ABSTRACT BOOK

DMS ANNUAL CONGRESS

2025

10 NOVEMBER 2025

MARMORHALLEN

COPENHAGEN · DENMARK

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Morning Keynote Speaker

Talk Title: Rediscovering the Drivers of Chronic Infections

Thomas Bjarnsholt[1]

[1]*Department of Clinical Microbiology, Rigshospitalet, & Department of Immunology and Microbiology, University of Copenhagen*

A chronic infection is a complex medical problem. Biofilms have over the last four decades been associated with chronic infections across the health field. It is believed that the bacterial aggregation into biofilms is the cause of the recalcitrance to antibiotic and the host defense in chronic infections. On the other hand, acute infections are supposedly caused by planktonic bacteria. Biofilms are easy to grow in the laboratory and biofilm specific mechanisms have been elucidated. Despite of this we still have difficulties both diagnosing and treating chronic infections, the biofilm explanation does not seem to alleviate the complex problem. A century ago, H. L. Mencken said “For every complex problem there is an answer that is clear, simple, and wrong.”. I suggest the biofilm explanation for chronic infections was too simple and is somewhat wrong? Based on recent publication both aggregated and single cell bacteria are observed in both chronic and acute infections. The difference between acute and chronic infections on the other hand seem to be the metabolic activity of the bacteria. This indicates that the biofilm dogma is wrong, but what is the truth then?

In this presentation, I discuss the biofilm dogma related to implant-related infections, wounds and airway infections, both in relation to treatment but also to the immune system. Furthermore, I will discuss the problems and pitfalls regarding diagnosis of these infections. I will discuss, what is a biofilm, do we all know what we talk about in vitro vs in vivo, and where did we might take the wrong turn? Also, what is the infectious microenvironment of infections, and why do you need to know about this?

Talk Title: Advanced CRISPR–Cas technologies for precise engineering of Pseudomonas

Pablo Ivan Nikel[1]

[1]Novo Nordisk Foundation Center for Biosustainability, Systems Environmental Microbiology, Technical University of Denmark

Pseudomonas putida KT2440, a soil bacterium well adapted to colonize plant roots and thrive in contaminated environments, has emerged as a robust platform for metabolic engineering. Innate stress tolerance, genetic accessibility, and enhanced capacity to host heterologous and artificial metabolic pathways are some of the features that make P. putida a candidate for sustainable bioproduction. Advances in CRISPR–Cas technologies have transformed the way we genetically manipulate P. putida and related species towards this end, enabling precise genome editing and tunable transcriptional control. Among other goals, these tools facilitated the integration of complex metabolic pathways for the synthesis of novel compounds, expanding the metabolic potential of P. putida beyond its native capabilities. In this talk, I will discuss how CRISPR–Cas systems support the rational metabolic "domestication" of this soil bacterium. Several case studies will highlight both the utility and challenges of implementing CRISPR-enabled technologies in Pseudomonas, from multiplexed genome modifications to dynamic gene regulation. As CRISPR–Cas toolkits continue to evolve, their application in engineering P. putida is poised to accelerate the development of next-generation microbial cell factories for diverse biotechnological applications.

Talk Title: Breaking the Biological Data Wall: Building the world's largest, ethically-sourced database of biological information to pioneer a new class of foundational AI models

Carla Greco[1]

[1]*Basecamp research*

Biological foundation models are revolutionizing the field of life science, from solving complex classification tasks to designing novel therapeutics. However, their effectiveness relies fundamentally on the availability of high-quality, diverse data. Without sufficiently broad and representative datasets, even the most advanced models struggle to generalize and deliver meaningful biological insight. Whilst public databases provide an immeasurably valuable resource, they capture only a fraction of global microbial diversity. Much of this diversity, particularly from underexplored environments, remains undiscovered. As a result, persistent geographical, taxonomic, and contextual biases continue to limit the development of truly comprehensive biological models.

BaseData™, Basecamp Research's comprehensive biological dataset, addresses these limitations. Fully grounded in ethical access and benefit-sharing agreements, it ensures transparent and compliant commercial use of all data. Supported by a self-reinforcing data supply chain, BaseData™ continues to grow rapidly, encompassing samples from more than 120 locations across 25+ countries and representing over half of all known biomes. BaseData™ has expanded classes of biological systems and biomolecules by 100 times compared to public databases, and this unprecedented novelty provides a foundation for biological AI models, enabling them to tackle highly complex tasks.

BaseData™ has already enabled advancements in protein structure prediction (BaseFold), enzyme function annotation (Hifi-NN), and programmable enzyme design (ZymCTRL), demonstrating how comprehensive, ethically grounded datasets can accelerate the next generation of predictive and generative biological models.

Talk Title: Leveraging Generative AI for Synthetic Biology Applications

Aleksej Zelezniak[1]

[1]*Life Science, Chalmers University of Technology, Gothenburg*

The central molecular dogma processes, foundational to life sciences, can be precisely controlled to catalyse advancements in biomedicine and biotechnology. Mastery of these processes can lead to transformative therapies and facilitate the transition from a fossil-centric to a bio-based society. To achieve this, our research merges computer and biological sciences by integrating generative machine learning (ML) techniques with synthetic biology. Our primary focus spans two directions: i) enhancing gene regulation for advanced protein expression systems, and ii) refining protein engineering to amplify biotechnology-relevant properties. Through applied science, we elucidate fundamental molecular mechanisms. Our development of quantitatively predictive ML models unveils the underlying mechanistic connections driving the observed molecular phenomena, e.g. such as gene expression control. In this talk, I will provide insights into the applications of generative ML within synthetic biology, share our team's recent results, and outline our forward-looking research trajectory.

Talk Title: Do we have the right tools and data to understand antimicrobial resistance epidemiology?

Patrick Munk[1]

[1]*National Food Institute, Technical University of Denmark*

We have more knowledge and data than ever and yet antimicrobial resistance keeps growing worse. What types of data and tools are needed to understand and perhaps even forecast AMR going forward?

Even seemingly similar routine surveillance produced in the Nordic countries, have confounding effects, and are hard to compare across borders. At least without careful adjustments.

The microbial genomics data shared openly has poor metadata and is hard to compare. It is also dwarfed by private data with restricted access.

Even when good metadata is captured, we seldom work together across disciplines to capture and combine contextual, sociological and ethnographic details important to AMR. Differences in methods and traditions make it notoriously hard to integrate and interpret qualitative and quantitative data produced across different disciplines.

How do we make AMR research truly One Health and work across species, national borders, professional backgrounds and sectors? Finding the answer is the goal of our NoMoReAMR network.

In the MULTIBIOMINE project, we are working to improve the bioinformatics analyses of microbiome data. But how do we best use our bioinformatics results in combination with that of other disciplines, sectors and regions, so results transcend academic interests and make a dent in the growing epidemic? I have a hypothesis that communication is key, and I will need your help to test it.

Talk Title: Ticks, hosts and human health

Karen Angeliki Krogfelt[1]

[1]*Department of Science and Environment, Roskilde University*

Ticks transmit bacteria, viruses, and parasites to humans and animals; changing climate, land use, and host populations expand their range.

Diagnosis combines exposure history with stage-appropriate tests: PCR early, serology later, microscopy for parasites. Co-infections can complicate clinical diagnosis. Prevention, vaccination where available, surveillance, and timely treatment reduce risk of infection.

Talk Title: Tick borne pathogens in Denmark

Nanna Skaarup Andersen[1]

[1]*Odense University Hospital, University of Southern Denmark*

The presentation covers tick-borne infections, focusing on Borrelia, Tick-borne Encephalitis, and Neoehrlichia. It includes clinical cases, diagnostics, treatments, and highlights the Clinical Center for Vector-Borne Infections (KCVI) at Odense University Hospital, alongside emerging research, collaborations, and public health efforts to improve understanding and management of these infections.

Talk Title: Potential for integrated monitoring of tick-borne diseases: Indices of tick activity, citizen science, and tick-borne Lyme neuroborreliosis infections in Denmark from 2017 to 2024

Lene Jung Kjær[1]

[1]*Department of Veterinary and Animal Sciences, University of Copenhagen*

This study developed an integrated approach to monitor tick-borne disease risk in Denmark using field data, predictive weather models, Google searches, and health records. Tick activity associated with temperature, humidity, and precipitation, predicted Lyme neuroborreliosis one month ahead, and matched search trends, demonstrating scalable, early-warning potential for public health surveillance.

Talk Title: Connectivity between microorganisms and their environment in the Ocean

Soeren Ahmerkamp[1]

[1]*Department of Biogeochemistry, Max Planck Institute for Marine Microbiology, Bremen, Germany*

Microorganisms are key players in ocean biogeochemical cycling, yet we know little about how their activity links to surrounding microenvironments. My talk presents insights from fieldwork, lab experiments, and modelling, showing how small-scale physical and chemical processes regulate microbial activity and influence carbon and nutrient cycling across the ocean.

Talk Title: Phage-bacteria eco-evolutionary dynamics in freshwater lakes

Maliheh Mehrshad[1]

[1]*Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden*

Dependence of phages on their bacterial hosts has defined the eco-evolutionary dynamics of both parties. My research combines high throughput methods such as MetaHiC and single-cell analyses based on semi-permeable capsules and cultured model systems to challenge the conventional one-virus-one-host paradigm revealing the complexity of viral ecology and co-evolution.

Talk Title: From resource use to respiration: chemico-biological links between phytoplankton and bacteria in carbon cycling

Lars Behrendt[1]

[1]*Department of Biotechnology and Biomedicine, Technical University of Denmark, Denmark*

Marine carbon cycling emerges from interactions between phytoplankton and bacteria, yet the mechanistic links from resource uptake to respiration remain poorly resolved. I will present perspectives from experiments and microfluidic approaches that highlight how chemical exchanges and cellular heterogeneity shape microbial activity, community interactions, and carbon flux in the ocean.

Talk Title: The Role of Penicillin Binding Proteins in Life and Death; New Clues to a still Unsolved Mystery

Dorte Frees[1]

[1]*Department of Veterinary and Animal Science, University of Copenhagen.*

Beta-lactams are the most widely used antibiotics for treatment of bacterial infections. They work by irreversibly binding to penicillin-binding proteins (PBPs), which are enzymes responsible for the crosslinking of peptidoglycan in the bacterial cell wall. According to a long-standing paradigm, bacterial killing is elicited by the mis-activation of cell wall-cleaving enzymes, the so-called autolysins resulting in explosive cell lysis. In the first part of my talk, I will challenge this paradigm using *Staphylococcus aureus* as a model. In this important pathogen, beta-lactams do not trigger cell lysis. In fact, these antibiotics instead prevent lysis of mutants overproducing autolysins. Based on chemical and genetic inactivation of PBPs, I propose that PBPs, beyond their enzymatic activity, act as checkpoints that coordinate cell wall synthesis and degradation during cell division. Specifically in *S. aureus*, the binding of beta-lactams to septal PBPs prevents activation of autolysins and arrest daughter cell separation eventually causing cell death.

Methicillin-resistant *Staphylococcus aureus* (MRSA) have acquired the *mecA* gene encoding a transpeptidase, PBP2a that can take over the cross-linking activity when native PBPs are inhibited by beta-lactams. Although antibiotic resistance is often portrayed as a black-and-white phenomenon, the *mecA* resistance gene often only confers low-level resistance with most cells in a population of *mecA*-positive *S. aureus* being phenotypically sensitive to β-lactams. In the second part of my talk, I will explain how high-level resistance develops. This process requires additional mutations in a small set of core chromosomal genes that are not directly related to the antibiotic's cell wall target.

Talk Title: Darwin meets the antibiotic crisis: How can we harness evolutionary insights to improve antibiotic therapy

Hinrich Schulenburg[1]

[1]*Christian-Albrechts-Universitaet zu Kiel*

Evolutionary processes are responsible for the current antibiotic crisis. Surprisingly, they are usually ignored during design of novel therapy, which mainly focuses on finding new drugs. In general, bacteria show an enormous potential to adapt to constant environments, even if extreme such as those defined by many antibiotics. Adaptation may however be more difficult, if conditions change fast. Therefore, rapid fluctuations in the application of drugs may enhance pathogen extinction and minimize resistance evolution. To date, such fast fluctuations are usually not considered for antibiotic therapy. My lab uses evolution experiments in combination with genomics, functional genetics, mathematical modelling, and also analysis of clinical material to explore novel, evolution-informed concepts for antibiotic therapy. I will present some of our findings with the model pathogen *Pseudomonas aeruginosa* that highlight the particular potency of fast sequential treatments, especially when these exploit evolutionary trade-offs like collateral sensitivity or transient physiological responses such as cellular hysteresis. Our work points to new treatment designs, which may help us to control the emergence and spread of antibiotic resistance.

Talk Title: Dermatophytes and the skin resistome

Prof. Jesper Larsen[1]

[1]Statens Serum Institut & University of Copenhagen.

The discovery of antibiotics more than 80 years ago instigated an era of drug innovation and implementation in human and animal health. The rapid emergence of resistance in many common pathogenic bacteria suggests that the evolution of antimicrobial resistance genes (ARGs) was driven by natural selection in the pre-antibiotic era. Soil bacteria have long been implicated as a potential source of ARGs, yet only 0.1% of soil ARGs are highly similar to those found in pathogenic bacteria. We have previously shown that methicillin-resistant *Staphylococcus aureus* (MRSA) – a notorious human pathogen – first arose in European hedgehogs in the pre-antibiotic era through horizontal acquisition of the *mecC* gene into a small number of *S. aureus* clones, whereby they became resistant to penicillins produced by the hedgehog dermatophyte *Trichophyton erinacei*. This raises the possibility that other dermatophyte species are involved in the selection and evolution of clinically relevant ARGs. To test this hypothesis, we sequenced and analyzed the genomes of dermatophyte species associated with different hosts and habitats (humans, companion animals, food-producing animals, wild animals, and environmental reservoirs), which showed that dermatophytes acquired penicillin and fusidic acid biosynthetic genes in an ancient horizontal acquisition event in connection with an ecological shift from a geophilic to a zoophilic and anthropophilic lifestyle. We also investigated whether hedgehogs constitute a reservoir of coagulase-negative staphylococci (CoNS), which are increasingly recognized as important sources of ARGs. The results showed that CoNS are abundant in hedgehogs and carry ancient ARGs against both penicillin and fusidic acid that can be transferred horizontally to other species. Collectively, this study shows that natural selection of β-lactam and fusidic acid resistance by zoophilic and anthropophilic dermatophytes is ancient and has contributed to the evolution of clinically relevant ARGs in the pre-antibiotic era.

Talk Title: Connecting the Laboratory, The Clinical Setting, and Public Health

Kirstine Kobberøe Søgaard[1], Marianne Voldstedlund[2], Rikke Thoft[3]

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This session explores how microbiological findings are transformed into clinically and epidemiologically meaningful insights.

The first presentation, *"The daily work of a clinical microbiologist: Tracing the infection from bug identity to primary focus,"* offers an inside look at the diagnostic reasoning and investigative process that links microbial identification to the source of infection. The second talk introduces *The Danish Microbiology Database (MiBa)*, a nationwide system compiling real-time data from all Danish laboratories, providing a powerful tool for national surveillance, infection control, and patient care. The final presentation, *"From test result to case count: Using laboratory data for infectious disease surveillance,"* examines the challenges of defining infectious cases for both national and international surveillance.

Together, these talks illustrate how clinical microbiology bridges laboratory science, clinical decision-making, and public health - transforming diagnostic testing into actionable knowledge that improves patient care and strengthens infection prevention at local, national and international levels.

Talk Title: Enhancing microbial nitrate ammonification for the green transition of agriculture

Sara Hallin[1]

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Dissimilatory nitrate reduction to ammonium (DNRA) can help retain nitrogen in soil, while also reducing nitrate losses through leaching and N2O emissions. DNRA bacteria are common in agricultural soils but they compete with denitrifying microbes for both nitrate and energy sources. The talk will outline current knowledge of these microbes and discuss if soil management and crop traits may strengthen DNRA in support of more nitrogen-efficient farming

Talk Title: Inhibition of microbial nitrification to reduce greenhouse gas emissions from agricultural soil

Søren O. Petersen[1]

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Meta-analyses have repeatedly shown a consistent 40-45% reduction of nitrous oxide (N_2O) emissions from synthetic fertilisers and manure. However, effects vary widely and include applications with much larger reductions as well as applications with little or no effect. This presentation will present evidence from field and laboratory studies for a possible mechanism to explain these observations and a strategy for cost-effective application of nitrification inhibitors.

Talk Title: Microbial and viral succession after soil rewetting: links to carbon sequestration

Ella Tali Sieradzki[1]

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Viruses have a significant effect on dissolved organic carbon stocks in marine environments, but in soil the activity of viruses is understudied. However, much of the carbon buried in soil comprises of microbial necromass, implying a potential link between viral predation on the soil microbiome and carbon sequestration. I will present results from rewetting experiments showing unexpected patterns of viral community succession, and introduce my current work which aims to link viral activity in soil to carbon sequestration.

Talk Title: Reproduction and Womens health – the role of vaginal dysbiosis and vaginal microbiome transplantation

Henriette Svarre Nielsen[1]

[1]*Amager-Hvidovre Hospital & Department of Clinical Medicine, University of Copenhagen*

This talk will give an overview of studies on the vaginal microbiome in relation to reproduction. It will also give an overview and discuss the rather novel field of vaginal microbiome transplantations

[O1] The Genome Taxonomy Database: Tenth release and transition to Aalborg University

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The Genome Taxonomy Database (GTDB; https://gtdb.ecogenomic.org) provides a phylogenetically consistent and rank-normalized genome-based taxonomy for prokaryotic genomes sourced from the NCBI Assembly database. Originally developed at the Australian Centre for Ecogenomics, GTDB is now officially hosted at the Center for Microbial Communities at Aalborg University. The database has become an essential taxonomic resource for microbiologists worldwide, and this year we offered the tenth release of GTDB (R10-RS226), supporting various applications in life sciences. R10-RS226 spans 715,230 bacterial and 17,245 archaeal genomes organized into 136,646 bacterial and 6,968 archaeal species clusters. We observed a gradual decline in the discovery of new major branches of prokaryotic life, which suggests that we are approaching the saturation of readily discoverable microbial diversity through culture-independent analyses. Nevertheless, species discovery continues unabated as >95% of bacterial and archaeal species remain to be genomically elucidated based on conservative projections. Here, we would like to present the growth of the GTDB, improvements to its methodology and website, policy changes, and major taxonomic and nomenclatural updates as well as future plans for the resource, including a fungal taxonomy and a nomenclatural extension to classify pathogens.

[O2] The Viral AlphaFold Database of monomers and homodimers reveals conserved protein folds in viruses of bacteria, archaea, and eukaryotes

Odai R[1], Leemann M[2], Al-Murad T[1], Abdullah M[1,3], Shyrokova L[1], Tenson T[3], Hauryliuk V[1,3,4,5], Durairaj J[2], Pereira J[6], Atkinson G[1,4]

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Viruses are among the most abundant and genetically diverse entities on Earth, yet the functions and evolutionary origins of most viral proteins remain poorly understood. Their rapid evolution often obscures evolutionary relationships, making it difficult to assign functions using sequencebased methods alone. Although conservation of protein fold can reveal deep homologies undetectable by sequence comparison, viral proteins remain vastly underrepresented in structural databases, limiting our ability to explore them at the structural level. Here, we address this gap by clustering all unique viral sequences from the NCBI RefSeq database and predicting the structures of ~27,000 representative proteins using AlphaFold2, creating a large-scale viral structural resource, the Viral AlphaFold Database (VAD). We uncover ~10,000 proteins belonging to clusters that share folds across viruses infecting bacteria, archaea, and eukaryotes, revealing shared protein folds across diverse host-infecting viruses. We also predict oligomeric states using AlphaFold2-based homodimer modelling, alongside structural comparisons to the Protein Data Bank, providing valuable new data on the potential for viral proteins to oligomerise. We further reveal that large regions of the viral protein universe remain functionally dark and report the discovery and experimental validation of a previously uncharacterised antiviral toxin-antitoxin (TA) system. VAD is a resource that provides a foundation for exploring viral structure–function relationships, including ancient folds that shape viral interactions across all life.

[O3] Bees as natural fermenters: The microbial context of honey making

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Floral nectar is one of the few natural habitats where sugar is freely available. While many animals consume this resource, bees accumulate it, concentrate it and generate one of the most energy-rich foods in nature: honey. Nectar is rich with microbial life, but the resulting honey is considered aseptic, and never spoils. The honeymaking process starts when forager bees carry floral nectar in their crops to the hive, where transport bees receive it, evaluate it, and deposit it in the cells, stirring and aerating this mixture until it becomes honey. Currently, there is no clear understanding of how bees manage the widely diverse nectar microbial communities to prevent microbes from overtaking the beehive environment. What is the fate of the microbes in floral nectaries when they are collected by bees? We hypothesise bees (Apis mellifera) actively manage the microbes in the collected floral nectar, through inoculation of their symbiotic 'good' bacteria.

