway modules also encode pathway-specific electron transfer proteins (if applicable), regulatory proteins and sometimes also transport proteins. These modules are highly similar with respect to gene organization and sequence identity to other *Desulfobacteraceae* members sharing respective catabolic capacities, which suggests transfer of these modules within the family. Differential proteomic analyses revealed a substrate-dependent regulation of the catabolic modules, demonstrating a high degree of adaptability.

The proteogenomic study suggests that the environmental success of *Desulfosarcina* is based on both, the large genomic potential, and the multifaceted regulatory network. The latter enables condition-specific adaptation of the protein complement and allows for a high level of energetic efficiency – a prerequisite for living at the thermodynamic limit.

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### P-PPM-034

#### Co-reduction of nitrate and oxygen in mycelium and spores of *Streptomyces coelicolor* during hypoxia

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**Introduction:** *Streptomyces coelicolor* A3(2) is a filamentous actinobacterium that requires oxygen for growth and uses a branched respiratory chain to respire with O<sub>2</sub>. A menaquinol:cytochrome *bcc* oxidoreductase and copper-*aa3*-type cytochrome *c* oxidase supercomplex form one branch, while the other branch includes a menaquinol:cytochrome *bd* oxidase. The formation of an electron-transferring supercomplex is necessary because *S. coelicolor* has a membrane-bound *c*-type cytochrome. *S. coelicolor* also synthesizes three related respiratory nitrate reductases (Nar). Although these do not support growth, Nar1 and hypoxia-induced Nar2 are presumed to help maintain a membrane potential under O<sub>2</sub>-limitation and nitrate is available. The Nar enzymes are synthesized and active in different stages of the complex developmental life cycle of the bacterium. Nar1 is exclusively present and active in spores, Nar2 mainly in exponentially growing mycelium and Nar3 in stationary mycelium. Anaerobic Nar1 activity depends on a functional cytochrome *bcc-aa3* supercomplex, but anaerobic Nar2 activity in mycelium is not totally dependent on the respiratory supercomplex. To date, nitrate reduction in the presence of oxygen has not been examined.

**Objective:** We wished to determine whether simultaneous oxygen and nitrate reduction occurs in mycelium or spores of *S. coelicolor* and, if so, whether it shows differential

dependence on the respiratory supercomplex in different developmental stages.

**Methods:** An oxygen electrode was used to measure O<sub>2</sub> reduction and samples were also taken to measure nitrate reduction in intact mycelium and spores. Assays were also carried out with defined mutants lacking Nar, the *bcc-aa3* supercomplex or the *bd* oxidase.

**Results:** Measurement of O<sub>2</sub> respiration in the presence of nitrate demonstrated that simultaneous O<sub>2</sub> and nitrate reduction occurs in both mycelium and spores. Nitrate reduction in the presence of O<sub>2</sub> in spores was dependent solely on the *bcc-aa3* supercomplex, while in mycelium it was dependent on both a functional *bcc-aa3* supercomplex and *bd* oxidase.

**Conclusions:** Our findings suggest that blocking O<sub>2</sub> reduction by mutating either of the two oxidase branches prevents hypoxic NO<sub>3</sub><sup>-</sup> reduction in mycelium. In contrast, NO<sub>3</sub><sup>-</sup> reduction in oxygen-limited spores is solely dependent on the cytochrome *bcc-aa3* supercomplex. These data indicate that the respiratory capacity of *S. coelicolor* is considerably more flexible than previously considered.

#### P-PPM-035

##### **Mechanisms for Modulation of Phosphoglucumutase Activity**

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Glycogen is the main storage polysaccharide in cyanobacteria and plays a critical role during the adaptation to environmental stresses. It is accumulated preceding dark periods and during the adaptation to nutrient-limiting conditions, such as nitrogen starvation. Phosphoglucumutases link the metabolism of this polysaccharide to central carbon metabolism through the interconversion between glucose-1-phosphate and glucose-6-phosphate. In the cyanobacterium *Synechocystis* sp. PCC 6803, PGM1 (slr0726) is responsible for 99% of the phosphoglucumutase activity. Regulation of PGM activity is essential to ensure proper management of glycogen stores and degradation. Here, we aim to investigate the different regulatory mechanisms that allow for the tight control of PGM1 activity. PGM1 has two phosphorylation sites, Ser47 and Ser 152, with the latter being in the catalytic site and is responsible for the phospho-group transfer to the glucose molecule. Our work showed that Ser47 is an inhibitory phosphorylation site, and its phosphorylation keeps PGM1 inactive during nitrogen starvation to prevent premature glycogen degradation. Our structural analysis shows that this residue is involved in interaction with the substrate and has a strong impact on the substrate specificity of the enzyme. This is supported by our mutational analyses, which show that the PGM1 variant S47A is insensitive to fructose-1,6-bisphosphate, a competitive inhibitor of the WT PGM1 reaction. Furthermore, we investigated, to our knowledge, for the first time the kinetics of the conversion of glucose-6-phosphate to glucose-1-phosphate reaction with an assay that was initially established for Glucose-1-phosphate adenyl transferase (GlcC). Lastly, we observed that *Synechocystis* PGM1 can form dimers in vitro. This is an uncommon feature that it shares with a few bacterial homologues and might represent an additional level of

regulation. However, the effect of dimerization on enzymatic activity and its physiological relevance remains to be elucidated.

#### References:

- [1] Doello, S., et al. The FEBS Journal 289.19 (2022): 6005-6020.
- [2] Neumann, N., et al. MBio 13.4 (2022): e01469-22.
- [3] Alford, Janette T., et al. Microbial Physiology 35.1 (2012): 50-64.

#### P-PPM-036

##### **The Show Must Go OFF: exploring ClpC roles in escape from the Heat Shock Response in *Bacillus subtilis***

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**Introduction:** Elevated temperatures can lead to protein misfolding and aggregation, which can be deleterious if not properly counteracted. To survive, bacteria employ the Heat Shock Response (HSR). HSR is a tightly regulated process involving changes in the transcriptome and the subsequent remodelling of the proteome. As a consequence, several members of the Protein Quality Control (PQC) system are highly expressed. This PQC system is mainly composed of chaperones and AAA+ protease complexes, where AAA+ unfoldases and peptidase complexes associate. In *Bacillus subtilis*, one of the major AAA+ unfoldase is ClpC. Other than unfolding activity, ClpC can eventually associate with the peptidase ClpP to form the proteolytic complex ClpCP and consequently gain proteolytic activity. ClpC bind ClpP through the tips of their ClpP-interacting loops (P-loops). The amino acid sequence of these loops determines the binding strength between the unfoldases and ClpP, affecting their degradation activity. For instance, the wt VGF loop of ClpC allows the complex to perform both refolding and degradation. Our lab generated 2 additional loop variants, GGR and IGF, which reduces or enhances binding to ClpP, respectively. This allows to obtain ClpC variants that will preferentially perform refolding or degradation activity.

**Goals:** This project aims to dissect the role of ClpC-mediated refolding and degradation during the shutdown of the HSR by specifically modulate ClpC activity.

**Materials and methods:** We performed a proteomic analysis of different genetic background: *wt*, *clpC*<sup>IGF</sup>, *clpC*<sup>GGR</sup> and *clpC*<sup>DWB</sup> (an ATP-hydrolysis-deficient mutant). Mass spectrometry was performed before the heat-shock, after 15 minutes to 50°C exposition, and in the subsequent 15, 30 and 60 minutes of recovery at 37°C. The total protein extracts as well as separated soluble and aggregated fractions were analyzed.

**Results:** First results show accumulation of known and previously proposed targets of ClpC such as CtsR and ClpE in *clpC*<sup>DWB</sup> and *clpC*<sup>GGR</sup> backgrounds. Ongoing in-depth analysis aims to identify novel substrates of ClpC and how this contributes to HSR shutdown.

**Summary:** Preliminary results show accumulation of known ClpC targets, suggesting that our approach represents an efficient way to identify ClpC targets in the specific conditions tested. Further effort aims to clarify the specific roles of ClpC

activities throughout proteome remodeling, especially during the shutdown of the HSR.

#### P-PPM-037

##### A novel glutamate fermentation pathway in *Anaerospira hongkongensis*

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Glutamate is a common metabolite in anoxic environments, serving as a carbon and nitrogen source for several strictly anaerobic bacteria. Previously, two fermentation routes, methylaspartate and hydroxyglutarate pathway, were described in bacteria (Buckel and Barker 1974). Additionally, it was shown that *Clostridia aminobutyricum* can degrade 4-aminobutyrate, the decarboxylation product of glutamate, but this organism cannot decarboxylate glutamate itself (Gerhardt *et al.*, 2000). Whilst studying the strictly anaerobic *Anaerospira hongkongensis* we found that this organism not only features the methylaspartate pathway for glutamate fermentation, but also a novel route sharing similarities to the 4-aminobutyrate fermentation pathway from *C. aminobutyricum* (Gerhardt *et al.*, 2000). Instead of decarboxylation to 4-aminobutyrate, glutamate is deaminated by glutamate dehydrogenase to 2-oxoglutarate. Afterwards, 2-oxoglutarate is oxidized by 2-oxoglutarate:ferredoxin oxidoreductase to succinyl-CoA followed by a reduction of succinyl-CoA to succinic semialdehyde, an intermediate of 4-aminobutyrate fermentation. Further degradation to butyrate and acetate occurs as described for 4-aminobutyrate fermentation (Gerhardt *et al.*, 2000). In addition to the methylaspartate pathway enzymes, we could show each individual step of this novel glutamate fermentation pathway in cell extract assays of glutamate grown *A. hongkongensis*, as well as overproduction of enzymes involved in this pathway when compared to *trans*-aconitate grown cells. Enzymes catalyzing two critical reactions, succinyl-CoA and succinic semialdehyde reduction, could not be identified based on in literature described proteins. Instead, we identified a novel, oxygen sensitive succinic semialdehyde reductase, belonging to the class IV alcohol dehydrogenases. Stable isotope labeling revealed that the main pathway for glutamate fermentation in *A. hongkongensis* was the methylaspartate pathway, with minor contribution of the novel route through 4-hydroxybutyrate. During acidic stress, an increased flux through the novel pathway was indicated, with this pathway most likely being favored due to less acid formation. Utilizing this pathway for glutamate fermentation may explain why key enzymes of this novel pathway are wide spread in anaerobic bacteria.

Buckel, W. & Barker, H. A. (1974). DOI:10.1128/jb.117.3.1248-1260.1974

Gerhardt, A. *et al.* (2000) DOI:10.1007/s002030000195

#### P-PPM-038

##### Molecular Mechanism of a Promiscuous Dinucleoside Tetraphosphate-Cleaving Enzyme

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Bacteria must find ways to thrive in environments that are sometimes unfavorable, thereby relying on signaling molecules to sense and respond to changes in their surroundings. Under stress conditions, signaling nucleotides known as alarmones accumulate inside the cell, triggering metabolic reprogramming to promote survival. One such alarmone is Diadenosine tetraphosphate (AP<sub>4</sub>A), a dinucleoside polyphosphate involved in stress responses and cellular regulation. Beyond AP<sub>4</sub>A, other related dinucleoside tetraphosphates, such as AP<sub>4</sub>G, AP<sub>4</sub>U, and AP<sub>4</sub>C, have also been detected in bacteria, suggesting that this class of molecules may play broader roles in nucleotide signaling. AP<sub>4</sub>A degradation is mediated by distinct enzyme families. In Gram-negative bacteria like *Escherichia coli*, Nudix hydrolases catalyze the asymmetric cleavage of AP<sub>4</sub>A into AMP and ATP, whereas ApaH performs symmetric cleavage to yield two ADP molecules. In Gram-positive bacteria, the enzyme YqeK, a member of the HD-domain superfamily, performs a similar symmetric degradation and is widely distributed among Firmicutes and other phyla. Despite its prevalence, the enzymatic properties and physiological significance of YqeK remain poorly characterized, and the consequences of elevated AP<sub>4</sub>A levels in *Bacillus subtilis* are not well understood. In this study, we investigated YqeK from *B. subtilis* as an AP<sub>4</sub>A hydrolyzing enzyme. Biochemical assays demonstrated that YqeK efficiently hydrolyzes AP<sub>4</sub>A, producing ADP as the main product. Enzymatic analyses revealed that YqeK is a relatively promiscuous enzyme with activity toward several dinucleoside polyphosphates, including AP<sub>4</sub>G, AP<sub>4</sub>U, AP<sub>4</sub>C, AP<sub>3</sub>A, and AP<sub>5</sub>A, suggesting flexibility in substrate recognition. To elucidate its catalytic mechanism, we determined crystal structures of *Geobacillus thermodenitrificans* YqeK in the apo form and bound to ADP and a non-hydrolysable AP<sub>4</sub>A analog. These structures reveal key features of the active site, including the positioning of the substrate and the coordination of metal ions essential for catalysis. Phenotypic analysis of the *yqeK* deletion strain demonstrated altered cell morphology, impaired biofilm formation and reduced survival under stress conditions, demonstrating that the absence of *yqeK* influences cell physiology. Together, these findings provide structural and functional insight into YqeK, and highlight its role in regulating AP<sub>4</sub>A metabolism and stress responses in *B. subtilis*.

#### P-PPM-039

##### Investigating the Mechanism of Acetophenone Carboxylase

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Acetone carboxylase (Acx) and acetophenone carboxylase (Apc) are members of a novel carboxylase class, which uses ATP for biotin-independent activation of chemically inert ketones [1,2]. First, Acx and Apc activate both of their substrates (*i.e.*, the ketone and HCO<sub>3</sub><sup>-</sup>) by phosphorylation

[1]. In Acx, both substrates are phosphorylated at the same activation site. Then, the activated substrates are transferred over a distance of 40 Å from the nucleotide binding site to the catalytic site via an internal channel [3]. In contrast, substrate phosphorylation occurs at two distinct sites in Apc. Phosphorylation sites are 50 Å apart from each other, which precludes direct interaction of the activated substrates. Free diffusion between the activation sites is not possible either as the activated substrates are readily decomposed in water. The Apc core complex ( $(\alpha\alpha'\beta\gamma)_2$ ) moreover lacks internal substrate channels for acetophenone-*enol*-phosphate transport [2]. However, in contrast to Acx, Apc possesses an additional Apc $\epsilon$  subunit, which is not part of the Apc complex, but is indispensable for acetophenone carboxylation [4]. The actual function of Apc $\epsilon$  is still at issue. It has been hypothesized that Apc $\epsilon$  forms a lid which shields the activated substrates from water and allows them to diffuse to the catalytic subunit or induces conformational changes in Apc to bring the substrate binding sites into close proximity [2].

In this project, we investigate the role of Apc $\epsilon$  in acetophenone carboxylation. We obtained the crystal structure of the isolated Apc $\epsilon$  subunit from *Aromatoleum aromaticum* and created an AlphaFold model of Apc $\epsilon$  in complex with the Apc core, which had been crystallized previously [2]. Next, we study the specificity of interactions between Apc $\epsilon$  and the core complex. We generate *Aromatoleum aromaticum*  $\Delta$ *apcE* deletion mutants and complement them with heterologous Apc $\epsilon$  subunits of high or low sequence identity (e.g., Apc $\epsilon$  from *Aromatoleum* spp. or *Nevskia ramosa*). Comparing the growth of these mutants on acetophenone, we evaluate which level of interaction specificity between Apc $\epsilon$  and core complex is required to sustain enzyme activity.

[1] Heider *et al.*, **J Mol Microbiol Biotechnol** 26:152-64 (2016).

[2] Weidenweber *et al.*, **Sci Rep** 7:39674 (2017).

[3] Mus *et al.*, **Sci Rep** 7:7234 (2017).

[4] Jobst *et al.*, **J Bacteriol** 192:1387-94 (2010).

## RNA biology

### P-RNA-001

#### Domesticating a new chromosome and RNA rewiring in *Vibrio cholerae*

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Approximately 10% of bacterial species possess more than one main chromosome, an architecture named multipartite genome. Such arrangement enables quicker adaptation to new environmental niches. *Vibrio cholerae*, for example, has two chromosomes: Chr1 and Chr2. While genes encoding for essential cell functions are located on Chr1, Chr2 encodes genes involved in specialized metabolism and pathogenicity. A novel giant replicon (named hereafter Chr3) has been found in a recent isolate of *Vibrio cholerae*. Despite being

present in various *Vibrionaceae*, the function of Chr3 remains elusive.

To shed light on the impact of Chr3 on its host, we performed transcriptional and post-transcriptional analysis. Comparative transcriptomics showed that Chr3 is conditionally expressed, influencing Chr1 and Chr2 gene expression mostly in the exponential growth phase. Global analysis of RNA duplex formation using RIL-Seq (RNA-interaction-by-ligation-and-sequencing) revealed a vast network of Hfq-assisted interactions between Chr3 and the other two chromosomes. In combination with RIP-Seq (RNA immunoprecipitation and sequencing) analysis, we found 74 sRNAs (small regulatory RNA) encoded on Chr3. Whilst the majority of the newly annotated sRNAs target genes encoded on Chr3, Vcr313 targets genes on primary chromosomes involved in tRNA metabolism. We further show that Chr3 impacts quorum sensing, possesses a functional type-F CRISPR-Cas, and contains a more specialized Hfq-like RNA-binding protein. Overall, our data shows ample interactions between all three chromosomes suggesting they are equally involved in post transcriptional gene control. Moreover, the new set of advantageous genes encoded on Chr3 makes it an important entity to expand the adaptation capacities of *Vibrio cholerae*.

### P-RNA-002

#### RNAylation as a Potential Modulator of Translation in Bacteriophage T4 Infection of *Escherichia coli*

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RNAylation is a novel discovered post-translational modification in which NAD-capped RNAs are covalently attached to host proteins, catalyzed by the bacteriophage T4 ADP-ribosyltransferase ModB. Among its known targets are ribosomal proteins such as S1 (rS1), and L2 (rL2) which play central roles in translation initiation and elongation. While RNAylation has been shown to occur during bacteriophage T4 infection, its impact on the translational process remains largely unknown. Infections in *Escherichia coli* with bacteriophage T4 carrying a catalytically inactive ModB mutant—lacking both ADP-ribosylation and RNAylation—result in delayed lysis, reduced burst size, and decreased phage adsorption (1). This suggests that ModB plays a crucial role on hijacking translational apparatus of *E. coli* (2). Building on these observations, this study investigates how RNAylation mediated by ModB during phage infection affects translation in *E. coli*, focusing on its impact on rS1–RNA affinity, the translation of RNAs attached to RNAylated proteins, and overall translational output. To further explain the biological role of ModB, we aim to characterize its structure to gain mechanistic insight into its catalytic activity and substrate recognition. Understanding how this modification affects translation will provide insight into phage–host interactions and will unveil novel strategies bacteriophage T4 use to manipulate *E. coli*.

(1) Wolfram-Schauerte, M., Pozhydaieva, N., Grawenhoff, J. et al. A viral ADP-ribosyltransferase attaches RNA chains to host proteins. *Nature* 620, 1054–1062 (2023).

(2) Yilmaz Demirel, N., Weber, M., & Höfer, K. (2024). Bridging the gap: RNAylation conjugates RNAs to proteins. *Biochimica et Biophysica Acta: BBA*, 1871(8): 119826



## P-RNA-003

### Two small RNAs with distinct temperature-dependent expression in *Yersinia pseudotuberculosis*

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The Gram-negative pathogen *Yersinia pseudotuberculosis* must adapt to changing temperatures while remaining viable under cold storage conditions, during the infection of warm-blooded hosts and after its secretion into the environment. As temperature changes cause stress, the bacterium responds with rapid regulation at DNA, RNA and protein level. Gene expression at RNA level is controlled, among other factors, by small regulatory RNAs. These sRNAs can bind to specific target mRNAs and either enhance or inhibit their transcription and/or translation. Post-transcriptional repression is initiated by the binding of the sRNA to the Shine-Dalgarno sequence and/or the start codon, thereby preventing the formation of the mRNA-ribosome complex. In contrast, interaction with highly structured mRNAs can induce structural rearrangements that liberate the Shine-Dalgarno sequence and consequently enable translation initiation.

Our study focuses on two inversely temperature-regulated sRNAs in *Y. pseudotuberculosis*, namely MicA and GlmY. While MicA expression is induced at 37 °C, GlmY is predominantly expressed at 10 °C. The regulatory function of MicA is well characterized in *E. coli*, where it primarily targets transcripts encoding outer membrane-associated proteins like the inner membrane protein HtrG or LpxT, which is involved in lipopolysaccharide synthesis. In contrast, GlmY, together with the sRNA GlmZ, plays a crucial role in cell wall biosynthesis by regulating the expression of *glmS* encoding the glucosamine-6-phosphate (GlcN6P) synthase.

Since most sRNAs regulate multiple mRNA targets, we aim to identify potential temperature-dependent targets of MicA and GlmY by computational predictions, reporter gene assays and Northern blot analysis. Furthermore, we seek to understand how these sRNAs influence gene regulation during rapid temperature shifts by performing heat and cold shock experiments.

## P-RNA-004

### Analysis of temperature-responsive regulation in *Yersinia pseudotuberculosis*

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During infection of warm-blooded hosts and subsequent excretion into the environment, pathogenic bacteria are exposed to fluctuating conditions. Consequently, they must be able to react to these changes rapidly and adapt their metabolism accordingly. Temperature is one of the key environmental parameters that has a significant impact on bacterial cells, which can consequently sense and respond to shifts in temperature at multiple levels of regulation.

*Yersinia pseudotuberculosis* is a food-borne human pathogen that can cause an infection of the gastrointestinal tract and the intestinal lymphatic system. Its optimum growth temperature is ~28 °C, but the cells can quickly react to an increase to host temperature (37 °C) and adapt their metabolism and virulence. In addition, *Y. pseudotuberculosis*

can survive cold temperatures, allowing growth on food stored in the refrigerator.

In the past, parallel analysis of RNA structures (PARS)<sup>1</sup> allowed us to identify a variety of different RNA thermometers (RNATs) that control translation initiation in a temperature-dependent manner. For example, several components of the T3SS, which are essential for virulence, are induced by RNATs. We discovered other promising RNATs candidates in *yopH*, *rpoA* and *rplN* where we were able to e.g. demonstrate post-transcriptional regulation by reporter gene studies. *In vitro* structure probing and follow up *in vivo* experiments will provide us with a better insight into the regulatory role of these candidates.