Metabarcoding sequencing of samples collected throughout the transformation of floral nectar into honey, confirmed that bee crop microbiomes outcompete the floral nectar microbial communities, safeguarding the beehive from potential microbial pathogens. Bee crop microbiomes are dominated by lactic acid (Lactobacillales) and acetic acid (Rhodospirillales) bacteria, which then go on to dominate the rest of the honeymaking process. Alpha diversity analyses suggest that the then environmental DNA of those dead floral nectar microbiomes is carried throughout the honeymaking process. Thus, the final honey accumulates the DNA of the bacterial communities present in the floral resources used for its making, as it does for the plant DNA itself. Beta diversity analyses confirm within-hive bee crop microbiomes as the most consistent, suggesting that the nectar in the transport bees is already "sterilised" and safe to handle. Within-hive dehydrating nectar, and honey are the most diverse, illustrating they act as a sink of the bacterial species accumulated in the honeymaking process. Metabolomic and microbiome evidence align. The forager plant choice is illustrated in the metabolomic similarities between a subset of floral nectar samples and forager crop content. Still, these two environments overall significantly differ from each other. Further, metabolomic analyses clearly distincts samples outside and inside the hive; within-hive dehydrating nectar and honey cluster separately, albeit significant differences based on colony are observed. This project demonstrates that bees control the microbial environment of honeymaking, relying on their symbiotic bacteria to ensure their most important food source: honey.

[O4] Optimised by evolution: nutritional profiling of the fungal crop cultivated by termites for 30 million years

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Termites, like all insects, require a balanced intake of macronutrients and amino acids to sustain growth, reproduction, and colony maintenance. To meet these nutritional demands, fungus-farming termites in the subfamily Macrotermitinae cultivate crop fungi in the genus Termitomyces within specialised fungus gardens. These fungal crops decompose lignocellulose-rich plant material and provide nutritious edible structures that form the dietary foundation of termite colonies. Despite extensive research on this 30-million-year-old symbiosis, it remains unclear how the nutritional composition of Termitomyces has been shaped by natural selection to match termite dietary requirements – particularly for protein and essential amino acids.

Here we quantified macronutrient (protein, lipid, carbohydrate) and amino acid compositions of Termitomyces mycelium in pure cultures and fungus gardens across at least six fungal species. Nutritional profiles were consistent across species, characterised by unusually high protein content that exceeds WHO/FAO recommendations with respect to essential and branched-chain amino acid. To test whether these profiles are nutritionally distinct and closer to termite dietary needs than other protein sources, we compared them to those of wood-decaying fungi commonly consumed by humans, animal-derived proteins, and insect nutritional requirements based on published data. Preliminary analyses support that Termitomyces more closely resembles animal amino acid composition than other fungi.

Our results support the hypothesis that millions of years of farming have shaped the nutritional value of crop fungi to suit termite hosts. This underlines the value of Termitomyces for animal nutrition – including humans – and supports efforts to conserve this ecologically important and nutritionally valuable symbiosis.

[O5] Exploring the Biosynthetic Potential of Marine Sediments Microbiomes: A Multi-omics Approach

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Microbial communities in marine sediments play a crucial role in global nutrient and carbon cycling. They also harbor a diverse biosynthetic potential which is important for understanding their ecology and for exploration of novel drugs. Despite this, we do not have a comprehensive overview of the Biosynthetic Gene Clusters (BGCs) within these microbiomes.
This study aims to characterize the full microbial BGC potential in marine sediments using long-read sequencing and determine their expression pattern as a function of environmental factors. We are investigating marine sediment from three locations across Denmark (Nivå, Jyllinge, and Amager) with varying salinity and nutrient levels, to examine their microbial community composition and BGC potential.

Our initial Long-read Nanopore sequencing of microbial DNA from Jyllinge Harbor sediment yielded 48 Metagenome-assembled genomes (MAGs) and 148 complete BGSs, most of which lack homologous sequences in antiSMASH. Full-length 16S rRNA gene sequencing revealed Pseudomonadota, Bacteroidota, Desulfobacterota, and Actinomycetota as the dominant phyla, aligning with the recovered MAGs. The taxonomic composition was similar at the three locations, indicating a common component of the bacterial community regardless of the sampling site. Using metagenomics, metatranscriptomics, metabolomics, and ecological modelling, we will explore how secondary metabolites function ecologically and influence marine sediment microbiomes.

[O6] Marine and Atmospheric Biogenic Ice-Nucleating Particles in Coastal Western Greenland

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Biogenic ice-nucleating particles (INPs) significantly influence mixed-phase cloud properties by initiating ice formation at high sub-zero temperatures, affecting precipitation patterns and cloud albedo. As Arctic snow and sea ice decline, expanding open-water may enhance the aerosolization of marine INPs, while terrestrial inputs from meltwater and rivers can increase coastal water concentrations. Yet, the contributions of marine versus terrestrial INPs to the atmosphere, and their microbial drivers, remain poorly constrained.

We present results from samples collected along a transect from the Davis Strait to Baffin Bay (August 2016) and from a sampling campaign at Disko Island, Greenland (summer 2023). We investigated biogenic INP and microbial communities in bulk seawater, the sea surface microlayer, freshwater, and ambient air, complemented by laboratory sea spray experiments.
We found that INP concentrations in seawater increased with latitude and peaked in summer and early autumn, with detectable concentrations to depths of 50 m. During the melt season, terrestrial runoff enhanced coastal INP concentrations, particularly in the surface microlayer. Correlations between INP abundance and specific microorganisms suggest that these microbes are potential INP contributors.

Source tracker analysis showed that marine microbes dominated the atmospheric microbiome under high wind speeds, while snow cover and terrestrial exposure affected atmospheric INP levels. Elevated atmospheric INP levels at higher latitudes aligned with INP-rich seawater, supported by sea spray experiments demonstrating transfer of INPs from seawater to aerosols.
Our results highlight the interplay of marine and terrestrial inputs of biogenic INPs, the strong seasonality of Arctic INPs, and the presence of potential unrecognized microbial INP producers. Our integrated approach advances our understanding of the Arctic coastal environments as reservoirs and sources of atmospheric INPs.

[O7] An alternating substrate access mechanism for the dual ABC-powered carbon-phosphorus lyase

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Phosphorus is an essential macronutrient for all microorganisms. E.coli encodes a 14-cistronic operon (phnCDEFGHIJKLMNOP) required for the carbon-phosphorus (C-P) lyase pathway, which allows extraction of phosphorus from a broad range of phosphonate (Pn) compounds1,2. Pns are characterised by their highly stable C-P bond and their close resemblance to carboxylates and phosphates lent to various industrial applications, one prime example being the herbicide glyphosate, which is commonly known as RoundUp®. Using single-particle cryogenic-electron microscopy (cryoEM), we previously resolved the structure of Phn(GHIJKL)2closed-core (1.93 Å) which features a 340 kDa enzyme complex, whereby the core complex, Phn(GHIJ)2, bearing the active site for Pns binds to a unique double dimer of ATP-binding cassette (ABC) proteins, PhnK and PhnL in a serial conformation, mediated by the PhnJ subunits 3. Here, we present a new state of the enzyme complex, Phn(GHIJKL)2open-core (2.45 Å) in which ATP hydrolysis by the ABC modules has resulted in a drastic structural remodelling of the core complex, exposing one of its active sites. This new state provides us a more complete mechanistic view of the enzyme, enabling us to propose the alternating substrate access model to explain the molecular cycle of C-P lyase. Intriguingly, despite being a non-ABC transporter, comparison of PhnK and PhnL from both the closed-core and open-core states of C-P lyase reveals striking similarities to the ABC modules of classical ABC transporters with some differences, which both confirms and expands the structural versatility of ABCs in adapting to different interacting partners. While we successfully resolved the open- and closed-core states of C-P lyase to high resolution, one key challenge remains that the key catalytic subunit PhnJ is in its apo form. In other words, the isolated enzyme complexes are incapable of catalysing the key catalytic step of homolytic cleavage of the C-P bond due to the lack of an intact [4Fe-4S] cluster which is known to be highly oxygen labile. Therefore, the challenge remains to constitute an anaerobic environment to express, capture and determine using cryoEM new states of the Phn-complexes with intact [4Fe-4S] clusters in the active site which is indispensable for the function of the enzyme complex and the breakdown of Pn compounds in bacteria.

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[O8] Discovery and characterization of cross-phylogenetically distributed antimicrobial lasso peptides

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We previously isolated and characterized the lasso peptide triculamin from Streptomyces triculaminicus (1-3). Initially, the biosynthesis of triculamin remained cryptic as no apparent lasso biosynthetic gene cluster (BGC) could be predicted. In our effort to elucidate this biosynthesis, we located an unusual lasso BGC lacking the well-characterized elements such as the leader sequence of the precursor peptide, the RiPP recognition element and the leader peptidase. Moreover, the isopeptide-forming lasso cyclase in this cluster was missing an N-terminal domain. We attempted heterologous expression of the unusual BGC in Streptomyces albus but were initially unsuccessful. However, we discovered that a nearby N-acetyltransferase was critical for achieving triculamin production. By heterologous expression and purification of the N-acetyltransferase from E. coli, we performed in vitro acetylation of triculamin and found it completely abolishes bioactivity. Thus suggesting a self-resistance mechanism.

Intriguingly, we found both the unusual and canonical BGCs (>200) across phyla encoding structurally almost identical lasso peptides. To further investigate, we characterized two of these lasso peptides and their BGCs. We successfully heterologously expressed a canonical BGC from Chitinasiproducens palmae in a Burkholderia host while we isolated a lasso peptide from fermentation of Brevibacillus gelatini.

The production of nearly identical lasso peptide despite key differences in biosynthesis and widespread occurrence across phyla raises questions about their evolutionary origin and highlights the critical function of triculamin-like lasso peptides.

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[O9] Opioid intervention causes immediate shift in microbiota composition and fermentation activity in healthy humans

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Opioid-induced bowel dysfunction (OIBD) is a significant clinical challenge associated with opioid therapy for acute and chronic pain management. Opioid-induced constipation (OIC) is the most prevalent and debilitating symptom, affecting more than 40% of patients receiving opioid therapy for chronic non-cancer pain, however, current treatment strategies are often insufficient in alleviating OIC symptoms. We present the results from a multi-omics study investigating the impact of opioids on the gut microbiome using a carefully selected cohort of otherwise healthy volunteers. Healthy volunteers (N=25) were treated in two separate five-day periods with orally ingested oxycodone or placebo in a double-blind, randomized, cross-over design and fecal samples were collected and subject to analysis in this study (N=96). This design isolates the direct effects of opioids while minimizing bias by controlling for inter-individual variability, and it enables within-subject comparisons of the immediate effects of treatment. The microbial community of collected fecal samples were analyzed using V1-V8 region 16S rRNA gene amplicon sequencing. Overall shifts in microbial community structure were observed along with significant reductions in alpha diversity and compositional changes, including the enrichment of Akkermansia muciniphila and Oscillospirales members, suggesting that opioid exposure induces early dysbiotic signatures associated with OIBD. Elevated short-chain fatty acid (SCFA) concentrations measured using HPLC-RI during oxycodone treatment were observed and combining with the detection of key functional genes for SCFA biosynthesis using metagenomics sequencing, we highlight the potential metabolic consequences of opioid-induced alterations. Together, these findings advance our understanding of OIBD pathophysiology and underscore the utility of applying multi-omics approaches in evaluating host-microbe-drug interactions.

[O10] Shaken batch cultures of Pseudomonas aeruginosa contain biofilm-like heterogeneity

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Antibiotic tolerance in Pseudomonas aeruginosa is thought to arise from slow-growing and aggregated subpopulations. To disentangle these effects, we tested the temporal dynamics of tolerance by exposing batch cultures to tobramycin, colistin, or both over 2–48 h of growth. Tobramycin was significantly more effective than colistin at early time points, while both converged in efficacy after 24–48 h. Combination therapy showed the strongest activity during late growth, suggesting the emergence of tolerant subpopulations eliminated by dual treatment. Transferring stationary-phase cells into fresh versus spent medium revealed that tolerance was partly reversible, implicating growth rate as a key factor.

We next compared planktonic and aggregated cells using a ribosome-dependent GFP reporter. Aggregates displayed significantly lower fluorescence intensity than single cells, consistent with reduced growth activity.

To test whether aggregation itself explained tolerance, we separated aggregates from planktonic cells by filtration and repeated antibiotic treatments. Here we found only minimal effects of aggregation status on antibiotic efficacy.

We then compared a wild-type with a hyper-aggregating ΔwspF mutant using isothermal microcalorimetry to measure metabolism. Although ΔwspF cultures contained significantly more and larger aggregates, antibiotic efficacy was only minimally influenced by aggregation. Linear mixed-effects modeling showed that antibiotic treatment accounted for >90% of observed variance in the effects of the antibiotics, whereas aggregation explained less than 10%. Microcalorimetry further revealed that metabolic activity during treatment was shaped primarily by the antibiotic used, with only modest contributions from aggregation to growth suppression or delay.

Together, these results demonstrate that growth rate and metabolic state, rather than aggregation per se, are the dominant drivers of antibiotic tolerance in P. aeruginosa batch cultures. This highlights metabolism as a central determinant of tolerant subpopulations and underscores the potential of combination therapy to eradicate them.

[O11] Eight-year effect of biochar amendment on soil properties, extracellular enzyme activity, microbiome structure and N-cycling genes in Danish fallow soils

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Biochar enhances the physicochemical properties of agricultural soils while promoting microbial diversity in the short term. However, biochar's long-term effects on microbiomes and soil health remain poorly understood. This study assessed the effects of 8-year field-aged biochar on microbiomes from two contrasting soils (a sandy clay soil and a coarse sandy soil) under temperate climate conditions. We hypothesize that over an 8-year period, biochar amendment persistently alters soil physicochemical properties, stimulates extracellular enzyme activity, increases the abundance of N-cycling genes, and shifts the prokaryotic and fungal community structure. In June 2015, the topsoil in field lysimeters was amended with 2% wt/wt straw biochar, and by August 2023, this biochar amendment significantly increased the activity of five key extracellular enzymes (α-glucosidase, β-galactosidase, cellobiosidase, phosphomonoesterase, and arylsulfatase) involved in C, P, and S cycling up to 57% in both soils. Further, biochar provoked a persistent shift in prokaryotic community structure and increased the abundance of the nitrite reductase (nirS) gene, indicating a sustained impact on the N cycle. Crucially, biochar enriched an ammonia-oxidizing archaeon of the family Nitrosophaeraceae in both soils, and, in the coarse sandy soil, boosted the abundance of prokaryotes (16S rRNA), key nitrification genes (AOA-amoA and AOB-amoA), and the denitrification gene nosZ I, which encodes the sole enzymatic pathway for N_2O reduction. The study clearly demonstrates that the effects of biochar persist over eight years, providing valuable insights into the long-term impact of biochar on soil health.

Key words: long-term effect, lysimeter, extracellular enzyme activity, N-cycling genes, community structure

[O12] Does the P status of plants select for a beneficial root microbiome?

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Terrestrial plants form mutually beneficial associations with soil microorganisms to overcome the biotic and abiotic limitations of the environment. In low phosphorous (P) soils, plants alter their root architecture and root exudation, while their rhizosphere is enriched in efficient P mineralizing bacteria. However, it remains to be determined how important soil P status contra plant P status is for the bacterial community composition and assembly. Disentangling this will help us understand to what extent plants actively recruit their microbiome to alleviate limitations.

We hypothesized that independent from the P status of the soil, plants recruit beneficial bacteria according to their need. Therefore, P sufficient plants will have similar microbiomes regardless of P-source (soil or foliar). To answer this, we utilized foliar fertilization to develop a setup for manipulating the P status of wheat. We compared wheat (Triticum aestivum L.) grown in low P soil receiving foliar P to wheat growing in the same soil, but with addition of P to the soil. We measured plant P content, plant biomass and plant exudate composition, and assessed the bacterial community with 16S rRNA amplicon sequencing and qPCR of bacterial P mineralization genes. Plant P content and biomass were similar independent of foliar and soil P treatment. Based on differential abundance analysis the rhizoplane microbiomes of the foliar and soil P treated plants were more similar to each other than to the low P soil without P amendment. In contrast, the functional potential for P mineralization was different between plants supplied with foliar or soil P. This indicates that the P status of the plant is the major driver in the recruitment of taxa during P limitation, but that this is not reflected in functional potential.

[P001] The role of VraT in vancomycin resistance, cell wall stress and bacteriophage susceptibility in Staphylococcus aureus

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Vancomycin is a last-resort antibiotic used for treating infections caused by methicillin-resistant Staphylococcus aureus (MRSA). However, when S. aureus is exposed to vancomycin, it often acquires stepwise mutations in genes encoding cell wall stress-related transcriptional regulators, mediating a vancomycin-intermediate S. aureus (VISA) phenotype. As an alternative to conventional antibiotics, phage therapy is gaining ground as a treatment strategy for S. aureus infections. It is critical to identify interactions between antibiotic resistance and phage susceptibility, for potential future phage or phage-antibiotic synergy therapies. This study investigated the role of the protein VraT from the VraTSR cell wall stress related three-component system in vancomycin resistance for S. aureus. We elucidated the impact of vraT single nucleotide variants (SNPs), found in VISA, on virulence, cell wall stress response and susceptibility to bacteriophages. To explore the role of VraT in vancomycin resistance and cell wall stress, we performed RNA sequencing on three vraT SNP strains, using USA300 S. aureus JE2 as control. We found that VraT contributes to S. aureus virulence by controlling the expression of spa, encoding Protein A. Two of the vraT SNPs resulted in a 14- and 10-fold downregulation of spa expression compared to the wild type, respectively. Furthermore, adsorption of several lytic phages was significantly increased in a spa transposon mutant. Cell wall stress related genes such as vraX and cwrA were significantly upregulated in all strains. Phenotypically, a cwrA mutant had smaller plaques than the wild type. By performing a one-step growth curve, we found that the cwrA mutant had a significantly reduced burst size. Finally, utilizing a protein-protein interaction split-luciferase system, we observed that VISA-acquired point mutations in vraT abolish the interaction with the histidine kinase VraS, indicating that VraT is a negative regulator of the VraRS system, and vraT mutations render the system constitutively active. Thus, VraT regulates virulence and stress tolerance by downregulating spa and upregulating vraX and cwrA. Additionally, lack of Protein A enhances adsorption of phages to S. aureus. A potential link between cell wall stress response and phage susceptibility in VISA strains is implied, which could impact future antibiotic-phage combination therapies.

[P002] Linking BNI and QS: The Potential of Sorghum Metabolites in Inhibiting Nitrification

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Synthetic fertilizers containing ammonium have boosted crop yields and food security worldwide. However, their use has also accelerated environmental challenges increasing microbe-driven nitrous oxide (N2O) emissions and nitrate leaching, both contributors to climate change and groundwater pollution. Biological nitrification inhibition (BNI) offers a sustainable alternative, by slowing the microbial conversion of ammonium into nitrate, thereby reducing nitrogen losses. This not only reduces environmental damage but also increases plant nitrogen-use efficiency by ensuring greater nitrogen availability for uptake.

Sorghum (Sorghum bicolor) is a drought tolerant, low input cereal crop native to Africa. It releases root exudates containing sorgoleone, a molecule known for its BNI activity. While the mechanisms underlying its effect on nitrifying bacteria remain unclear, evidence suggests a potential link between BNI and quorum sensing (QS) in nitrifiers. This project aims to investigate that link by constructing a novel bioreporter library in E. coli. The library will incorporate LuxR homologs along with QS-regulated and nitrogen metabolism-related promoters derived from ammonia- and nitrite oxidizing bacteria. These promoters will drive the expression of the luxCDABE operon generating biosensors that emit bioluminescence in response to QS signals. Using these tools, sorghum-derived metabolites, including sorgoleone, will be tested for their ability to inhibit QS and nitrogen metabolic pathways, as well as their potential to reduce N2O emissions.

By linking the effects of BNI molecules to QS inhibition and subsequent regulation of nitrogen metabolism, this project will provide new insights into molecular interactions between plant derived metabolites and soil nitrifiers. These findings will contribute to the development of climate-ready sorghum cultivars that improve nitrogen-use efficiency, mitigate greenhouse gas emissions, and support sustainable agriculture.

[P003] Implementation of interventions to reduce bioaerosol exposure among waste collection workers: A cross-disciplinary study

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Occupational bioaerosol exposure remains a health concern among waste collection workers. With the ongoing green transition, emphasis on sorting and recycling of waste is growing, leading to lower waste collection frequencies and thus a potential increase in exposure to fungi, bacteria, and endotoxin. Previous studies have shown that factors such as frequent hand sanitation, standing beside the truck when emptying waste containers, and maintaining a clean vehicle can have an impact on occupational exposure. However,
implementation of effective safety practices remains a challenge.

This study aims to implement behavioural interventions in order to reduce occupational bioaerosol exposure of
waste collection workers. This will be done through participatory observations, along with collection of interview and questionnaire data - revealing a realistic view of their daily work, practical obstacles, and challenges, which can thus be identified and managed appropriately. To assess the efficacy of the interventions, measurements post-implementation of interventions will be compared to baseline measurements.

We have collected 60 personal baseline measurements, which are split between two companies and three locations (29 control and 31 intervention). Exposure was measured using personal air samplers and hand swabs. Bacteria and fungi were cultured and enumerated. Microorganisms grown at 37°C were identified to the species level using MALDI-TOF MS. Endotoxin concentrations were measured using the Limulus assay and inhalable dust was weighed.

As of now, baseline measurements of exposure have been conducted, and they resemble previous measurements from waste collection workers. Additionally, initial observational, interview and questionnaire data has been collected. In the spring of 2026, exposure measurements will resume to assess the efficacy of the interventions.

This project is funded by AMFF

[P004] Analysis of microbial DNA in blood compartments for improved sepsis diagnosis

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Sepsis is an acute and life-threatening condition, characterized by a dysregulated host response to an infection that leads to organ dysfunction. It represents a major global health burden and is a leading cause of deaths worldwide with 48.9 million cases of sepsis and 11 million deaths annually. Successful treatment requires fast and correct initiation of antimicrobials. However, the current gold standard for diagnosis of sepsis relies on culturing of blood samples, which have a turnaround time of pathogen identification of approximately 2 days, contradicting the need for rapid effective antimicrobial treatment. Due to the long turnaround time, intravenous broad-spectrum antimicrobials are administrated within one hour of patient admission. However, the use of broad-spectrum antibiotics contributes to the rising prevalence of antimicrobial resistance.

This study proposes a method to enhance the detection of microbial DNA from a blood sample by assessing the quantity of microbial DNA in different blood compartments. We hypothesize that microbial DNA is present in different concentrations across blood compartments, and targeting specific compartments may improve the specificity and sensitivity of this diagnostic tool. This will in turn lead to a faster pathogen identification and possibly enable physicians to give patients targeted, narrow antimicrobial treatment. This will be explored by extracting and sequencing DNA from the different blood compartments from a clinical cohort of septic patients and from healthy donors as references.

[P005] Interplay between bacterial metabolism and survival during interspecies interactions between Acinetobacter baumannii and Staphylococcus aureus

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Bacterial interspecies interactions are strongly influenced by the shared environment. During infection, these dynamics are critical for maintaining the polymicrobial communities and shaping the bacterial adaptation and survival. The interactions may be antagonistic, including growth inhibition or active killing, or cooperative, such as mutualistic synergism. Nutrient availability and quorum sensing signals largely determine their outcome.

Here, we investigate the mechanisms behind the interspecies interactions between Acinetobacter baumannii and Staphylococcus aureus; two ESKAPE pathogens frequently isolated together from polymicrobial infections in immunocompromised individuals. We show a significant inhibitory effect of A. baumannii towards the survival of S. aureus during in-vitro sessile growth within mixed species macrocolonies. The outcome of the competition is highly dependent on the growth medium. Confocal fluorescence microscopy revealed distinct biofilm structures within mixed species colonies showing a clear spatial segregation where S. aureus is confined to the peripheral area of the macrocolony during inhibitory conditions.

To investigate the underlying mechanisms of the bacterial interaction, we analyzed the transcriptomic profiles of S. aureus during mono- and co-culture growth conditions. In the presence of A. baumannii, multiple key virulence factors and exoproteins were down-regulated in S. aureus, whereas genes associated with iron homeostasis and carbohydrate metabolism were strongly up-regulated. Altogether, our data indicate that the nature of the bacterial interaction is highly shaped by the environment, and the inhibition of S. aureus is dictated by nutrient availability rather than a direct killing mechanism.

[P006] Polyphenols non-covalently bound to artificial plant cell walls are readily accessible to fecal microbiota and transiently alter fermentation activity

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Polyphenols (PPs) readily associate with plant cell wall (PCW) polysaccharides. In fruits and vegetables, most PPs remain bound to PCWs after cell rupture during eating or processing. PCWs therefore act as carriers that deliver PPs through the gastrointestinal tract to the proximal colon, where microbes can release and metabolize both PCWs and PPs. However, the fate of PCW-bound PPs and their sustained effects on gut microbiota remain unclear. To address this, we engineered multicomponent cellulose hydrogels with Komagataeibacter xylinus and incorporated pectin and xyloglucan to emulate the apple cell wall matrix. We quantified PP binding dynamics and partitioning during soaking in an apple PP extract by UHPLC-ESI-QTOF-MS/MS, identifying 45 PPs; flavonol glycosides showed the strongest overall retention. We then performed in vitro fermentations of selected three-component PCWs, with and without bound PPs, using fecal slurries as inoculum. Microbiota composition and fermentation activity were tracked by 16S rRNA gene sequencing and short-chain carboxylic acid (SCCA) analysis. Eleven PPs, including three aglycones, were detected at multiple timepoints. PPs observed only in fermentations with PP-soaked PCWs declined rapidly early in fermentation and were completely depleted by 5 h. SCCA formation was transiently modulated by PCW-bound PPs compared with controls. The presence of PCW polysaccharides and PPs also altered the relative abundance of several microbial families, with the magnitude and direction of change strongly dependent on the starting community. This work clarifies how non-covalently bound PPs interact with food polysaccharide matrices, reveals donor-dependent microbial metabolism of these complexes, and highlights how food composition can steer PP delivery to the colon — informing the design of prebiotic formulations that pair dietary fibers with non-covalently bound PPs.

[P007] The Small Intestine MICrobiota model (SIMIC): a continuous in vitro model to simulate the ileum microbiota

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The gastrointestinal tract is a highly compartmentalized part of the human body and harbors different microbial populations. While microbial communities in the mouth and distal colon have been studied thoroughly, the same cannot be said for the microbiota of the small intestine. Studies on the microbiota of the small intestine remain a significant challenge. This is primarily due to the invasiveness of procedures for collecting samples, such as colonoscopy or gastroenterostomy. These techniques raise ethical concerns when applied to healthy individuals and can lead to cross-contamination from neighboring districts.

To gain insight into the functionality of the small intestine ecosystem, we designed the Small Intestine MICrobiota model (SIMIC), a continuous in vitro model system that aims at simulating the ileum microbiota. We designed a consortium of eight bacterial species representative of the small intestine microbiota and a custom-made medium fine-tuned to mimic the nutrient availability in the ileum. This consortium is grown in a benchtop bioreactor that mimics feeding and fasting states. The SIMIC supported the growth of the selected bacteria within a range of 10^3 to 10^8 bacteria/mL, which is reported to be the standard range in vivo. Fermentation profiles were stable, characterized by the presence of acetate, propionate, and formate as the primary fermentation products, in concentrations similar to those found in vivo in the terminal ileum of adult humans.
These findings suggest that the SIMIC is suitable for studying community dynamics and fermentation networks of the ileum microbiota.

Building on these findings, further studies will implement different kinds of perturbations to the system by modifying the nutrient administration and altering the initial community to determine the community's resistance and resilience, as well as the role of individual members within it.

[P008] Zooming into Livestock Microbiomes via Micro-Scale Spatial Metagenomics

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Understanding and engineering microbial communities is increasingly central to advancing animal health, productivity, and sustainability. While shotgun metagenomics is a powerful tool for investigating the composition and function of gut microbiomes at the strain level, conventional bulk sampling approaches do not capture microbe–microbe interactions and localised community structure. To overcome this limitation, we applied a novel methodology for reconstructing microbial communities at micron-scale resolution. Our Micro-Scale Spatial Metagenomics approach combines microsampling via laser microdissection, tissue lysis, and Illumina shotgun sequencing, with bioinformatic workflows for quantitative community reconstruction and strain-resolved spatial modelling.

Using this novel method, we investigate the impact of diet composition, pre- and probiotic supplementation, infection, and vaccination on the gut microbiomes of livestock animals, namely poultry and swine. These experiments are part of the 3D'omics EU project, which aims to reconstruct three-dimensional, holo'omic landscapes to reveal host-microbiota interactions shaping animal production.

In this study, we present how these treatments alter microbial community composition at both the micro- and the macro-scale, drawing on a dataset of over 6,000 metagenomic samples. We expand on the potential for capturing spatial signals in the organisation of the gut bacterial communities, as well as the functional potential of the community members.

Overall, we introduce a novel approach of micro-scale metagenomic profiling and demonstrate its application in exploring and guiding microbiome engineering in livestock systems.

[P009] Prophage induction stimulates ribosomal RNA operon recombination and facilitates genome mobility

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Bacterial genomes contain multiple ribosomal RNA operons that by homologous recombination facilitate genome rearrangements. Here we show that a Staphylococcus aureus temperate bacteriophage stimulates homologous recombination between the rRNA operons flanking that prophage and promotes a novel route of horizontal gene transfer we term rrn-linked lateral transduction. By PCR, sequencing of phage-packaged bacterial DNA, and phage transduction assays, we show that upon induction of the prophage, large circles of DNA formed by rRNA operon recombination are packaged and transduced by the phage via the mechanism of lateral transduction. This phenomenon is likely to be widely occurring, with prophage-linked rRNA operon recombination also shown here in Salmonella. Our results challenge the concepts of mobile and core genomes and establish rrn-linked lateral transduction as a new form of gene transfer involving rRNA operons.

[P010] PCR screening and multiplex ddPCR for the detection of carbapenemase-positive Klebsiella pneumoniae in wastewater samples

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Infectious diseases and the associated antimicrobial resistance (AMR) are a major global health concern. Carbapenemase-producing Enterobacteriaceae, particularly Klebsiella pneumoniae, are classified by the World Health Organization (WHO) as critically important pathogens due to their increasing resistance to antimicrobial agents. Wastewater-based epidemiology (WBE) has emerged as a valuable tool to complement traditional surveillance systems by enabling population-wide monitoring of pathogens and resistance markers.

In this study, K. pneumoniae was isolated from influent samples of four wastewater treatment plants (WWTPs) between July and November 2024. A total of 259 isolates were screened for carbapenemase production phenotypically and by PCR targeting blaOXA-48, blaNDM-1, blaKPC, blaVIM, and blaIMP. The blaOXA-48 gene was detected most frequently (n=125), followed by blaNDM-1 (n=99) and blaKPC (n=53), with distribution patterns varying among WWTPs. In parallel, a multiplex droplet digital PCR (ddPCR) assay was developed to allow rapid detection of K. pneumoniae and blaOXA-48 directly from wastewater without prior culture. While detection was feasible in both pure cultures and wastewater samples, the assay performance was close to the detection limit, and inhibition effects were observed, indicating the need for further optimization (e.g., filtration of wastewater).

Together, these findings confirm wastewater as a reservoir for carbapenemase-producing K. pneumoniae and demonstrate the potential of WBE combined with molecular methods such as ddPCR for fast, population-level AMR surveillance.

[P011] Testing for tissue-specific foraging by a developing insect-pathogenic fungus

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Entomopathogenic fungi infect living insects and complete their life cycle within their host. They entirely depend on the nutrients provided by the insect and are exposed to various challenges by its immune defences. In-vitro studies have shown that the nutrient availability differs within the host's body and that the nutritional needs of these fungi change throughout their development. However, it remains unknown whether free-ranging fungal pathogens practice tissue-specific foraging.

We hypothesise that entomopathogenic fungi forage in an optimal, sequential, organ-specific manner inside their living host, which ultimately increases their fitness.

Wild type Drosophila melanogaster were infected with spores of wild type Metarhizium brunneum. For tracking the fungal growth in a temporal and spatial manner, cryosectioning was performed on flies at different stages of the infection. The microbal growth was visualized using fluorescence microscopy.

Entomopathogenic fungi are responsible for the majority of infections found in insects. Examining their foraging behaviour will help understand the evolution of pathogen-specific disease progression and host adaptation.

Keywords: insect-pathogenic fungi, foraging, host adaptation, Metarhizium, Drosophila

[P012] Production of Value-added Products from Hemicellulosic Hydrolysate

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Lignocellulosic biomass is considered one of the most abundant sources of biomass found on the planet and is full of fermentable sugars that can be valorized in fermentation processes. However, a pretreatment process of the lignocellulosic biomass is needed to release these fermentable sugars. During this process, inhibitory compounds such as furan aldehydes, weak acids and aromatic compounds are also released. To remove these compounds, several detoxification methods can be used, but most of them are either expensive or considered as not being environmentally friendly. However, a strategy involving microbial degradation of the inhibitory compounds, could be a solution to this issue.

In this study, we aim to establish bacterial cell factories capable of degrading toxic compounds present in hemicellulosic hydrolysates through heterologous expression of degradation pathways. Several plasmids, encoding genes for the degradation pathways of furfural and aromatic compounds such as vanillin and syringaldehyde will initially be generated through USER cloning. The generated plasmids will subsequently be tested in our pre-engineered Escherichia coli cell factories through growth coupling, HPLC and LC-MS. Non-functional pathways will be further investigated using a dual-reporter biosensor system, after which we will attempt to resolve potential issues with bottleneck enzymes.

When the pathways have shown to function in the heterologous host, they will be integrated into the genome of a pre-engineered host organism, after which a fermentation will be performed using hemicellulosic hydrolysate derived from wheat straw.

[P013] Microplastics as Accelerators of Antibiotic Resistance Propagation in Marine Environments

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Antimicrobial resistance is a critical global health crisis, with environmental reservoirs playing an underexplored role in the spread of antibiotic resistance genes (ARGs) [1,2]. Recent evidence shows that DNA adsorbed to mineral surfaces can remain stable over geological timescales and be horizontally transferred to bacteria, suggesting a previously unrecognized evolutionary pathway [3,4]. Building on this insight, we investigate whether microplastics— a novel surface ubiquitous in marine ecosystems [5,6]—serve as vectors that accelerate ARGs dissemination.

We deployed beads of polyethylene (PE) and polylactic acid (PLA), and mineral particles in Kalø Bay (Denmark) in both surface waters and at the bottom of the water column. During the two months in the bay the samples developed complex plastisphere assemblages composed of biofilms, minerals, and heavy metals. Scanning electron microscopy confirmed the presence of minerals and colonization by diverse microorganisms, including bacteria, diatoms, and fungi. However, the composition of the plastisphere varied across the sample types and with the position of the plastic within the water column.

Subsequent laboratory experiments revealed that the ARG adsorption capacity of the collected samples had increased significantly and that inoculated bacteria were able to genetically transfer the adsorbed ARGs. Addition of heavy metal further enhanced the uptake efficiency. We conducted surface geochemical analyses of the beads and show that surface properties of the samples influence plastisphere composition which seem to be key steps for plastisphere development.

Our findings highlight microplastics as novel hotspots for horizontal gene transfer and ARG propagation. By integrating geomicrobiology, environmental geochemistry, and health perspectives, this study advances understanding of how anthropogenic substrates reshape microbial evolution.

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[P014] Tree species shape ectomycorrhizal fungal traits and community composition through litter quality and resultant soil properties

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Ectomycorrhizal (ECM) fungi are key components of the nitrogen (N) and carbon (C) cycles in temperate and boreal forests soils. However, it is still unclear how differences in soil properties driven by litter quality shape ECM fungal diversity and composition. To test this, we used a unique common garden experiment in Denmark which included three common European tree species, lime, beech, and Norway spruce, each associated with contrasting litter quality from fast- to slow-turnover respectively. We found that soil physicochemical properties varied significantly among tree species, with spruce soils having higher soil organic matter (SOM), phosphorus (P), inorganic N and lower pH, than beech and lime. A total of 1,248 ECM root tips were selected for Sanger sequencing and individually assessed for the presence of hyphae and/or rhizomorphs protruding from the fungal mantle. Sequencing revealed 133 fungal amplicon sequence variants (ASV's), clustered in 86 fungal operational taxonomic units (OTU's), from which 61 corresponded to ECM fungi. Compared to spruce, lime and beech supported a higher ECM fungal richness and diversity. The ECM fungal community composition differed significantly between broadleaved species and spruce, with Russula dominating in lime and beech. Regarding fungal traits, in addition to the direct observations of hyphae and rhizomorphs, species were also scored based on literature for exploration types and hyphae hydrophobicity. Tree species had a specific association with ECM fungal exploration types, with broadleaved species favoring contact and short-distance types and spruce associating with long-distance types. The results suggest that tree species via foliar litter quality have a direct impact on soil properties and indirect to the ECM fungal community, where broadleaved tree species with nutrient-rich and rapid decomposing litter support a more diverse ECM community with limited soil exploration capabilities, while spruce supports an ECM fungal community more adapted to soil exploration.

[P016] Exploring Fungal Enzymes for Flavor Improvement in Plant-Based Protein Ingredients

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Off-flavors are a common challenge in the utilization of protein-rich industrial side-streams from plant-based sources. These compounds limit the sensory appeal and consumer acceptance of otherwise valuable protein ingredients, such as the potato protein side-stream from the industrial starch production. Potato protein has high nutritional value and functional properties, but its use in food is limited by off-flavors, such as the bitter-tasting glycoalkaloids α-chaconine and α-solanine. Although microbial degradation of these compounds has been reported, the responsible enzymatic mechanisms remain largely uncharacterized.

In this study, we investigate a diverse set of filamentous fungi for their ability to produce enzymes capable of degrading glycoalkaloids. Several fungal pathogens of potatoes have evolved metabolic strategies to circumvent the antimicrobial activity of glycoalkaloids. By exploring these metabolic processes, we seek to uncover enzymes with potential for the industrial scale degradation of potato off-flavors to improve the potato protein side-streams sensory quality and thereby permit its wide use as a food ingredient.

To support enzyme discovery, we are developing a robotics-assisted high-throughput screening platform to identify and compare fungal enzymatic activities. This approach integrates applied microbiology, enzymology, robotics, and bioinformatics to accelerate the identification of candidate enzymes for industrial food processing.

Our work contributes to the development of microbial-enzyme solutions for flavor optimization in plant-based proteins and is part of the REFINES project, funded by Plant2Food (https://projects.au.dk/plant2food/funded-projects/refines).

Keywords
Filamentous fungi, Fungal enzymes, Off-flavor degradation, Plant-based proteins, Glycoalkaloids, High-throughput screening, Applied microbiology

[P17] Biotechnological valorisation of keratin-rich animal wastes by Aspergillus aculeatinus isolated from abattoir dump soils.

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Abattoir effluents and solid wastes contain blood, fats and proteins, especially complex and recalcitrant keratin-rich proteins, which are resistant to conventional treatment methods and pose serious environmental and public health risks. These wastes contribute to water pollution, greenhouse gas emissions and nutrient imbalances in receiving environments when improperly treated before disposal due to their high organic load composition. This study investigated the biodegradation potentials of microbial isolates indigenous to abattoir waste dump soil to mitigate bulk accumulation of these wastes with concurrent recovery of valuable resources to promote circular economy. Microbial community analysis targeting fungal ITS region identified the isolate as Aspergillus aculeatinus KU378102. Bio-valorisation of the substrates (cow hair, feather, horn and human hair) were carried out under controlled mesophilic temperature of 30 ± 2 oC. This process showed corresponding protease activity increase of 2.81 ± 0.13 - 68.16 ± 2.59, 5.92 ± 0.45 - 118.14 ± 0.78, 3.42 ± 0.37 - 76.85 ± 0.78 and 2.84 ± 0.07 - 47.11 ± 1.29 U/ml, determined by casein hydrolysis method for the respective substrates. Also, soluble protein content determined by Lowry method increased from 3.91 ± 1.17 - 19.00 ± 0.06, 5.57 ± 0.11- 36.96 ± 0.74, 4.60 ± 0.13 - 16.31 ± 0.83 and 3.74 ± 0.38 - 14.06 ± 0.34 mg/ml, respectively. Ninhydrin assay protocol determination of the free amino nitrogen (FAN) yielded 302.25 ± 10.35, 953.80 ± 32.54, 362.91 ± 22.18 and 216.49 ± 22.19 mg/L, while sulphate concentration estimated by turbidimetric method produced 93.91 ± 0.33, 171.88 ± 0.17, 81.52 ± 0.00 and 60.57 ± 0.34 mg/L from the respective isolates. These analytes confirmed the keratinolytic potentials of the isolate on recalcitrant protein composition of abattoir wastes which reduces its organic load for enhanced nutrient recovery. The study further highlights the potential benefits of integrating biotechnological animal wastes treatment systems in livestock production to enhance soil health and bioresource recovery which aligns with circular bioeconomy goals.

[P18] Linking genes for genes for nutrient acquisition to evolutionary and ecological traits in fungi

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Genes regulating nutrient acquisition such as nitrate and phosphate transporters and ones regulating organic matter degradation are common in many fungal clades and play a pivotal role in the growth and survival of these fungi. Although these protein-coding genes may be present in most of the species, evolution and ecology have played an important part in shaping their diversity and number. It is not entirely clear however what the magnitude of these two factors are.

In this study, we took the same approach as Romero-Olivares et al. (2021)* and collected 240 genomes of ectomycorrhizal fungi purchased from different online databases (NCBI and mycocosm). We selected genera fungal genera that associated with three tree species, namely Norway spruce (Picea abies), European beech (Fagus sylvatica) and small-leaved lime (Tilia cordata), recorded from three Danish common gardens sites (Quian, Kjøller et al., unpublished). The genomes were filtered by completeness via BUSCO (Benchmarking Unique Single Copy Orthologs) thus offering a strong base for phylogenetic analyses. A table containing the frequency of gene families related to the metabolism of nitrogen, carbon and phosphorus for each are in progress. For the latter we use the programs Augustus and InterProScan for structural and functional annotations of genomes and the Mycocosm searching browser, whenever the genomes were already annotated.

The results will be statistically analysed via R, testing whether ecology, phylogeny or other important factors such as substrate composition play a significant part in variation of gene frequencies across genomes, tree species and location.

Our hypothesis is that the number of nutrient uptake genes will mainly be influenced by the ecological role and substrate of the single species rather than just on the phylogeny, thus demonstrating how ecological lifestyles are the mainly drivers of evolutionary processes.

*Romero-Olivares et al. (2021) Linking Genes to Traits in Fungi. Microbial Ecology 8

[P019] Culturable Fungi from Portuguese Karst Cave Ecosystem Reveal Hidden Diversity

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Caves are important subterranean ecosystems that harbour unique oligotrophic environments, known to have high levels of endemic biodiversity. Microbial ecology in caves is still underexplored, particularly when it comes to species richness and community interactions. Previous studies investigating culturable fungal diversity, have shown Penicillium, Aspergillus, and Cladosporium to be the most common genera. The aim of this study was to obtain and compare culturable fungal diversity across three cave compartments in the Vale Telheiro cave in Portugal. This karst cave is characterised by low oxygen- and high carbon dioxide levels and it is recognised as a biodiversity hotspot, comprising three compartments that differ in light input, ventilation, nutrient availability, and concentrations of the radioactive element radon. We isolated fungi using multiple media and a dilution series to obtain pure cultures that we then identified through DNA barcoding of the ITS gene. A total of 77 fungal cultures were obtained, dominated by Penicillium, Aspergillus, and Pseudogymnoascus. Culture medium and dilution affected the diversity and identity of fungal isolates, but there was no significant effect of cave compartment on the number of isolates. Barcoding indicated the possibility of novel taxa, but further investigation is underway to robustly place the fungal isolates among known fungal diversity. Even so, our findings suggest that karst cave ecosystems are promising for the discovery of so far unknown microbial life and highlight the importance of cave ecosystem conservation to prevent the loss of hidden fungal biodiversity.