While RNATs play an important role for the uptake of pathogenic bacteria by the host, we are also interested in determining how the bacterium reacts when it gets released back into the environment. For this, RNA-seq was performed, resulting in a list of interesting targets that could play an important role for *Yersinia* within 30 min after temperature shifts from 37 °C to 25 °C, 17 °C or 10 °C. We assigned up- and downregulated genes to different categories and are working on investigating interesting targets in more detail. To do this, we generate deletion mutants, analyze transcript levels, examining mRNA and sRNA stabilities and more to identify important genes that are required for optimal survival of the cells.

<sup>1</sup> Righetti et al., *PNAS* 113: 7237–7242 (2016)

## P-RNA-005

### The RNA atlas of the biotechnological chassis *Vibrio natriegens*

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*Vibrio natriegens* is a rapidly growing, non-pathogenic marine bacterium, renowned for its remarkably short generation time, making it one of the fastest-growing organisms known. This unique trait, together with an exceptional metabolic versatility, has garnered significant interest in biotechnology and molecular biology. Although previous whole genome sequencing revealed a wealth of rRNAs and tRNAs in *V. natriegens*, an in-depth analysis of its global transcriptional output, including the role of non-coding RNAs (ncRNAs) and small RNAs (sRNAs), was previously missing. In this work, we provide an integrated sequencing-based approach to chart an atlas of the transcriptional and post-transcriptional regulatory networks in *V. natriegens*. Differential RNA sequencing (dRNA-seq) and terminator sequencing (term-seq) revealed the primary transcriptome of *V. natriegens*, allowing the identification of transcriptional start sites (TSS), rho-dependent or -independent termination sites, operon structures, and RNA regulatory elements across the genome. RNA immunoprecipitation followed by sequencing (RIP-seq) was then performed on Hfq, an RNA-binding protein primarily involved in the sRNA-mediated post-transcriptional regulation, to identify all of its potential ligands. Alongside 15 homologs previously described in *Vibrio cholerae*, we found 935 mRNAs and 107 sRNAs associated with Hfq. Finally, RNA interaction by ligation and sequencing (RIL-seq) shed light on the Hfq-mediated sRNA-target interactions *in vivo*, revealing a reshaping of gene expression at low and high cell densities and linking post-transcriptional regulation to collective behaviours such as biofilm formation. We expect that studying the transcriptional and post-transcriptional

landscape of *V. natriegens* will allow us to expand its utility as a biotechnology platform, promote targeted metabolic engineering approaches, and enable the design of novel synthetic biology circuits.

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#### P-RNA-006

##### **CauloSOEP 2.0 – A sRNA overexpression plasmid library for *Caulobacter crescentus***

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Small non-coding RNAs (sRNAs) play a key role in regulating gene expression in bacteria at the post-transcriptional level and typically act through short base-pairing interactions with target mRNAs. Hundreds of sRNA candidates have been identified in diverse species, including the Alphaproteobacterium *Caulobacter crescentus*. Its synchronizable dimorphic life cycle has made *C. crescentus* a well-established model organism for studying bacterial cell biology and development. The role of sRNAs for the progression of the cell cycle has however not been addressed in much detail.

We have previously established the library CauloSOEP (*Caulobacter* sRNA overexpression plasmids) which facilitates conditional induction of 49 individual sRNAs using a vanillate-inducible promoter. Screening efficiency of plating and cell morphology in response to CauloSOEP induction revealed that sRNA AbnZ strongly affected cell viability and membrane integrity, highlighting the potential of the library to study the physiological roles of individual sRNAs.

To also systematically study the influence of sRNAs on the development of *C. crescentus*, we have now extended CauloSOEP to include sRNAs which show differential regulation over the course of the cell cycle. We characterize the distinct features and expression of these candidate sRNAs, and assess their activity as post-transcriptional regulators.

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## **Signal transduction & gene regulation**

#### P-STGR-001

##### **Illuminating the mechanism behind c-di-GMP-dependent activation of chloramphenicol biosynthesis in *Streptomyces venezuelae***

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#### **Introduction**

The majority of antibiotics we use in medicine today derived from members of the phylum Actinomycetota, especially from soil bacteria of the genus *Streptomyces*. Genome sequencing revealed that *Streptomyces* sp. typically harbour 30-40 natural product biosynthetic gene clusters (BGCs) within their genomes. However, most of these BGCs are

silent under laboratory conditions, as molecular tools are needed for their activation. The nucleotide second messenger c-di-GMP has been shown to affect antibiotic biosynthesis in *S. ghanaensis*<sup>[1]</sup>.

#### **Goals**

Our central goal is to understand the mechanism behind c-di-GMP-dependent activation of antibiotic production by using the chloramphenicol producer *S. venezuelae* as a model.

#### **Methods**

LC/MS analysis was used to study the natural products profile of *S. venezuelae* after overexpression of genes encoding for enzymes that either produce c-di-GMP (CdgB, diguanylate cyclase) or degrade c-di-GMP (PdeH, phosphodiesterase), respectively. In addition, expression of chloramphenicol genes was analysed using RT-qPCR. Electrophoretic mobility shift assays (EMSAs) were used to study protein-DNA interactions in a c-di-GMP-dependent manner.

#### **Results and Conclusion**

Our data indicate that the chloramphenicol titre is increased upon overexpression of *cdgB*, encoding a c-di-GMP-producing diguanylate cyclase. We found that expression of *cmIR*, the transcriptional activator of chloramphenicol genes<sup>[2]</sup>, is not affected by c-di-GMP modulation. In contrast, expression of two core biosynthesis genes *cmIP* and *cmIA*, which are under CmlR-control, is clearly increased in the strain overexpressing *cdgB*. Furthermore, our EMSAs revealed enhanced CmlR binding to promoter DNA of the core chloramphenicol biosynthesis genes in presence of c-di-GMP.

Our data show that c-di-GMP activates antibiotic biosynthesis in *S. venezuelae*. We propose that CmlR is a novel c-di-GMP effector that binds c-di-GMP. Ligand-binding increases its affinity to the promoter within the chloramphenicol gene cluster and ultimately leads to activation of gene expression and antibiotic biosynthesis.

<sup>[1]</sup>Makitynsky, R., *et al.* (2020). Secondary nucleotide messenger c-di-GMP exerts a global control on natural product biosynthesis in streptomycetes. *Nucleic acids research*, 48(3), 1583–1598

<sup>[2]</sup>Fernández-Martínez, L. T., *et al.* (2014). New insights into chloramphenicol biosynthesis in *Streptomyces venezuelae* ATCC 10712. *Antimicrobial agents and chemotherapy*, 58(12), 7441–7450

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#### P-STGR-002

##### **A novel player in c-di-GMP signalling and type IV pilus-dependent functions in a model cyanobacterium**

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The model cyanobacterium *Synechocystis* sp. PCC 6803 exhibits type IV pilus-dependent (T4P) functions such as motility (including phototaxis), aggregation and natural competence, all of which are regulated by the second messenger c-di-GMP. To understand how cyanobacteria adapt to their environment, we must understand the molecular mechanisms that underpin these processes. In this study, we investigated the role of CdgR, a recently identified c-di-GMP receptor in cyanobacteria [1], in controlling motility, aggregation, and natural competence in *Synechocystis*.

It has been demonstrated that the CdgR proteins specifically bind c-di-GMP with high affinity [1,2]. Genetic and phenotypic analyses revealed that CdgR is involved in type IV pilus-dependent functions, including phototactic motility and natural competence. The inactivation of the *cdgR* gene resulted in an altered expression of minor pilin genes, which are essential for these processes. Utilising a combination of co-immunoprecipitation and mass-spectrometry, we identified interactions between CdgR and the CRP-family transcription factors SyCRP1 and SyCRP2. The disruption of these CdgR-SyCRP1 and CdgR/SyCRP2 complexes is initiated by elevated levels of c-di-GMP. Moreover, the assembly and stability of these complexes are influenced by other cyclic nucleotides, such as cAMP and c-di-AMP. These observed interactions imply a complex regulatory mechanism by which CdgR influences gene expression in response to cyclic nucleotide messenger signalling, particularly c-di-GMP.

The present findings highlight the importance of CdgR in c-di-GMP signalling and its role in regulating type IV pilus-dependent functions in *Synechocystis*. It has been demonstrated that the modulation of the expression of specific minor pilin genes by CdgR, through interactions with SyCRP1 and SyCRP2, contributes to the establishment of the multiple T4P functions and adaptive behaviours of cyanobacteria. This study has enhanced our understanding of the molecular mechanisms underlying c-di-GMP signalling and its impact on cellular processes in cyanobacteria.

[1] Zeng et al. (2023). "A c-di-GMP binding effector controls cell size in a cyanobacterium." *PNAS* **120**(13): e2221874120.

[2] Samir et al. (2025). "The second messenger c-di-AMP controls natural competence via ComFB signaling protein." *Cell Discovery* **11**(1): 65.

### P-STGR-003

#### Extraintestinal *Escherichia coli* employ the membrane protein RcrB to protect against reactive chlorine species

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While some pathogenic *E. coli* cause intestinal diseases, extraintestinal *E. coli* (ExPEC) can colonize and infect environments outside the gut, including the urinary tract. Upon entry of the urinary tract, ExPEC must counter a multitude of bactericidal host defense strategies, which require specialized genetic adaption for survival from this *E. coli* pathotype. For instance, ExPEC must defend highly antimicrobial oxidants such as hypochlorous acid (HOCl), a potent reactive chlorine species (RCS) generated during neutrophil-mediated phagocytosis and by enzymes in

uroepithelial cells to control bacterial colonization. The increasing rate of ExPEC infections in humans due to changing infection dynamics demonstrate the critical need for a better understanding of ExPEC pathogenesis, which is desperately needed to improve approaches for infection prevention and treatment given the rise in antibiotic resistance spreading among *E. coli*.

Our lab has reported that members of the ExPEC pathotype are more resistant to RCS *in vitro* and to neutrophil-mediated phagocytosis when compared to non-pathogenic and enteropathogenic *E. coli*. We identified the defense system responsible for these phenotypes and characterized its regulation during RCS stress: the RcrR regulon consisting of the previously uncharacterized *rcrARB* genes is controlled by the RCS-sensing transcriptional repressor RcrR, which reversibly loses its repressor activity upon oxidation by RCS, resulting in de-repression of its downstream targets. Induced expression of RcrB contributes significantly to ExPEC's increased RCS resistance, however, the precise mechanism was unclear.

Using sucrose gradient ultracentrifugation and western blotting, we identified RcrB as an inner membrane protein. We also show that ExPEC cells that lack RcrB are characterized by elevated protein and DNA damage, indicating increased RCS influx into the cell compared to wildtype or complemented  $\Delta rcrB$  strains. Interestingly, RcrB-expressing cells can protect  $\Delta rcrB$  cells from RCS. We found that this is mediated through RcrB's ability to quench RCS in the media, which it does through select redox-active amino acids located on its periplasmic side. Further, we found that RcrB requires the presence of glutathione for its activity. Overall, our data suggest that RcrB works as an RCS reductase, which is novel in the field. Thus, RcrB plays a crucial role for ExPEC in maintaining bacterial redox homeostasis in RCS-rich environments.

### P-STGR-004

#### Microbial single-cell transcriptomics for environmental samples

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Transcriptomics is a powerful tool to study gene expression, enabling new insights into biological processes. While methods for single-cell transcriptomics (SCT) in eukaryotes are well established, application to prokaryotes is still challenging. Reasons are the rigid and diverse cell walls hampering lysis, the lack of polyadenylated transcripts impeding mRNA enrichment, and the minute amounts of RNA<sup>1,2</sup>. Nevertheless, several SCT approaches for bacteria have been recently published<sup>3,4</sup>. Those methods bypass physical cell separation by applying multiple rounds of cell pooling and barcoding to acquire unique barcode combinations for each single cell that later enable demultiplexing through bioinformatics. Despite being high throughput, these methods are not readily applicable to the

analysis of microbial dark matter in environmental samples, since they rely on the availability of reference genomes.

In this study, we solved this problem by sorting and lysing individual cells, and employing a multiple displacement amplification (MDA) step after reverse transcription to obtain sufficient cDNA amounts for sequencing. We first generated data of *Escherichia coli* cells that underwent heat shock or were grown at normal temperature. The median number of detected protein-coding genes per single cell was comparable to studies using protocols without MDA. Furthermore, analysis of differentially expressed genes revealed cellular heterogeneity not detected in the bulk transcriptome and showed that heat shocked and non-heat shocked cells could be distinguished based on their expression profiles.

To demonstrate the method's feasibility to environmental microbial communities, the workflow was applied to a wastewater sample. Metagenomic and -transcriptomic data, as well as eight high-quality single cell transcriptomes were generated. Several biological processes were found to be exclusively detected in the single cells but not in the corresponding metatranscriptome. Although further improvement of the method is needed, it demonstrates the ability to unravel cellular heterogeneity and metabolism that can otherwise be veiled in bulk data, representing the first study applying SCT to an environmental sample.

1. Münch *et al.* *Adv Appl Microbiol.* 2023. doi:10.1016/bs.aambs.2023.04.002
2. Magold & Kaster. *Springer Nature.* 2025. doi:10.1007/978-3-032-07527-7\_7
3. Blattman *et al.* *Nat Microbiol.* 2020. doi:10.1038/s41564-020-0729-6
4. Kuchina *et al.* *Science.* 2021. doi:10.1126/science.aba5257

## P-STGR-005

### Genomic adaptation of *Bacillus subtilis* to nature's most versatile cofactor: involvement of a TetR-like transcriptional regulator in the export of the B<sub>6</sub> vitamer pyridoxal

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Vitamin B<sub>6</sub>, consisting of the six vitamers pyridoxal (PL), pyridoxine (PN), pyridoxamine (PM) and their phosphorylated derivatives pyridoxal 5'-phosphate (PLP), pyridoxine 5'-phosphate (PNP) and pyridoxamine 5'-phosphate (PMP), is an essential micronutrient for animals and humans. PLP, the most important B<sub>6</sub> vitamer, is a key cofactor for over 160 enzymatic reactions and especially involved in the amino acid metabolism (1). Vitamin B<sub>6</sub> is synthesized by two mutually exclusive pathways, the deoxyxylulose 5-phosphate (DXP)-dependent pathway that is present in  $\alpha$ - and  $\gamma$ -proteobacteria and the evolutionary older DXP-independent pathway that is present in plants, fungi and bacteria like the Gram-positive model bacterium *Bacillus subtilis*. In contrast, animals and humans cannot synthesize Vitamin B<sub>6</sub> (2-4). Due to the accumulation of toxic metabolites, including the reactive vitamers PL and PLP, efficient production of vitamin B<sub>6</sub> has so far not been competitive in comparison to chemical synthesis (5). In this study, we investigated the adaptation of *B. subtilis* to toxic PL concentrations. The genome of two suppressor mutants with restored growth in the presence of toxic amounts of PL were

sequenced. In both suppressor mutants, the same gene, *pksA*, was affected. *pksA* encodes for the TetR-like transcriptional regulator that has been shown to not be involved in the regulation of the downstream *pks* operon (6). The *pksA* gene is divergently transcribed to the *ymcC* and *ymzD* genes, encoding small membrane proteins of unknown functions. Using *in vivo* and *in vitro* approaches, we could show that PksA indeed directly regulates the expression of *ymcC* and affects the expression of *ymzD*. Interestingly, PL seems to affect this regulation, but not to the extent expected from the effector molecule of a TetR-like repressor. Furthermore, deletion of *ymcC* results in decreased tolerance towards toxic PL concentrations and we therefore hypothesize that it encodes an efflux pump capable of decreasing intracellular PL concentrations.

- (1) Percudani R, & Peracchi A. (2009) doi: 10.1186/1471-2105-10-273.
- (2) Tanaka T, *et al.* (2005) doi: 10.1093/molbev/msi011.
- (3) Fitzpatrick TB, *et al.* (2007) doi: 10.1042/BJ20070765.
- (4) Richts B, *et al.* (2019) doi: 10.3389/fmolb.2019.00032.
- (5) Commichau FM, *et al.* (2014) doi: 10.1016/j.ymben.2014.06.007.
- (6) Vargas-Bautista C, *et al.* (2014) doi: 10.1128/JB.01022-13.

## P-STGR-007

### Molecular basis of target gene recognition by the LysR-type transcriptional regulator LsrB in *Agrobacterium tumefaciens*

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The facultative plant pathogen *Agrobacterium tumefaciens* employs rapid response systems to transition between its soil-associated and host-associated lifestyles. In this adaptation, the LysR-type transcriptional regulator (LTTR) LsrB has been identified as a key player. Deletion of *LsrB* leads to the misregulation of over 1,200 genes which manifests in pleiotropic phenotypes, such as reduced virulence as well as increased sensitivity to reactive oxygen species (1-3). Recent results from our group identified LsrB as a thiol-based redox switch (3); however, the precise mechanism of LsrB target gene regulation and recognition remains elusive. Here we present the results of our investigation into the binding sites and DNA-binding domain of LsrB.

Promoter sequences of genes that are misregulated in  $\Delta$ *LsrB* were investigated using the *in silico* tool MEME Suite. The analysis revealed putative LsrB binding sites that contain an extended T-N11-A motif with dyad symmetry, consistent with other LTTR consensus sequences. Genes containing the putative LsrB binding site in their promoter were validated as direct target genes via electrophoretic mobility shift assay (EMSA), including the sRNA AbcR1. Northern blot analysis

showed that AbcR1 expression is under positive regulation by LsrB. Subsequent investigation of the AbcR1 promoter included I) EMSAs with promoter truncations and II) site-directed mutagenesis of a promAbcR1:*egfp* reporter plasmid which revealed the precise LsrB binding site. Furthermore, structure modelling of the LsrB-DNA complex revealed an amino acid motif that may specifically bind the LsrB binding site, recognizing distinct nucleobases. Subsequent mutagenesis and phenotypic characterization of LsrB variants confirmed that individual amino acids within the putative binding motif are required for LsrB function.

Taken together, we present novel insights into LsrB-mediated target recognition and expand our understanding of LTTRs - the largest family of transcriptional regulators in bacteria.

(1) Tang, G. et al. *Mol. Plant. Microbe Interact.* **2018**, DOI: 10.1094/MPMI-02-18-0041-R.

(2) Eisfeld, J. et al. *Mol. Microbiol.* **2021**, DOI: 10.1111/mmi.14695.

(3) Schmidt, J. J. et al. *Nucleic Acids Res.* **2025**, DOI: 10.1093/nar/gkaf267.

## P-STGR-008

### Investigating the role of the CreBC system during treatment with colicin M

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**Introduction:** Antibiotics not only act on bacterial targets, but cause secondary cellular responses. Whether these responses can influence antimicrobial resistance/sensitivity independently from the inhibition of the major target is still unclear. Previous studies on the transcriptional alterations of *Escherichia coli* treated with colicin M (ColM) showed an upregulation of the CreBC core regulon genes *cbrA*, *cbrB*, *cbrC* and *creD1*. CreBC is a two-component system (TCS) known in *E. coli* as a global regulator responding to carbon switches, but not associated with antimicrobial responses so far. Colicins are bacteriocins that *E. coli* can produce to kill surrounding sensitive strains. ColM blocks the peptidoglycan biosynthesis by cleaving lipid II, which ultimately leads to cell lysis.

**Goal:** Based on the previously published data, we aim to confirm the activation of the CreBC system in ColM-treated *E. coli* and investigate the requirements for this trigger. We further aim to understand if the CreBC core regulon genes contribute to resistance/sensitivity to ColM.

**Methods:** We created a luciferase reporter plasmid controlled by the *creD* promoter, which responds to activated CreBC, and we investigated the activation of CreBC in *E. coli* BW25113 upon ColM treatment as well as the growth alterations caused by the drug using a microplate reader for 24 h. We tested both the wild type and knockout mutants of genes involved in the TCS and ColM metabolisms. Moreover, we studied the growth fitness and ColM sensitivity of mutants of the CreBC core regulon genes with survival assays in liquid medium and on plate.

**Results:** The CreBC system is activated directly after ColM addition in *E. coli* BW25113. The luciferase assays showed activation also in resistant cells and using sub-MIC drug concentrations, indicating that cellular lysis is not required for triggering the TCS. However, ColM still needed to enter the cells to activate CreBC, as the signal was not detected in transporter mutants. Mutants of the CreBC core regulon showed altered fitness to the antibiotic presence.

**Summary:** Treatment with ColM activates the CreBC system in *E. coli*, and some of the thereby regulated genes have a role in the sensitivity to the drug. This supports the idea that secondary cellular responses to antibiotics are relevant and need to be further studied.

1. Kamenšek, S., Žgur-Bertok, D. *BMC Microbiol*2013;13:42

## P-STGR-009

### New Layer of Regulation by Bacterial uORFs: Ribosome Recruitment at Upstream Sites Directly Occludes Primary Translation Sites

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Upstream open reading frames (uORFs) in the mRNA leader can regulate gene expression in both eukaryotes and bacteria. In bacteria, genome-wide detection of uORFs is still limited due to ribosome profiling challenges. Bioinformatic prediction of ribosome binding sites (RBS, composed of a Shine-Dalgarno sequence and a start codon) upstream of annotated genes, combined with experimental data, offers a promising approach to identify new uORFs in bacteria. We predicted upstream RBSs (uRBSs) for 1066 genes in the *Sinorhizobium meliloti* 2011 and studied 15 of them experimentally using reporter constructs and integrating existing RNA-seq and Ribo-seq data. At 13 of the studied uRBSs, translation of *egfp* reporter was detected. However, we show that the two remaining uRBSs, for which no translation was detected, impact the downstream gene expression, in line with their ribosome coverage in Ribo-seq. In total, we demonstrate positive and negative effects of 8 uRBSs and their associated uORFs on downstream genes, among them a T1SS operon, the RNA chaperone gene *hfq*, and metabolic genes. Our data suggest a new layer of gene regulation by inhibiting uRBSs, which display 70S ribosome footprints overlapping the mRNA region expected to be covered by a ribosome initiating translation of the main gene. We propose that main RBSs occlusion by a ribosome covering an upstream translation initiation site is a mechanism for downregulation of gene expression in bacteria.