[P020] Evaluation of the Diagnostic Accuracy of the Xpert MTB/RIF Ultra Assay for Detecting Mycobacterium tuberculosis complex in Routine Samples in Denmark, 2020–2024

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Category: 02. Bacterial infection & disease
Sub-category: 02a. Tuberculosis and other mycobacterial infections
Keywords: Mycobacteria, Rapid molecular diagnostics

Background
WHO recommends the use of next-generation Xpert MTB/RIF Ultra ('Ultra', Cepheid, USA), to rapidly detect Mycobacterium tuberculosis complex and rifampicin resistance in sputum, cerebrospinal fluid, lymph node aspirates, and lymph node biopsies (1). However, evaluation of the Ultra assay in different patient specimens from patients in TB low-incidence countries is limited (2). We evaluated the diagnostic accuracy of the Xpert MTB/RIF Ultra assay for detecting Mycobacterium tuberculosis complex, using culture confirmed tuberculosis as the reference standard, in routine samples collected in Denmark.

Methods
All samples analysed using the Xpert MTB/RIF Ultra assay at the International Reference Laboratory of Mycobacteriology, Statens Serum Institut, during year 2020 to 2024 were included in this retrospective analysis. We assessed sensitivity and specificity against microbiologically confirmed tuberculosis, defined by a positive liquid culture (MGIT), Löwenstein-Jensen culture, or Dubos media within three months of the Xpert MTB/RIF Ultra assay test was performed. In secondary analysis, we stratified diagnostic accuracy by specimen type (sputum, fluids, biopsies).

Results
A total of 633 samples were tested using the Xpert MTB/RIF Ultra. 44 samples were excluded. Specimens included sputum (215/589, 36,5%), fluids (116/589, 19,7%), and biopsies (258/589, 43,8%). The TB prevalence among included samples was 74/589 (12,6%) Ultra's sensitivity and specificity for detecting Mycobacterium tuberculosis complex were 94,9% and 97.1% respectively. Among eight Ultra-positive but culture-negative samples (false positives according to the reference test), 62.5% (5/8) were from individuals previously diagnosed with culture positive TB (range 0-5 years prior). The remaining 3/8 (37.5%) samples came from a patient with prior non-microbiologically confirmed miliary TB, one with multiple PCR-positive but culture-negative samples, and one IGRA-positive patient with a single cerebrospinal fluid sample

Conclusions
Ultra demonstrated good performance against a culture-based reference standard among routine samples in Denmark, a low TB incidence country.

1.World Health Organization. WHO consolidated guidelines on tuberculosis - Module 3: Diagnosis - Rapid diagnostics for tuberculosis detection. Geneva, https://www.who.int/publications/i/item/9789240029415; 2021 update.

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[P022] epsSMASH predicts exopolysaccharide pathways in environmental and human microbial communities

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Polysaccharides perform several critical functions in the extracellular space of microbial communities. Lipopolysaccharides, which are covalently bound to the cell, aid pathogens in host evasions and enable attachment to surfaces or other cells. Capsular polysaccharides form a protective layer around the cell, protecting it from toxins and desiccation. Other polysaccharides are secreted further into the extracellular space, where they contribute to the spatial structure of the biofilm matrix while also adsorbing toxins, retaining water and acting as a communal carbon and energy reservoir for the biofilm community. The biosynthesis of extracellular polysaccharides (exopolysaccharides) is often performed by complex pathways which can differ even at strain level. Even so, most exopolysaccharide biosyntheses can be grouped into four major pathways, with the corresponding genes typically colocalised into biosynthetic gene clusters (BGCs). Despite the ubiquity of exopolysaccharides in microbial communities, attempts at mapping the genomic potential for their production has been limited.

Here we present epsSMASH, a bioinformatic tool capable of detecting known exopolysaccharide BGCs in bacterial genomes and predicting novel ones. epsSMASH detects 28 distinct exopolysaccharide BGCs using strict rules inspired by those from antiSMASH, a renowned tool for detecting BGCs encoding secondary metabolites. Less stringent rules are used to predict novel versions of known exopolysaccharide BGCs, while a third set of rules is employed to detect uncharacterised exopolysaccharide BGCs. We apply epsSMASH to four large genomic databases representing the microbiomes of activated sludge, soils, the ocean and the human gut, revealing that the vast majority of exopolysaccharide BGCs in environmental communities are uncharacterised.

To highlight the ability of epsSMASH to detect and explore novel exopolysaccharide BGCs, we pick the most phylogenetically widespread exopolysaccharide BGC in the global activated sludge microbiome. We show that the synteny and gene composition of this so far uncharacterised BGC is remarkably conserved across the Sphingomonadales order. Gene annotations and homology to an exopolysaccharide BGC from the order Burkholderiales suggests that the Sphingomonadales BGC is involved in the exopolysaccharide-associated exosortase/PEP-CTERM system.

Upon publication, epsSMASH will be released as both a publicly available web-service as well as a command-line tool for more experienced bioinformaticians. We believe that the exploratory capabilities of epsSMASH coupled with its ease of use will make it an essential tool for investigating exopolysaccharide production in microbial communities.

[P023] Comparative metabolomic profiling of the brown rot fungi Monilinia fructicola, Monilinia fructigena and Monilinia laxa

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Brown rot and twig blight caused by Monilinia species are devastating diseases that lead to significant yield losses in stone and pome fruits orchards. This study aimed to investigate the genetic and metabolic differences among the three main Monilinia species (M. fructicola, M. fructigena, and M. laxa) to enhance our understanding of these phytopathogenic fungi and develop effective strategies for disease management.

Genomic analysis using the antiSMASH prediction tool for fungi revealed varying numbers of biosynthetic gene clusters (BGCs) for each species. A total of 63 correlated BGCs were identified among the three species, and M. fructigena exhibited a greater distance in terms of shared clusters compared to the other two species. Annotation and literature review highlighted nine clusters associated with the production of siderophores, melanin, chloromonilinic acid, botcinic acid, solanapyrone, and retinal.

A metabolomic analysis using UHPLC-MS/MS was conducted on multiple strains of each species to establish a molecular fingerprint, highlight differences in their metabolomes and identify secondary metabolites. Noteworthy compounds included retinal, scytalone, solanapyrone, botcinic acid derivatives, and chloromonilinic acid, accordingly with some of the identified BGCs. M. fructicola exhibited the most significant differences in the metabolic profiles among the three Monilinia species, while the three species showed unique patterns when the analysis was focused on the production of identified secondary metabolites.

As the last part of the study, the reference strain of M. fructicola was cultivated in three different conditions (in vivo and in vitro monoculture, in vitro co-culture with P. expansum) to reveal differences in selected metabolites production with the aim of understanding their biological role. Further analyses, including transcriptomic analyses, molecule purification and bioactivity assays are necessary to validate these differences and deepen our knowledge of Monilinia secondary metabolism. This research contributes to a broader understanding of the mechanisms underlying brown rot disease and provides potential targets for the development of new more sustainable disease control approaches.

[P024] Spatial constraints foster divergent evolution in phage-infected communities

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Bacteriophages (phages) are potent evolutionary drivers whose constant infection promotes diversification within bacterial communities. The interaction between bacteria and phages has commonly been studied in homogenous liquid cultures, where evolution often favors complete resistance via specific loss-of-function mutations. However, the evolutionary outcomes in environments where spatial constraints limit mixing and thus multiple bacteria-phage encounters, remain poorly understood. Here, we investigated the evolution of resistance in E. coli against two distinct phages (Phage L, a Tequintavirus, and Phage G, an Alphatrevirus) in homogenous batch cultures and fixed plate environments. We isolated and characterized phage-resistant bacterial mutants from each condition to assess phage infectivity, growth rates, and biofilm formation.

Whereas all liquid culture-evolved mutants uniformly displayed complete phage resistance, those from the plate environment exhibited a diverse spectrum of phenotypes. Notably, for Phage L, resistance levels in mutants isolated from plates spanned 10 orders of magnitude, ranging from complete immunity to only slightly reduced susceptibility. This diversity was linked to distinct phenotypes, likely reflecting fitness trade-offs and we grouped the mutants based on these. For instance, some mutants with partial phage resistance were associated with enhanced biofilm production, whereas others achieved higher resistance at the cost of biofilm-forming capacity. Our results demonstrate that spatial constrains fundamentally alter phage-host coevolution, promoting a wider range of resistance strategies than observed in standard liquid culture experiments.

[P025] Characterization and functionalization of a type IV-A3 CRISPR-Cas system for bacterial engineering

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CRISPR-Cas systems have revolutionized genome engineering technologies, but type IV CRISPR-Cas systems and their genome engineering potential have been largely unexplored. In this study, we identified a type IV-A3 CRISPR-Cas system from a clinical Klebsiella pneumoniae isolate and characterized its plasmid clearance activity and capacity to suppress gene expression. We revealed the pivotal role of Csf3 (Cas5) and the dispensable roles of Csf1 (Cas8-like) and Csf4 (DinG helicase) subunits in IV-A3 CRISPR-Cas complex formation. The system prevents plasmid propagation via interplay between DinG helicase activity and strategic protospacer positioning relative to plasmid replication and maintenance components. We enabled the IV-A3 CRISPR-Cas system to introduce lethal, sequence-specific dsDNA breaks in the E. coli chromosome by fusing the nuclease domain of the I-TevI nuclease to the Csf1 N-terminus. Further, we functionalized the system for base editing, obtaining various editing efficiencies and windows. Our study provides new insights into type IV-A3 CRISPR-Cas systems and highlights their potential in genome engineering applications.

[P026] Onsite pathogen and AMR detection using mobile ONT metagenomics sequencing of gastrointestinal infections in refugee and hospital settings in Burundi

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Background: Rapid diagnostics are essential for surveillance and outbreak response, particularly in remote and resource-limited settings. In this study, we evaluated a mobile laboratory workflow for onsite detection of gastrointestinal bacterial pathogens and antimicrobial resistance (AMR) without reliance on grid power or internet access.

Methods: Using long-read Oxford Nanopore Technologies (ONT) sequencing, laboratory equipment powered by external battery, and our newly developed laptop-based tool, CGELabs, we performed pathogen detection and antimicrobial resistance profiling in the field within 10 hours from faecal samples from individuals with gastrointestinal (GI) symptoms. Field metagenomic sequencing was conducted in Burundi, Africa, at the Cishemere refugee camp in Cibitoke and at the Rugombo Health Centre.

Results: Onsite diagnosis revealed comparable bacterial profiles from individuals from the same refugee camp and health centre indicating the validity of the sequencing pipeline as the microbiomes were homogeneous from the same site. Additionally, patients from the health facility with cholera diagnoses (based on symptoms) showed several bacterial reads identified as Vibrio cholerae in their gut microbiomes using our onsite metagenomics approach, while their healthy household companion (e.g., a child mother) were negative for V. cholerae in their faeces. In parallel, bacterial isolates were cultured from the same faecal samples and subjected to whole-genome sequencing.

Conclusion: This study demonstrates the potential of a mobile, self-sufficient sequencing laboratory as a powerful tool for frontline diagnostics, particularly during outbreak scenarios in low- and middle-income countries and remote regions.

[P027] Overlooked Signals: Highly Stable Quorum Sensing Molecule in Phage Lysates Induces Quorum Sensing Response

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The outcome of phage infection in the opportunistic pathogen Pseudomonas aeruginosa is strongly influenced by quorum sensing (QS), a bacterial cell-to-cell communication system, which regulates both virulence, phage defences, and phage receptor abundance. Phage lysates, commonly used to study phage-bacterial interactions, contain residual small molecules released from the propagation host, which are co-transferred to recipient cells alongside phage. However, the influence of these molecules on experimental outcomes is often overlooked.

Here, we show that the P. aeruginosa QS molecules 3OC12-HSL and C4-HSL are rapidly degraded in phage lysates, while the hydrophobic QS molecule PQS is remarkably stable (Eichler et al. 2025). PQS remains abundant and bioactive in phage lysates for at least one year, due to its binding within outer membrane vesicles. Strikingly, the concentration of PQS in a standard lysate exceeds 30 µM and is sufficient to induce expression of PQS-regulated traits when the lysate constitutes as little as 1% of the total culture volume. Additionally, we show that PQS carried over in phage lysates significantly enhances virulence of P. aeruginosa in a Galleria mellonella larvae burn wound infection model.

These findings highlight that unintentional carryover of PQS in membrane vesicles can significantly confound phage-bacterial interaction studies, as PQS is involved in both phage defence mechanisms and virulence factor production. To avoid these misinterpretations, phage particles should be purified or propagated on QS-deficient mutants. As other bacteria also produce membrane vesicle-associated QS molecules, these findings may have important implications for phage research in other bacterial species.

Eichler, A.H., Mauritzen, J.J., Koonce, K.C., Kaur, G., and Høyland-Kroghsbo, N.M. (2025) ‘Overlooked signals: Highly stable quorum sensing molecule in phage lysates induces quorum sensing response’, bioRxiv, 2025.04.11.648453. https://doi.org/10.1101/2025.04.11.648453.

[P028] Deoxyhexose Metabolism in Lactobacillaceae and Characterization of a Novel Pathway in Liquorilactobacillus ghanensis

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Deoxyhexose sugars are found in seaweeds and are also components of the cell walls of various terrestrial plant polysaccharides. Recently, Lactobacillaceae species were identified that metabolize the deoxyhexose L-rhamnose and L-fucose to the antimicrobial propionate via 1,2-propanediol (1,2-PD), which is of interest for food biopreservation. This study aimed to investigate the ability of different Lactobacillaceae to utilize deoxyhexoses and to produce propionate.

We screened nine Lactobacillaceae strains that were identified in BacDive as deoxyhexose utilizers for their ability to produce propionate. Strains were grown with L-rhamnose and L-fucose at 30°C in anaerobic conditions. Bioreactor runs were performed with Liquorilactobacillus ghanensis, in the presence of L-rhamnose for 72 h at 30°C and pH 6.5 in both aerobic and anaerobic conditions. Substrate utilization and fermentation metabolites were determined by liquid chromatography with refractive index detection. Expression of key genes related to deoxyhexose metabolism was monitored using quantitative PCR. For food application, propionate formation from pectin was evaluated in wheat sourdough using L. ghanensis as the starter culture.

Seven strains grew on L-rhamnose and produced 1,2-PD or propionate. L. ghanensis exhibited a distinct metabolite profile characterized by low 1,2-PD (5.0 mM), the formation of propionate (11.6 mM), and a high amount of lactate (36.8 mM). In the bioreactors, L. ghanensis produced up to 6.4 mM propionate anaerobically, with minimal production at aerobic conditions. Similarly, gene expression data showed that 1,2-PD utilization genes were upregulated under anaerobic conditions but downregulated in the presence of oxygen. Based on genome analysis, L. ghanensis possessed a gene encoding a glycyl radical enzyme to metabolize the intermediate 1,2-PD, rather than the more common glycerol/diol dehydratase enzyme.

This study shows for the first time that a member Lactobacillaceae possesses an active and oxygen-dependent glycyl radical enzyme for deoxyhexose/1,2-PD metabolism. Using propionate-producing Lactobacillaceae, such as L. ghanensis, could be a natural strategy for food biopreservation, including sourdough.

[P029] BE-SALT: Nano-optodes for quantifying oxygen consumption by Sphingomonas echinoides biofilms in response to osmotic stress

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Biofouling is a major challenge in reverse osmosis (RO) water treatment systems, which is driven by microorganisms adapting to constant salinity conditions inside continuous RO elements and forming biofilms that block the membrane during continuous mode operation. In batch mode operation, salinity oscillations may prevent microbial adaptation and reduce biofilm formation if the oscillations are fast and large enough.

To investigate this, we are developing a confocal microscopy-based method for quantifying oxygen consumption in real time by bacterial biofilms to assess their metabolic activity in response to salinity oscillations. This method employs luminescent nanoparticle optodes that can be quantified ratiometrically to convert ratios of luminescent emission intensities into local oxygen concentrations. We first validated the biocompatibility of nano-optodes with bacterial systems by assessing their impact on planktonic growth of Aliivibrio fischeri, and identified several formulations which did not inhibit bacterial growth. We have also confirmed that nano-optodes incorporate into Sphingomonas echinoides biofilms using confocal microscopy.

We are currently working towards applying this method to measure oxygen consumption by S. echinoides biofilms when exposed to oscillating salinities and are characterising planktonic growth under a range of salinity conditions. Our data will support the development of optimised salinity cycling parameters to mitigate biofouling in RO systems.

[P030] Effect of food additives on the conjugative transfer of a blaCTX-M-14-carrying plasmid in Escherichia coli

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The horizontal transfer of antimicrobial resistance genes through conjugative plasmids is a significant concern for both food safety and public health. Plasmids serve as key reservoirs of antimicrobial resistance genes, conferring adaptive advantages to their bacterial hosts and enabling their dissemination via horizontal gene transfer.

In this study, we evaluated the effect of several food additives on the conjugative transfer of a 193 kb plasmid carrying the blaCTX-M-14 gene in Escherichia coli. The donor strain, E. coli HV40, originally isolated from goat milk, harbors the conjugative plasmid and was maintained under cefotaxime selection. The recipient strain, E. coli CECT 516-STR, derived from the Spanish Type Culture Collection ("Colección Española de Cultivos Tipo", CECT) was obtained and maintained under streptomycin selection. Conjugation experiments were performed in the presence of a wide range of additives, including natural colorants (cochineal, carminic acid, carmines), organic acids and salts (sorbic acid, sodium sorbate, sodium sulfite, potassium metabisulfite, potassium nitrite, sodium nitrite, sodium nitrate, potassium nitrate, acetic acid, sodium diacetate, ascorbic acid, sodium ascorbate, sodium lactate, citric acid, sodium citrate), hydrocolloids (carrageenans, locust bean gum, xanthan gum), sugar alcohols (sorbitol), phosphates (diphosphates, triphosphates, polyphosphates), and glutamate. Each additive was tested at two concentrations: the maximum level permitted by legislation and fivefold lower.

For the conjugation protocol, both strains were grown in the presence of their respective selective antibiotics (streptomycin, 16 µg/ml; cefotaxime, 4 µg/ml) at 37 °C. Cultures were mixed when they reached an optical density of 0.30 ± 0.05 at 610 nm, and then incubated in 96-well plates with the tested additives for 18 hours at 37 °C. Afterwards, cultures were plated on selective media containing cefotaxime (0.5 µg/ml) for donor isolation, streptomycin (32 µg/ml) for the recipient, and a combination of both antibiotics to recover transconjugants. Plates were incubated at 37 °C for 24 hours before colony enumeration.

Our results indicate that the average conjugation rate was markedly affected by certain additives. Polyphosphate and diphosphate showed a significant reduction in conjugation rates, whereas sorbitol gave rise to a slight increase in them. For these additives, a wider range of concentrations was tested to unravel dose-dependent effects: 1000, 2500, 5000, and 6000 µg/mL for polyphosphate and diphosphate, and 2000, 5000, 10,000, and 15,000 µg/mL for sorbitol. Polyphosphate showed the strongest inhibitory effect. The conjugation rate in the control was 0.0288 (log_{10} = -1.63) and the addition of polyphosphate decreased the conjugation rate in a dose-dependent manner: 0.00650 (log_{10} = -2.21) at 1000 µg/mL, 0.00299 (log_{10} = -2.56) at 2500 mg/L, 0.00142 (log_{10} = -3.13) at 5000 µg/mL, and 0.00120 (log_{10} = -2.98) at 6000 mg/L. The reduction was statistically significant at all concentrations tested, except at the lowest dose (1000 µg/mL). In the case of diphosphate, a significant decrease in conjugation was observed only at 5000 µg/mL, where the average rate dropped to 0.00638 (log_{10} = -2.26). Conversely, for sorbitol, only the highest dose (15,000 µg/mL) showed a statistically significant increase compared to the control, with an average conjugation rate of 0.04428 (log_{10} = -1.38).

In conclusion, this study highlights the potential role of common food additives in shaping the spread of antibiotic resistance within the food chain. Additional research is needed to understand the mechanisms underlying these effects.

[P031] Beyond bacteria: the overlooked role of fungi in plastisphere carbon cycling

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Plastics introduce a new and abundant source of organic carbon into aquatic carbon cycles, fragmenting and degrading in the environment and acting as host for microbial colonization that forms the plastisphere. While bacteria and fungi are known members of plastisphere communities, the role of fungi remains poorly characterized. Fungi are universal degraders that assimilate carbon into biomass, retain it in the biological cycle and contribute to marine snow, trophic transfer and sedimentary burial. But it is still unclear which fungal taxa colonize plastics, how fungi adhere to plastic surfaces, and through which mechanisms fungi degrade polymers to drive the mobilization of plastic-derived carbon within aquatic ecosystems. In coastal environments, which serve as the entry point of land-based plastics to the sea, fungal communities may differ across salinity gradients, habitats (water column vs sediments), or plastic types. Plastics become entrapped in coastal and marine plants, but it is unclear whether rooting zones act as hotspots of microbial activity that foster degradation.

To address these questions, polyethylene (PE) and polylactic acid (PLA) plastics were deployed for two months in a coastal estuary of western Denmark across freshwater, brackish, and marine sites. Plastics were placed in the water column, sediment, and rhizosphere across sites. In total, about 900 fungal isolates were obtained from the exposed plastics, and an enrichment experiment yielded an additional 250 strains. The fungi were identified by Sanger sequencing, while metagenomics provided community-level profiles. SEM imaging documented fungal colonization and surface alteration. Current and future work includes NanoSIMS approaches to trace carbon assimilation and transport in cultures. This work reveals the remarkable diversity of plastisphere fungi and will track their potential to mediate carbon transformation and recycling in coastal ecosystems.

[P032] Life on a Leaf: Bacteriophage Diversity and Interactions in the Wheat Phyllosphere

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Bacteriophages regulate leaf-surface microbiomes, yet their diversity, lifestyle, and host links on the phyllosphere remain poorly resolved. We expanded our initial wheat phyllosphere survey (Phyllovir 1.0) with a replicated, multi-year design (Phyllovir 2.0) and paired viromes with microbial whole-genome shotgun (WGS) metagenomes. The primary analysis set comprised 36 non-MDA viromes, 35 with matched microbial WGS, collected from flag and penultimate leaves and spikes in Denmark (2020–2023) and from flag leaves in Idaho, USA (2022). In total, 89 viromes were sequenced (2×150 bp), including technical replicates. Standardized assembly, de-replication, and read-mapping yielded a nonredundant catalog of 7,958 vOTUs, a tenfold increase over Phyllovir 1.0 (876). 1,211 vOTUs (15.2%) were high-quality or complete, enabling robust lifestyle, taxonomy, and host inference; 52.4% of viral reads mapped back to the catalog. Only 1.2% of high-quality vOTUs matched an ICTV genus (e.g., Sendosyvirus, Uliginvirus, Murrayvirus), underscoring novelty.

Because virion-enrichment can carry bacterial DNA, we defined putatively active vOTUs (pavOTUs) as contigs exceeding 1.5× the trimmed-mean abundance of any genomic sequence detected in the microbial fraction in at least one sample. This conservative subset contained 663 vOTUs (8.3%) yet accounted for 45.3% of clean viral reads. pavOTUs were longer on average (mean 35.5 kb), included 291 high-quality/complete genomes and 4 jumbo phages (>200 kb), and showed a temperate dominance among high-quality genomes (51.5% temperate vs. 23.8% in Phyllovir 1.0). Microbial-fraction WGS (n=35) recovered 560 species across 126 genera and 7 phyla. Six genera were ubiquitous: Frigoribacterium, Candidatus Williamhamiltonella, Erwinia, Pantoea, Pseudomonas, and the fungus Zymoseptoria. Pseudomonas was consistently abundant (mean 25.2%), and Sphingomonas was often dominant when present. Host predictions for high-quality/complete pavOTUs mirrored prevalent bacteria. By abundance, temperate pavOTUs infecting Erwinia dominated (21.0% of total viral relative abundance), followed by temperate pavOTUs predicted to infect ‘Ca. Williamhamiltonella’ (10.2%) and Pantoea (8.6%).

Conclusion. Wheat leaf viromes are temperate-dominated, with active temperate phages targeting the most prevalent bacterial taxa. The expanded vOTU catalog and host-linked, putatively active subset provide a framework to test how prophage induction and lysogenic conversion shape phyllosphere ecology and to identify candidate phage–host systems for sustainable crop protection.

[P033] Barleygram: A Molecular Message to the Microbiome

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Quorum sensing (QS) is a bacterial cell-cell communication enabling regulation of gene expression in response to cell density. In Gram-negative bacteria, LuxI enzymes synthesize AHLs, which accumulate in step with increasing cell density. AHLs bind and activate LuxR receptors, which in turn activate the expression of hundreds of group behaviour genes, including those governing virulence and biofilm production. Many organisms have evolved to interfere with or modulate bacterial QS. For example, plants produce potent QS inhibitors and activators. This not only shapes direct interactions between plants and bacteria, we hypothesize that the plant derived molecules may interact with QS at the gut-microbial interface as well.

To assess such interactions, this project aims to develop a bioreporter stain using the native LuxR receptor protein SdiA from Escherichia coli, a known gut commensal. We constructed an E. coli SdiA bioreporter strain and its ΔsdiA mutant. Specifically, we cloned the SdiA-activated gadW promoter in front of lux genes, creating a reporter that produce light in response to AHL-mediated activation of SdiA. We confirmed that SdiA senses a broad range of AHLs. In our ongoing work, we assess the effect of health-promoting barley-food-based molecules and extracts on E. coli QS.

Future work with these bioreporters will investigate the application of dietary products as supplements in treating disorders linked to the gut-microbiota-brain axis.

[P034] Discovery of Kinase-Based Anti-Phage Systems in Bacteria

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Toxin-antitoxin (TA) systems have emerged as important components of bacterial immunity against phage predation [1]. Hip kinases, which are usually part of TA systems, have been studied primarily in stress response contexts [2], but their anti-phage defense roles remain unexplored.

Here, we present a comprehensive bioinformatics pipeline to identify and characterize Hip-like kinases in prophage-encoded defense systems in Escherichia spp. and Pseudomonas spp. Our approach integrates multiple computational tools: PHASTEST for prophage region prediction, a custom Hidden Markov Model for Hip kinase homolog detection, and Operon Mapper for genetic context analysis. Additionally, protein structural modeling using AlphaFold provided insights into kinase domain architecture and potential binding sites. This pipeline identified seven candidate anti-phage systems in Pseudomonas spp. and 11 in Escherichia coli. Experimental validation against the CEPEST [3] and BASEL [4] bacteriophage collections confirmed functionality of at least one system so far. We are currently implementing AlphaFold Pulldown for in silico prediction of Hip kinase targets, motivated by known Hip kinase interactions with tRNA synthetases [5] [6], which suggests these kinases may broadly target translation machinery components. This computational approach enables prediction of substrate specificity without experimental screening. Our bioinformatics framework demonstrates how integrated computational approaches can accelerate discovery of novel defense systems, providing a scalable method for identifying kinase-based immunity systems across bacterial genomes and predicting their molecular targets.

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[P035] CO2MOS: CO2 Metabolism Optimisation Strategy to prevent CO2 loss during methanol assimilation

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Methanol is a promising alternative feedstock for bio-production due to its large-scale availability and potential for sustainable synthesis from renewable resources. Among methylotrophic bacteria, Bacillus methanolicus shows significant promise for industrial applications. However, approximately 50% of the carbon is lost as CO2 during methanol-based growth, limiting its efficiency as a bio-production host.

This study aims to enhance carbon utilization in B. methanolicus and deepen our understanding of its metabolic regulation. To this end, flux balance analysis (FBA) was performed using the reconstructed genome-scale metabolic model (GEM) of B. methanolicus MGA3. During the analysis, three distinct formaldehyde dissimilatory pathways were identified as contributors to CO2 loss, therefore these pathways were considered as potential targets for strain engineering. Since two of the pathways are NADPH-generating, their deletion during FBA increases flux through the putative isocitrate dehydrogenase in B. methanolicus. Through a crude enzyme extract assay, isocitrate dehydrogenase was confirmed to be a NADP-dependent variant with a specific activity of 50.17 U/mg and 201.71 U/mg for forward and reverse reaction, respectively. To further understand methylotrophic gene regulation, a growth experiment with formaldehyde supplementation of B. methanolicus was conducted. A logistic decay correlation between formaldehyde concentration and growth rate was observed, with IC50 estimated at 1.57 mM. Using this information, the regulation of formaldehyde metabolism and detoxification in B. methanolicus was investigated through comparative transcriptomic analysis.

In conclusion, deletion targets have been identified to potentially optimize carbon utilization in B. methanolicus, and the regulation of genes involved in formaldehyde metabolism and detoxification was investigated.

[P036] Mapping Bacterial Growth Activity with Dual-Reporter Strains in Human Lung Epithelia at Microscale Resolution

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Understanding how bacterial subpopulations behave during infection requires tools that combine spatial and temporal resolution. Here, we applied a dual-reporter system in Pseudomonas aeruginosa PAO1 and two pathoadaptive mutant strains with a PAO1 background; ΔmexZ and ΔpscC, in order to quantify growth activity during infection of in vitro human lung epithelia. The reporters enabled high-resolution image- and computational analysis, allowing us to assess strain- and aggregate size-specific growth activity across distinct tissue sites at the microscale.

Subsequent analyses revealed that each strain reached peak growth in distinct micro-niches, underscoring how pathoadaptation shapes unique growth preferences. Aggregate size-stratified analyses added further nuance to this pattern.

Our findings demonstrate the power of dual-reporters as a tool to dissect infection dynamics at microscale resolution, being able to provide overarching trends while also uncovering context-dependent details. This approach holds promise for elucidating pathogen-host interactions at microscale and for pinpointing potential therapeutic intervention targets. Moreover, extending this strategy to reporters of other traits, such as toxin production or biofilm formation, could provide an even more versatile platform for pathogenesis research and support the development of novel antimicrobial strategies.

[P037] From Soil to Solution: Actinomycetes metabolites reversing MRSA Resistance

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Methicillin-resistant Staphylococcus aureus (MRSA) is a major public-health concern due to its resistant to β-lactam antibiotics, which are among the safest and most widely used antibiotics. Rather than to undergo on the lengthy and difficult discovery of entirely new drugs, identifying MRSA-resensitizing compounds can preserve the use of these existing antibiotics. MRSA cells can be re-sensitized to β-lactams by blocking the synthesis of proteins involved in cell division or the synthesis of the cell-wall polymers teichoic-acids (TA). Therefore, compounds that inhibit cell division or TA synthesis could be combined with β-lactams to restore MRSA sensitivity.

To identify MRSA-resensitizing compounds, we screened extracts from actinomycetes, a well-known source of diverse bioactive molecules and natural antibiotics. A total of 1,020 extracts from various actinomycete species were screened. Extracts were assessed for two activities: (i) promotion of clpX mutant growth at 25 °C, as this mutant displays a cold-sensitive phenotype which can be chemically or genetically rescued by inhibiting cell division proteins or TA synthesis and (ii) inhibition of wild-type (WT) growth at 37 °C in the presence of 1/20 MIC oxacillin. This approach was designed to identify metabolites that act synergistically with β-lactams.

Two extracts were identified as hits as they both promoted growth of the clpX mutant and inhibited growth of the WT. An additional 35 extracts promoted clpX growth but did not inhibit WT growth. All 37 active extracts were fractionated by HPLC, and the bioactivity of the fractions was retested. Fractions from two extracts retained biological activity, and mass spectrometry revealed an active-fraction-unique metabolite in one extract.

Through this screening, we identified an uncharacterized metabolite that promotes growth of the cold-sensitive clpX mutant and inhibits the growth of the WT in the presence of sub-inhibitory levels of β-lactams. Further experiments are needed to isolate this metabolite and determine its mode of action. Our findings highlight the potential of natural products for discovering new active compounds and demonstrate the usefulness of the clpX mutant as a screening tool for identifying potential MRSA-resensitizing compounds.

[P038] Ecology of biofilms colonising outdoor stone monuments: learning outcomes from a year of the Assistens Kirkegård project.

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Stone monuments are endangered by multiorganism biofilms, well-structured communities of bacteria, fungi and algae, enclosed in a protective matrix. Biofilms are particularly problematic when monuments are outdoors, because of no possibility of controlling climate and biofilms' growth. To protect our cultural heritage, conservators clean the monuments, but biofilms promptly grow back. To design more effective and sustainable solutions, we investigate how multiorganisms biofilms respond to cleaning now, and with the future environmental conditions expected by climate change.

To this aim, in September 2023 we established the Assistens Kirkegård project, a longitudinal study to monitor the evolution of bacterial and fungal biofilms colonizing the stone surface of six tombstones at the Assistens Cementery in Copenhagen. With a conservation treatment in October 2023, the biofilm was removed from the six tombstones, but it is expected to grow again in few years. To monitor the biofilm development, we have been collecting samples for microbiome analysis just before the cleaning, a year after, and we will continue for the next few years. The first results describe complex communities of both phototrophic and heterotrophic microorganisms, that respond to cleaning in diverse ways.

In addition, pre-conservation samples have been processed for isolation of phototropic and heterotrophic microorganisms, that have been identified as well-known soil and stone inhabitants. Their response to commonly used biocides and to the climate change-induced increase of temperature has been investigated, and they will soon be used to reproduce multiorganism biofilms in the laboratory.

[P039] Facilitating transformation of bacteria through mimicking of DNA methylation patterns

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Transformation of DNA into cells is a pre-requisite for enabling the development of synthetic biology tools. However, transformation of DNA into bacteria is often impeded due to the restriction modification (RM) system, a defense mechanism against foreign DNA in bacterial cells. The RM system consists of endonucleases that recognize and degrade foreign DNA based on differences in DNA methylation patterns and DNA methyltransferases that protect DNA native to the cell. The RM defense mechanism can be circumvented if the correct DNA methylation pattern is enabled through the expression of the proper DNA methyltransferases in the cloning host. In this project we work on constructing a diverse library of MTs from various organisms for proper expression in the most often used cloning host, E. coli. The methylation pattern of DNA methyltransferases will be identified in both isolates and metagenomic samples using nanopore sequencing. To analyze the patterns we will use the newly developed Nanomotif[1] tool, which enables identification and exploitation of DNA methylation Motifs in metagenomics. Correct translation and folding of the heterologously expressed methyltransferases will be ensured through the use of a dual-reporter system[2], prior to functional testing. The goal of this project is to create a diverse plug-and-play DNA methyltransferase library, which will enable the development of synthetic biology tools for previously unconsidered microbes, potentially unlocking new and unexplored sustainable microbial applications.

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[P040] 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_2O. 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.

[P041] Selenoproteome dynamics in the gut microbiome across disease severity in ulcerative colitis

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Selenoproteins incorporate the rare amino acid selenocysteine, commonly referred to as the 21st amino acid. They play key roles in maintaining redox homeostasis, regulating energy metabolism, and mediating cellular responses to oxidative stress. In this study, we characterised the selenoproteome within gut microbial metagenomes from patients with ulcerative colitis, comparing fecal samples from individuals in clinical remission (Mayo score 0) with those from patients experiencing severe inflammation (Mayo score 3). Using DeepSep, a BERT-based deep learning network, we predicted both known and novel selenoproteins with high confidence. In Mayo 0, 53 selenoproteins were identified in a total of 19 meta assembled genomes (MAGs) out of 158, encompassing reductases (e.g., glycine/sarcosine/betaine and D-proline), thioredoxin-like proteins, Fe-–S cluster assembly proteins (IscA/HesB family), and cofactor-synthesizing enzymes (e.g., CofE). Notably, SelD (selenophosphate synthase) was widespread across multiple taxa, demonstrating both the capacity for selenoprotein biosynthesis and adaptation to selenium-dependent catalysis. In contrast, Mayo 3 samples contained fewer selenoproteins (13 sequences), originating from 6 MAGs out of 93, largely represented by SelD and select reductases, suggesting reduced selenium-associated metabolic potential with increasing disease severity. Our findings highlight a microbial strategy centered on energy conservation, redox homeostasis, and cofactor maintenance, which may decline in advanced ulcerative colitis. The integration of deep learning-based prediction with functional annotation provides a comprehensive view of selenoprotein diversity and its potential link to disease state.

[P042] A microbial surprise: Methanogens are more oxygen-tolerant than expected

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Methanogens are considered strictly anaerobic archaea. Using highly sensitive oxygen (O_2) optodes this study aims to investigate the physiological adaptations of methanogens to O_2 exposure. Two methanogens were chosen based on their genetic potential to produce antioxidant enzymes and their ability to grow without reducing agents added to the medium as this might affect the O_2 concentration in the medium. They were grown in batch cultures with the addition of different O_2 concentrations in the headspace. O_2 was constantly monitored by optode measurements and growth as well as activity were measured by the change in optical densities and methane production. We found that the methanogens were not only able to withstand exposure of the inoculum to air prior to inoculation but also grew and continued to produce methane in the presence of several micromolar of O_2. Our results suggest that methanogens are by far not as sensitive to oxygen as previously thought. The measured amount of O_2 was decreasing within 30 min after the addition back to anoxic levels, suggesting the chosen methanogens are either able to actively consume the O_2 or that they excrete a strong reducing agent. The specific mechanisms remain to be determined. This is specifically interesting when considering that aquatic zones with reduced O_2 concentrations and thus potential habitats for methanogens are expanding. As methane is a potential greenhouse gas, this impacts our understanding of how greenhouse gas emissions are controlled.

[P043] Differentially Expressed Infection-associated genes in Shewanella algae from a Deceased Northern Bottlenose Whale

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Pathogens are an underexplored but potentially significant driver of marine ecosystem health. However, little is known about the virulence mechanisms of bacteria infecting wild marine mammals. Here, we investigated the transcriptomic response of Shewanella algae, a putative pathogen isolated from the brain tissue of a deceased northern bottlenose whale (Hyperoodon ampullatus). To simulate host-associated conditions, bacterial cultures were grown in Tryptic Soy Broth (TSB) with or without 5% whale brain homogenate (BH). High-resolution transcriptome profiling was performed using Oxford Nanopore RNA sequencing (direct cDNA), followed by differential gene expression analysis.

Of the 7,213 actively expressed genes, 23 were significantly differentially expressed between the two conditions. Upregulated genes included those linked to protein translocation (e.g., sSRP), flagellar motility, chemotaxis, and iron uptake, traits commonly associated with bacterial virulence. Conversely, genes related to carbohydrate and inorganic ion transport were downregulated. Functional pathway analysis revealed a shift toward enhanced amino acid metabolism and motility in the presence of BH, indicating that host-derived cues can modulate bacterial virulence programs. This study demonstrates that host-tissue can induce measurable virulence-associated transcriptional responses in S. algae. Furthermore, our work highlights the potential of Oxford Nanopore RNA sequencing as a tool for exploring prokaryotic transcriptomes under host-simulated conditions, providing new insights into pathogen-host interactions in marine environments.

[P044] Wheat responds to lack of root hairs with recruitment of bacteria harboring P mineralization genes and increased arbuscular mycorrhizal colonization

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Plant roots simultaneously rely on root hairs and the rhizosphere microbiome to acquire phosphorus (P) from the soil, but the full potential of the plant to utilize its microbiome for nutrient capture has yet to be fully understood. We hypothesized that a wheat mutant defective in root hair elongation would recruit more P-cycling bacteria and AMF compared to its wild-type background to compensate for the loss of root hairs. Through quantitative polymerase chain reaction and staining, we quantified the enrichment of bacterial P-cycling genes and arbuscular mycorrhizal fungi (AMF) colonization across different stages of wheat development and in differentially P fertilized soils in the presence and absence of root hair elongation. The loss of root hair elongation led to an enrichment of P-cycling bacteria only during early seedling development, and P mineralizing bacteria dominated over P solubilizing bacteria. The AMF symbiosis was only fully established after several weeks of growth, and the loss of root hair elongation promoted mycorrhizal formation, especially under low-P. Our results demonstrate that wheat can recruit bacteria with P mineralization genes to meet its P needs before the AMF symbiosis is established, but the colonization of these bacteria is no longer prioritized once the AMF symbiosis is developed.

[P045] Unveiling possible association of gut microbiota with anxiety and depression disorders and profiling their antibiotic degradation potential

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Abstract
In present work, association of human gut microbiota and its role in anxiety and depression disorders was studied. The metabolic potential of the gut bacteria for degradation of some antibiotics was also determined. The microbiological profiles of patients with clinical depression (CD; n=30) and social depression (SD; n=30) were compared with the healthy controls (HC; n=30). The findings revealed increased culturable bacterial counts (2.9×1010) in the SD group than in the HC group (2.9×1010). The Lactobacillus was significantly lower ($p < 0.05$) in CD and SD groups with 4.58×1005 and 8.82×1005 cfu/g respectively in comparison to HC (8.80×1007). The resistance patterns of the bacteria indicated 90% of the isolates were resistance to Nalidixic acid, 70% to Moxifloxacin and 60% against Amoxicillin in all groups. Proteobacteria were abundant in CD and SD groups while members of Firmicutes were prevalent in the HC. The CD and SD groups demonstrated low relative abundance of beneficial bacteria such as Faecillibacterium, Lactobacillus and Bifidobacterium. The antibiotics degradation profile of different microbiotas indicated that SD group degraded 54% of the antibiotics followed by 34 % by CD group 12% by the healthy control. The CD and SD groups indicated higher percentage of antibiotics degrading bacteria in their guts. In conclusion, the depressed patients harbor higher percentage of pathogenic bacteria, more prevalence of antibiotic resistant and antibiotics degradation profile as compared to the healthy control, establishes a strong link between gut microbial flora and human health. The better insight of human gut microflora, its dynamics and interaction with human systems could be very helpful to modulate gut microbiomes to manage health and depression issues.