## Reference

Scheuer, Robina et al. "Incoherent dual regulation by a SAM-II riboswitch controlling translation at a distance." *RNA biology* vol. 19,1 (2022): 980-995. doi:10.1080/15476286.2022.2110380

Hadjeras, Lydia et al. "Unraveling the small proteome of the plant symbiont *Sinorhizobium meliloti* by ribosome profiling

Kothe, Jennifer A F et al. "Early posttranscriptional response to tetracycline exposure in a gram-negative soil bacterium reveals unexpected attenuation mechanism of a DUF1127 gene." *RNA biology* vol. 22,1 (2025): 1-16. doi:10.1080/15476286.2025.2521887

## P-STGR-010

### Sensing the Environment: MoHog1p Phosphorylation Dynamics and Localization in the fungal pathogen *Magnaporthe oryzae*

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One of the big questions in microbiology is how living cells sense and respond to a multitude of environmental cues with only a limited set of signaling components. To address this question, we work on the High Osmolarity Glycerol (HOG) signaling pathway in the filamentous fungus *Magnaporthe oryzae*. The HOG pathway is composed of relatively few core proteins, yet it routes and coordinates many different stress signals from osmotic and oxidative stress to heat stress as well as fungicide action.

Here, we focus on the MAPK-cascade of the pathway, and in particular on its terminal MAP kinase, MoHog1p. Activation of the HOG MAPK module leads to MoHog1p phosphorylation at the T171-G-Y173 motif, promoting nuclear translocation of MoHog1p and reprogramming of gene expression and metabolic processes. Transcriptomic analysis reveals signal-specific transcriptional responses, even when all signals converge on MoHog1p.

To explore signal encryption, we investigate MoHog1p phosphorylation characteristics as well as MoHog1p subcellular localization. Mutants with amino acid substitutions at T171 or Y173 display distinct growth phenotypes under osmotic and fungicide stress, indicating that phosphorylation at single specific residues of the motif affects pathway output. Preliminary nanoUPLC-MS results further reveal stimulus-dependent spatial and temporal differences in MoHog1p phosphorylation intensity, supporting the idea that dynamic phosphorylation patterns may encode signal identity.

In addition, we follow MoHog1p nucleocytoplasmic trafficking to find out how it contributes to signal specificity. The importin  $\beta$  family protein MoNmd5p emerged as a promising candidate since  $\Delta$ MoNmd5 mutants show reduced growth under KCl stress. These findings are underpinned by AI-driven Molecular Dynamics simulations which show promising MoNmd5p–MoHog1p interactions. Fluorescence microscopy of MoHog1p-GFP and MoNmd5p-mCherry expressing strains is running to further clarify the mechanisms of MoHog1p translocation under stress conditions.

Our findings suggest that *M. oryzae* can use a single MAPK pathway to coordinate distinct stress responses by fine-tuning the regulation of MoHog1p - potentially encoding information in both its dynamic phosphorylation pattern and its subcellular localization.

## P-STGR-011

### Two regulatory feedback loops drive heterogeneous expression of the flagellar master regulator *flhDC* in *Salmonella enterica*

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Flagella-mediated motility plays a crucial role for *Salmonella* pathogenicity by enabling directed movement. The flagellar master regulator FlhD<sub>4</sub>C<sub>2</sub> resides on top of the flagellar synthesis cascade. Expression of *flhDC* is mainly driven from two promoters (P1<sub>flhDC</sub> and P5<sub>flhDC</sub>) and is under extensive control by various positive and negative regulators involving feedback loops. In particular, we previously showed that RfI<sub>flm</sub> and RcsB form a heterodimeric complex to repress *flhDC* transcription from P1<sub>flhDC</sub> [1]. Further, the flagellar protein FlhZ increases activity of the main virulence activator HilD, which in turn directly activates *flhDC* transcription from P5<sub>flhDC</sub> [2].

Here, we found that expression of the flagellar master operon is heterogeneous and we elucidated the underlying regulatory mechanisms.

Using single-cell time-lapse microscopy on agarose pads and in mother machines as well as flow cytometry we found that *flhDC* expression is heterogeneous resulting in *flhDC*-positive and *flhDC*-negative subpopulations. Heterogeneous *flhDC* expression was driven by two distinct regulatory feedback loops. First, the negative auto-regulatory FlhD<sub>4</sub>C<sub>2</sub>-RfI<sub>flm</sub> feedback loop involving the global Rcs system was responsible for heterogeneous expression of *flhDC* from the P1<sub>flhDC</sub> promoter during early growth phases. Furthermore, we demonstrated that RfI<sub>flm</sub>-dependent repression of *flhDC* exhibits hysteresis, suggesting that the system is bistable. Second, we showed that the double-positive FlhD<sub>4</sub>C<sub>2</sub>-FlhZ-HilD feedback loop was responsible for driving heterogeneous expression of *flhDC* from the P5<sub>flhDC</sub> promoter at later growth phases.

In summary, we propose that heterogeneous expression of *flhDC* might allow *Salmonella* to fine-tune initiation of flagellar synthesis and to rapidly adapt to changing environments during infection by maintaining stable flagellated and non-flagellated subpopulations.

[1] Kühne C et al. (2016) *Mol Microbiol* 101:841-855

[2] Singer HM et al. (2014) *J Bacteriol* 196:1448-1457

## P-STGR-012

### Genome-wide identification of Fis binding sites in *Yersinia*

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Regulation of bacterial gene expression at the DNA level is often mediated by nucleoid-associated proteins (NAPs), which act as nucleoid-structuring factors. These proteins alter DNA topology locally and globally, thereby regulating gene expression and shaping overall chromosomal architecture. Among the primary NAPs, the factor for inversion stimulation (Fis) is one of the most abundant proteins in Gram-negative bacteria. Fis regulates gene expression by binding to and bending DNA, thus orchestrating large-scale structural and transcriptional changes. In this study, we aimed to determine how temperature fluctuations influence Fis-mediated gene regulation in *Yersinia pseudotuberculosis*. This human pathogen is an established model organism due to its extensive repertoire of regulatory mechanisms that enable rapid adaptation to temperature changes, when it enters and exits the host.

We present the first *in vivo* genome-wide analysis of Fis binding profiles at ambient (25°C) and host-specific (37°C) temperatures using chromatin affinity purification coupled with sequencing (ChAP-seq). This analysis revealed more than 700 binding sites across the chromosome and the virulence plasmid. By integrating transcriptomic data (RNA-seq), we uncovered a broad spectrum of direct and indirect Fis regulons, demonstrating the crucial role of Fis in diverse metabolic pathways, hemin uptake, and anaerobic respiration. Moreover, at lower temperatures, Fis extensively represses virulence gene expression through direct binding to type-III-secretion system-related loci on the virulence plasmid. Direct binding of selected targets was further confirmed *in vitro* by electrophoretic mobility shift assays. Collectively, these findings underscore the central role of Fis in mediating transcriptional reprogramming during the transition between free-living and virulent states in *Y. pseudotuberculosis*.

#### P-STGR-013

##### Regulatory Crosstalk Between Daptomycin and Bacitracin Responsive Pathways Coordinates the Cell Envelope Stress Response of *Enterococcus faecalis*

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*Enterococcus faecalis* exhibits intrinsic resistance to several cell envelope-active antibiotics, including daptomycin (DAP) and bacitracin (BAC). Understanding how this organism senses and responds to cell envelope damage is key to combating multidrug resistance. Recent work demonstrated that the LiaFSR system, previously known as the main regulator of daptomycin resistance, also controls the expression of the bacitracin-responsive system SapRS. In turn, SapRS activates resistance determinants including rapAB (against bacitracin) and dltXABCD (against daptomycin), creating a regulatory cascade that ensures an efficient and coordinated adaptive response to cell envelope stress. Interestingly, we observed that even in a LiaR/SapR double deletion strain, the dltXABCD operon still maintained a partial response to daptomycin challenge. This points towards additional regulatory systems contributing to envelope integrity and antibiotic resistance. To identify these additional regulators and resistance determinants operating within this network, we combine transcriptional reporter assays with targeted deletions of annotated regulatory systems in the *E. faecalis* genome. To further expand on these findings, we employ whole-genome transcriptomic analysis to refine the existing model and to identify potential

components of the regulatory network linking LiaFSR–SapRS signaling to other stress response pathways. Together, these findings highlight the complexity and connectivity of the *E. faecalis* cell envelope stress response and lay the foundation for identifying novel regulatory elements that could serve as future targets to restore antibiotic susceptibility.

#### P-STGR-014

##### The role of the putative diadenosine tetraphosphate hydrolase YqeK in biofilm formation and virulence regulation in *Staphylococcus aureus*

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**Introduction:** Diadenosine tetraphosphate (Ap4A) is a nucleotide second messenger, proposed to act as an alarmone in bacteria. The intracellular concentration of AP4A has been shown to increase in response to stress and starvation, resulting in altered sensitivity to antibiotics and decreased biofilm formation, thereby linking Ap4A metabolism to the bacterial capacity to cope with stress and the regulation of virulence traits [1,2]. However, the physiological roles of Ap4A and the putative Ap4A hydrolase YqeK are unknown in *Staphylococcus aureus*. **Goals:** Therefore, we aimed to investigate the function of Ap4A in *S. aureus* by characterizing the phenotypes of the *S. aureus* *yqeK* mutant under stress and virulence conditions. **Materials & Methods:** We constructed the *S. aureus* *yqeK* deletion mutant and the *yqeK*+ complemented strain to study the phenotypes under stress and antibiotics, biofilm formation and staphyloxanthin levels. Transcriptional analyses were used to analyze changes in gene expression in the *yqeK* mutant. **Results:** While the growth of the *yqeK* mutant was delayed in LB and RPMI medium, we did not observe any phenotypes of the *yqeK* mutant in response to oxidative stress and antibiotics treatments. However, the *yqeK* mutant showed increased biofilm formation using crystal violet staining. Furthermore, macrocolonies of the *yqeK* mutant exhibited reduced pigmentation, consistent with a significant decrease in staphyloxanthin levels. Northern blot analyses further revealed reduced expression of the SigmaB and AgrA regulons in the *yqeK* mutant. **Summary:** Phenotype analyses revealed increased biofilm formation and reduced expression of SigmaB and AgrA regulons in the *yqeK* mutant, suggesting a link between AP4A levels and virulence regulation in *S. aureus*. In future studies, we aim to study the molecular mechanisms of how YqeK and AP4A regulate biofilm formation and virulence in *S. aureus*.

#### References:

- [1] Cervoni M, Sposato D, Ferri G, Bähre H, Leoni L, Rampioni G, Visca P, Recchiuti A, Imperi F. (2024) The diadenosine tetraphosphate hydrolase ApaH contributes to *Pseudomonas aeruginosa* pathogenicity. PLoS Pathog. 20: e1012486.
- [2] Young MK, Wang JD. (2024) From dusty shelves toward the spotlight: growing evidence for Ap4A as an alarmone in maintaining RNA stability and proteostasis. Curr Opin Microbiol. 81:102536.

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## Systems and synthetic microbiology

### P-SSM-001

#### Functional reconstruction of the iron reduction pathway of *Acidithiobacillus ferrooxidans* in *Escherichia coli*

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Exoelectrogens harness defined extracellular electron transfer (EET) pathways to shuttle electrons beyond the cell membrane, which can be applied in microbial fuel cells, electrosynthesis, and biosensing. Engineering well-characterized microbial hosts with functional exoelectrogenic capabilities enhances our understanding of EET and accelerates biotechnological innovation. *Acidithiobacillus ferrooxidans*, an extreme acidophile, reduces Fe(III) extracellularly under anaerobic conditions, yet the components and pathways are not experimentally validated. To test the proposed iron reduction cascade – comprising the c-type cytochromes CycA2, Cyc1A, Cyc2A, and the copper redox protein rusticyanin (Rus) – we expressed these proteins in four heterologous hosts: *Escherichia coli* C43(DE3), BL21(DE3), T7 Express, and *Vibrio natriegens* Vmax X2. Both Vmax X2 and C43(DE3) successfully produced all four redox proteins in a redox-active state and in the correct cell compartment. T7 Express failed to produce holo-CycA2, while BL21(DE3) yielded only low levels of holo-Cyc2A per OD<sub>600</sub>. Introduction of the *At. ferrooxidans* iron reduction pathway into the heterologous hosts enhanced Fe(III)-citrate reduction 2-fold in C43(DE3) and 1.4-fold in BL21(DE3) compared to wildtype controls. The inner membrane cytochrome CycA2 was essential for efficient iron reduction in the modified *E. coli* strains. Although Vmax X2 expressed the full pathway, it did not reduce Fe(III)-citrate, suggesting host-specific limitations in electron channelling or redox coupling. In contrast, the recombinant pathway in C43(DE3) was redox-active under turnover conditions and enabled a 5-fold increase in current generation in an electrochemical H-cell using a carbon felt electrode as terminal electron acceptor. This study provides the first experimental validation of the *At. ferrooxidans* iron reduction pathway, demonstrating functional reconstitution in *E. coli* and highlighting C43(DE3) as a robust chassis for engineering EET pathways from extremophiles.

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### P-SSM-002

#### A cyclic multiplex co-transformation approach to customize *V. natriegens* for protein production

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*V. natriegens*’ fast growth, high carbon uptake rates and versatile metabolism render this bacterium a promising host for industrial applications [1]. Additionally, natural transformation, a native mechanism for linear transforming DNA (tDNA) uptake yielding homologous recombination events, make this chassis a formidable host for genome and metabolic engineering [2, 3]. Several studies utilized this host for protein production [2], however, some with moderate success which could be attributed to an incomplete understanding of the overall protein turnover in *V. natriegens*.

Addressing this, we mapped over 100 putative proteases and peptidases on the two chromosomes. An optimized time efficient tDNA design and transformation protocol was developed, using unpurified fusion PCR products. With this approach we successfully deleted 106 targets separately in an mScarlet-I expressing strain with an average transformation efficiency of >20 % of the total cells, without the need of a selection marker. These strains served as platform to test the impact of each individual knockout regarding fluorescence and growth rate. To find the best combination of target proteases, we amplified a tDNA library from the knock-out strains. In a cyclic approach of co-transforming the tDNA library alongside replaceable selection markers we enriched combinations of protease deletions with increasing cycles. Fluorescence markers are used to validate a positive impact on heterologous protein expression. Promising candidates are selected by single cell screening and sorting for later validation. Applying this approach under different growth conditions, we aim to identify relevant proteases combinations to customize strains for target protein production and gaining a better understanding of the degradation machinery in *V. natriegens*.

### References

1. Hoffart E, Grenz S, Lange J, Nitschel R, Müller F, Schwentner A, et al. High substrate uptake rates empower *Vibrio natriegens* as production host for industrial biotechnology. Appl Environ Microbiol. 2017;83:e01614-17. doi:10.1128/AEM.01614-17.
2. Hädrich M, Schulze C, Hoff J, Blombach B. *Vibrio natriegens*: Application of a fast-growing halophilic bacterium. Adv. Biochem. Eng. Biotechnol. 2024. doi:10.1007/10\_2024\_271.
3. Dalia TN, Hayes CA, Stolyar S, Marx CJ, McKinlay JB, Dalia AB. Multiplex Genome Editing by Natural Transformation (MuGENT) for synthetic biology in *Vibrio natriegens*. ACS Synth. Biol. 2017;6:1650–5. doi:10.1021/acssynbio.7b00116.

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### P-SSM-003

#### Scalable *In Vivo* DNA Assembly for Synthetic Genomic

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Advances in DNA sequencing technologies have revolutionized our ability to read and interpret genetic information, generating vast datasets which lead to a wealth of hypotheses about genome function and evolution. However, it becomes increasingly clear that testing these hypotheses requires the capacity to *write* DNA at scale — assembling large, complex, and context-rich synthetic constructs that go far beyond the limits of conventional cloning and gene editing approaches. While synthetic DNA has become increasingly accessible as a commodity, scalable strategies for assembling long DNA molecules remain a critical bottleneck in the still nascent field of synthetic genomics.

Here, we present an iterative *in vivo* DNA assembly platform in *Saccharomyces cerevisiae* that is rapid, robust, and highly scalable. Our approach is **sequence-agnostic** and enables **hierarchical** and **combinatorial** construction of long DNA constructs, allowing the assembly of large genomic regions with high efficiency. We demonstrate the versatility of this system through the assembly of model reporter gene arrays and complete bacteriophage genomes. Furthermore, we extend its



application to the construction of **synthetic genome landscapes** derived from diverse organisms, paving the way for systematic functional characterization of genomic elements and large-scale exploration of genome design principles.

This technology represents a significant step toward scalable genome writing, unlocking new possibilities for synthetic biology, functional genomics, and the creation of engineered biological systems.

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#### **P-SSM-004**

##### **Synthetic Small RNA Arrays as a Modular Toolkit for Controlling Multiple Phenotypes**

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Synthetic biology is an emerging discipline focused on the rational design of novel biological systems with programmable functions. Within this field, synthetic small RNAs (sysRNAs) have gained attention as versatile tools for post-transcriptional regulation. These regulatory molecules typically consist of two modules: a seed region mediating target recognition and base pairing, and a scaffold region contributing to stability and interaction with RNA chaperones such as Hfq. By rationally combining these elements, synthetic sRNAs can be engineered to achieve predictable regulation of virtually any gene. In this study, we aimed to construct synthetic sRNAs and evaluate their regulatory performance using phenotypic screening in *Escherichia coli*. Our goal was to develop a sysRNA array capable of modulating multiple phenotypes simultaneously. Synthetic sRNA expression plasmids were assembled via Golden Gate and the Modular Cloning (MoClo) system, enabling rapid and reliable construction of transcriptional units. Functional assays revealed that regulatory activity depends strongly on the specific combination of seed and scaffold regions. While the seed sequence determines target specificity and repression efficiency, the scaffold selection markedly influences regulatory outcomes, highlighting the importance of module compatibility. From initial screenings, we identified functional sysRNAs suitable for multiplexed regulation. A sysRNA array was constructed to simultaneously repress antibiotic resistance through *acrA* (encoding an efflux pump component) and flagella-mediated motility via *fliC* (encoding flagellin). Additionally, we developed an array targeting *ampC*, which encodes  $\beta$ -lactamase. Interestingly, repression of *ampC* could not be effectively achieved with a single sysRNA. However, combining up to three synthetic sRNAs enhanced *ampC* downregulation substantially. Based on these results, we propose that our multiplexed sRNA-based regulatory framework provides a powerful and versatile tool for synthetic biology applications requiring precise, simultaneous modulation of multiple genetic targets.

## P-SSM-005

### Synthetic Biology approaches to study passive regulation as a consequence of transcription in budding yeast

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DNA topology plays an important role in modulating transcription and chromosome organization. According to the twin-supercoiled-domain model, positive supercoils are accumulated downstream and negative supercoils upstream of a transcribed gene [1]. DNA topoisomerases help in resolving this supercoiling by transiently breaking, releasing the tension and rejoining the DNA [2]. Failure in resolving tension or in rejoining the DNA would have deleterious effects on cellular life. Nevertheless DNA torsion can persist and travel through the surrounding chromatin [3][4]. The consequences are torsional stress of the DNA molecule which can affect transcription of nearby genes. While the active mechanisms regulating transcription are well characterized, the passive modulation of transcription by DNA topology remains underexplored. This project aims to elucidate the role of transcription-induced DNA supercoiling as a passive regulator of gene expression in *Saccharomyces cerevisiae*. In this interdisciplinary project funded by the NCN and DFG, we hypothesise that passive regulation by DNA torsion influences gene expression and may have driven the evolution of gene synteny and genome architecture in modern genomes. By integrating quantitative fluorescence-based assays with synthetic biology and controlled transcriptional perturbations, we will examine how changes in DNA topology influence local transcriptional activity. The results will provide new insights into how DNA topology contributes to the spatial coordination of gene expression within the eukaryotic genome.

## References

[1] Liu, L. F., & Wang, J. C. (1987). Supercoiling of the DNA template during transcription. *Proceedings of the National Academy of Sciences*, 84(20), 7024–7027. <https://doi.org/10.1073/pnas.84.20.7024>

[2] Roca, J. (2011). The torsional state of DNA within the chromosome. *Chromosoma*, 120(4), 323–334. <https://doi.org/10.1007/s00412-011-0324-y>

[3] Kouzine, F., Sanford, S., Elisha-Feil, Z., & Levens, D. (2008). The functional response of upstream DNA to dynamic supercoiling in vivo. *Nature Structural & Molecular Biology*, 15(2), 146–154. <https://doi.org/10.1038/nsmb.1372>

[4] Kouzine, F., Gupta, A., Baranello, L., Wojtowicz, D., Ben-Aissa, K., Liu, J., Przytycka, T. M., & Levens, D. (2013). Transcription-dependent dynamic supercoiling is a short-range genomic force. *Nature Structural & Molecular Biology*, 20(3), 396–403. <https://doi.org/10.1038/nsmb.2517>

## P-SSM-006

### Establishing Novel Molecular Tools for Rumen Microbes

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Extreme weather events caused by the ongoing climate crisis emphasize the urgent need for innovative solutions to reduce and capture environmentally harmful carbon emissions, and mitigate future impacts. Methane, one of the most potent greenhouse gases, is produced in large quantities by rumen microbes in domestic livestock. Even a moderate reduction of these methane emissions could positively impact our climate. Achieving this goal requires the development of genetic tools to manipulate key species to deepen our understanding of the rumen microbial community.

As part of a consortium in the Global Methane Hub, we investigate the genetic accessibility of key microbial players in the rumen: methane-producing archaea (methanogens) and CO<sub>2</sub>-consuming bacteria (acetogens). Our goal is to establish robust and harmonized genetic engineering tools to explore potential mechanisms for mitigating methane emissions. Initially, we focused our experiments on cultivating key methanogens and identifying antibiotic resistance and counter-selection markers.

We cultivated all methanogens in liquid and solid media and determined the minimum inhibitory concentrations of various antibiotics. Furthermore, we identified suitable counter-selection targets for genomic deletions using suicide plasmids. Based on these results, we constructed a modular suicide-plasmid library containing antibiotic resistance genes controlled by promoters of varying strength and homology arms targeting respective genetic regions.