Key words: Gut Microbiota, Antibiotic Resistance, Depression, Drug Metabolism

[P046] Highly Active Biogenic Ice-Nucleating Particles in the West Greenland Phyllosphere Imply Potential Impacts on Arctic Weather and Climate

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Biogenic ice-nucleating particles (INPs) are widely detected in the Arctic atmosphere, where they play a key role in modulating relatively warm Arctic mixed-phase clouds, significantly influencing the regional climate. However, limited data on source environments, abundance, diversity, and atmospheric concentrations of biogenic INPs introduce substantial uncertainties into climate models. As Arctic warming reduces snow- and ice cover and accelerates greening, it may also promote emission of plant-associated biogenic INPs, with potential feedbacks on cloud formation. To better understand the role of epiphytic microbiota in the Arctic climate, we collected quantitative data on INP and microbial population density and community composition across sites in western Greenland (Kangerlussuaq, Ilulissat and Disko Island) between June and September. We combined freezing assays with bacterial quantification and community profiling as well as cultivation-based approaches. Highly active INPs were detected across all locations, with onset freezing temperatures ranging from -3°C to -7°C. The highest concentration of INPs active at -10°C per gram of plant material was observed in Kangerlussuaq in September, suggesting that environmental factors, such as temperature, trigger INP production. Pseudomonas was consistently present in plant samples, and cultivation studies resulted in eight ice-nucleation-active (INA) isolates, all affiliated with Pseudomonas. Whole genome sequencing of three isolates revealed novel INA species and confirmed the presence of ina genes. Our findings identify the Arctic phyllosphere as a previously underrecognized source of highly active biogenic INPs with the potential to significantly influence regional atmospheric INP levels and thereby cloud properties and lifetime. Biogenic INPs derived from the Arctic phyllosphere are thus essential to include in atmospheric models to improve predictions on the Arctic climate feedback.

[P047] Physiology and Cooperation of Methylotrophs grown on Methane

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Microbial methane and nitrogen transformations play central roles in ecosystem function and climate regulation, yet the metabolic strategies that control these processes remain incompletely understood.

This study investigates the respiratory flexibility of the methylotroph Methylotenera mobilis, with focus on its ability to aerobically thrive on methylamines while simultaneously being able to perform anaerobic methanol-dependent denitrification. This could be a potential strategy to transition between aerobic and anaerobic metabolism, while utilizing methane derived methanol, thought to be provided by a cryptic cooperation with the methanotroph Methylobacter tundripaludum.

We show that M. mobilis performs highly efficient aerobic respiration on methylamine, drawing oxygen concentrations down to the sub-nanomolar range, while methanol supports both aerobic respiration and anaerobic denitrification. Observed denitrification was methanol dependent and only activated under low oxygen conditions, with a complete reduction from nitrate (NO3-) to di-nitrogen (N2), confirmed by 15N-isotope labelling, contradicting earlier genomics-based assumptions of an incomplete pathway. Cyanide addition was followed by nitric oxide (NO) accumulation, implying an unexpected inhibition of the nitric oxide reductase. Attempts to optimize culturing of M. tundripaludum were made, laying the foundation for future coculturing and respiration experiments.

Together these findings expand our understanding of methylotrophic respiration under variable redox conditions and help to further explain the intricate cooperation of Methylotenera and Methylobacter species, which could have long reaching implications for nitrogen cycling with direct links to methane oxidizers.

[P048] Environmental risk assessment at the microbial level: utilizing total RNA sequencing to evaluate the non-target impact of fungicides in agriculture.

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Biochemicals are widely used in agriculture to meet the global demand for food production, however, their non-target effects on soil microbial communities remain poorly understood. Total RNA sequencing provides a powerful tool to address this gap by capturing both rRNA- and mRNA-based signals, thereby linking activity of specific taxa with gene expression indicating ecosystem functions.

In the GENEPEASE II project, we assessed the impact of Prosaro across a concentration gradient (0.35–235 ppm) on soil microcosms maintained over a six-month incubation period. rRNA trancripts revealed shifts in microbial community activity, including a decline in fungi and an increase in Proteobacteria and Actinobacteriota. While taxonomic diversity was only significantly altered at 61 ppm, functional diversity responded at much lower concentrations (15 ppm), indicating that ecosystem functions could be affected by a long-term accumulation of the compound in agicultural soils. Carbon degradation genes show a higher affinity to cellulose degradation rather than lignin degradation, likely reflecting the decrease of fungal decomposers that, along with fungal pathogens, were supressed by the fungicide treatment. After 60 days, functional diversity and carbon degradation were similar across treatments up to 15 ppm, indicating the community's ability to recover ecosystem functions over time. Higher concentrations (61 and 235 ppm), however, did not allow full recovery of taxonomic or functional diversity even after 200 days of incubation, suggesting potential limits to the community's resilience to the fungicide.

This study represents a first step toward integrating total RNA analysis into environmental risk assessments, demonstrating how microbial community data, including active community profiles and functional gene expression, can inform more sustainable agricultural practices.

[P049] Combat-related wound infections in Ukraine: Emerging threats from multidrug-resistant Gram-negative bacteria

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Keywords: Multidrug-resistance (MDR); Carbapenem-resistance; Virulence; Combat-related wounds; Ukraine invasion

The war in Ukraine has resulted in extensive military and civilian casualties, overwhelming front line medical services and complicating evacuation and infection-control. In such settings, delayed definitive care, contaminated wounds, and widespread empirical antibiotic use can promote colonization and infection by multidrug-resistant (MDR) bacteria, posing therapeutic and public-health challenges. In September 2024, the World Health Organization warned of the escalating MDR threat in the country.

In this study, 35 Gram-negative isolates representing eight species (A. baumannii, A. xylosoxidans, C. freundii, E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, and S. marcescens) were recovered from combat-related wounds in Ukrainian patients. Species identification was performed using MALDI-TOF, and antimicrobial susceptibility was assessed by disc diffusion according to EUCAST guidelines. Preliminary analyses revealed resistance to multiple antibiotic classes in several isolates, with the majority demonstrating carbapenem resistance, underscoring the clinical relevance of these pathogens in conflict-related infections.

Whole-genome sequencing (WGS) is currently underway to characterize resistance determinants, identify plasmid replicons and determine clonal relatedness. Planned work will focus on phenotypic assessment of virulence traits, including biofilm formation, virulence factor production, serum resistance, and host-pathogen interactions.

By integrating phenotypic resistance profiles with genomic and virulence data, this study aims to provide a comprehensive understanding of the pathogenic potential of MDR Gram-negative wound isolates and to strengthen strategies for managing MDR infections in war-affected settings.

[P050] The Role of Tolerance in Recurrence of Urinary Tract Infections

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Antimicrobial resistance (AMR) is a well-known challenge in treating bacterial infections. Beyond resistance, evidence shows additional challenges to treatment outcomes such as bacterial tolerance and persistence which allow bacteria to transiently survive antibiotic exposure. Additionally, tolerant strains have been reported to precede antibiotic resistance development, as a means of gaining more time to confer resistance. Unlike resistance, tolerance remains difficult to detect in routine diagnostics. In this study, we assessed tolerance in clinical urinary tract infection isolates. We compared recurrent E. coli isolates (rUTI) to non-recurrent E. coli isolates (cured) to determine whether the presence of tolerance is associated with recurrence. Tolerance was evaluated using the Tolerance Disk test (TDtest), a modified disk diffusion assay to detect tolerance against ciprofloxacin, mecillinam, and gentamicin. Our results show that the TDtest can determine tolerance among clinical E. coli isolates from UTIs. From both patient groups, isolates demonstrated tolerance against ciprofloxacin, mecillinam, and gentamicin. Although we currently do not find significant differences between rUTI and cured isolates, our findings suggest specific mechanisms used for tolerance against different classes of antimicrobials. The lack of association with recurrence warrants a larger dataset or is suggestive of the influence of host-related factors, which merits further investigation.

[P051] Methanol-based production of 3-hydroxypropionate in recombinant Bacillus methanolicus MGA3

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Methanol is gaining an increasing recognition as feedstock for biotechnological processes thanks to its unique properties such as low reduction state, easiness of transport and storage and its sustainability potential [1]. Majority of methanol is currently produced from syngas. In recent years large progress has been made in the development of sustainable technologies for production of renewable methanol, for example from captured CO2 by Power-to-X technology. The feasibility of methanol as feedstock for biotechnology depends on existence of methylotrophic microbial cell factories capable of its conversion to value-added compounds.

Here, we employed methylotrophic metabolism of Bacillus methanolicus to establish methanol-based production of organic acid, 3-hydroxypropionate (3HP). 3HP is a platform chemical for various compounds and a building block of polymers, plastics, resins and other substances [2]. There are multiple pathways for microbial 3HP production. In our research, we explored conversion of malonyl-CoA, naturally produced by B. methanolicus, to 3-HP. This reaction is catalysed by the heterologous bifunctional malonyl-coenzyme A reductase (MCR) or MCR together with malonic semialdehyde reductase (MSR). We tested B. methanolicus strains heterologously overproducing MCR or MCR/ MSR from different donors, and identified the most promising strain which produced 0.08±0.01 g L-1 3HP in flask cultivation. This titer was further improved by previously used strategy of pyruvate regeneration through activity of malic enzyme together with glyoxylate shunt enzymes, leading to final 4-fold increase of 3HP titer to 0.32±0.04 g L-1 [3].

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[P052] Metagenomics provides enhanced detection of pathogens, antimicrobial resistance and virulence genes in pig diarrhoea as a complement to conventional methods

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Gastrointestinal infections represent a major health problem in animals and humans and a significant economic burden in farm animals. Metagenomic sequencing using Oxford Nanopore Technology (ONT) offers a rapid, culture-independent approach to detect the full spectrum of causative agents of infections. By profiling the entire microbial community, ONT metagenomics provides genome-wide insights into antimicrobial resistance genes (ARGs) and virulence factor (VF) repertoires, complementing conventional diagnostic methods.

The objective of this study was to evaluate whether ONT sequencing can detect enterotoxigenic Escherichia coli (ETEC) and Clostridium perfringens directly from diarrheal pig feces and to compare its performance with conventional diagnostics (culturing and qPCR). We combined culture-based isolation and whole-genome sequencing (WGS) of 26 haemolytic E. coli and 9 C. perfringens isolates with ONT metagenomic sequencing using PromethION (research-based approach) and GridION (rapid diagnostics approach) workflows. The capacity of each approach was assessed to detect pathogens, ARGs, VFs and intestinal parasites. While we detected significantly larger repertoire of pathogens, ARGs and VFs using the research-based PromehION sequencing, the rapid GridION diagnostics approach could detect the major bacterial taxa, including E. coli and Clostridium sp. and broadly captured the resistome, making it a valuable tool for delivering actionable information when rapid response is required. For several of the cases, the reconstructed metagenome assembled genomes (MAGs) and the direct mapping of the reads to the genomes of the recovered E. coli isolates were highly similar, up to 96% coverage of the direct mapping was achieved for six of the samples and for seven of the cases up to 90% alignment between the MAGs and the genomes of the E. coli isolates. In conclusion, ONT metagenomics is a valuable complement to conventional diagnostics, providing a broader overview of pathogens, resistance and virulence determinants that are not strict to targeted culturing or PCR. Its dual capacity, comprehensive profiling via PromethION and rapid actionable insights via GridION, supports both in-depth investigations and timely outbreak responses.

[P053] Comparing Line Probe Assay and Whole-Genome Sequencing for Accurate Identification of Nontuberculous Mycobacteria

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Infections caused by nontuberculous mycobacteria (NTM) are increasingly recognized worldwide. Their intrinsic resistance challenges the prolonged multidrug treatment required to cure these infections. Accurate species identification is essential for guiding treatment, as regimens differ between species due to species-specific resistance patterns. Although the GenoType Mycobacterium CM Line Probe Assay (LPA) is widely used in routine diagnostics, its utility is limited by the number of species it can identify and by potential misclassification of closely related species due to conserved regions in the 23S rRNA region used by this LPA.

In this study, we compared LPA-based species determination with whole-genome sequencing (WGS)-based identification using average nucleotide identity (ANI) to reference type strains in 40 clinical NTM isolates spanning eight species. Concordance and discordances between the two methods were assessed, with a focus on clinically relevant misidentifications. Six species (M. avium, M. xenopi, M. fortuitum group, M. intracellulare group, M. chelonae, and M. abscessus) showed concordance between the LPA and WGS, with either identical species or expected subspecies identified. However, discordance was observed in two cases: 1) isolates reported as M. kansasii by LPA were identified as both M. persicum and M. kansasii by WGS and 2) all isolates identified as M. malmoense by LPA were identified as M. parascrofulaceum by WGS. These misclassifications could have clinical impact, as treatment regimens for the LPA-identified species are not suitable for true species identified by WGS due to their resistance patterns.

Our findings highlight the importance of accurate species determination of clinically relevant NTM. Misclassification may result in inappropriate treatment, promote resistance development, and worsen patient outcomes.

[P054] Microbial Origins of Biogenic Ice Nucleating Particles in Agricultural Soils Are Driven by Site Conditions Rather Than Management

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Atmospheric ice nucleating particles (INP) trigger ice formation in supercooled water, influencing cloud microphysics and precipitation. While mineral particles are abundant in the atmosphere, microbially produced compounds have been linked to INP with an ability to nucleate ice at high subzero temperatures (>10 °C). Agricultural soils, which cover roughly 37% of Earth's land surface, have been identified as reservoirs of these potent biological INP (BioINP). However, it remains unclear how site conditions and common agricultural practices, such as tillage, cover cropping, and liming, influence BioINP abundance and whether these effects relate to the microbial community composition. To address this, we quantified BioINP concentrations and properties in soils from five long-term Danish agricultural field trials and characterized the microbial communities using high-throughput sequencing of 16S rRNA and ITS marker genes. BioINP concentrations varied over several orders of magnitude, with particularly high levels in Flakkebjerg, the most clay-rich site. Soil management practices influenced microbial community composition but had limited and inconsistent effects on BioINP abundance. Bacterial taxa previously reported as ice nucleation active, including Pseudomonas and Lysinibacillus, were detected at low relative abundances and weakly correlated with BioINP activity. In contrast, fungal community composition was a stronger predictor: the relative abundance of Fusarium spp. and Linnemannia associated with elevated BioINP concentrations. Our results suggest fungi, particularly Fusarium, are the dominant contributors to BioINP concentration in agricultural soils. This highlights the need to consider fungal ecology when linking agricultural management to atmospheric processes. Future work will quantify Fusarium abundance using qPCR, determine fluxes of soil-derived BioINPs under varying wind and rainfall, and assess impacts on cloud dynamics and climate.

[P055] A wax-based model for studying microbial colonisation on plant surfaces

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Understanding microbial interactions on plant surfaces is key to improving microbial biocontrol and microbiome engineering. Artificial models of plant surfaces create controlled environments to investigate microbial interactions, yet such replicas often lack the nutritional or physical plant characteristics which impact microbial behaviour. This work addresses this limitation by developing a wax-based model that integrates both the nutritional composition and structural features of one of the world's most important crops, potatoes (Solanum tuberosum). Microbial adhesion and growth of the soft rot bacterium, Pectobacterium, which causes soft rot and consequential significant post-harvest losses, was tested using the potato replica model. Bacterial growth curves were measured on nutrient media and wax models to validate their ability to support Pectobacterium. The similarities in the physical structure of the potatoes and the wax models were demonstrated with surface analysis techniques, including Scanning Electron Microscopy (SEM), Optical profilometry (OP), Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR), Energy Dispersive X-ray Spectroscopy (EDX), and water contact angle measurements. The model provided a controlled environment for reproducible investigation of colonisation patterns, biofilm formation, and interspecies interactions on potato surfaces. Overall, the model offers a biologically relevant tool for exploring microbial behaviour on plant surfaces and can support future efforts to reduce spoilage and improve crop resilience.

[P056] MAGnifying biogas production efficiency: high-quality metagenome-assembled genome recovery in anaerobic digesters reveals abundant novel populations with key functional roles

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Anaerobic digestion (AD) plays a key role in moving towards a climate-neutral energy economy framework, as it transforms organic waste into methane-rich biogas for both heat and power generation. However, the efficiency of this process is limited, with nearly half of the potential biogas yield lost mainly due to the presence of inhibitors that negatively affect the microbial community. Recent studies show that a substantial portion of this community remains undescribed. Here, we applied nanopore long-read sequencing to generate 1,978 high-quality (HQ) MAGs from 20 Danish anaerobic digesters, including seven treating wastewater sludge and 13 biogas plants primarily processing manure and crop waste. Among the dereplicated HQ MAGs, 28.5% lacked species-level assignments based on GTDB R226. Incorporation of these MAGs into the GTDB R226 dataset increased the median relative abundance (RA) captured at the species level to 81%. Genome annotation revealed that species involved in hydrolysis, acidogenesis, acetogenesis, and methanogenesis accounted for 72.0%, 71.6%, 41.3%, and 9.27% median RA, respectively. Notably, by integrating DNA stable isotope probing, we confirmed the syntrophic acetate-oxidizing function of midas_g_112, an abundant species across biogas plants (~16% RA), as suggested by its genomic potential in MAGs, thereby advancing efforts to design optimize microbial consortia. This research not only uncovers key microbial actors driving biogas production but also lays the foundation for enhancing the efficiency and sustainability of this vital energy process.

[P057] Phage-derived CRISPR therapeutic designed to reduce E. coli in haematological cancer patients

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Patients with hematological malignancies frequently develop bloodstream infections due to translocation of E. coli and other bacteria from the gut. Antibiotic treatment to prevent these infections has detrimental effects on the microbiome and immune tonus and is further hampered by increasing antibiotic resistance, in particular towards fluroquinolones. We screened a library of 162 wild-type (WT) phages identifying 8 phages with broad coverage of E. coli. Selected phages were engineered with novel tail fibers and a CRISPR-Cas machinery targeting clinically relevant E. coli. Engineered phages target bacteria in biofilms, reduce the emergence of phage-tolerant E. coli and out-compete their ancestral WT phages in co-culture experiments. SNIPR001 comprises four engineered bacteriophages with broad host-range across the E. coli phylogeny, including multi-drug resistant strains. SNIPR001 is well-tolerated in animals and reduces E. coli load in the murine gut better than its constituent components. SNIPR001 represents a novel CRISPR-Cas-therapeutic designed to selectively target E. coli which may cause fatal infections in hematological cancer patients. A clinical study to evaluate the ability of SNIPR001 to ascertain safety and its ability to reduce E. coli in the gut without perturbing the overall gut microbiome is currently ongoing in the US (NCT05277350).

[P058] The puzzle of assembly - full genomes from incomplete data

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Background: Epithelial-associated microbial communities represent a critical yet under-characterized niche within the gastrointestinal tract, particularly relevant in the context of chronic inflammatory conditions such as ulcerative colitis (UC). Conventional approaches relying on fecal samples provide limited insight into these mucosal communities, which are often more directly involved in disease pathogenesis.

Results: We systematically evaluated three DNA extraction protocols differing in their use of mechanical disruption, enzymatic host DNA depletion, and detergent-based bacterial enrichment, for their ability to recover high-quality microbial DNA from intestinal biopsies of ulcerative colitis patients. The findings revealed that enzymatic host depletion combined with chemical lysis yielded the highest proportion of non-host DNA while maintaining microbial diversity, though metagenome-assembled genomes could not be retrieved from individual samples due to low sequencing depth. To overcome this, we constructed a high-quality, environmentally specific MAG reference database (esMAGdb) from faecal samples of the same cohort (n=31), comprising 4,396 MAGs, including hundreds of novel species representatives. Alignment of co-assembled biopsy contigs (>1 kb, ≥99% identity) to esMAGdb enabled the identification of 72 species, most of which were undetected by 16S rRNA gene sequencing.

Conclusions: This study demonstrates that high-resolution characterisation of epithelial-associated microbial communities is achievable through a novel alignment approach leveraging cohort-specific reference databases and co-assembly strategies, when DNA input is optimised. . This approach recovered over several bacterial species from the epithelial compartment—far exceeding what was captured by 16S profiling—and included numerous low-abundance taxa. Here we introduce robust yet adaptable thresholds for contig-based species assignment: 7 kb with ≥99% identity or at least three contigs >1 kb at ≥99% providing a scalable framework for future studies in low-biomass systems.

[P59] Outer Membrane Disruption Reshapes the Proteome of Acinetobacter baumannii Outer Membrane Vesicles

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Acinetobacter baumannii is an opportunistic, nosocomial pathogen that is known to cause infections such as pneumonia, meningitis, blood stream infections and implant-associated infections in immunocompromised patients. The rapid rise in multidrug resistance (MDR) in A. baumannii has positioned this bacterium in recent years as a serious global threat to human health. In 2024, The World Health Organization nominated MDR A. baumannii as one of the top priority pathogens, which urgently require new treatment strategies.

Outer membrane protein A (OmpA) is a highly conserved protein among Acinetobacter species and is considered to play an important role in the virulence of clinical Acinetobacter strains, including low permeability of the outer membrane, attachment and biofilm formation, host cell invasion and host cell cytotoxicity. Deletion of ompA causes disruption to the integrity of the outer membrane, which leads to an increase in outer membrane vesicle (OMV) production. OMVs are vesicles derived from the outer membrane of Gram-negative bacteria in response to different stimuli and are known to deliver encapsulated content, such as a wide range of proteins and nucleic acids, to host cells. OmpA in OMVs has been shown to induce host cell cytotoxicity and mitochondrial fragmentation. This study aims to investigate how deletion of ompA, and the resulting disruption of the outer membrane, affect the composition and enrichment of the proteome in OMVs. Furthermore, we will explore what these changes can reveal about the mechanism of OMV production in A. baumannii.