Developing a transformation protocol for rumen methanogens lays the foundation for future genetic engineering of rumen microbes. Ultimately, this work is an essential step toward understanding and mitigating methane emissions from livestock.

## P-SSM-007

### Development of *Cutibacterium acnes* as a Chassis for Skin-Delivered Biologics

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*Cutibacterium acnes* is a key commensal in the human skin microbiome, exhibiting several beneficial traits. It thrives in microaerophilic, lipophilic, and acidic environments deep within the pilosebaceous unit of the skin, making it well-suited to its specific niche and an attractive chassis to create skin-delivered therapeutics<sup>1,2,3,4</sup>. We show the efficient engineering of this bacteria and its potential as a living drug by producing and secreting therapeutic molecules *in vivo*. Topical application of the engineered strain led to durable engraftment on mouse skin without triggering inflammation. Finally, we present biocontainment strategies based on auxotrophic dependencies, supporting the safe deployment of *C. acnes* as a therapeutic platform<sup>5</sup>.

<sup>1</sup>Grice, E. A. et al. Topographical and temporal diversity of the human skin microbiome. *Science* 324, 1190 (2009).

<sup>2</sup>Conwill, A. et al. Anatomy promotes neutral coexistence of strains in the human skin microbiome. *Cell Host Microbe* 30, 171–182 (2022).

<sup>3</sup>Zouboulis, C. C. Acne and sebaceous gland function. *Clin. Dermatol.* 22, 360–366 (2004).

<sup>4</sup>Gribbon, E. M., Cunliffe, W. J. & Holland, K. T. Interaction of *Propionibacterium acnes* with skin lipids in vitro. *J. Gen. Microbiol.* 139, 1745–1751 (1993).

<sup>5</sup>Knödlseider, N., Fábrega, M.J., Santos-Moreno, J. et al. Delivery of a sebum modulator by an engineered skin microbe in mice. *Nat Biotechnol* 42, 1661–1666 (2024).

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### P-SSM-008

#### Synthetic ATP auxotrophy enables control over cellular carbon and electron flow

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Replacing linear petrochemical processes with circular bioprocesses requires efficient microbial bioproduction strains. Current industrial bioprocesses exploit anaerobic fermentation to achieve electron and carbon conservation. Naturally, fermentative processes limit substrates to sugars and products to stem from glycolytic end points. To enable control over cellular carbon and electron flow in a substrate-agnostic manner, we engineered MaxKat as an *E. coli* platform strain that enables novel types of growth-coupled bioproduction. MaxKat can neither feed electrons into the respiratory electron transport chain nor use fermentative pathways and is thus ATP-auxotrophic. Reactions restoring either oxidative or substrate-level phosphorylation reinstate growth allowing for selective oxidative or reductive bioconversions. We experimentally validated this precise control over intracellular carbon and electron flow by switching between different ATP generation modes relying either on enzymatic NADH oxidation or quinone reduction. To showcase the potential for bioproduction, we demonstrated the growth-coupled oxidation of 10 different sugars into their respective sugar acids. Notably, we demonstrated D-xylonate production from D-xylose with ~100% of the theoretical yield. We foresee applications of MaxKat for efficient bioconversions from various feedstocks. Equally, the concept of ATP auxotrophy can serve as a tool for the screening and evolution of enzymes to drive biotechnological innovations.

### P-SSM-009

#### Gas fermentation with *Cupriavidus necator* for lactate production

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#### Introduction:

Lactate is a valuable C3 compound widely used in food, pharmaceutical, and biodegradable plastic industries, yet conventional lactic acid production involves high feedstock costs and relies on agricultural resources, raising both economic and sustainability concerns<sup>1</sup>. As a non-food and carbon-neutral alternative, carbon dioxide has attracted attention as a renewable feedstock for biomanufacturing. The chemolithoautotrophic *Cupriavidus necator* presents a sustainable platform for lactic acid biosynthesis through gas fermentation using CO<sub>2</sub> as the sole carbon source.

#### Goals:

This study aims to engineer *C. necator* for efficient production of enantiopure L-lactate under autotrophic gas fermentation conditions.

#### Materials & Methods:

Nine L-lactate dehydrogenases (L-LDHs) from different lactic bacteria were introduced into *C. necator* for heterologous expression and evaluated for L-lactate production. This was assessed in fed-batch gas fermentation supplying CO<sub>2</sub>, H<sub>2</sub>, and O<sub>2</sub>, and under varying nitrogen concentrations. Lactate and pyruvate production titers were quantified by high-performance liquid chromatography equipped with a refractive index detector.

#### Results:

All nine L-LDHs were heterologously expressed in *C. necator*, but only 4 of the 9 LDHs, which are from *Lactococcus lactis* subsp. *cremoris* MG1363, *Lactiplantibacillus plantarum*, *Lactobacillus helveticus*, and *Bos taurus*, can produce a remarkable amount of L-lactate from CO<sub>2</sub> under autotrophic gas fermentation, with the top variant reaching a titer of 959.9 mg/L. Nitrogen limitation led to a remarkably increased pyruvate accumulation in culture medium, indicating a bottleneck in downstream carbon flux from pyruvate.

#### Summary:

This study demonstrates L-lactate production from CO<sub>2</sub> using *C. necator* expressing heterologous and provides a basis for optimization of metabolic pathways and fermentation conditions toward high-yield lactate and lactate-derived biopolymers. Future work will assess the synthesis of the biodegradable copolymer poly(3HB-co-L-lactate) through targeted enzyme and pathway engineering.

**Reference:** 1. Abdel-Rahman, M. A., Tashiro, Y., & Sonomoto, K. Recent advances in lactic acid production by microbial fermentation processes. *Biotechnol. Adv.* 31, 877–902 (2013).

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## P-SSM-010

### Stress-induced ribosome degradation in *B. subtilis* is mediated by the Y-complex

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**Introduction:** Bacteria have a remarkable capacity to survive stressful conditions. This adaptation is achieved by inducing stress response programs that modify the transcriptomic, proteomic, and metabolic landscape. This response is facilitated by the immediate detection and response to environmental changes. For example, protein folding stress induce the synthesis of chaperones and proteases to handle the increase in misfolded aggregated proteins. However, another key aspect of this response is the concurrent downregulation of protein biosynthesis. In *Bacillus subtilis*, this can be achieved through the coordinated action of the alarmones (p)ppGpp and the stress-responsive transcription factor Spx, which are part of a broader network of stress regulators.

**Goals:** In this project, we aimed to discover new pathways involved in stress adaptation.

**Materials and Methods:** We combined high-throughput genomic screening, transcriptomics, and proteomics to identify and characterize the role of the Y-complex in RNA processing and decay during stress.

**Results:** We identified the Y-complex, which confers specificity to the endonuclease RNase Y, as a critical player during stress conditions, such as heat or transition into the stationary phase. This protein complex is required for processing diverse RNAs, notably the maturation of mRNAs encoding proteins involved in translation and metabolism. We further demonstrate that the Y-complex and RNase Y initiate the degradation of mature ribosomes, lowering their abundance upon stress. The lack of the Y-complex in a strain unable to synthesize (p)ppGpp leads to a strong growth defect, accumulation of ribosomes, and even an increased amount of protein aggregation during proteotoxic stress, suggesting that both systems are key for downregulating the cell's protein synthesis capacity.

**Conclusion:** The Y-complex-RNase Y system serves as a central regulatory hub, orchestrating both gene expression modulation and ribosome decay, thereby cooperating with (p)ppGpp and Spx to finely tune the cell's translation capacity for optimal stress adaptation.

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## P-SSM-012

### Introduction of a stable CRISPRi *crtB* knockdown and *crtN* / *sqs* overexpression strain in *Synechocystis* sp PCC 6803 for increased Squalene production

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The production of terpenes has gained more interest, based on diverse fields of application. Phototrophic organisms naturally direct metabolic flux towards C40 terpenoids, which

functions as essential photosynthetic pigments. While C30 terpenoids can be utilized in industrial, pharmaceutical and cosmetic products. *Synechocystis* sp. PCC 6803 is one of the best characterized model organisms for cyanobacterial biosynthesis, making it a perfect candidate for metabolic engineering to target terpenes. Previous studies have successfully enhanced the production of C30 squalene (Dietsch et al., 2021), providing a foundation to build on.

This study aims to generate a stable C30 squalene production strain that is balanced in its altered metabolism towards squalene production. The CRISPRi system is applied to silence *crtB*, an essential gene for C40 carotenoid biosynthesis. In addition, *crtN*, which is known to convert squalene into C30 carotenoids in other organisms, is utilized as a squalene reporter and as an essential gene for the survival of *Synechocystis* (Santana-Molina et al., 2022). Evolutionary pressure through light is applied to select for a higher metabolic flux through FPP. Ideally, the metabolism of *Synechocystis* will balance on the non-native CrtN pathway to produce essential carotenoids. Cultivation experiments will be performed to test the fitness of the strains and apply different light conditions to cause stress. The adaptive progress will be monitored by the growth rate, fitness, gene expression and formation of pigments. In the long term, this approach may provide a sustainable and cost-effective alternative to current industrial squalene production methods, since traditional squalene production relies on extraction from shark liver oil, which leads to serious ecological consequences.

## References:

Dietsch, M., Behle, A., Westhoff, P., & Axmann, I. M. (2021). Metabolic engineering of *Synechocystis* sp. PCC 6803 for the photoproduction of the sesquiterpene valencene. *Metabolic Engineering Communications*, 13, e00178. <https://doi.org/10.1016/J.MEC.2021.E00178>

Santana-Molina, C., Henriques, V., Hornero-Méndez, D., Devos, D. P., & Rivas-Marin, E. (2022). The squalene route to C30 carotenoid biosynthesis and the origins of carotenoid biosynthetic pathways. *Proceedings of the National Academy of Sciences of the United States of America*, 119(52). <https://doi.org/10.1073/PNAS.2210081119/-/DCSUPPLEMENTAL>

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## P-SSM-013

### A Standardized Golden Gate-Based Cloning and Integration System for *Corynebacterium glutamicum*

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To simplify and standardize genetic engineering in *Corynebacterium glutamicum*, we have expanded and refined a modular cloning toolbox, providing a blueprint for establishing cross-organism applications. Building on the Marburg Collection (Stukenberg et al., 2021) designed for *Vibrio natregens*, we have created a set of guidelines for integrating host-specific vectors into the Golden Gate-based cloning system for *C. glutamicum*. The expansion of the *C. glutamicum* toolbox enables the flexible construction of transcription units and gene clusters within both episomal and integrative (pK19mobsacB) backbones. In particular, the pK19mobsacB system has been equipped with connection

modules which allows targeted genomic integration via homologous recombination at previously described landing pads in *C. glutamicum* (Lange et al. 2018). These landing pads were systematically characterized under various conditions. To support hierarchical genetic assembly, the system enables the assembly of level 1 transcription units consisting of promoters, ribosome binding sites (RBS), and terminators of varying strength. These regulatory elements were characterized in different media using a high-throughput cultivation platform, enabling context-specific tuning of gene expression. Level 2 assembly enables the combination of transcription units into functional gene clusters, while Level 3 supports the integration of multiple clusters into complex metabolic pathways. To demonstrate the versatility of the toolbox, we created a library of transcription units encoding a CoA-dependent reduction pathway from succinate to 1,4-butanediol. These were assembled into Level 2 gene clusters for the biosynthesis of the target molecule, integrating genes encoding enzyme variants with different cofactor requirements.

Overall, this expanded and standardized toolbox not only simplifies genetic engineering in *C. glutamicum* but also initiates a framework for organism-spanning cloning strategies, enhancing reproducibility and accelerating pathway design and optimization in industrial biotechnology.

Stukenberg, Daniel; Hensel, Tobias; Hoff, Josef; Daniel, Benjamin; Inckemann, René; Tedeschi, Jamie N. et al. (2021): DOI: 10.1021/acssynbio.1c00126.

Lange, Julian; Müller, Felix; Takors, Ralf; Blombach, Bastian (2018): DOI: 10.1111/1751-7915.12879.

#### P-SSM-014

##### Engineered Bacterial Coacervates as Engineered Living Materials for biotechnological applications

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Engineered Living Materials (ELMs) represent an emerging innovative platform for the design of novel adaptive materials, using living cells to impart biologically active functionalities to synthetic materials. Here, we present Engineered Bacterial Coacervates (EBCs) as a novel hybrid living material. Polymer-based coacervates function as membrane-less cages to spatially confine engineered bacteria, thereby providing 'on demand' properties that can be modulated by genetic engineering. Coacervates might function as protective compartments of encapsulated bacteria against toxic compounds, which could be useful for biotechnological applications, e.g. for biocatalytic processes in the presence of heavy metals. Protection can be assessed by monitoring bioluminescence over time because this process is a certified ecotoxicological indicator for metabolic activity and viability.

In this study, we report the encapsulation of two biotechnologically relevant bacteria, *Escherichia coli* and *Pseudomonas putida* both carrying genes for fluorescence proteins in their chromosomes and plasmid-based bioluminescence genes. Fluorescence microscopy showed that cells of *E. coli* were encapsulated inside the coacervates, while *P. putida* cells adhered to their external

surface. The encapsulated *E. coli* cells maintained both bioluminescence and fluorescent protein expression for several hours. In co- cultures of *E. coli* and *P. putida*, the cells maintained their distinct spatial location.

Upon exposure to toxic CuSO<sub>4</sub> concentrations, the encapsulated *E. coli* continued to exhibit bioluminescence in comparison to non-encapsulated cells. In contrast, the *P. putida* cells located on the surface of EBCs showed a decline in bioluminescence upon CuSO<sub>4</sub> addition. This result indicates, that coacervate encapsulation supports protection against copper ions. Similar assays using alternative chemical stressors are currently being tested to further validate the general applicability of the EBCs under different environmental stimuli. Moreover, spatial separation of bacterial species due to coacervates offers a new opportunity to utilize the properties of EBCs. Current efforts are focused to develop a potential two-species bacterial consortium, consisting of *E. coli* and *P. putida*, to study metabolic interactions through the coacervates.

#### P-SSM-015

##### Development of an expression platform for nonribosomal peptides in symbiotic cyanobacteria of the genus *Nostoc*

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Cyanobacteria are a diverse group of oxygenic phototrophic bacteria that are prolific producers of secondary metabolites. They hold great promise as sustainable producers of a wide range of chemical compounds [1]. Currently, mainly unicellular cyanobacteria of the genera *Synechococcus* and *Synechocystis* are established as chassis for the production of fine chemicals and commodities. However, these model organisms are not perfectly suited as hosts for the production of complex NRPS-type natural products. In contrast, filamentous cyanobacteria of the genus *Nostoc* have the potential for high-titer production of NRPs as they naturally encode a high number of NRPS BGCs. The filamentous cyanobacterium *Anabaena* sp. PCC 7120 has emerged as the most effective heterologous host for cyanobacterial natural products [2]. In our recent work, we were able to heterologously express the NRPS-derived natural product pyreudione A from *Pseudomonas fluorescens* in high titers in *Nostoc* sp. KVJ2. In this study, we aim to establish a new filamentous cyanobacterium as a heterologous host for the production of nonribosomal peptides (NRPs). Different strategies will be compared for peptide expression, and both inducible and constitutive promoters, as well as plasmid-based and genomic integration approaches, will be evaluated. Additionally, spatial imaging will be employed to visualize metabolic phenotypes between wild-type and mutant strains. Our goal is to expand and optimize our expression strategy for the production of bioactive NRPS-type peptides from different heterologous hosts and to broaden the heterologous expression toolkit in *Nostoc* strains.

#### References

[1] Koot, C. J. et al. J. Biol. Chem. 2018, 293, 5044-5052. <https://doi.org/10.1074/jbc.R117.815886>

#### P-SSM-016

##### Vnat sysRNAs: Synthetic post-transcriptional regulation in *V. natriegens*

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Recent advancements in molecular biology techniques and RNA deep sequencing methods have expanded our understanding of regulatory RNAs in prokaryotic cells. Trans-encoded small RNAs (sRNAs) play a crucial role in modulating gene expression at the transcriptional level by targeting messenger RNAs (mRNAs). They typically exhibit limited and imperfect base pairing with multiple targets. This feature enables sRNAs to fine-tune diverse cellular pathways, including stress responses, metabolism, and virulence. Researchers have extensively studied trans-encoded sRNAs and their potential as synthetic gene regulators in model organisms such as *E. coli*, *Salmonella*, and *Vibrio cholera*. Over the past decade, *Vibrio natriegens*, a Gram-negative marine bacterium with a rapid reported doubling time of less than 10 minutes, has received significant attention. Recent developments in establishing reliable genetic engineering tools and methods for *V. natriegens* have made it possible to utilize the full potential of this microorganism as a new chassis organism in biotechnological and industrial applications. In this study, we aim to investigate the regulatory network of *V. natriegens* promoted by endogenous trans-encoded sRNAs under applied stress conditions (e.g., salt and temperature) and to develop strategies to characterize constructed synthetic sRNAs.

#### P-SSM-017

##### Growth-coupled bioproduction of anthranilate in a pyruvate auxotrophic *Escherichia coli* strain

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Anthranilate is a highly valuable industrial compound, because it is a precursor in the synthesis of non-opioid analgesics such as Ibuprofen and Paracetamol, as well as aroma compounds. The industrial synthesis of anthranilate involves the use of phthalimide, which is extracted from either crude oil or coal tar in several steps.

The aim of this project is to produce anthranilate via growth-coupling in a pyruvate auxotrophic *E. coli* strain. Since the last enzymatic step in the synthesis of anthranilate produces pyruvate as a byproduct it can release the auxotrophy. This strain can then be used for the synthesis of anthranilate, to establish a sustainable production method. Growth-coupling is a method, where the bacteria are genetically modified in a way that it is essential for them to use a provided pathway or enzyme to produce the desired component as a byproduct of active metabolism. The pyruvate auxotrophic strain is engineered to produce anthranilate while feeding on glycerol, an industrial waste product. Furthermore, the strain is evolved via adaptive laboratory evolution (ALE) to enhance the anthranilate production.

#### P-SSM-018

##### Optimization of heterologous sMMO production for growth-coupled CH<sub>4</sub> bioconversion in *E. coli*

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#### Introduction

Methane (CH<sub>4</sub>) is an abundant C1 carbon source with high potential for sustainable biotechnological applications. Methanotrophs utilize CH<sub>4</sub> as their sole carbon and energy source, but complex genetics and growth requirements limit their broader use<sup>[1]</sup>. Synthetic methanotrophy overcomes these constraints by engineering microbes to convert CH<sub>4</sub> into value-added products, with soluble methane monooxygenase (sMMO) as key enzyme<sup>[2,3]</sup>.

#### Goals

This work aims to advance synthetic methanotrophy by focusing on three objectives:

I. Simplification and optimization of heterologous sMMO production in *E. coli*.

II. Use of methylotrophic sensor strains<sup>[4]</sup> to establish CH<sub>4</sub>-dependent growth in *E. coli*.

III. Characterization of MmoE<sup>[5]</sup>, a putative auxiliary protein across *Methylobacter*.

#### Methods

The sMMO operon from *M. methanica* MC09, previously produced heterologously in *E. coli*, was redesigned to consolidate the original four-plasmid system<sup>[5]</sup> into a single-plasmid construct, enabling streamlined production and purification of active sMMO. Growth of an sMMO-producing methylotrophic sensor strain was monitored to evaluate sMMO activity *in vivo*. Salt-dependent stability of MmoE was assessed in thermoshift assays, and binding to ions, DNA targets, and the hydroxylase MMOH was tested by microscale thermophoresis (MST).

#### Results

Purified sMMO from the single-plasmid system was catalytically active, as shown by activity assays using the model substrate nitrobenzene. Indications of CH<sub>4</sub>-dependent growth were observed in an sMMO-producing sensor strain, suggesting functional activity *in vivo*. Thermoshift assays revealed increased MmoE stability at higher salt concentrations, but MST has so far detected no binding to ions, DNA targets, or MMOH.

#### Summary

By enabling functional heterologous sMMO production and demonstrating initial *in vivo* activity, this work, establishes a framework for engineering synthetic methanotrophs for sustainable C1 bioconversions.

[1] K. K. Sahoo, G. Goswami, D. Das, *Front Microbiol.* **2021**, 12, 636486.

[2] C. Tahiraj, L. Hille, K. Brunsbach, M. Macgregor-Fairlie, V. Steier, S. N. Lindner, R. Banerjee, L. Lauterbach, *Trends Biotechnol.* **2025** (accepted 15 Oct 2025)

[3] F. J. Tucci, A. C. Rosenzweig, *Chem. Rev.* **2024**, 124, 1288–1320.

[4] H. Kim, S. An, Y. R. Park, H. Jang, H. Yoo, S. H. Park, S. J. Lee, U. S. Cho, *Sci. Adv.* **2019**, 5.

[5] D. Zill, E. Lettau, C. Lorent, F. Seifert, P. K. Singh, L. Lauterbach, *ChemBioChem.* **2022**, 23, 4–8.

## P-SSM-019

### MaxKat: Harnessing energy and redox auxotrophy for selective bioconversions in an engineered *Escherichia coli* strain

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A sustainable alternative to conventional petrochemical industry is the biological conversion of waste streams or sugars from crops into bulk chemicals. Synthetic biology aims to establish efficient biocatalysts which potentially can replace fossil-based processes.

Natively, *E. coli* is a facultative anaerobe. In the absence of oxygen, it transfers its electrons onto an intermediate as terminal electron acceptor to balance the intracellular redox state. This results in a fermentation product, like ethanol or lactate. However, substrate and product combinations are inherently limited due to the required redox balance.

In this work, a genetically engineered energy-auxotroph *E. coli* strain "MaxKat" was investigated as a chassis to produce platform chemical 2,3-butanediol from glucose and glycerol via the recently developed mode of production called "controlled respiration-fermentation"<sup>1</sup>.

In MaxKat, all fermentation routes and links to the respiratory chain are deleted. This results in an unbalanced intracellular redox state through NADH accumulation and therefore glycolysis cannot operate to produce ATP. We could demonstrate that co-supplementation of intermediate acetoin restored growth of MaxKat by transferring electrons from NADH onto acetoin to form 2,3-butanediol. This enabled glycolysis and the production of ATP via substrate-level-phosphorylation.

To demonstrate the respiration-fermentative production of 2,3-butanediol from glycerol, MaxKat was further engineered to express another respiratory module. Besides the aerobic glycerol 3-phosphate dehydrogenase it expressed the quinone-dependent D-lactate dehydrogenase to allow re-balanced fermentation of glycerol. However, the strain grew in minimal medium containing glycerol regardless of 2,3-butanediol pathway expression. We hypothesized that the expression of Dld resolved the energy auxotrophy via ATP production without NADH generation.

So far, this work highlights MaxKat's redox-coupling strategy as a reliable mechanism for testing enzymatic reactions in a

fermentative pathway that consumes reducing equivalents and thus releases the energy-auxotrophy.

1. Schulz-Mirbach, H. *et al.* Engineering new-to-nature biochemical conversions by combining fermentative metabolism with respiratory modules. *Nat. Commun.* **15**, 6725 (2024).

## P-SSM-020

### Optogenetic Multicolor Light Control of Gene Expression in Gram-Negative Bacteria

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Precise temporal and spatial control of gene expression is crucial for analysing regulatory processes and engineering metabolic pathways in bacterial systems. Photocaged inducers enable a versatile optochemical approach for light-controlled gene expression by remaining biologically inactive until protecting groups are cleaved upon illumination with specific wavelengths. This enables targeted, non-invasive activation of gene transcription with high temporal resolution. UV-A- and blue-light-sensitive photocaged inducers have been successfully applied to control gene expression in various bacteria [1,2,3]. Despite these advances, there is a great interest in expanding the spectral range by including green-light-sensitive photocaged inducers to improve the flexibility of the system and enable wavelength-specific control of multiple genetic circuits within the same cell.

Photocaged inducers are particularly important because they can be combined with established and commonly used regulator/promoter systems, such as LacI/P<sub>tac</sub>, Rha(R)SP<sub>rhaBAD</sub> or AraC/P<sub>BAD</sub>, to create novel optogenetic switches. In this study, we aim to establish and implement new BODIPY-based green-light-responsive photocaged inducers to expand the optochemical toolbox. We started to synthesize the caged inducers BODIPY-I-arabinose and I-rhamnose and initially analysed their spectral properties and (photo)toxicities in *P. putida*, *G. oxydans* and *E. coli*. Furthermore, we started to evaluate the photoreceptor-based BLADE (blue light-inducible AraC dimers in *E. coli*) as an orthogonal system. The recombinant optogenetic switch comprises the DNA-binding domain of the transcription regulator AraC and the blue light-sensitive dimerization domain VIVID [4], which can be combined with the same P<sub>BAD</sub> promoter systems. These approaches combine a proven bacterial expression system with a direct optogenetic component, which facilitates complex synthetic biology applications such as regulatory networks investigation of or the targeted modulation of cell functions. Our work thus represents an important extension of the optogenetic toolbox and supports the development of programmable, light-controlled systems for advanced bacterial biotechnology and synthetic biology.

[1] Hogenkamp, F., *et al.*, *Chembiochem* **2021**, 22(3): 539-547.

[2] Hogenkamp, F., *et al.*, *Chembiochem* **2022**, 23(1): e202100467.

[3] Binder, D., *et al.*, *Integr. Biol.* **2014**, 6(8), 755–765.

[4] Romano, E., *et al.*, *Nat. Chem. Biol.* **2021**, 17(7): 817–827.

## P-SSM-021

### Enhanced recombinant laccase expression in *Aspergillus niger* by designing a gene expression cassette and reducing the extracellular proteases of chassis cells

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*Coprinopsis cinerea* laccase Lcc9 has significant potential for industrial applications, such as the decolorization of indigo-like dyes. However, the incomplete genetic manipulation system and underdeveloped fermentation process of *C. cinerea* hinder high-level production of Lcc9, limiting its large-scale industrial use. This study utilizes *Aspergillus niger*, an industrial microorganism known for its high protein synthesis and secretion capabilities, as the chassis cell to develop an engineered strain for efficient recombinant expression of Lcc9. Firstly, we identified high-expression integration sites for the lcc9 expression cassette in the *A. niger* genome and examined how co-expressing fusion partner molecules and intermediate linker sequences affect the recombinant extracellular expression of Lcc9. The recombinant strain AnXynBKA, created by integrating the lcc9 expression cassette mediated by the xylanase XynB and the linker sequence PTANVISKRAPV at the agdA locus, exhibited an extracellular laccase activity of 798.4 U/L in shake flask fermentation, which is 2.98-fold higher than that of the original strain. Then, knocking out the extracellular protease transcription factor PrtT increased the recombinant extracellular laccase activity to 1062.7 U/L, which is 3.97 times that of the original strain. Finally, increasing the copy number of the lcc9 to 3 resulted in a recombinant strain AnPXynBKA3 with an extracellular laccase activity of 1821.2 U/L after shake flask fermentation, representing a 6.80-fold increase over the original strain. After scaling up to a 3-L fermenter, AnPXynBKA3 achieved an extracellular laccase activity of 2338.9 U/L, which is 1.28-fold higher than its shake flask fermentation level. To our knowledge, this represents the highest level of recombinant expression of *C. cinerea* laccase Lcc9 in *A. niger*. The research implementation has established a theoretical foundation for the efficient production of Lcc9 and offered a strategic reference for increasing other exogenous protein yields in *A. niger*.

## Secondary metabolites and natural products

### P-SMNP-001

#### Fighting pathogens: Antimicrobial and antibiofilm activities of plant latex extracts

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Bacterial biofilm formation represents a major challenge in medical and environmental settings, contributing to chronic infections and antibiotic resistance. Disrupting quorum sensing, the bacterial cell–cell communication system controlling virulence and biofilm development, offers a promising strategy to counteract these processes. Plant latex is a rich yet largely unexplored source of bioactive secondary metabolites with potential quorum quenching activity. In this study, latex samples from 50 plant species were extracted using a methanol–cyclohexane mixture and screened for antimicrobial and antibiofilm activities against selected examples of human or plant pathogenic bacteria, i.e. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Actinomyces naeslundii*, and *Xanthomonas campestris*. Biofilm formation was quantified using the crystal violet assay under optimized culture conditions. Several latex extracts significantly inhibited biofilm formation, while others showed neutral or enhancing effects, revealing the complex bioactivity of latex-derived compounds. Extracts from *Mammillaria compressa*, *Macleaya cordata*, and *Euphorbia milii* showed the strongest antibiofilm effects, with bioactivity persisting in individual HPLC fractions after separation of the latex extracts. No antifungal activity against *Fusarium culmorum* was observed, suggesting low general cytotoxicity. These findings demonstrate that plant latex harbors diverse quorum quenching and biofilm-modulating activities, highlight its value as a source of novel antimicrobial agents, and establish an efficient workflow for systematic latex screening for novel therapeutics.

### P-SMNP-002

#### The two faces of cystargolides: Concentration-dependent modulation of the Clp protease of *Kitasatospora cystarginea* by its natural product cystargolides

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Antibiotic resistance is on the rise. The discovery and evaluation of novel antibacterial scaffolds and alternative targets are essential to avoid a post-antibiotic era. The bacterial Clp protease system, composed of the proteolytic ClpP core and associated Clp-ATPases, is a promising, underexploited antibacterial target due to its essential role in the virulence of numerous bacterial species. In actinobacteria, it is even essential for growth. Up to now, only a few ClpP-targeting agents have been described that can act either as activators, such as acyldepsipeptides, or as inhibitors, such as clipibicyclenes. The naturally produced  $\beta$ -lactones cystargolide A and B form a new class of ClpP inhibitors and were shown to covalently inactivate the catalytic serine of *Staphylococcus aureus* ClpP (Illigmann *et al.*, 2024). However, how cystargolides function within their own actinobacterial producer strain, *Kitasatospora cystarginea*, remains elusive. To address this question, we purified all components of the ClpP protease machinery of *K. cystarginea*, comprising four Clp analogs and three Clp-ATPases. We analyzed various combinations of these components in activity and interaction assays in the absence and presence of cystargolide. In peptide degradation assays, we observed that ClpP1P2, representing the proteolytic core of the housekeeping Clp protease, is inhibited by cystargolide at stoichiometric concentrations. In contrast, a sub-stoichiometric amount of cystargolide activates the ClpP1P2 core. Biochemical analyses showed a shift in the assembled ClpP1P2 complex from a smaller 14-mer to a larger conformation in the presence of cystargolide. In summary, our data shed light on the composition, functionality and conformational dynamics of *K. cystarginea*



CIP1P2 and suggest a concentration-dependent dual mode of action of cystargolide.

#### P-SMNP-003

##### Discovering the mode of action of an underexplored polyketide harboring an uncommon amino acid

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With the increasing emergence of multidrug-resistant bacteria, the need for novel antimicrobial agents has become urgent. While target-based strategies were used to overcome this problem, by screening and rational design of (semi)synthetic compounds, these often face limitations, including poor cell entry or efflux. In contrast, natural products have evolved and frequently demonstrate efficient target engagement, multipronged antibacterial mechanisms, and good cellular uptake and retention. Enduracyclinone, a polyketide featuring the rare amino acid enduracididine, has been identified in various strains of *Nonomuraea* species. It exhibited reduced bioactivity against cell wall-deficient *Staphylococcus aureus* L-forms, suggesting a mode of action targeting the bacterial cell wall. In previous studies, strong antibacterial activity with minimal cytotoxicity toward eukaryotic cells was reported, but the mode of action remained elusive<sup>[1]</sup>. Interestingly, teixobactin, which also contains enduracididine, was reported to bind cell wall precursors, thereby inhibiting cell wall biosynthesis<sup>[2]</sup>. However, neither binding to cell wall precursors nor membrane lipids could be confirmed for enduracyclinone. We explore the effects of enduracyclinone on bacterial cells using fluorescence microscopy, combined with other microbiological methods.

[1] P. Monciardini, A. Bernasconi, M. Iorio, C. Brunati, M. Sosio, L. Campochiaro, P. Landini, S. I. Maffioli, S. Donadio, *J Nat Prod* **2019**, 82, 35-44.

[2] L. L. Ling, T. Schneider, A. J. Peoples, A. L. Spoering, I. Engels, B. P. Conlon, A. Mueller, T. F. Schärerle, D. E. Hughes, S. Epstein, M. Jones, L. Lazarides, V. A. Steadman, D. R. Cohen, C. R. Felix, K. A. Fetterman, W. P. Millett, A. G. Nitti, A. M. Zullo, C. Chen, K. Lewis, *Nature* **2015**, 517, 455-459.

#### P-SMNP-004

##### Identification of a novel bioactive peptide and its unique biosynthesis in *Pseudomonas entomophila*

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Natural products derived from microorganisms have long been a valuable source of potential new drugs. Non-ribosomal peptides (NRP), polyketides (PK) and hybrids of both, represent large groups of natural products, which are produced by multi-functional enzymes encoded by biosynthetic gene clusters (BGC). The growing threat of multi-resistant bacteria underscores the urgent need for novel compounds with novel mechanisms of action. To address this challenge, a thorough understanding of these

compounds is essential, requiring a comprehensive elucidation of their biosynthetic pathways to identify all key components involved in their production.

*Pseudomonas entomophila* is an entomopathogenic bacterium, which produces a wide range of different compounds including siderophores, hemolytic lipopeptides and antibacterial alkaloids. In the present study, a yet unknown biosynthetic gene cluster encoding for a NRP-PK hybrid was investigated in terms of its biosynthetic pathway and bioactivity.

In order to decipher the biosynthesis, genes within the corresponding BGC were deleted and the promoter upstream of the BGC was exchanged against an inducible *P<sub>BAD</sub>* promoter. This enabled the detection of several new compounds by HPLC-MS analysis. By applying this method, the incorporation of the non-proteinogenic amino acid 2,3-diaminopropionate (Dap) was confirmed. Furthermore, a unique biosynthetic mechanism involving an NRPS with an N-acyl-transferase domain was revealed. The full-length compound functions as a pro-drug, which is cleaved by an amidase leading to the final bioactive compounds exhibiting antibacterial activity against Gram-positive and Gram-negative bacteria.

Our findings strongly suggest a novel type of natural product with antibacterial activity generated by a unique biosynthesis.

#### P-SMNP-005

##### Investigation of calcimycin analogs produced by *Streptomyces chartreusis*

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Calcimycin, a natural product produced by the soil bacterium *Streptomyces chartreusis* NRRL 3882, is a polyether ionophore, which forms lipophilic complexes with divalent cations. It has an antibiotic effect on some fungi and gram-positive bacteria, for example *Bacillus subtilis*, and causes an increase in intracellular Ca<sup>2+</sup> and a decrease of intracellular Mg<sup>2+</sup> and Fe<sup>2+</sup> levels in exposed *B. subtilis* [1]. Furthermore, it is used as a tool to investigate calcium signaling. Since *B. subtilis* is a soil bacterium sharing the habitat with *S. chartreusis*, it can be used as model organism to test the effects of calcimycin. It was shown, that under iron-limited conditions calcimycin extracts iron from *B. subtilis*. The semisynthetic calcimycin analog 4-Br-A23187 was shown to transport Cu<sup>2+</sup> instead [2]. A number of natural analogs have been shown to be produced in media with different amino acid supplements [3]. It is unknown, which metals are transported by natural analogs and which effects these analogs have on the ion homeostasis of *B. subtilis* or *S. chartreusis* itself. We purified calcimycin analogs from cultures of *S. chartreusis* wildtype or deletion mutants grown under different conditions to then characterize the effects of these calcimycin analogs. We are interested in the antibacterial effects of the analogs, as well as their effects on *S. chartreusis* physiology.

1. Raatschen *et al.* (2013) Extracting iron and manganese from bacteria with ionophores – A mechanism against competitors characterized by increased potency in environments low in micronutrients. *Proteomics* 13: 1358-1370
2. Senges C. H. R. *et al.* (2022) Effects of 4-Br-A23187 on *Bacillus subtilis* cells and unilamellar

vesicles reveal it to be a potent copper ionophore. *Proteomics* 22: e2200061

- Arend K. I. et al. (2021) Influence of Amino Acid Feeding on Production of Calcimycin and Analogs in *Streptomyces chartreusis*. *International Journal of Environmental Research and Public Health* 18: 8740

#### P-SMNP-007

##### Implementation of a heterologous screening system to unravel phycocyanobilin biosynthesis in red algae

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Red algae are eukaryotic phototrophs performing oxygenic photosynthesis. Besides chlorophyll, they contain open-chain tetrapyrroles covalently bound to phycobiliproteins, forming phycobilisomes as light-harvesting complexes that expand their absorption spectrum - a trait gained via endosymbiosis with a cyanobacterial ancestor. [1]. The open-chain tetrapyrroles, or phycobilins, are synthesized from heme-derived biliverdin IX $\alpha$  and further reduced by ferredoxin-dependent bilin reductases (FDBRs) [2]. This activity was first detected in *Galdieria sulphuraria* (formerly mistaken for *Cyanidium caldarium*), where biliverdin (BV) was found to be reduced to phycocyanobilin (PCB) through phycoerythrobilin (PEB) [3]. In cyanobacteria, PCB is produced via a four-electron reduction catalyzed by phycocyanobilin:ferredoxin oxidoreductase (PcyA) [4]. In contrast, phycoerythrobilin (PEB) synthesis involves two sequential two-electron reductions catalyzed by 15,16-dihydrobiliverdin:ferredoxin oxidoreductase (PebA) and phycoerythrobilin:ferredoxin oxidoreductase (PebB) [2]. Proteomic analysis of *G. sulphuraria* revealed the lack of PcyA and presence of only PebA and PebB, suggesting PCB is synthesized from PEB via a putative isomerization. In contrast, the related species *Cyanidioschyzon merolae* possesses *pcyA*, indicating a direct PCB pathway. Although *G. sulphuraria* extracts convert PEB to PCB, the responsible PEB:PCB isomerase remains uncharacterized [5]. To identify the PEB:PCB isomerase, proteomic comparisons between *C. merolae* and *G. sulphuraria* were conducted, revealing candidate isomerase-like genes unique to *G. sulphuraria*. Isomerase activity was tested in a heterologous *E. coli* system using the cyanobacterial phytochrome Cph1, which binds both PCB and PEB. PCB-bound Cph1 exhibits 15Z/15E photoisomerization without fluorescence, whereas PEB-bound Cph1 is fluorescent and lacks photoisomerization. Fluorescence and culture color (PCB: blue-green; PEB: red-pink) served as readouts. Candidate *G. sulphuraria* genes, together with *cph1*, heme oxygenase (*ho1*), and FDBRs (*pcyA*, *pebS*), were co-expressed in *E. coli* BL21(DE3) for screening.

[1] Glazer, A. N. *Mol Cell Biochem.* 1977 Dec 29;18(2-3): 125–140.

[2] Frankenberg, N., et al. *Plant Cell.* 2001 Apr;13(4):965-78.

[3] Beale SI, Cornejo J. *J Biol Chem.* 1991 Nov;266(33):22328-32.

[4] Frankenberg N, Lagarias JC. *J Biol Chem.* 2003 Mar;14;278(11):9219-26.

[5] Beale SI, Cornejo J. *J Biol Chem.* 1991 Nov 25;266(33):22333-40.

#### P-SMNP-008

##### High-Throughput Engineering and Modification of Non-ribosomal Peptide Synthetases Based on Golden Gate Assembly

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Non-ribosomal peptide synthetases (NRPS) are multimodular enzymes that produce complex peptides with diverse biological activities, potentially being used as clinical drugs to treat infectious diseases, cancer and other health conditions.<sup>1</sup> However, the pharmaceutical applications of such natural peptides often require further derivatisation and modification of the peptide backbone, mainly performed by synthetic/medicinal chemistry.<sup>2</sup> Conversely, a sustainable alternative resembles the *in vivo* engineering of NRPS to alter and modify the enzyme properties rationally and consequently the produced products.<sup>3–5</sup> The novel NRPS engineering strategy, the eXchange Unit Thiolation domain (XUT) concept, facilitates the efficient modular exchange and, therefore, the assembly of different natural NRPS fragments to form hybrid NRPS that produce defined peptides.<sup>3</sup> In this work, we developed an efficient Golden Gate Assembly-based method/syntax for generating novel and engineered NRPS libraries utilising the XUT concept in high-throughput. This method was applied to generate over 100 novel NRPS, allowing for the exchange of starter, elongation, and termination modules, respectively. Additionally, we applied this method for the targeted modification of the xenoamycin biosynthetic gene cluster (*xabABCD*) from *Xenorhabdus doucetiae*, generating 25 novel xenoamycin derivatives.

#### References

- Süssmuth, R. D. & Mainz, A. Nonribosomal Peptide Synthesis—Principles and Prospects. *Angewandte Chemie Int Ed* 56, 3770–3821 (2017).
- Süssmuth, R. D., Kulike-Koczula, M., Gao, P. & Kosol, S. Fighting Antimicrobial Resistance: Innovative Drugs in Antibacterial Research. *Angew. Chem. Int. Ed.* e202414325 (2025) doi:10.1002/anie.202414325.
- Bozhüyük, K. A. J. et al. Evolution-inspired engineering of nonribosomal peptide synthetases. *Science* 383, eadg4320 (2024).
- Kries, H., Niquille, D. L. & Hilvert, D. A Subdomain Swap Strategy for Reengineering Nonribosomal Peptides. *Chem. Biol.* 22, 640–648 (2015).
- Crüsemann, M., Kohlhaas, C. & Piel, J. Evolution-guided engineering of nonribosomal peptide synthetase adenylation domains. *Chem. Sci.* 4, 1041–1045 (2012).

## P-SMNP-010

### Evaluating the activity of acyldepsipeptide antibiotics against extra- and intracellular *Listeria monocytogenes*

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Increasing emergence of antibiotic-resistant pathogens urgently calls for the discovery of novel antibiotic classes and unique targets. Acyldepsipeptide (ADEP) antibiotics feature an unusual mechanism of action. They dysregulate ClpP – the proteolytic core of caseinolytic protease Clp – towards destructive proteolysis while also inhibiting its natural functions (Brötz-Oesterhelt et al., 2021). Clp proteases are ubiquitous in bacteria and, besides being responsible for regulatory proteolysis and protein homeostasis, they are often crucial for virulence of pathogens such as the facultative intracellular food pathogen *Listeria monocytogenes* (Gaillot et al., 2000). Although ADEPs have shown a wide-ranging anti-Gram-positive antibiotic activity (Brötz-Oesterhelt et al., 2005), their effect on *L. monocytogenes* has not yet assessed.

Here, we characterize the activity of different ADEP congeners against both free-living and intracellular *L. monocytogenes* that propagate in a monolayer of eukaryotic MDCK cells. High-resolution microscopy further reveals a dual mode of action, including growth inhibition at higher concentrations and a filamentous phenotype at concentrations below the minimal inhibitory concentration. Further work will aim to analyze whether elongated, non-dividing *L. monocytogenes* result from degradation of the cell division factor FtsZ by ADEP-stimulated ClpP, just as it was shown in *Bacillus subtilis* (Sass et al., 2011).