The absence of OmpA leads to a significant increase in OMV release, likely due to a compromised outer membrane integrity. Proteomics on isolated OMVs followed by differential and functional analysis revealed a pronounced change in the protein composition and relative abundance between the parental strain OMVs and the ompA deletion mutant OMVs. Proteins predominately associated with translation and energy production were more abundant in OMVs from the parental strain, while cell wall and membrane associated proteins were more abundant in ΔompA OMVs, notably the proteins of the BAM complex. Approximately 23 % of proteins present in parental strain OMVs were not secreted in the mutant OMVs. However, we identified 98 proteins that were unique to ΔompA OMVs. This discrepancy may result from disruptions in the pathways involved in cargo selection during OMV production.

Our findings indicate that disturbances in the outer membrane integrity can shift the proteome of OMVs, possibly reflecting the involvement of a specific and targeted mechanism for cargo selection during OMV production in A. baumannii.

[P060] Comparison of Anaplasma phagocytophilum diagnostics

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A. phagocytophilum is an obligate intracellular Gram-negative prokaryote that infects neutrophils. It is the causative agent of anaplasmosis, a vector-borne disease, in Europe. It is primarily transmitted by the tick species Ixodes ricinus, which feeds on ruminants, pets, and humans alike.

In particular, deer and sheep are common targets for I. ricinus and, therefore, A. phagocytophilum as well, with numbers as high as 300,000 infected lambs in Norway, and direct loss of up to 30% of a flock (Stuen et al., 2002). In Denmark, a 94% seroprevalence in roe deer was observed in 2019, which is a doubling from 2009 (Andersen et al. 2019).

This underlines the importance of fast and efficient clinical diagnostics, which presents a crucial challenge. Currently, monitoring is performed through a serological test using IFA (indirect immunofluorescence assay) or ELISA and there seems to be a large discrepancy between the positive results of the two tests.

Therefore, this master's thesis aims to establish PCR verification of A. phagocytophilum infections using primers for the msp2 and compare this to serological methods, which will also allow the establishment of A. phagocytophilum seroprevalence in I. ricinus.

[P061] Characterization of fungal exposure in biomass facilities

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While energy from biomass is considered sustainable, the materials can harbor fungi, thus constituting a potential source of exposure for workers. Fungal exposure can lead to health effects including the onset or worsening of asthma and allergy, while infections can affect predisposed individuals. This study aimed to assess fungal exposure at biomass facilities in Denmark combusting either straw, wood chips or wood pellets. Potential risks associated with the exposure including, respiratory deposition, occurrence of pathogenic fungi, and resistance among key pathogens were investigated. To this end, 48 whole-workday personal exposures were measured, and 36 nasal lavage (NAL) samples were obtained from workers, while potential respiratory deposition was measured using 6-stage Andersen Cascade Impactor (ACI). Samples were cultivated on fungi-selective media and species identified using MALDI-TOF MS. Furthermore, Aspergillus fumigatus isolates from personal exposures and NAL samples were tested for Azole resistance according to EUCAST guidelines. Significant differences between biomass facilities in overall concentrations of fungi and fungal species compositions were observed for both the personal exposures and in NAL samples. A positive association between exposure and nasal deposition of fungi was found, while species associations were less clear. Risk group 2 fungi were abundant in personal exposures, and three isolates of the infectious species A. fumigatus exhibited multi-resistance against Itraconazole, Voriconazole, and Posaconazole; resistant A. fumigatus was only observed for isolates from the straw facility. The study indicates that the type of biomass impacts workers risk of fungal exposure and nasal deposition, for overall concentrations and species specific exposure. It further highlights additional risks associated with exposure, with potential implications for workers health. Finally, the study demonstrates and assesses the potential value of NAL as a tool in exposure assessment.
This work was supported by the Danish Working Environment Authority; Focused Research Effort on Chemicals in the Working Environment (FFIKA- Green) from the Danish Government

[P062] Engineering Phages to Outsmart Bacteria

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Antibiotic-resistant infections are a growing global health threat, and new therapeutic strategies are urgently needed. Phage therapy—using bacteriophages to infect and kill bacteria—is re-emerging as a promising alternative due to phages' host specificity and ability to amplify at infection sites. However, pathogens like Pseudomonas aeruginosa have evolved quorum sensing (QS)-regulated anti-phage defenses that limit phage efficacy.

QS is a bacterial communication system involving the production and detection of signaling molecules. When these accumulate to a threshold concentration, they gear bacterial gene regulation towards virulence and anti-phage defense. Targeting the versatile pathogen P. aeruginosa, we developed a dual-action therapeutic approach that combines phage therapy with QS inhibition. We have engineered anti-QS phages that not only infect and kill P. aeruginosa but additionally produce anti-QS enzymes that degrade QS signal molecules. This suppresses virulence and sensitizes neighboring cells to phage attack, enhancing antibacterial efficacy.

In vitro, our phages reduce QS signal molecule concentrations, even in the presence of phage-resistant mutants. We tested their therapeutic potential in vivo using a Galleria mellonella burn wound infection model. Larvae infected with P. aeruginosa and treated with anti-QS phages show significantly improved survival compared to those treated with unmodified phages. This effect also holds true when infecting with mixed populations of phage-susceptible and phage-resistant cells, highlighting the ability of anti-QS phages to disarm resistant subpopulations.

Currently, we are engineering an anti-QS phage cocktail broadly targeting clinical P. aeruginosa isolates. Since many pathogens rely on QS, our long-term goal is to expand this strategy to other critical bacteria. This work lays the foundation for next-generation antibacterial therapies that address both phage resistance and the broader challenge of antibiotic resistance.

[P063] Uncovering the Early-Life Dynamics of Phages and Plasmids in a Non-Western Infant Gut Cohort

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Early-life gut microbiome development is critical for the development of the immune system and metabolism. While bacterial succession has been extensively studied, the dynamics of mobile genetic elements (MGEs), such as phages and plasmids, remain underexplored, especially in underrepresented non-Western populations. Previous studies suggest that viral populations change over time, particularly during the first years of life.

We hypothesize that mobile genetic elements (MGEs) undergo distinct shifts in diversity and persistence during early gut colonization, shaping microbial community development and host interactions. To test this, we investigate the temporal dynamics of MGEs in the infant gut microbiome from a non-Western cohort, focusing on phage lifestyle transitions, phage–host associations, and plasmid mobility. We also analyze CRISPR–Cas system abundance and targeting patterns to explore host–MGE interactions and immune dynamics.

We analyzed 1347 metagenomic samples from ~223 infants collected at 7 timepoints from birth to 24 months. We observed a lifestyle shift of virulent phages overtaking temperate phages around the 20th month. Approximately 99% of viral families were novel, highlighting the need for more thorough viral analysis. Viral prevalence patterns revealed early transient families and late persistent ones. Host analysis showed a transition from E. coli dominance in early months to Prevotella comprising ~50% of viral hosts by month 24, following bacterial abundance trends. Plasmid contig counts peaked at month 6 and declined thereafter, following a different trend compared to the viral counts.

Our findings suggest that MGE dynamics in non-Western infant guts differ substantially from those reported in Western cohorts. These differences underline the importance of inclusive sampling in microbiome research and may have implications for understanding early-life microbial ecology and gene flow.

This ongoing study provides novel insights into the temporal behavior of MGEs during gut microbiome maturation in a non-Western infant cohort. Our analyses will improve understanding of the functional roles and ecological impacts of these elements in early life.

[P064] Engineering a Marine Microbial Cell Factory for Novel Antibiotic Discovery

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Antimicrobial resistance poses a growing global threat, highlighting the urgent need for new antibiotics. While the majority of current antibiotics originate from microbial natural products, the vast genetic potential of marine microorganisms remains inaccessible due to their limited cultivability under laboratory conditions. To overcome this limitation, we propose to engineer Photobacterium galatheae S2753 as a bacterial cell factory capable of directly absorbing environmental microbial DNA (eDNA) and expressing biosynthetic gene clusters (BGCs). This strategy aims to access the vast untapped marine microbial genetic potential and accelerate the discovery of novel antibiotics.

Microbial cell factories are powerful tools for producing bioactive molecules, yet their role in drug discovery is often limited by challenges in cloning BGCs. This PhD project seeks to harness the natural transformation system of Photobacterium galatheae to establish a robust, cloning-free workflow for S2753 to uptake eDNA and express BGCs. Proof-of-concept studies will employ genomic DNA containing BGCs of known antibiotics and pigments, followed by application to eDNA isolated from marine bioactive microbiomes. Combined with antimicrobial activity screening and HPLC-MS/MS, this project is expected to yield a functional marine microbial cell factory capable of screening for novel antibiotics from the ocean. If successful, this strategy also offers a scalable path for accessing untapped marine biodiversity and contributes to addressing other global challenges.

[P065] Small but mighty: A Small ORF Effector Powers Retron-Mediated Phage Defense

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Bacteria and phages are locked in an evolutionary arms race, driving the emergence of diverse defense and counter-defense strategies. Among them, retrons - systems containing a signature reverse transcriptase, ncRNA, and an effector have emerged as a diverse class of defense systems. But what's so interesting about "phage-defending tripartite toxin–antitoxin systems"? In recent studies, various retrons have shown a vast diversity in their mode of defense, their molecular assembly, as well as their protective capacities. While the interplay of effectors, ncRNA, and RT can be studied with the help of molecular imaging technologies, crucial hints to unravel phage sensing and infection dynamics are gathered by phage biology work: anti-phage screening, system mutagenesis, and studying how phages evolve to overcome immune targeting. In my work, I investigate a new minimal retron system—encoding a small protein effector of unknown function—and aim to uncover how such a streamlined system mounts an effective defense against phages.

[P066] The urban air microbiome across major railway stations of Delhi, India: Seasonal variations and public health links

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Public transit systems such as the railway stations in Delhi, India, are semi-sheltered, outdoor settings with frequent train and passenger movement, causing dust resuspension, and variable temperature and humidity, which shape airborne particle dynamics and human exposure. These stations represent high-density urban environments with intense human traffic from all over the country, handling a daily footfall of hundreds of thousands, leading to sustained and repeated exposure to bioaerosols. At the same time, India faces a substantial and growing antimicrobial resistance (AMR) burden, driven by high antibiotic use and environmental dissemination. Yet metagenomic data on airborne microbes, in such environments, remain sparse, especially in the Indian context, limiting risk assessment related to opportunistic pathogens, antimicrobial resistance genes (ARGs), and virulence factors. The study profiled the urban air microbiome at four major stations, Anand Vihar Terminal (ANVT), Delhi Junction (DLI), New Delhi (NDLS), and Hazrat Nizamuddin (NZM), to identify seasonal variations in diversity, resistome, and virulome, and assess relevance tolic health.

Bioaerosols were collected at each station during the autumn (Oct-Nov 2023) and summer (June-July 2024) seasons, with 8 samples in total, using an SKC BioSampler operated for 16 hours (8 hours each day: peak hours) at 12.5 LPM, following a previously optimized protocol. Shotgun metagenomics facilitated a comprehensive taxonomic and functional characterisation of the urban bioaerosol samples.

Airborne communities were dominated by Pseudomonadota and Actinomycetota, with several opportunistic genera prominent, including Moraxella (19.3-62.52%), and Sphingomonas (2.09-14.13%) in autumn, and Stutzerimonas (6.30%-66.82%) in summer, as well as Acinetobacter (3.14%-48.40%) in all samples. Across the two seasons, alpha diversity differences were not statistically significant (Shannon index: median = 2.8; Mann–Whitney U, p = 0.68). In contrast, beta diversity measured by Bray-Curtis distance showed clear seasonal variations: ordination separated samples by season, and PERMANOVA indicated a significant seasonal effect (R2 = 0.3975, p = 0.033). LEfSe highlighted season-representative genera such as Moraxella and Barrientosiimonas in autumn, and Stutzerimonas and Caulobacter in summer.

Functionally, ARG classes associated with common antibiotics, including beta-lactams, aminoglycosides, and macrolide-lincosamide-streptogramin, and virulence-related categories from VFDB, such as adherence, motility, and effector delivery system, were identified. The presence of these functional genes alongside opportunistic pathogens suggests plausible exposure pathways relevant to overall health for commuters, staff, and other frequent users.

The results highlight the need to integrate microbial monitoring into air quality management and public health policy frameworks in rapidly expanding urban regions like Delhi. Future work should expand temporal coverage and replicates, and integrate co-measured meteorology and particulate matter to strengthen exposure models and support scalable environmental health surveillance in urban transit settings.

[P067] Spatial heterogeneity in Streptococcus thermophilus milk fermentations and the effect on a high throughput assay

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The industry is increasingly focused on developing new food products that meet criteria such as sustainability, clean labeling, and health benefits. At Novonesis, a large strain collection is screened to identify strains suitable for specific food matrixes and applications. One key area of interest is Streptococcus thermophilus (ST) for dairy applications including yoghurt and mozzarella-type cheeses made from bovine or plant-based milks.

Small scale milk acidification data from a 2 mL high throughput assay and larger scale applications do not always align. Spatial heterogeneity has been hypothesized as one of the reasons for this. In the small-scale screening setup, pH is measured at the bottom of polypropylene 96-well plates whereas the larger scale 200 ml setup relies on pH probe measurements in the middle of the milk liquid in glass bottles.

Scale-down and microenvironments are discussed when developing production strains within the biotech industry [1], however it is poorly characterized for dairy fermentation. In this study, testing more than 150 ST's, in small (2ml) and large (200 ml) scale, we find that there are several different strain dependent acidification responses at the top and bottom of the 2 ml microtiter plate wells, suggesting different microenvironments. A strain with a faster acidification at the bottom was shown to have a higher CFU count at the bottom compared to the top. Another strain that showed faster acidification at the bottom in the 2 mL scale but not at the 200 mL scale, was shown to have a lower CFU count at the bottom of the polystyrene plate compared to both the top of the polystyrene plate and the top and bottom in the 200 mL milk fermentation. Future analysis of the 150+ ST strains, using both milk acidification phenotypes and genotypes will expectedly allow for a better understanding of how genetic differences may lead to spatial heterogeneity and acidification differences across small and large scale.

[P068] Etiology and Pathogenesis of Nasal Polyps Elucidated by Histopathological Studies of ”Fully Developed Polyps” and Small Polyps and Their Place of Origination

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Key words; Prevalence, Cells, epithelia, glands, “Epithelial Rupture”- and “Glandular new formation Theory”, biofilm, staphylococci,

We have attempted to shed light on the etiopathogenesis of nasal polyps based on studies of polyps of different sizes and the mucous membrane from the place of origin of small nasal polyps.
Supra- (goblet cells and epithelia) and infrastructure (glands and cells) was examined in “Fully Developed” nasal polyps (>15 mm.) and supra- (epithelia and glands) and infrastructure (cells and biofilm) in small nasal polyps (<5 mm.), their place of origination and the corresponding mucosa.
The suprastructure (epithelia and goblet cells) was examined in 29 large “Fully Developed” nasal polyps. The mucous membrane was fine dissected and placed in a chamber with anise-oil colophonium sealed with paraffin after PAS-Alcian Blue whole mount staining. The goblet cell density was determined using a projection microscope.
After counting the goblet cells, 5 µm thick sections were made, allowing epithelial types to be identified by hematoxylin eosin and PAS-Alcian blue staining.
Glands from fully developed polyps were examined in Pas and Pas-Alcian blue stained whole mount specimens.
Glands were also examined after immunohistochemical staining for growth factors and myoepithelial basket cells.
Mast cells were stained with a Naphtol-AS-D-Chloracetat staining.
Biofilm with DAPI - 4´,6-diamidino-2-phenylindole (DNA-staining).
Goblet cell density was investigated in 10 counting fields each measuring 0.01768 mm² in three horizontal directions and in proximal, middle, and distal parts of anterior and posterior polyps.
The goblet cell density was extremely irregular on different polyps and in different areas on the same polyp. Median density in total; 280-10.000 cells / mm2, 3.450 cells / mm2 in anterior polyps and 6.050 / mm.2 in posterior polyps.
After counting the goblet cells, 5 µm thick sections were made for examination of epithelia.
Different densities of goblet cells and different types of epithelia (pseudostratified, columnar, transitional, squamous transitional, stratified squamous) were found within the same polyp.
A gradual or abrupt transition between epithelial types could also be seen.
The results are thought to be caused by constant changes under the influence of airflow, contact with other polyps, infection, growth and age of the polyp, as well as other unknown factors.
Glands could be studied in whole polyps also using the PAS-Alcian Blue whole mount method.
Most of the glands were localized in the distal part of the polyp.
The density was much lower (0.1 – 0.5 glands per mm²) than in the nasal mucosa.
The structure and architecture were different from the glands in the nasal mucosa.
The secretion was mucous.

Myoepithelial basket cells were found around glands in the nasal mucosa but not associated with glands in the polyps.
After studying the glands in the whole mount specimens, we proposed a “Glandular New Formation Theory.”
Mast cells, eosinophils, neutrophils, and lymphocytes were found.
An unbiased uniform random sampling technique was used for quantification of cellular elements, including eosinophils.
Differentiation between T- and B-lymphocytes was done by immunohistochemical staining for CD3+ and CD20+.
There was a strong relationship between clinical recurrence of nasal polyps and eosinophilic accumulation, but not T- or B-lymphocytes.
Additionally, we conducted a clinical study on Vascular Cellular Adhesion Molecules, Inter Cellular Adhesion Molecules and eosinophils in nasal polyps.
T-cells can initiate IL-5 production, further adhesion of eosinophils and recruit eosinophils to the polyp tissue.
New biologics can reduce nasal polyps by blocking IL-5.
But when treatment stops, some patients experience recurrence of polyps.
Three studies on the frequency and origin of nasal polyps in autopsies were performed.
By anterior rhinoscopy on 300 autopsies we found polyps in 2%.
En-bloc removal of 19 naso-ethmoidal complexes without prior examination revealed polyps in 5, corresponding to 26%.
As preliminary preparation for endoscopically guided sinus surgery, we performed systematic endoscopic examination of the nose and sinuses and endoscope-assisted sinus surgery, which enabled us to detect small polyps and remove them along with the adjacent nasal mucosa / polyps’ origin site, providing an opportunity to study the initial stages of polyp formation in the hope of achieving more optimal treatment for nasal polyps.
The examination and sinus surgery was performed on 150 autopsies. Polyps were found in 48, corresponding to 32%.
Preferred places of origin were the mucous membrane in the sinus outlets.
Several pathogenetic theories have been proposed:
Billroth proposed the “adenoma theory.”
We proposed the epithelial rupture and glandular new formation theory.
Cross sections of small nasal polyps showed an epithelial defect on the apical part of the polyp, which could be a sign of epithelial rupture.
The small nasal polyps were covered by a very low epithelium, and different epithelia were observed on opposing sides of the same polyp, indicating epithelialization following a defect.
Initial stages of gland formation and early gland formation were evident as small epithelial “buds” and secretory cells within the surface epithelium of the distal polyp region.
Growth factor KI67 was found.
Inflammatory cells were present.
Semiquantitative counts showed a high density of eosinophils at the site of origin.
It has been proposed that major basic proteins from eosinophils can be the cause of epithelial defects.
If polyps were removed by scissors and the tissue slowly dehydrated and fixed in an oxygenated medium, no epithelial defects were found.
Our experimental studies in rats have shown that polyps can form in the middle ear if drainage conditions are changed by tubal occlusion combined with bacterial infection and bacterial stasis.
Studies on infected sinuses in rats were also performed but did not show signs of polyp formation, possibly because rats have very broad sinus outlets that may prevent secretion stasis and bacterial biofilm formation.

As mentioned before, preferred sites for polyp formation in humans are the mucosa of the sinus outlets.
In small nasal polyps with corresponding nasal mucosa, a bacterial biofilm could be seen in the polyp stalk, and staining for staphylococci revealed that the bacteria were staphylococci.
Staphylococcus aureus enterotoxin (SAE) can induce T-lymphocyte production of IL-5, which activates eosinophils and prolongs their survival.
The results seems to indicate that;
Nasal polyps mainly originates in the mucosa of sinus outlets and are new formations in the mucosa.
Nasal polyps can be caused by a local inflammatory process.
More extensive sinus surgery has been proposed for removal of nasal polyps:
“Reboot Surgery” aims to remove all inflamed mucosa down to the periosteum of the ethmoidal, sphenoid, and maxillary sinuses.
In the future, a combination of medical treatment with IL-5 inhibitors, antibiotics, and surgery could be used in the hope of “hitting the target” and breaking the vicious cycle.
Early intervention seems to be important.

Fig. 1
”Fully Developed” Nasal Polyp, removed by a snare.

Fig. 2 a) Endoscopic picture of a small nasal polyp which originates in the mucous membrane of the sinus outlet.

Fig. 2 b) Small nasal polyp removed with polyp stalk and corresponding mucosa.
P and small arrows; polyp. N; nasal mucosa.
Big arrow; polyp stalk.

[P069] Linking plant-based diet, gut microbiota composition and inflammation in individuals with inflammatory bowel disease

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Diet, gut microbiome and human health are strongly connected. The microbiota has a huge impact on the health of the host and acts through the dietary components and metabolites produced from these food-derived substrates. Inflammatory bowel disease (IBD) is a condition thought to be modulated by diet, with high animal protein intake and low consumption of fruits and vegetables being contributing factors. Studies indicate that the western diet is associated with a negative development of microbiota diversity and health.

This study aims to investigate how a plant-based diet combined with physical training affects gut microbiome composition and intestinal inflammation in individuals with IBD.
Fecal samples were collected (week 0 and 4) from a cohort of 47 participants with IBD. Dietary adherence was assessed through self-reported food diaries and validated by amplification of the chloroplast TrnL gene and genes targeting, cow, pig and chicken. Composition of the microbiota was characterized by 16S rRNA gene amplicon sequencing of the V1-V8 region, and intestinal inflammation was assessed by fecal calprotectin levels.
Associations between the plant-derived dietary components, microbiota and reduction in intestinal inflammation provide a direct link between diet and microbial compositions. These findings will contribute to understanding diet-microbiota interactions in IBD and may help identify microbial biomarkers with potential for disease monitoring and management.