#### References:

Brötz-Oesterhelt, H., Beyer, D., Kroll, H.-P., Endermann, R., Ladel, C., Schroeder, W., et al. (2005). Dysregulation of Bacterial Proteolytic Machinery by a New Class of Antibiotics. *Nat. Med.* 11, 1082–1087. doi:10.1038/nm1306

Brötz-Oesterhelt, H., and Vorbach, A. (2021). Reprogramming of the caseinolytic protease by ADEP antibiotics: molecular mechanism, cellular consequences, therapeutic potential. *Front. Mol. Biosci.* 8, 690902. doi:10.3389/fmolb.2021.690902

Gaillot, O., Pellegrini, E., Bregenholt, S., Nair, S., and Berche, P. (2000). The ClpP serine protease is essential for the intracellular parasitism and virulence of *Listeria monocytogenes*. *Mol. Microbiol.* 35(6), 1286–1294. doi:10.1046/j.1365-2958.2000.01773.x

Sass, P., Josten, M., Famulla, K., Schiffer, G., Sahl, H.-G., Hamoen, L., et al. (2011). Antibiotic Acyldepsipeptides Activate ClpP Peptidase to Degrade the Cell Division Protein FtsZ. *Proc. Natl. Acad. Sci.* 108, 17474–17479. doi:10.1073/pnas.1110385108

## P-SMNP-011

### Specialized specialists – The role of strain-specific biosynthetic gene clusters in *Photorhabdus* and *Xenorhabdus*

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Bacteria of the genus *Photorhabdus* and *Xenorhabdus* are motile, Gram-negative bacteria that live in symbiosis with entomopathogenic nematodes. Due to their complex life cycle, they produce several natural products encoded in biosynthetic gene clusters (BGC). Pangenomic analyses revealed not only a shared core set of BGCs, but also the occurrence of strain-specific "singleton" clusters [1]. Our goal was to activate and characterize individual BGCs in order to investigate their contribution to competitive interactions in ecological contexts. We used a modular genetic toolkit based on CRISPR/Cpf1 to replace native regulatory elements with artificially inducible promoters paired with heterologous expression [2]. We analyzed the metabolites using LC-MS and evaluated their bioactivity in bacterial interaction assays. Many singleton clusters were silent under standard laboratory conditions and required multiple genetic interventions for detectable expression. Activated singleton-derived metabolites showed antimicrobial activity not only against unrelated bacteria but also against other *Photorhabdus* and *Xenorhabdus* strains lacking the respective clusters. Our findings suggest that these metabolites play a role in fine-tuning competitive interactions and host occupation within these entomopathogenic bacteria.

[1] Shi, Y.M., Hirschmann, M., Shi, Y.N. *et al.* Global analysis of biosynthetic gene clusters reveals conserved and unique natural products in entomopathogenic nematode-symbiotic bacteria. *Nat. Chem.* 14, 701–712 (2022).

[2] Rill, A., Zhao, L. & Bode, H.B. Genetic toolbox for *Photorhabdus* and *Xenorhabdus*: pSEVA based heterologous expression systems and CRISPR/Cpf1 based genome editing for rapid natural product profiling. *Microb Cell Fact* 23, 98 (2024). <https://doi.org/10.1186/s12934-024-02363-8>

## P-SMNP-012

### Nutrient-media dependent antimicrobial effects of *Limosilactobacillus reuteri* and *Lactobacillus casei* against *Salmonella* Enteritidis

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**Introduction** Probiotic *Lactobacillus* strains are known for their ability to inhibit pathogenic bacteria through various mechanisms. This study aimed to determine the conditions under which *Lactobacillus* strains exert antibacterial activity against *Salmonella* Enteritidis.

**Goals** To identify the environmental and nutritional conditions influencing the anti-*Salmonella* activity of *Lactobacillus* isolates and to verify the mechanism of inhibition using well diffusion, enzyme sensitivity, neutralization, and competition assays.

**Methods** Six *Lactobacillus* strains were first tested against *S. Enteritidis* using the well diffusion method. The probiotics were cultivated in MRS, 2×MRS/2×BHI, BHI adjusted to pH 7.4 and 6.0, and TSB media, and their cell-free supernatants (CFSSs) were evaluated for antibacterial activity. Among them, *L. reuteri* and *L. casei* showed clear inhibition and were selected for further experiments. CFSSs were treated

with Pronase and Proteinase K to assess protein-based inhibition, and their pH was neutralized to 6.5 to test acid dependence. Competition assays were performed in 2×MRS/2×BHI and BHI media (both at pH 6.0) at 37 °C under aerobic and microaerophilic conditions, using probiotic-to-*Salmonella* ratios ranging from 1000:1 to 1:1 and inocula of 10<sup>8</sup>–10<sup>5</sup> CFU/mL.

**Results** In well diffusion assays, *L. reuteri* and *L. casei* inhibited *S. enteritidis* only when grown in MRS-containing broth, indicating a strong dependence on medium composition. Neutralization eliminated inhibition, and enzyme treatment had no effect, confirming that the antibacterial activity was due to acidification rather than bacteriocins. In competition assays, *Salmonella* was inhibited in 2×MRS/2×BHI, with >4 log CFU/mL reduction after 24 h, while both probiotics increased by about 4 log CFU/mL. No inhibition occurred in BHI, although its pH was equal to that of 2×MRS/2×BHI, demonstrating that the effect was medium-specific rather than pH-dependent. Probiotic concentration did not influence the extent of inhibition.

**Conclusion** *Lactobacillus reuteri* and *L. casei* exhibit strong antibacterial activity against *Salmonella* Enteritidis only when grown in MRS-containing media. Their inhibition is medium-specific, independent of cell concentration, and driven by acid production rather than bacteriocin secretion. These findings highlight the importance of nutrient composition in probiotic–pathogen interactions and provide insight into optimizing conditions for probiotic efficacy.

#### P-SMNP-013

##### Characterization of Mac-acyltransferases from different mannosylerythritol lipid-producing basidiomycetes

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Compounds synthesized by microorganism are utilized in our daily life since decades, one of these microbial compounds are biosurfactants. Among these biosurfactants are mannosylerythritol lipids (MELs), a class of glycolipids that are naturally synthesized by various basidiomycetes mainly under nitrogen starvation conditions. MELs are known for their biological activity and their excellent interfacial properties, making them interesting for the industry. These properties were shown to be dependent on the length of fatty acids decorating the mannose-moiety of the MEL. During MEL-biosynthesis the acyltransferases Mac1 and Mac2 incorporate these fatty acids in a species-specific manner. Previous studies demonstrated, that the length of incorporated fatty acids through Mac1 and Mac2 depends on the organism they are derived from. This specificity was analyzed to get a better overview over different Mac-acyltransferases, since a better understanding of fatty acid specificity of Mac1 and Mac2 could improve tailor-made MEL-synthesis and therefore potential industrial application.

Three less-characterized MEL-producer, *Sporisorium cruentum*, *Sporisorium consanguineum* and *Sporisorium exsertum*, were closely examined regarding their synthesized MELs, after identification of their MEL-gene-cluster. Their fatty acid specificity was verified via HPLC-MS/MS-analysis using MELs from complementation strains in *Ustilago maydis* and from the corresponding wildtypes. MEL-properties were analyzed regarding antibiotic activity, impact on the surface tension of water and emulsifying activity.

Furthermore, Alphafold3 predictions of Mac1 and Mac2 protein structures showed strong similarity between the Mac-acyltransferases of different basidiomycetes. While sequence homology analysis revealed two strongly conserved motifs, HxxxD (Mac1) and HxxxG (Mac2), in the acyltransferases of different basidiomycetes. These motifs had been identified as catalytically important and function as putative active center of Mac1 and Mac2. The selection mechanism of the Mac-acyltransferases leading to a certain fatty acid specificity of the acyltransferases remains unknown.

#### P-SMNP-014

##### Laboratory-based natural habitat mimicry with soil modulates the secondary metabolism of *Aspergillus niger*

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Soil microorganisms possess an immense but largely untapped potential for secondary metabolite (SM) production, which is highly regulated in response to environmental needs and the interaction with the co-inhabiting microorganisms [1]. Many SM biosynthetic gene clusters remain silent under axenic laboratory conditions, and current co-cultivation systems – while promising – either have to be performed *in situ* or still poorly mimic the complexity of natural habitats and multi-species microbial communication [2,3,4,5].

To address this, we developed a complex co-cultivation (COCO) method, a simple, robust, and reproducible system that mimics soil-like conditions and enables stable co-cultivation of a diverse set of microorganisms with culturable natural soil microbiota. The established workflow allows separate and repeated sampling of biomass and metabolites over time for multi-omics analyses.

Using the soil fungus *Aspergillus niger* as a model, we demonstrated (using RT-qPCR and LC-MS<sup>1</sup>/MS<sup>2</sup>/MRM) that co-cultivation with soil dynamically alters its SM cluster core gene expression and SM profile. While several known SMs were downregulated, previously unknown metabolites were induced, suggesting activation of cryptic biosynthetic pathways through interkingdom interactions. COCO was readily adapted to diverse fungi (*S. cerevisiae*, *C. albicans*, *K. phaffii*) and bacteria (*E. coli*, *B. subtilis*, *M. luteus*) as well as alternative microbiomes (from organic oat flakes, peanuts, grapes, tomatoes), confirming its broad applicability and reproducibility.

Overall, the COCO method provides a versatile platform to study microbial interactions in controlled yet ecologically relevant settings. It facilitates systematic exploration of chemical communication in soil communities and holds strong potential to uncover novel bioactive compounds for next-generation drug discovery.

[1]: Macheleidt *et al.* (2016). <https://doi.org/10.1146/annurev-genet-120215-035203>

[2]: Ling *et al.* (2015). <https://doi.org/10.1038/nature14098>

[3]: Netzker *et al.* (2015). <https://doi.org/10.3389/fmicb.2015.00299>

[4]: Nai and Meyer (2018).  
<https://doi.org/10.1016/j.tim.2017.11.004>

[5]: Brakhage and Schroeckh (2011).  
<https://doi.org/10.1016/j.fgb.2010.04.004>

#### P-SMNP-015

##### **A novel, commensal derived, three-peptide antimicrobial to combat *Staphylococcus aureus***

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Careless prescription and overuse of antibiotics in healthcare, as well as their application in livestock, create a strongly selective environment for bacteria to acquire various antibiotic resistances to survive. One of these, the human pathogen *Staphylococcus aureus*, was solemnly responsible for over 1 million deaths worldwide in 2019 alone. In recent years, the microbiota of humans has received increased attention as a potential source for the discovery of new antimicrobials. This has led to the discovery of a variety of different, commensal-produced antimicrobial compounds, generally summarized under the term of 'bacteriocins'. By screening bacterial isolates from nasal swabs of volunteers, we identified a commensal *S. epidermidis* strain that showed potent antimicrobial activity against various bacteria, including the methicillin-resistant strain *S. aureus* USA300 LAC. Genomic analysis of the producer strain identified a biosynthetic gene cluster (BGC), which encodes a hitherto unknown bacteriocin. The BGC encodes eight putative modification enzymes, three putative structural peptides, and a bipartite ABC-transporter. The identified modification genes indicate that the antimicrobial compound is a 'Ribosomally synthesized and post-translationally modified peptide' (RiPP) belonging to the class of linear azole-containing peptides (LAP) or TOMMs (thiazole-oxazole modified microcins). To our knowledge, no three-peptide antimicrobial RiPP has previously been described. Knockout of the three structural peptides leads to complete loss of activity, indicating that this BGC encodes the compound of interest. We found that the producer strain shows antimicrobial activity against many gram-positive bacteria, including various clinically relevant pathogens, including MRSA and vancomycin resistant Enterococci (VRE). Heterologous expression of the BGC-encoded ABC-transporter in the sensitive strain *S. aureus* USA300 conferred resistance, indicating that the ABC-transporter is necessary for producer self-immunity. Deleting any of the three precursor genes led to loss of antimicrobial activity, indicating that it is truly a unique, three-component antimicrobial. Additionally, co-cultivation of the producer strain with *S. aureus* USA300 showed almost complete eradication of *S. aureus* USA300 within 24 hours. Overall, this novel antimicrobial can effectively kill multiple clinically relevant pathogens, supporting a continued study of the compound and its potential applications.

#### P-SMNP-016

##### **Characterization of a class II lanthipeptide from the cyanobacterium *Nostoc punctiforme* that is implicated in symbiotic interactions with the liverwort *Blasia pusilla***

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Cyanobacteria are prolific producers of a broad range of natural products.[1] While classes of RiPPs such as

lanthipeptides have been identified in cyanobacteria, their biological functions remain mostly unknown. Additionally, the analysis of lanthipeptides in cyanobacteria presents significant challenges. To date, cyanobacterial lanthipeptides like prochlorosins have predominantly been studied in *E. coli*. [2] To address these issues, we chose to investigate a class II lanthipeptide biosynthetic gene cluster (BGC) from *Nostoc punctiforme* PCC 73102, a terrestrial filamentous cyanobacterium capable of forming symbiotic associations with diverse hosts, including the bryophyte *Blasia*, cycads, and *Gunnera* sp.

Our previous studies demonstrated early upregulation of this BGC during chemical and physical interactions with the liverwort *Blasia pusilla*. Despite the observed upregulation, the lanthipeptide product was not analytically detectable in *N. punctiforme*. Therefore, we heterologously expressed parts of the BGC in *E. coli* and successfully characterized its product, an unusual lanthipeptide harboring two lanthionine rings, a free dehydro amino acid as well as a free cysteine. Apart from the RiPP monomer that is produced when the precursor peptide is coexpressed with the lanthionine synthetase in *E. coli*, we observed a pronounced tendency toward peptide oligomerization ( $\geq 200$  kDa), especially at elevated pH values. We are currently testing the hypothesis that these oligomers are formed by covalent cross-linking of the lanthipeptides. His-tag labeling of precursor peptide copies in *N. punctiforme* provided evidence that oligomers could be the dominant species in cyanobacteria.

We assume that the biological function is conferred by a lanthipeptide surface layer rather than peptide monomers. Constitutive expression of the lanthipeptide BGC in a second symbiotic *Nostoc* strain lead to a non-motile phenotype and a significant delay in symbiotic interactions particularly affecting the early stages of the symbiosis between *Nostoc* and *Blasia pusilla*. We therefore postulate that the lanthipeptide plays a pivotal role in symbiotic negotiations of *Nostoc* strains.

#### References

[1] Dittmann, E. *et al.* Trends Microbiol. 2015, 23 (10) 642–652. <https://doi.org/10.1016/j.tim.2015.07.008>

[2] Li, B. *et al.* Proc. Natl. Acad. Sci. U.S.A. 2010, 107 (23) 10430–10435. <https://doi.org/10.1073/pnas.0913677107>

#### P-SMNP-017

##### **Identification and Characterization of Novel Metallophores from *Nostoc Cyanobacteria***

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Metallophores are specialized metabolites secreted by microorganisms to facilitate the acquisition of essential metallic elements under nutrient limitation or to detoxify the environment by chelating excess metals. These metal-binding properties can be exploited for diverse applications, including clinical use as a component of antimicrobial drug

conjugates [1]. Genome mining studies predicted that approximately 41% of cyanobacterial taxa contain at least one non-ribosomal peptide metallophore; however, only few have been thoroughly described, indicating that their full potential remains undiscovered [2]. Consequently, the genus *Nostoc* has revealed numerous cryptic hybrid polyketide synthase and non-ribosomal peptide synthetase (PKS/NRPS) biosynthetic gene clusters (BGCs) that are predicted to encode innovative metallophore pathways.

Following studies to uncover the specialized metabolism of *N. punctiforme* PCC73102, we selected the orphan metallophore PKS4 BGC for further investigation. This BGC exhibits a unique combination of tailoring enzymes, featuring a fatty acid acyl-CoA ligase domain, genes encoding for  $\beta$ -OH-aspartate and an expected modified histidine, and the presence of acylase and glucosyltransferase enzymes. Comparative genomic analysis led us to also select the *Nostoc* sp. KJVJ3 strain, which contains a similar BGC. The KJVJ3 strain showed robust production of a major compound under iron-deprived conditions, which we predict to be the PKS4 metallophore. In contrast, *N. punctiforme* did not show a major change in its metabolome under these same conditions. While several candidate peaks showed potential to be the PKS4 product, a clear major compound was not observed. The distinct lack of production in *N. punctiforme* suggests that the PKS4 BGC may be silenced or that the pathway is activated by alternative environmental triggers, indicating that other regulatory mechanisms are involved in their expression.

This research integrates a multi-omics platform to discover novel *Nostoc* metallophores. Current efforts focus on optimizing culture conditions, isolation, and structural characterization. Future experiments will elucidate the enzymatic details of this pathway. Understanding these biosynthetic specific steps may enable the awakening of dormant BGCs with analogous enzymatic mechanisms in related or distant microorganisms.

[1] Schalk, Isabelle. J. Nat. Rev. Microbiol. 2025; 23: 24–40

[2] Reitz, Z. L. et al. J BioRxiv. 2025; 10.1101/2022.12.14.519525

#### P-SMNP-018

##### Relieving Repression to Unlock a Silent Biosynthetic Gene Cluster in *Nostoc punctiforme*

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During the "Golden Age" of antibiotic discovery, the focus was mainly put on abundant antimicrobial compounds that were easily identified using bioactivity-guided isolation. However, as a result of increasing antibiotic usage, multi-resistant bacteria strains have developed, now posing a major threat to global health. In contrast, the discovery rate of novel compounds has slowed [1]. Our need for new antibiotics has not abated, but our strategy to discover these compounds has adapted with the emergence of genomic mining tools for the prediction of BGCs (biosynthetic gene clusters), increasing our capability to find compounds beyond the scope of "low-hanging-fruits". Cyanobacteria have long been known as producers of a diverse range of complex bioactive compounds, but many of their BGCs are silent under standard cultivation conditions. One organism that is

of particular interest in this adapted strategy is the terrestrial cyanobacterium *Nostoc punctiforme*, as there are still many secondary metabolite BGCs that remain orphaned.

The main aim of this project is the discovery and characterization of the product from the *pkS2* BGC in *Nostoc punctiforme*. The BGC is predicted to be negatively controlled by a TetR repressor, so that the BGC remains silent until its repression can be relieved. This can be achieved by finding either the correct external or internal stimuli. In this study, we will therefore employ different approaches to relieve this repression, including a genetic repressor binding site decoy approach, aiming to dilute effectively available repressor. Both transcriptomic analysis and metabolomic profiling will serve to elucidate the effectiveness of these approaches, furthering our understanding of negative regulation in *Nostoc*. We hope that the results from this project will prove valuable not only as a viable strategy for upregulating this particular BGC, but that they can be transferred to other similarly regulated BGCs that are present in the *Nostoc*, but also in different genera of microorganisms.

#### Reference

[1] Lewis K. The science of antibiotic discovery. *Cell* **181**, 29–45 (2020).

#### P-SMNP-019

##### Genome mining leads to the discovery of a new dioxolanone compound

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Fungal non-reducing Non-Ribosomal Peptide Synthetase (nrNRPS)-like enzymes represent an interesting and diverse class of secondary-metabolite-related enzymes. In contrast to canonical NRPS, they appear to be strictly monomodular and do not utilize amino acids as substrates. Instead, they typically catalyze the condensation of two identical aromatic  $\alpha$ -ketoacids forming small cyclic scaffolds<sup>1,2</sup>. The minimal domain structure of fungal nrNRPS-likes consists of an Adenylation (A), a Thiolation (T) and a Thioesterase (TE) domain. As these enzymes lack a Condensation (C) domain and cannot form peptide bonds, the TE domain is ultimately responsible for the cyclization of the bound substrates and the release of the final product<sup>1,3</sup>. All nrNRPS-like products reported to date derive from the aromatic amino acids tryptophan, tyrosine and phenylalanine, which implies a rather limited substrate scope<sup>3</sup>. Although a few compounds, such as Guignardic acid, which is formed from the  $\alpha$ -ketoacids of phenylalanine and valine, are suspected to be produced by nrNRPS-likes, no definitive proof for the incorporation of non-aromatic substrates has been reported to date<sup>3</sup>.

This study aimed to investigate the product of a yet uncharacterized nrNRPS-like enzyme, which was discovered through genome mining in an ascomycete. Expression of the NRPS-like enzyme in *Aspergillus oryzae* led to the discovery of a compound that, to our knowledge, has not been described previously. This yet unnamed dioxolanone shares

some structural similarities with Guignardic acid, displays branched side chains and completely lacks the phenyl groups that are typical for most nrNRPS-like products. Based on the observed structural characteristics and the number of carbon atoms, we propose that this compound could be formed through the cyclization of two molecules of  $\alpha$ -ketoisocaproic acid. If confirmed, this would be the first report of a fungal nrNRPS-like enzyme incorporating non-aromatic substrates.

1. Wieder, C. *et al.* Allantofuranone Biosynthesis and Precursor-Directed Mutasynthesis of Hydroxylated Analogues. *J. Nat. Prod.* **88**, 1191–1200 (2025).

2. Kalb, D., Lackner, G. & Hoffmeister, D. Fungal peptide synthetases: an update on functions and specificity signatures. *Fungal Biology Reviews* **27**, 43–50 (2013).

3. Wieder, C. *et al.* Characterisation of ascocorynin biosynthesis in the purple jellydisc fungus *Ascocoryne sarcoides*. *Fungal Biol Biotechnol* **9**, 8 (2022).

## P-SMNP-020

### Developing a heterologous production of azaphilone colourants in *Aspergillus niger*

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Colour is an immanent attribute of almost all items of our daily live. However, most colourants used are of synthetic origin. Due to political bans and social rethinking, the demand for natural alternatives has been growing.

Fungi produce a wide range of secondary metabolites (SMs), potentially serving as natural colourants in various industries<sup>1</sup>. In contrast, parallel production of mycotoxin, a relatively slow growth rate or SM production just under specific, potentially unfavourable, conditions, make them irrelevant for an industrial production. A solution is the shift of production into an industrially established heterologous host, i.e. *Aspergillus niger*. *A. niger* has proven to be an efficient heterologous producer for non-ribosomal peptide- based SMs with high titers<sup>2</sup>. Therefore, we postulate that *A. niger* should be capable to provide high quantities of polyketide precursors, rendering it an optimal choice for heterologous gene cluster integration.

Given the capacity of *A. niger* to produce diverse polyketides<sup>3</sup>, we aim to establish *A. niger* as a heterologous producer of azaphilones, a promising class of natural colourants within the yellow to red colour spectrum, from the genus *Monascus*. Therefore, a refactoring of the *Monascus* azaphilone biosynthetic gene cluster (BGC) with an iterative integration approach for all genes encoding catalytic enzymes in the respective BGC was applied. Gene expression is validated on transcriptomic as well as proteomic level while product and intermediate formation is analysed by mass spectrometric methods.

Thereby we were able to observe heterologous production of polyketide based azaphilone core structures in *A. niger*. For the heterologous production native promoters of the azaphilone BGC under control of the native transcription factor were used, showing very strong expression in *A. niger*. Gene expression and product formation is benchmarked

against optimal production conditions for *Monascus* species. Results will be presented accordingly.

1. Kalra, R., Conlan, X. A. & Goel, M. Fungi as a Potential Source of Pigments: Harnessing Filamentous Fungi. *Front Chem* **8**, 369 (2020).
2. Richter, L. *et al.* Engineering of *Aspergillus niger* for the production of secondary metabolites. *Fungal Biol Biotechnol* **1**, 4 (2014).
3. Nielsen, K. F., Mogensen, J. M., Johansen, M., Larsen, T. O. & Frisvad, J. C. Review of secondary metabolites and mycotoxins from the *Aspergillus niger* group. *Anal Bioanal Chem* **395**, 1225–1242 (2009).

## P-SMNP-021

### Influence of cellulose as a solid particle on diverse *Streptomyces* spp. growth, morphology and product spectrum

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Microorganisms have long served as a source of medically and biotechnologically relevant natural products. Within this context, Actinomycetes, and in particular the genus *Streptomyces*, are of major importance, producing roughly 80% of currently known natural-product antibiotics. However, industrial production remains challenging due to their filamentous cell morphology. In submerged cultivation, *Streptomyces* form macromorphologies ranging from dispersed mycelia to clumps and compact pellets, and the morphology that favors optimal secondary metabolite formation can vary substantially between strains. Consequently, controlled morphology engineering has become a key strategy in strain and process development.

Particle-enhanced cultivation represents one such approach. Yet commonly applied particles, such as glass beads or talc, can complicate downstream processing and raise environmental sustainability concerns. To address these limitations, we investigate cellulose as a renewable, biodegradable microparticle capable of modulating morphology in submerged *Streptomyces* cultures.

We assess the impact of cellulose addition on growth behavior, macromorphology, and secondary metabolite profiles across 13 *Streptomyces* strains. Because cellulose interferes with scattered-light-based biomass monitoring, we further evaluate flavin autofluorescence (Ex 450 nm / Em 520 nm) as an alternative method to determine initial, substrate-unlimited growth rates. Morphological responses are quantified by comparing pellet feret diameters with and without cellulose, while LC-MS/MS metabolomic analyses are used to identify conserved shifts in metabolite production.

This work demonstrates that cellulose can act as an environmentally friendly morphology-modifying agent and highlights autofluorescence-based monitoring as a viable strategy in particle-containing bioprocesses. Together, these findings support the development of more sustainable and controllable cultivation processes for natural product-producing *Streptomyces*.

## P-SMNP-022

### **Pseudotetraivprolides from *Pseudomonas entomophila* provide insight into the biosynthesis of detoxin/rimosamide-like anti-antibiotics**

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Novel variants of known natural product (NP) classes can provide valuable insights into their biosynthesis, mechanisms of action, and potential as drug leads across the entire class. Here, we describe a novel member of the widespread detoxin/rimosamide-like (DRL) natural products, named pseudotetraivprolide, produced by *Pseudomonas* strains. Pseudotetraivprolide exhibits the characteristic DRL-activity of protecting *Bacillus cereus* against the antibiotic blasticidin S. Through the generation of multiple deletion and complementation mutants, heterologous expression experiments, identification and structure elucidation of several derivatives, chemical synthesis of main derivatives, enzymatic characterization of individual biochemical steps, and detailed homology modelling of enzyme complexes, we elucidated key aspects of its biosynthesis. Our findings demonstrate that the primary metabolism-derived malonyl CoA:ACP transacylase (FabD) functions as a *trans*-AT in the biosynthesis pathway. Furthermore, we suggest an order for all late-stage modifications and assign a function for the three conserved hypothetical proteins PipDFG acting as last-step acetylation complex responsible for stabilization and activation of the final product.

## **Industrial microbiology**

### P-IM-001

#### **Development of an Indirect Immunofluorescence Assay for Canine Distemper Virus Antibody Surveillance in Ghanaian Dogs**

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**Introduction:** Canine distemper virus (CDV) is a highly contagious morbillivirus that poses a major threat to domestic dogs and diverse wildlife species. Despite its global significance, epidemiological data remain scarce in many regions, particularly in Africa, limiting effective control strategies. Addressing these gaps is crucial for understanding the true burden of canine distemper and informing effective control interventions and vaccination strategies.

In this study, we established an indirect cell-based immunofluorescence assay (IFA) to generate serological data in Ghana, where there have been no previous reports.

**Methods:** CDV viral RNA extracted from a PCR-positive Ghanaian dog was reverse transcribed, cloned into a mammalian vector, and transfected into Vero-B4 cells to generate recombinant proteins. The expression of viral proteins provided native antigenic targets for antibody

detection. This approach allows sensitive and specific measurement of host antibody responses while reducing reliance on purified viral protein preparations. To demonstrate the assay's utility and generate data on past exposure through IgG detection, we applied it to cross-sectional sera from 700 unvaccinated domestic dogs from Northern and Southern Ghana, and additionally checked for cross-reactivity with human sera.

**Results:** The IFA revealed an overall CDV seroprevalence of 2.74% (95% CI: 1.78–4.19), with higher exposure in Northern Ghana (5.67%) compared to the South (0.70%). A mean endpoint dilution titre among positive sera was 640 (range: 160–5120), indicating robust immune responses in seropositive dogs. Risk factor analysis showed increased exposure among free-ranging dogs, younger age groups (1–2 years), and non-local breeds. Importantly, the assay exhibited cross-reactivity with human sera, reflecting conserved morbillivirus epitopes.

**Conclusion:** This study presents the first CDV seroprevalence data from Ghana and demonstrates a versatile IFA platform for monitoring morbillivirus exposure. Beyond the current application, the assay holds promise for broader adaptability to other wildlife species for emerging morbillivirus surveillance.

### P-IM-002

#### **Transformation of hard-to-transform fungi and bacteria - implications from membrane biology and applications for filaments and biofilms**

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**Introduction:** A wide range of microorganisms live on human skin or mucosa surfaces as commensalists or do only interact with humans accidentally. Examples include staphylococcus, toxic cyanobacteria or different species of microspora. Opportunistic bacteria inhabiting the skin will not inflict harm on their host unless virulence factors will be activated upon environmental signals. Antimicrobial peptides have long been discussed for their feasibility to replace antibiotics. Their capacity to not simply disrupt, but in some cases manipulate the membrane, can be used to transport cargo into a target cell. In collaboration with several partner work groups, BPP successfully transformed cyanobacteria and fungi that usually resist transformation. Not only does this open up the opportunity to externally manipulate the virulence phenotypes of aforementioned microorganisms. The experiments shed light on the similarities in protein-membrane interactions of different taxa of (micro)organisms.

**Project Goals:** Transformation can be limiting factor in many projects. Many microbial organisms – be they bacterial, fungal or algal – form filaments, spores or biofilms. In order to make these organismal setups utilizable for industrial processes, they need to be transformed. The goal of current BPP Bioprotide™ projects in filamentous cyanobacteria, ascomycetes and gram-positive bacteria is to successfully transform biologically protected forms of these organisms.

**Methods:** The BPP Bioprotide transformation was compared to standard transformation protocols for single cell and filamentous cyanobacteria, for protoplasts and mature ascomycetes and for certain gram-positive bacteria and their respective biofilms.



**Results:** In cyanobacteria, BPP Bioportide™ mediated transformation is especially efficient in single cell strains, also in type IV pili deficient mutants. In fungal systems, efficiency of BPP Bioportide™ mediated transformation in protoplasts is temperature dependent. For fungal filaments and biofilms, similar results are expected but are currently being under investigation.

**Summary:** Although, we are still currently working on verification of certain subsets of experiments, we can already conclude that certain BPP Bioportides™ were identified that are to be used in filaments and biofilms for certain microorganisms. Transformation efficiency is only dependent on the interaction between the cell penetrating domain and the cells' barriers, not on the nucleic acid cargo.

#### P-IM-003

##### **BIOFILMS in Orbit: Assessing Antimicrobial Copper Surfaces for Biofilm Prevention in Microgravity**

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Biofilms present significant challenges in various environments, including hospitals and industrial facilities, as well as space habitats. Biofilm formation can lead to microbial-induced corrosion, biofouling and blocked pipes, all of which pose a serious risk to the structural integrity of spacecrafts. Furthermore, biofilms can harbour opportunistic pathogenic microorganisms, which pose additional health risks to astronauts, especially given the compromised immune function they experience during space missions. One promising strategy to mitigate microbial biofilm formation is to apply antimicrobial surfaces. Copper, which has been recognised for its antimicrobial properties since ancient times, has recently received renewed attention thanks to advancements in surface modification techniques, such as Direct Laser Interference Patterning (DLIP), that enhance its antibacterial effectiveness. However, the unique conditions of space, characterised by altered fluid dynamics, mass transport and sedimentation in microgravity, necessitate further investigation into the complex interactions between microbial cells, their environment and surfaces in these conditions.

In response to this need, the "BIOFILMS"-experiment aimed to evaluate the effectiveness of copper-containing DLIP antimicrobial surfaces against bacterial adhesion and biofilm formation in conditions of reduced gravity. The BIOFILMS experiment comprised four separate flights to the ISS between August 2021 and May 2025. During these missions, the bacterial species *Staphylococcus capitis*, *Cupriavidus metallidurans* and *Acinetobacter radioresistens* were incubated on the different surfaces within the BIOFILMS hardware to ensure a controlled environment for attachment, growth and biofilm formation. Findings from the experiment suggest that reduced gravity does not negatively impact the

antimicrobial effectiveness of copper. Additionally, the influence of gravity on adhesion and biofilm formation on stainless steel reference surfaces appears to be negligible. These initial results suggest that antimicrobial strategies can remain effective in microgravity, paving the way for future space mission applications. Ultimately, the BIOFILMS results will provide valuable insights into selecting suitable surfaces and functionalisation techniques for reducing biofilm formation in spaceflight settings.

#### P-IM-004

##### **Detection of Shigatoxin-producing *E.coli* in flour – recap of a proficiency test**

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Shigatoxin-producing *E. coli* (STEC) pose a threat to human health, causing severe intestinal symptoms up to kidney malfunction and haemorrhagic uraemic syndrome (HUS). Despite the natural occurrence of STEC in ruminants and consequently in contaminated food derived from these animals, plant-based foods can also harbour STEC. In the last years, triggered by several STEC-outbreaks in northern America and France flour came into spotlight of research and diagnostic. Since efficient microbiological diagnostic procedures are a prerequisite to generate trustful and comparable data on prevalence, survival and contamination level of STEC in flour. As a reference laboratory, we conducted a proficiency test focussing on detection and isolation of STEC in flour to compare laboratory performance, generate knowledge and determine the efficiency of the used methods for this rather dry food matrix. We used a set of nine samples consisting of two times four samples inoculated approx. 59 CFU and 9 CFU per samples, respectively and one plain flour sample as negative control. Twenty-eight Laboratories analysed these samples by either a protocol according to national standard (64 LFGB, L 25.00-6) originally validated for fresh produce and/or other methods (like ISO/TS13136:2012). Overall detection of STEC leads to successful isolation in over 90% of samples. However, the limit of detection (LOD50) was calculated to be approx. 26 CFU per sample, whereas the method L 25.00-6 performed slightly better. Noteworthy, the usage of antibiotics and bile acids in the enrichment broth hindered STEC detection in the proficiency test. This implies that laboratories should consider method adaptation when investigating flour for STEC. Here methodological improvements like increased sample volume could lower the LOD, especially to detect the low contamination levels of STEC in flour. The further improvement of methods as well as method standardization are the ongoing next steps.

#### P-IM-005

##### **CO<sub>2</sub>-neutral co-culture of *Cupriavidus necator* and *Bacillus subtilis* for biopolymer production**

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Replacing fossil-based polymers with CO<sub>2</sub>-neutral biopolymers is a key step toward sustainable material production. Mixed microbial cultures (MMCs) offer a promising approach by combining distinct metabolic

capabilities within one system. In particular, the coupling of autotrophic carbon fixation and heterotrophic biosynthesis enables fully CO<sub>2</sub>-based polymer formation.

#### Goals:

This study aims to establish a defined co-culture of *Cupriavidus necator* and *Bacillus subtilis* for the CO<sub>2</sub>-neutral production of  $\gamma$ -polyglutamic acid ( $\gamma$ -PGA). The objective is to design a stable metabolic interaction in which an autotrophic strain provides a carbon substrate for a heterotrophic producer, thereby creating a synthetic consortium for sustainable polymer synthesis.

#### Methods:

A genetically engineered *C. necator* strain lacking the polyhydroxybutyrate (PHB) synthesis genes[1] grows lithoautotrophically on CO<sub>2</sub> and H<sub>2</sub>. The knockout redirects carbon flux toward the secretion of pyruvate as an overflow metabolite, particularly under nitrogen-limiting conditions. The secreted pyruvate serves as a carbon source for a tailored *B. subtilis* strain engineered for enhanced  $\gamma$ -PGA biosynthesis[2].

#### Results:

*B. subtilis* demonstrates robust growth on pyruvate as the sole carbon source. Under heterotrophic conditions, co-cultivation of both species does not significantly affect growth performance. In preparation for studies under autotrophic conditions, *B. subtilis* expressing GFP and *C. necator* expressing mCherry are being developed to enable fluorescence-based tracking of both species within the co-culture.

#### Outlook:

These findings establish the basis for a CO<sub>2</sub>-driven co-culture linking autotrophic carbon fixation to heterotrophic polymer synthesis. Future experiments will focus on assessing metabolic exchange and  $\gamma$ -PGA production under lithoautotrophic conditions to evaluate the system's potential for truly carbon-neutral biopolymer production.

#### References:

- [1] C. F. Budde, S. L. Riedel, L. B. Willis, C. Rha, A. J. Sinskey, *Applied and Environmental Microbiology* **2011**, 77, 2847-2854.
- [2] B. Halmschlag, F. Völker, R. Hanke, S. P. Putri, E. Fukusaki, J. Büchs, L. M. Blank, *Frontiers in Food Science and Technology* **2023**, Volume 3 - 2023.

#### P-IM-006

##### Trehalose lipid production by *R. erythropolis* CWB12

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Surface active compounds, composed of both hydrophilic and hydrophobic moieties, are synthesized by different organisms. They exhibit a variety of chemical structures, including glycolipids, lipopeptides, phospholipids, fatty acids,

and polymeric biosurfactants. Glycolipids, consisting of a carbohydrate head and a lipid tail, are one of the most extensively studied classes of biosurfactants. In addition to fungal biosurfactants such as sophorolipids, bacterial glycolipids such as rhamnolipids from *Pseudomonas* sp. are also known. In the phylum Actinomycetota, *Rhodococcus* is well reported to produce trehalose lipids, with trehalose occurring as the head group and acyl chains varying in number and length. These trehalose lipids are basic part of the outer mycolic acid layer, but are released into the medium under certain conditions. The use of biosurfactants as an alternative to synthetic surfactants offers significant advantages, as synthetic surfactants tend to be more toxic and pose environmental risks. Trehalose lipids are therefore promising candidates for applications in bioremediation, as well as for use as emulsifiers, foaming agents, and wetting agents.

The trehalose lipid production of *R. erythropolis* CWB12 was characterized using minimal media under nitrogen rich and limited conditions. Various carbon sources were tested to determine whether or not it produces trehalose lipids. Two assays were performed for trehalose lipid detection, first the emulsification activity assay and second the extraction of lipids in the supernatant and detection on the TLC.

It was observed that the production of biosurfactants by *Rhodococcus* is dependent on nitrogen availability, as 5-fold higher biosurfactant production of *R. erythropolis* CWB12 was observed under nitrogen limitation. Furthermore, the strain *R. erythropolis* CWB12 showed higher production of trehalose lipids, when reaching the stationary phase. The head group of the extracted lipid from *R. erythropolis* CWB12 could be verified as trehalose using GC-MS.

Understanding the factors that influence the production rate of trehalose lipids in *Rhodococcus* is an important milestone for potential applications. This study demonstrates the impact of the carbon sources and nitrogen availability on the production of biosurfactants and the production of trehalose lipids depending on the growth stage.

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#### P-IM-007

##### Virtual reality vs. video lecture: A randomised study on the influence of innovative hygiene training on learning success, practical relevance and microbiological kitchen hygiene

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**Background:** Many foods naturally contain microorganisms, such as bacteria, viruses and fungi. Improper storage, poor hygiene, and cross-contamination can quickly cause foodborne illnesses. Although simple preventive measures such as handwashing and cleaning utensils are highly effective, they are often neglected in daily life. Practice-oriented training methods, including virtual reality (VR), offer a promising approach to improving hygiene behaviour.

**Aim:** Our conceptually designed study investigates whether immersive virtual reality simulations are more effective than traditional video-based lectures in improving participants' microbiological literacy and reducing pathogenic contamination in domestic kitchens. The research will also assess the influence of individual characteristics on learning

outcomes, as well as the impact of different training formats on motivation and perceived safety regarding microbiological hazards.

**Methods:** At the beginning, microbiological swab samples will be collected from defined kitchen surfaces in participants' homes to determine initial contamination levels. On the training day, participants will complete questionnaires assessing their microbiological knowledge, personality traits, and psychological well-being. The intervention group will undergo practical VR training in a virtual kitchen, while the control group will receive a theoretical video lecture. Follow-up sampling and surveys will be conducted two weeks later to evaluate changes in hygiene behaviour and microbial contamination.

**Expected Outcome:** By comparing innovative VR training with traditional methods, this study will provide insights into the most effective approach for promoting sustainable hygienic practices. The results are expected to inform the development of future educational strategies in both domestic and professional settings, potentially contributing to a reduction in foodborne illnesses.

#### P-IM-008

##### **From Monocultures to Communities: a Multi-Species Platform for Phototrophic Biomanufacturing**

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Cyanobacteria are a versatile platform for sustainable bioproduction, combining photosynthetic efficiency with metabolic plasticity. To expand the performance of axenic cultures, we utilize "hybrid photosynthetic microbiomes," in which selected native phototrophs are replaced with engineered strains. This approach preserves the ecological integrity of the community while introducing synthetic functions, providing a robust platform for phototrophic biotechnologies.

Our model system uses *Synechocystis* sp. PCC 6803 PPT1, previously optimized to produce polyhydroxybutyrate (PHB) up to 60% of cell dry weight under photoautotrophic conditions [1]. While these titers are promising, monocultures face robustness and stability limitations for large-scale application. We introduced PPT1 into a stable, cyanobacteria-rich microbiome previously enriched under nutrient-limiting conditions, likely promoting community members to produce carbon storage polymers as well, making it an ideal system to test [2].

In this hybrid community, we observed enhanced resilience to abiotic fluctuations, likely due to heterotrophic partners mitigating oxidative stress, and increased metabolic flexibility, with PHB production occurring not only under light but also in the dark upon acetate addition. In this system, PPT1 functions as a metabolically dominant "leading species", orchestrating community dynamics and triggering PHB biosynthesis in supporting members in response to metabolic cues.

This work highlights a paradigm shift in metabolic engineering from single-species optimization to multi-species design, demonstrating how enhanced community resilience and metabolic flexibility enable scalable and robust phototrophic biomanufacturing.

#### References:

[1] Koch, M., Bruckmoser, J., Scholl, J. et al. Maximizing PHB content in *Synechocystis* sp. PCC 6803: a new metabolic engineering strategy based on the regulator PirC. *Microb Cell Fact* 19, 231 (2020).

[2] Altamira-Algarra B, Lage A, Meléndez AL, Arnau M, Gonzalez-Flo E, García J. Bioplastic production by harnessing cyanobacteria-rich microbiomes for long-term synthesis. *Sci Total Environ*, 176136 (2024).

#### P-IM-009

##### **Impact of commercially available DNA extraction kits on the microbiota composition of leafy greens**

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The popularity of ready-to-eat (RTE) salads has increased significantly in recent years, reflecting toward nutritious, healthier and more convenient food products. Fresh RTE leafy greens harbor high counts of bacteria and can also be contaminated with foodborne pathogens. The high moisture content, along with a pH of 6.0–7.0, makes the products highly perishable and promotes the growth of foodborne pathogens. This study aimed to evaluate the effects of different DNA extraction methods on (i) DNA yield and (ii) microbiota composition across various RTE leaf species at retail level. In parallel, (iii) bacterial load in all leaf samples were analyzed using culture-dependent methods.

A total of 38 packaged RTE leaf samples were analyzed using two DNA extraction kits from Macherey-Nagel (GmbH & Co. KG): the NucleoSpin Tissue Mini Kit (for DNA isolation from cells and tissues) and the NucleoSpin Plant II Mini Kit (for plant material). Additionally, ten samples were processed using the DNeasy PowerSoil Pro Kit (Qiagen GmbH). DNA yield was quantified fluorometrically with the Qubit assay (Thermo Fisher Scientific Inc.). Purified DNA samples were subjected to amplicon-based metagenomic sequencing using the Illumina platform (targeting the bacterial 16S rRNA gene, V3–V4 region). Sequencing was performed by Microsynth (AG). In parallel, the aerobic mesophilic count (AMC) and presumptive *Bacillus* (*B.*) *cereus* counts were determined for each batch. For DNA extraction, 50 g of lettuce per sample were diluted 1:5. Pellets obtained after centrifugation were subsequently used for DNA isolation. AMC and presumptive *B. cereus* counts were determined from 10 g of sample material.

DNA yields ranged from 3.9 ng/μL (arugula) to 73.5 ng/μL (iceberg lettuce) with the NucleoSpin Tissue Kit. The NucleoSpin Plant Kit gave higher yields in lamb's lettuce (24.3–87.1 ng/μL). The DNeasy PowerSoil Pro Kit produced the highest concentrations, consistently exceeding 20 ng/μL for all leaf samples and reaching up to 396 ng/μL in arugula.

Total aerobic mesophilic counts ranged from 6.0 to 8.6 log CFU/g, but no correlation with DNA yield was observed. Presumptive *B. cereus* counts were low across all samples ( $\leq 2.0$  log CFU/g).

In summary, the DNeasy PowerSoil Kit demonstrated consistently high DNA yields for all examined leafy varieties. Nevertheless, a conclusive assessment of its suitability for

bacterial DNA extraction remains pending, as the comparative evaluation has not yet been completed.

## P-IM-010

### Investigation of Expression-Guided Feeding Strategies for Bioprocess Development

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#### Introduction

Fed-batch fermentation is the most widely applied cultivation strategy in industrial biotechnology. Feedforward control approaches rely on *a priori* fixed feeding strategies. However, such simple and robust strategies lack real-time feedback from the current state of the process. In contrast, feedback-based control strategies adjust the feed based on real-time process parameters such as dissolved oxygen or pH as indirect measure of cellular activity. Given the complex interplay between bioreactor conditions and cellular behavior, this study investigates expression-guided feeding strategies as direct measure of cellular states. (Chmiel et al., 2018)

#### Goals

This project aims to design and validate *Corynebacterium glutamicum* reporter strains to monitor cellular states during fed-batch fermentation. Transcriptional fusions of target promoters to fluorescent reporter genes allow the semi-continuous monitoring of stress responses, nutrient depletion and oxygen limitation. The goal is to utilize the resulting expression patterns for tailored expression-guided feedback feeding strategies.

#### Methods

Three plasmid-based reporter systems are characterized in bench-top bioreactor experiments under defined substrate and oxygen gradients: (i) the native promoter regions of *sigH* and *sigB* as general stress response targets (Pátek and Nešvera, 2011), (ii) *ldhA* promoter for monitoring of overflow metabolism (Bott and Niebisch, 2003) and (iii) the *nar* promoter to analyze intracellular oxygen limitation (Nishimura et al., 2011).

#### Results

Ongoing experiments characterize the reporter strains under different feeding regimes mainly substrate gradients and dissolved oxygen profiles to evaluate reporter system kinetics and dynamic ranges. The goal is to identify reporter systems that detect and respond to cellular deviations from optimal growth or production conditions within minutes to hours, enabling feed rate adjustments that harmonize the microbial population, minimize stress, and maintain cells in a productive physiological state.

#### Conclusion

The aim of this study is to lay the groundwork for expression-guided feeding control by integrating molecular biosensors into bioprocess control.

## References

Bott and Niebisch 2003; DOI: 10.1016/S0168-1656(03)00144-5

Chmiel et al. 2018; DOI: 10.1007/978-3-662-54042-8

Nishimura et al. 2011; DOI: 10.1099/mic.0.044552-0

Pátek and Nešvera 2011; DOI: 10.1016/j.jbiotec.2011.01.017

## P-IM-011

### Online monitoring of methanol as a substrate in lactic acid fermentations of the methylotrophic yeast *Ogataea polymorpha*

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#### Introduction

Methanol as a substrate for microbial processes opens up opportunities for a land-free alternative in the biotechnological sector through its production from CO<sub>2</sub> and green energy. However, microbial toxicity to methanol limits the implementation of efficient feeding regimes in reactor level.

#### Goals

This project aims to explore fermentations of the methylotrophic yeast *Ogataea polymorpha* for the production of lactic acid from methanol using novel Volatile Organic Compounds (VOC)-based feeding to avoid over and underfeeding.

#### Materials & Methods

Cultivations of *O. polymorpha* strains were carried out in 2-L reactors for the production of lactic acid from methanol in semi-defined medium. The fermSYS Buddy system consisting of a sensor for VOC, an associated pump and a web-based implementation software was used for the Internet of Things (IoT)-based regulation of the methanol feeding to the reactors. Fermentation outlines were analyzed offline through OD600 and HPLC for biomass monitoring and metabolite quantification.

#### Results

The cultivation of *O. polymorpha* was successfully established in reactor level for the production of lactic acid from methanol in a fed-batch operation mode. Manual feedings with adapted high cell density inoculum followed similar trends as those observed in shake-flasks. However, periods of starvation and sudden stress were present with each feeding, possibly inducing metabolic changes in the cells reflected in periods of adaptation following each bolus where cell activity was reduced. The implementation of the fermSYS Buddy system allowed for the smart control of the

methanol concentrations in the reactor, thereby minimizing the concentrations that might cause toxic stress to the cells, and further avoiding the periods of starvation observed after methanol depletion during the manual feeding regime. Methanol uptake rates and cell growth rates were thereby observed to be superior in the VOC-based feeding strategy compared to discrete bolus feeding.

## Summary

This study represents the advantage of the implementation of an IoT-based sensor-and-effector system for the monitoring and administration of methanol in lactic acid fermentations of *O. polymorpha* in order to avoid the typical "Feast-Famine" cycles connected to common bolus fed-batch processes, thereby improving the performance of the microbial conversion of methanol to lactic acid.

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## Open Topics

### P-OT-001

#### How much microbiology is taught at schools in Germany? Analysis of school curricula

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Bacteria and other microorganisms belong to the oldest and most successful living organisms on our planet affecting all parts of our daily life. However, children and also older persons associate mainly dirt and disease with microorganisms. The beneficial sites of them are usually neglected. These alternative conceptions can lead to germophobia, excessive hygiene, inappropriate antibiotic use, and an impediment of potential benefits (e.g., genetic engineering). Therefore, microbiologists demand a stronger microbiology literacy to deal with the challenges of the 21st century. To develop a microbiology literacy, microbiology has to be a substantial part of the school curricula. However, due to the federalism in Germany the 16 single federal states have different school curricula with different microbiological content. This study aims to analyse the curricula (middle, secondary, comprehensive, and high schools) of all 16 federal states in terms of microbiological content. The results show that negative aspects of microorganisms as pathogens are prevalent in all curricula. However, the curricula from Bavaria, Mecklenburg-Western Pomerania, Rhineland-Palatinate, and Saxony-Anhalt consider positive aspects of microorganisms such as biotechnological or ecological aspects, proving that microbiology can be a substantial part of biology lessons in all competence areas. The release of new educational standards for secondary schools in 2024 presents an opportunity to strengthen the presence of microbiology in school curricula.

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### P-OT-002

#### Pre-service teachers' ideas about microorganisms and microbiological concepts

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Ideas about microorganisms have been investigated in pupils and the general population since the early 1950s. However, the ideas haven't changed much over time independent of

the investigated country. Pupils (and also older persons) associate microorganisms mainly with disease, dirt, and food spoilage. Positive aspects of microorganisms such as biotechnological or ecological aspects are usually neglected, which can lead to germophobia, excessive hygiene, inappropriate antibiotic use, and an impediment of potential benefits (e.g., genetic engineering). Therefore, microbiologists demand a stronger microbiology literacy to deal with the challenges of the 21st century. As teachers are the intermediary of microbiology literacy, they need a profound knowledge about microbiology for proper teaching this subject. Beside factual knowledge, own ideas about the taught subject have to be considered as it has been shown that these ideas affect instruction and learning. However, pre-service teachers' ideas about microorganisms haven't been investigated so far. Therefore, we asked pre-service biology teachers in different phases of their studies (undergraduate/master) for their ideas about microorganisms and microbiological concepts using open questions, drawings, and concept maps. We can show that ideas about microorganisms are varying in the different study-phases. Undergraduate students show similar ideas about microorganisms as pupils (e.g., microorganisms as little insects). Master-students have more profound ideas (e.g., drawing of a typical bacterial cell). However, for both groups we can show that some microbiological concepts – especially technological applications – are often neglected. Our results open possibilities for improving microbiological education for pre-service teachers.

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### P-OT-003

#### New approaches to Science Communication – Insight from Berlin

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In the light of ever faster dissemination of information (and misinformation), the perception of science is changing in the public eye. At the same time, societies are facing complex global and regional challenges that demand public understanding and trust in science. Against this backdrop, Berlin is experiencing a surge in new science outreach formats, largely driven by its many international scientists who are both establishing original formats and bringing new and innovative and contemporary outreach and science communication formats to Berlin's stages. Laughing Matter, for instance, puts on a monthly improvisational comedy show inspired by a scientific talk, attracting non-researchers and researchers alike. The show first premiered as part of the Berlin Science Week in November of 2023 and has become one of the best selling shows at one of the main English-speaking improv comedy venues in the German capital, the Comedy Café Berlin. To date, Laughing Matter is the only group to put on a science comedy show of this kind. Soapbox Science, a format first established in London in 2011, showcases research conducted by women and non-binary scientists, bringing it to school children and young adults, thereby increasing the visibility of women and non-binary researchers in STEM. Pint of Science (first established in 2012 and born out of King's College London) is an international event that takes place in over 450 cities globally, Berlin being one of the more than 20 participating German cities. In the Berlin version of the event, scientists are invited into pubs to present their original research to a lay

audience in either English or German. Lastly, Sceptics in the Pub, one of the newest formats to appear on the Berlin science outreach horizon that is, again, based on a format first inaugurated in London in 1999, tasks scientists with busting myths, pseudoscientific constructs, and deconstructing conspiracy ideologies using science, empirical evidence, and critical thinking. This format also takes place in pubs, making it accessible to the widest possible audience. By introducing these innovative and contemporary formats to Berlin's science outreach landscape, science communicators are ensuring that knowledge, data literacy and critical thinking skills are disseminated through multiple voices and perspectives, making it more diverse, accessible, inclusive, and relevant to all communities.

#### P-OT-004

##### **Alg4Nut: Algae in diary cow nutrition – microbiological and functional fundamentals**

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Algae receive increasing attention as source for sustainable biomass since as marine organisms they have the potential to reduce need for land area in food and feed production [1]. Despite this, local production in Germany remains underexploited. Recent studies reported that the use of algae as feed additive for ruminants can have beneficial effects on animal health and also reduce methane emission. These effects are attributed to algae-specific polysaccharides, unsaturated fatty acids as well as to secondary metabolites [2]. Macro-algae *Ulva* sp. and *Porphyra* sp. can be cultivated in Mecklenburg-Western Pomerania in the Baltic Sea. Both contain a high content of their specific polysaccharide ulvan respectively porphyran and are rich in protein, which makes them applicable as regionally produced feed additives for dairy cows. Since ruminants rely on their microbiome for the breakdown of feed components and fermentation to SCFAs, microbial breakdown of algae in the rumen is crucial for the release of bioactive compounds and subsequent effects.

The joint project Alg4Nut combines a feasibility study for algae cultivation in MV, feeding trials with dairy cows and accompanying microbiological investigations: (i) To assess the influence of algae on the rumen microbiome, comprehensive metagenomic, transcriptomic, and proteomic approaches will be utilized to elucidate the metabolic pathways involved in degradation of algal polysaccharides. (ii) Another approach focuses on isolating and characterizing key algae-utilizing bacteria from rumen and faeces of experimental animals. (iii) In parallel, genetically accessible model strains will be used for the production of new polysaccharide degrading enzymes and investigated as platform organisms for the biotechnological utilization of algal polysaccharides.

Alg4Nut aims at establishing a basis for using sustainably and locally produced algal biomass as feed additive for dairy cows. Understanding the underlying microbiological processes in the animals' digestive tract thereby plays a crucial role. Combined -omics, cultivation approaches and biochemical analysis will reveal metabolic pathways involved in conversion of algal components and production of health promoting compounds.

[1] FAO and WHO (2022) *Food Safety and Quality Series*.

[2] McGurrin et al. (2023) *J Animal Sci Biotechnol*.

#### P-OT-005

##### **Investigating antagonistic bacteria as biocontrol agents against *Erwinia amylovora* infection**

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Plant pests are one of many challenges encountered in commercial pome fruit production. Major threat is the phytopathogenic bacterium *Erwinia amylovora*, the causative agent of fire blight disease. Within the EU only a few plant protection products such as copper compounds or yeast-based biocontrol agents are registered against fire blight and those available are only effective before infection is established. Once the pathogen invades the host, only pruning or clearing of infected plants are effective in containing the infection (1). Thus, the research project ABBA (apple, bee, bacteria, antagonist) onfire (fire blight) aims to investigate new treatment approaches, based on the application of naturally tailocin-producing antagonists (TPAs) (2).

Tailocins are phage-tail-like bacteriocins. They are naturally produced by multiple bacteria and prevent growth of kin strains, which compete in the same biological niche. Similar to phages, tailocins are highly specific and efficient. Binding to the target membrane leads to the disruption of the proton motive force, which kills the cell (3).

In three interdependent working packages the project focuses on the identification, characterization as well as the risk assessment and non-target effects of potential *E. amylovora* antagonists. The project thereby aims to provide a thorough evaluation of TPAs capability and present an alternative to conventional fire blight control measures.

Here we present data on the identification of potential antagonists from various source materials and the investigation of TPAs persistence and efficacy against *E. amylovora* *in vitro* and on host plant material. Furthermore, this poster provides an outlook for the application of bacterial antagonists in field trials.

1. Sundin G.W., Castiblanco L.F., Yuan X., Zeng Q., Yang C.H. Bacterial disease management: challenges, experience, innovation and future prospects: Challenges in Bacterial Molecular Plant Pathology. *Mol Plant Pathol*. 2016;17(9):1506-18.
2. Becker M., Becker Y., Werner S., Schrader G., Wensing A., Erler S., et al. ABBAonFire – An innovative treatment approach based on natural tailocins for fire blight control in organic pome fruit orchards. *Journal für Kulturpflanzen* 2024;76(2):88-93.
3. Patz S., Becker Y., Richert-Pöggeler K. R., Berger B., Ruppel S., Huson D.H., et al. Phage tail-like particles are versatile bacterial nanomachines – A

#### P-OT-006

##### **Bacterial cell wall biosynthesis as a target for novel antibiotic compounds**

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Antimicrobial resistance (AMR) represents a major global health threat, as treatment options for infections caused by (multi-)drug-resistant pathogens are becoming increasingly limited. Most antibiotics currently in clinical use are derivatives of molecules discovered during the early to mid-20th century, underscoring the urgent need for novel compounds with new modes of action or molecular targets (1).

The bacterial cell wall biosynthesis pathway, first exploited for antibiotic intervention, remains one of the most successful targets despite the emergence of resistance. Polyketide antibiotics constitute a structurally diverse class of natural products with potential to provide novel scaffolds for targeting this essential pathway. Their structural diversity and modular biosynthesis render them a promising source for next-generation antibiotics with unprecedented mechanisms of action. Whole-cell experiments using *Bacillus subtilis*- and *Staphylococcus aureus*-based bioreporters confirmed interference with cell wall biosynthesis, and subsequent biochemical analyses elucidated the compounds' mode of action.

#### P-OT-007

##### **Integrative imaging and processing technologies for tackling complex biological samples**

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Fluorescent imaging of biological samples faces many challenges. Complex environments and inherent imperfections often give rise to aberrations and background which compromise image quality. In this presentation, we offer a comprehensive solution designed to overcome these obstacles, extending its benefits from confocal to stimulated emission depletion (STED) super-resolution microscopy. At the heart of our approach is the integration of adaptive optics (RAYSHAPE) with array detection (MATRIX) and our new deconvolution technology (TRUESHARP), uniting superior optics with innovative computational algorithms. Key features include:

Aberrations from sample inconsistencies or inadequate immersion are dynamically corrected by RAYSHAPE. Together with spatial light modulators (SLM) and versatile objective lenses, RAYSHAPE's adaptive optics ensures the generation of high-quality raw data. TRUESHARP then identifies and removes noise and background without making any assumptions about the sample's properties and without introducing artifacts by regularization or overfitting.

MATRIX complements this process by directly measuring background and in-focus signals, providing information that improves TRUESHARP's deconvolution accuracy (Figure 1).

This significantly increases optical sectioning and signal-to-background ratio and enables high-resolution 2D and 3D STED microscopy on thick samples and densely labeled structures. Furthermore, TRUESHARP uses temporal data from TIMEBOW fluorescence lifetime imaging to remove background.

The integration of TIMEBOW with STED introduces lifetime modulation. By using the spatial information encoded in fluorescence lifetimes, TRUESHARP offers an additional boost in resolution. Furthermore, the implementation of low-power STED illumination, combined with FLEXPOSURE3,4 adaptive illumination technology, minimizes phototoxicity by optimizing light exposure. This approach protects live and sensitive samples and facilitates long-term and volumetric imaging.

Taken together, our multi-layered approach boosts sharpness, clarity, and resolution in confocal and STED super-resolution microscopy, delivering superior image quality. Thus, empowering the advanced exploration of living samples, complex tissue and organoids.

[1] Hell S.W., Wichmann, J., 1994. Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. Opt. Lett. 19, 780 [2] Saal S.J., Hell, S.W., Jakobs, S., 2017. Fluorescence nanoscopy in cell biology. Nat Rev. 18

[3] Heine et al., PNAS 114 (37), 9797-9802 (2017)

[4] Staudt et al., Opt Express 19(6), 5644-5657 (2011)

#### P-OT-008

##### **Potential of mycelium cultures from the basidiomycete *Fomes fomentarius* to produce sustainable composite materials**

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The building sector, responsible for approximately 40% of anthropogenic CO<sub>2</sub> emissions, faces growing pressure to meet sustainability requirements. Fungal biotechnology offers a compelling contribution by enabling the replacement of several fossil- or mineral-based materials with biologically derived mycelium composites. In particular, the medicinal polypore *Fomes fomentarius* has emerged as a promising candidate for producing bio-based composites from renewable lignocellulosic biomass, which are effective as carbon sinks and can be recirculated due to their biodegradability.

*F. fomentarius* can efficiently grow on a variety of agricultural by-products, yielding lightweight composite materials with properties that challenge conventional, petroleum-based materials such as expanded polystyrene (EPS). Uncompressed composites made from *F. fomentarius* and hemp shives show comparable compressive strength to EPS, while performing better at higher strain levels. Their



thermal conductivity ( $0.044 \text{ W} \cdot (\text{m} \cdot \text{K})^{-1}$ ) and water vapour permeability (1.72) align with common plant-based insulators. Heat-pressing further transforms these composites into rigid particleboards, offering an alternative to conventional wood-based particleboards with synthetic additives.

Low-vacuum scanning electron microscopy reveals that, when enclosing insulating mycelium composites between load-bearing clay or hardened concrete layers for interior construction, the hyphae can form stable interfaces with these materials. Moreover, extrusion-based additive manufacturing of living fungal mycelium enables the precise fabrication of complex, lightweight materials, while 3D printing of patterned biopolymers can be used to reinforce fungal materials. In addition, X-ray microcomputed tomography provides high-resolution imaging of the fungal hyphal growth on and within the substrate, offering insights into the microstructural characteristics that contribute to the material's strength and resilience.

As research continues to refine these materials and address scalability challenges, fungal-based composites have the potential to significantly reduce fossil fuel dependency and contribute to a more sustainable building sector.

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#### P-OT-009

##### NFDI4Microbiota: Making Microbiology Data FAIR and Open

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NFDI4Microbiota is a consortium within the DFG-funded German National Research Data Infrastructure (NFDI) that focuses on managing, standardizing, and sharing microbiological and microbiome-related research data. Its mission is to enhance data accessibility, interoperability, and reproducibility for researchers working with microbiota across diverse domains, including health, agriculture, and the environment.

The vision of NFDI4Microbiota is to enable microbiology researchers, including those in bacteriology, virology, protistology, mycology, and parasitology, to translate research data into a deeper understanding of microbial species and their molecular interactions. To achieve this, the consortium aims to serve as central hub for supporting the microbiology community through access to data, analysis services, data and metadata standards, and training. NFDI4Microbiota actively promotes the FAIR (Findable, Accessible, Interoperable, and Reusable) principles and Open Science.

NFDI4Microbiota supports microbiology researchers throughout the entire research lifecycle (from study design to data publication) by providing tools, infrastructure, and expert guidance that ensure data are FAIR and reproducible. The consortium develops and offers computational infrastructure and analytical workflows for storing, accessing, processing, and interpreting diverse microbiology-related data types. Secure, scalable object storage combined with guided metadata templates ensures consistent recording of technical and biological metadata. For data analysis, NFDI4Microbiota provides standardized, containerized workflows, software tools, and access to curated databases. A comprehensive Knowledge Base offers training materials

and best practices, while a dedicated helpdesk provides personalized consultation on all aspects of data management and analysis. In addition, NFDI4Microbiota organizes training courses covering topics such as metabolomics, programming, research data management, and electronic lab notebooks. To engage with early-career researchers, the consortium established an ambassador program that helps identify community needs. All relevant information and services are available through the NFDI4Microbiota web portal: <https://nfdi4microbiota.de/>

In conclusion, NFDI4Microbiota is building a robust data infrastructure to advance microbiome research across disciplines, following its guiding principle: Making Microbiology Data FAIR and Open.

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#### P-OT-010

##### Applicability and efficacy of different disinfectants for the disinfection of personal protective equipment

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In scenarios of biological or chemical threats it is vitally important to ensure a fast and effective disinfection of personal protective equipment (PPE). To this end, disinfectants must be sufficiently effective to inactivate highly pathogenic microorganisms, in particular environmentally resistant *Bacillus* spores, as well as chemical warfare agents. Currently 2% Wofasteril®, a liquid peracetic acid product, is used by fire brigades in Germany against biological threats. Although this product is highly effective in the disinfection of PPE, the application has several disadvantages such as a pungent odor, storage instability, and material corrosion. In the BBK-funded project BIOCHEM-PSA, we therefore have tested different disinfectants with chlorine or peracetic acid as active component for their sporicidal efficacy against *Bacillus* spores on PPE, to be used as possible alternatives. These results should be compared with the data obtained for chemical agents at the end of the project.

A standardized procedure, dubbed "covering with mechanical action", was used to test the efficacy of disinfectants on PPE. *Bacillus thuringiensis* spores were used as a surrogate for *Bacillus anthracis*. Spores were used as contaminants, because they show a much higher resistance against disinfectants than vegetative bacteria. Since short disinfection times are highly recommended, these have been set to up to 10 minutes. Additionally, the influence of temperature variation as well as protein contamination was analyzed to achieve sufficient reduction by 5 - 6 log levels.

So far, two disinfectants were tested regarding the disinfection of PPE material. A concentration of 4 % chlorine in HAZ-TAB Tablets with an exposure time of 10 minutes did not reduced *B. thuringiensis* spores sufficiently. The same effect was observed for different temperatures. However, a concentration of 6 % chlorine could reduce spores to  $\geq 5$  log levels in 10 minutes. A concentration of 1 % peracetic acid in the granulate ULTRASOL® ACTIVE did not reduced *B. thuringiensis* spores sufficiently in suspensions tests. The disinfectant was therefore not tested on PPE materials.

Thus, one of the disinfectants that has been analyzed so far was tested and found to be sufficiently sporicidal against bacterial spores. It could therefore be considered an



alternative for decontaminating PPE in the field. Other disinfectants containing chlorine or peracetic acid will be tested during the course of the project.

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