Keywords: Plant based diet intervention, Inflammatory bowel disease, nutrition, trnL, 16S rRNA.

[P070] Bacterial ubiquitin-like conjugation of viral receptor binding proteins prevents phage spread

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Ubiquitin-like (Ubl) conjugation is central to eukaryotic regulation, but its role in bacterial immunity is largely unexplored. Here we describe a novel bacterial defense system restricts phage propagation at the population level. Genetic and structural analyses show that conserved Ubl glycines and catalytic residues in the E1–E2 are essential for conjugation, whereas DUF6527 acts as a negative regulator. Phage escapers carry mutations in lateral tail fiber operons, and infection yields progeny with defective tail structures. These findings reveal a bacterial Ubl pathway that targets phage virion components, broadening the repertoire of antiviral strategies and highlighting evolutionary links to eukaryotic ubiquitin signaling.

[P071] Utilization of brown juice for short chain carboxylic acids production by co-cultures of lactic acid bacteria

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Brown juice is a nutrient rich by-product from green biomass biorefining, e.g., of grass, clover, alfalfa. Despite its availability, brown juice still remains largely unexplored for microbial bioproduction in biotechnology [1]. This study investigates the potential of brown juice as a carbon source to produce antimicrobial compounds. We will harness microbial cross-feeding interactions to facilitate the production of short-chain carboxylic acids (SCCAs), such as propionate and caproate as alternative to chemical preservatives in food applications [2]. Special emphasis is placed on the metabolic conversion of lactate into SCCAs by facultative anaerobes [3], [4].

Brown juice composition was analysed to identify fermentable substrates and assess its suitability as a microbial habitat. UHPLC-RI/DAD was used for SCCA quantification, while HPLC-RI was employed to analyse substrate utilization. Preliminary results include the detection and quantification of glucose, fructose and arabinose as well as succinate. Fermented brown juice shows high amounts of lactate, indicating natural lactic acid bacteria. Furthermore, the classification of the native microorganisms through colony-forming unit (CFU) counts and 16S rRNA gene sequencing gives insight into the brown juice microbial diversity. Co-culture experiments will be conducted in both small-scale dynamic systems and controlled bioreactors.

This study contributes to the development of sustainable bioprocesses for agricultural side-stream valorisation, aligning with circular bioeconomy principles. By elucidating the microbial and metabolic mechanisms for efficient SCCA production from brown juice, the project provides a foundation for the rational design of robust microbial consortia and fermentation processes.

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[P072] Fungal and Bacterial Species and Inflammatory Potential of Sedimented Dust in Danish Homes: A Cross-sectional Study

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The WHO notes that the relationships between dampness, microbial exposure, and health effects cannot yet be precisely quantified; therefore, no quantitative health-based guideline values are currently recommended for indoor microbial exposure. The aim of this study is to obtain knowledge about potential exposure via the air to fungal and bacterial species in homes with and without self-reported 'visible mould' problems. Airborne dust was sampled in 256 homes using the passive sampler, EDC, for 14 days. Fungal and bacterial species were identified using MALDI-TOF MS, and inflammatory potential (TIP) was assessed in an HL-60 cell line. In a linear model, the impact of season and 'visible mould' on fungi, bacteria, and TIP was analysed. Concentrations of fungi correlated positively with bacteria ($r=0.29$, $p<0.0001$) and TIP ($r=0.16$, $p=0.048$). Concentrations of fungi and TIP of samples were associated with season, with highest fungi in the summer and highest TIP in summer and autumn. Seasonality was also found for 16 fungal species and 5 bacterial species. Among 256 homes, 56 reported 'visible mould'; these homes showed higher concentrations of fungi and TIP. In homes with 'visible mould', Aspergillus ochraceus, Aureobasidium pullulans, Paecilomyces variotii, and Penicillium brevicompactum were found more often than in other homes. Risk class 2 pathogens were found during all seasons, with 27 different bacterial species and 8 different fungal species. This study provides quantitative evidence to support indoor air quality risk assessment and offers a foundation for developing indicative benchmarks or action levels for microbial exposure in residential environments.

Thanks to Grundejernes investeringsfond for økonomisk støtte.

[P073] Proteome Profiling of Salmonella enterica, Vibrio cholerae, and Escherichia coli in response to AI-3 by LC-MS/MS

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Background: The global rise of multidrug-resistant bacteria threatens the efficacy of conventional antibiotics, necessitating new approaches that reduce selective pressure for resistance. A promising strategy targets bacterial quorum sensing (QS), an intercellular communication system that regulates virulence, biofilm formation, and stress responses. AI-3 metabolites, including 3,5- and 3,6-dimethylpyrazin-2-ol (3,5-DPO and 3,6-DPO), modulate these pathways across multiple pathogens: 3,6-DPO activates virulence in Escherichia coli, while 3,5-DPO suppresses virulence and biofilm formation in Vibrio cholerae. In Salmonella enterica serovar Typhimurium, transcriptomics has shown broad genome remodelling, but the downstream proteomic responses remain unclear in all three pathogens.

Aim: To address this gap, we performed the first comparative proteomic profiling of 3,5-DPO and 3,6-DPO responses in E. coli, V. cholerae, and S. Typhimurium.
Methods: Wild-type strains were cultured with or without 100 µM DPOs, harvested at the stationary phase, and analysed by LC–MS/MS. Label-free quantification was applied for E. coli and V. cholerae, and isobaric labelling for S. Typhimurium. Differential protein abundance was assessed by statistical testing ($|\log_2 FC| > 1$, $p < 0.05$).

Results: Proteomic analysis revealed both shared and species-specific effects. In E. coli, both molecules downregulated λ integrase Int, while 3,6-DPO reduced WaaQ, affecting LPS assembly. V. cholerae displayed broader changes, including upregulation of ArgE under both conditions and 3,6-DPO-specific induction of FlaD, linked to motility. S. Typhimurium showed the strongest response to 3,5-DPO, with downregulation of SPI-2 T3SS proteins SsaN and SseA, while both molecules upregulated FljB, suggesting antigenic variation. Functional annotation revealed diverse proteins, from DNA repair enzymes to membrane-associated factors relevant under stress.

Conclusion: Our findings show that 3,5-DPO and 3,6-DPO drive distinct yet overlapping proteomic shifts in three pathogens, modulating virulence, stress, and metabolic pathways. These results show AI-3 metabolites as potential anti-virulence agents that can affect the bacterial physiology without driving antibiotic resistance.

[P074] Molecular insights into microbial community structure and function in Danish “mini-wetlands”: preliminary observations and ongoing campaigns

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Microbial communities are central to ecosystem processes necessary for soil health and water quality. Constructed wetlands, specifically Danish “mini-wetland”, widely implemented in 2015 to treat agricultural runoff, offer an opportunity to study how microbial communities mediate nutrient cycling, micropollutant degradation, and overall ecosystem functionality. While these systems were initially designed for nutrient removal, emerging concerns, including micropollutant accumulation, metal retention, and antibiotic resistance, require a deeper understanding of microbial dynamics and functional resilience.

Within the framework of the EU Path4Med project, we are conducting an integrative molecular ecology study combining total RNA sequencing with chemical analyses (LC-MS/MS and LC-HRMS) to characterize microbial communities and their functions in mini-wetland sediments and water. By capturing both taxonomic and functional signals from bacteria, fungi, algae, and other eukaryotes, this approach provides high-resolution insight into microbial diversity, rhizosphere interactions, functional gene expression, and ecosystem service provision, including nutrient buffering and micropollutant removal. Moreover, since the system has been operated for 10 years, it represents a unique testbed to assess for the first time whether such systems function as effective and sustainable remediation strategies or act as sinks for metals, micropollutants, and Antibiotic Resistance Genes (ARGs).

We have designed two complementary sampling campaigns to address both long-term accumulation and short-term resilience of microbial communities in these systems:

1. Sediment Transect Campaign (August 2025)

This campaign targets the historical accumulation of nutrients, metals, and micropollutants after ten years of wetland operation. Twenty-four sediment cores were collected along the flow path of a representative mini-wetland, with top and bottom layers separated for analysis. The top 5 cm are expected to host the most active microbial communities, while deeper layers likely contain accumulated micropollutants and metals. Key research questions include:

- Are constructed wetlands hotspots for ARGs?
- Do the sediments act as sinks for metals and micropollutants?
- How are microbial community structure and function distributed along the sediment gradient?

Samples were preserved in liquid nitrogen for RNA extraction, and analyses being carried out include total RNA sequencing, nutrient profiling, micropollutant quantification, and metal analysis. Preliminary screening of micropollutants has been conducted, but sequencing and functional analyses are ongoing. Preliminary data for this campaign will be shown at the conference.

2. Fertilization Campaign (Spring 2026)

The second campaign will evaluate the resilience of microbial communities under episodic high nutrient and micropollutant loads. Water samples will be collected using an autosampler triggered by rainfall events before, during, and after fertilization of surrounding fields. Inlet and outlet concentrations of nutrients, micropollutants, and microbial RNA will be monitored to determine short-term system responses. Research questions include:

- How quickly do key microbial nutrient-cycling functions recover after disturbance?
- Do micropollutant loads affect these functions?
- Can the wetland maintain its treatment performance under combined nutrient and micropollutant stress?

By integrating RNA-based microbial profiling with chemical measurements, we aim to uncover functional responses and potential bottlenecks that affect ecosystem services in mini-wetlands. This dual approach allows us to link accumulated impacts over a decade with short-term functional resilience, providing a holistic understanding of microbial ecology in treatment wetlands and how those systems cope with such loads.

Although data collection has only recently begun, preliminary micropollutant screening will be shown at the conference. The ongoing RNA sequencing and functional analyses will enable identification of key microbial taxa and genes involved in nutrient cycling, pollutant degradation, and resistance, allowing us to assess the interplay between community composition, function, and system performance.

Our study exemplifies how modern molecular ecology tools can expand the conceptual framework of ecosystem health and water quality in engineered ecosystems. By addressing both long-term accumulation and short-term stress response, we hope to provide new insights into microbial processes that sustain ecosystem services and inform monitoring and management strategies in constructed wetlands and similar treatment systems.

[P075] Persisters are primed for CRISPR-Cas adaptation

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In the evolutionary battle between bacteria and mobile genetic elements, including bacteriophages and plasmids, bacteria have evolved intricate defense systems. While the bacterial immune system CRISPR-Cas has been extensively studied and exploited as a revolutionary gene editing tool, fundamental questions on when this microbial immune system acquires genetic CRISPR memory and immunity against invaders remain largely unanswered.

Naïve CRISPR adaptation is generally rare, but occurs more frequently when bacteria are challenged with replication-deficient phages. In this scenario, the bacteria are not faced with a ticking bomb and have ample time to adapt to the phage DNA, without risking cell death. Accordingly, slow growth caused by low temperatures, low aeration, or bacteriostatic antibiotics promotes CRISPR adaptation, possibly by allowing the Cas complexes more time to adapt before being outpaced. Persister cells are transiently antibiotic-tolerant dormant subpopulations of cells, which are genetically identical with the remainder of the antibiotic-sensitive bacterial population. When an invader infects a persister cell, its replication is arrested until the host cell resumes growth, creating an ideal opportunity for CRISPR adaptation.

Our preliminary data show that CRISPR adaptation is 10-fold more frequent in transiently dormant Escherichia coli persister cells compared to the general bacterial population. Thus, persister cells, in addition to being notoriously antibiotic tolerant, are primed for CRISPR-Cas adaptation and may be in a state of heightened immune capacity and evolution, securing the survival of the population.

[P076] From Cell to Community: Unlocking Microbial Diversity with Single-Cell Genomics

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Single-cell DNA sequencing complements the metagenomic analysis of uncultured bacteria by revealing cell-to-cell variation, linking host genomes to extrachromosomal DNA, and providing strain-level taxonomic resolution. However, current techniques are limited to processing fewer than 1,000 cells and produce single-amplified genomes (SAGs) of low completeness due to biases in whole genome amplification (WGA).

We present an innovative, cost-effective approach to sequence up to 10,000 SAGs with superior genome recovery. Individual microbial cells are isolated into 70 µm semi-permeable capsules (SPCs), enabling compartmentalized multi-step processing - including lysis, WGA, and barcoding - of all cells simultaneously at a cost of less than $1 per cell. Using well-characterized E. coli and B. subtilis, we demonstrate >90% genome recovery per SAG at sequencing depths below 10x and <1% cross-contamination. Additionally, we processed a commercially available microbial community standard and were able to detect all species within the mixture.

The advantages of SAG sequencing are especially valuable for uncovering the diversity and unique adaptations in the context of microbial ecology. We processed soil and aquatic samples to generate matched SAG and MAG datasets, with the latter obtained through bulk metagenomics. SAG assemblies produced longer contigs and revealed detailed genomic features, most notably the linkage between viral and plasmid sequences and their hosts. The linkage information was lost in the MAG dataset.

Our high-throughput SAG sequencing workflow provides a detailed view of microbial communities, offering unmatched resolution and scalability. This capsule-based single-cell sequencing technology opens new horizons for microbial genomics research.

[P077] Phage resistance impacts antibiotic susceptibility and virulence in Staphylococcus aureus

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As antibiotic resistance continues to rise worldwide, phage therapy offers a promising solution by harnessing viruses that infect and kill bacteria. However, phage-resistance may develop, compromising therapy options. Phage-resistance has primarily been linked to changes in major phage receptors that for the human pathogen Staphylococcus aureus are the cell-wall linked wall teichoic acids (WTA). To identify additional factors contributing to phage-resistance, S. aureus was evolved under selective pressure from lytic phages, including candidates from therapeutic phage cocktails, to obtain resistant clones. A third of the phage-resistant clones acquired mutations associated with cell-wall changes previously linked with phage resistance, namely in femA, involved in peptidoglycan crossbridge formation, and tagO, encoding the initiator of the WTA biosynthesis. The remaining clones had mutations in pathways not previously associated with phage-resistance, including nucleoside catabolism (deoC1), polyamine import (potAB) and DNA/RNA replication (cshA, ligA), suggesting that phage-resistance may be associated with phage-driven host takeover. Antibiotic susceptibility and virulence were diversely affected by the mutations; mutations in tagO increased β-lactams sensitivity and attenuated virulence, whereas mutations in femA/ligA or cshA increased staphylococcal virulence in Galleria mellonella. This work demonstrates the involvement of several novel bacterial pathways in phage resistance, highlighting the complexity behind phage-bacterial interactions.

[P078] Coil'n'Roll: harnessing bacteriophage replication for eDNA synthesis in the biofilm extracellular space

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We are used to thinking that DNA replication in bacteria is confined inside a cellular membrane. How can it be otherwise? Bacteriophages (phages) invade bacteria and replicate hundreds of copies inside single bacterial cells bursting after the phage capsid assembly. Some phages, e.g. phage λ in E. coli and phage phi29 in B. subtilis, replicate via rolling circle replication (RCR) [1,2]. Unlike bacterial DNA replication, a superior phage replication relies on a polymerase, e.g. phi29 [3], with a strand displacing activity that can unwind double-stranded DNA without helicase or other proteins. We wondered if phage polymerase and nucleotide triphosphates (NTPs) are available after bursting the cell to continue replication of an exogeneous circular DNA in the extracellular space.

Wild-type (WT) S. oneidensis MR-1 is natural hub of phage λSo encoded in the chromosomal DNA as a pro-phage. The role of the pro-phages has been linked to the production of extracellular DNA (eDNA) since the ΔλSo ΔMuSo mutant (Mut) S. oneidensis lacking all pro-phages is particularly known to be poor in eDNA [4]. The aim of this study was to measure the activity of exogenous DNA replication in response to phage λSo replication and cellular burst, using the WT and Mut strains as well as phage λSo reporter strains of S. oneidensis.

First, we constructed and used the following reporter strains of S. oneidensis: S2 (WT, tn7::CFP, cro::Venus) [4] and S5 (ΔMuSo, SO_2963::GFP). To construct the S5 reporter for the λSo capsid production in S. oneidensis, we integrated a second copy of the capsid-encoding gene SO_2963 fused to sfgfp into the λSo chromosome by sequential crossover. A synthetic version of the gene (SO_2963EC) was synthesized by Genscript (Rijswijk, NL) and was codon-optimized for E. coli, resulting in a gene with 75% DNA sequence identity to the original λSo gene. The Venus fluorescence in S2 and the signal of GFP fused to λSo capsid in S5 peaked after the CFP signal produced by the living intact cells decreased starting from ≈200 min, indicating S. oneidensis cellular burst in response to phage proliferation.

Next, to measure extracellular synthesis of DNA (via RCR), we designed an exogeneous circular DNA template "encoding" G-quadruplex (GQ) motifs. The synthesis of GQ-DNA was monitored by N-methyl Protoporphyrin (NMP) fluorescent probe. The templated synthesis of eDNA via rolling circle was indicated by an increase in the NMP fluorescence due to accumulation of GQ-DNA in S. oneidensis planktonic cultures harboring pro-phages (in WT, S2 and S5) after ≈300 min lag phase. On the contrary, no increase of NMP fluorescence was detected in the Mut strain lacking pro-phages. From the tn7::CFP, cro::Venus, SO_2963::GFP and NMP trends, we concluded that GQ-DNA accumulated in response to phage λSo proliferation and lysis of the S. oneidensis cells.

Finally, after 3-days of the DNA templated biofilm growth of S. oneidensis (WT) the G-rich products of RCR phase-separated, yielding amorphous spherical crystals resembling "DNA flowers" [3]. These were stained by NMP and BG4 antibody, indicating GQ, and were analyzed by CLSM and SEM-EDX. Consequently, we confirmed the large concentration of GQ-DNAzymes with peroxidase activity as well as carbon, sodium and chloride ions in the DNA superstructures. Moreover, differential pulse voltammetry measurements revealed convoluted redox reactions in the templated biofilms containing the DNA “flowers” with the 3-fold increase in the average current density compared to the non-templated biofilms.

In conclusion, our results demonstrate that phage λ can be harnessed for the unlimited synthesis of DNA superstructures containing large concentrations of custom sequence motifs such as G-quadruplex, which are in demand for applications such as performing extracellular electron transfer and driving electromethanogenesis in a methanogenic archaeon [5]. There are an estimated 10^{31} to 10^{32} phage particles on Earth, making them the most abundant organisms on the planet. Potentially tens of millions of different phage species may exist in natural environments, infecting all known strains of bacteria and even some archaea. This work funded by Villum Fonden (project #00050284) will establish a new dimension in the bacteria-phage interactions.

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[P079] Mucofilm: A novel ecological framework for bacterial communities within the human microbiome

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We propose mucofilm as a new framework for understanding phage–microbiome interactions in the gut. By treating mucus and biofilm as a single integrated system, this concept reshapes how we view microbial resilience, diversity, and host responses in the gut ecosystem.

The human gut is a dynamic ecosystem where bacteriophages (phages) and bacteria interact within a complex mucosal environment. To capture the interactions between bacteria embedded in both self-produced extracellular polymeric substances (EPS) and host-derived mucin, we introduce the concept of mucofilm – a hybrid matrix of mucus and biofilm – as a distinct ecological niche in the gut and on other mucus-covered surfaces. Rather than treating mucus and biofilm as separate entities, we emphasize that their interwoven nature provides unique ecological properties that influence microbial behavior, phage dynamics, and host responses.

Considering mucofilm as a single, integrated structure highlights its dual role as both a protective barrier and a reservoir for microbial communities. Within this environment, bacteria can enhance their persistence and resilience, while phages face obstacles to infection but may also benefit from localized retention and increased opportunities to shape bacterial populations. This interplay is central to understanding how microbial communities adapt, diversify, and maintain stability in the gut.

By recognizing mucofilm as a distinct ecological unit, we provide a framework for reinterpreting phage–microbiome interactions in ways that link basic mechanisms to translational opportunities. Applying this perspective highlights the potential of mucofilm-focused research to refine microbiome studies, improve predictions of microbial behavior, and inspire new phage-based therapeutic strategies. At the conference, we will expand on how this conceptual approach can guide experimental design and open promising avenues for targeting gut-associated diseases.

[P080] Task-Specific Exposure to Airborne Microbes and Dust Among Biofuel Plant Workers in Denmark

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Introduction: The increasing use and production of bioenergy leads to a growing workforce within the sector, making it increasingly important to uncover possible health effects and safety issues related to working at biofuel plants. Our aim was to determine the employees' exposure to dust and microbial agents at Danish biofuel facilities.

Methodology: The study included occupational measurements at different biofuel plants. Personal airborne samples of workers and stationary indoor air samples were collected from biofuel plants. A reference sample was collected outside each plant to account for the influence of outdoor air. Bacterial and fungal species were identified using MALDI-TOF, while dust was measured using gravimetric analyses.

Results: Personal samples showed higher microbial loads compared to indoor and outdoor reference samples (Kruskal–Wallis, $p < 0.05$). Geometric mean (GM) in personal samples were 1.5×10^4 Colony-Forming Units (CFU)/m³ (bacteria) and 7.3×10^3 CFU/m³ (fungi). For Indoor stationary sampling, the corresponding GM were 1.3×10^4 and 5.5×10^3 CFU/m³, respectively; the GM for outdoors were 1.2×10^3 and 8.3×10^2 CFU/m³. Task-based variation was evident: bacterial and fungal concentrations were highest during straw handling and cleaning, and lowest among office workers at the same plant (Kruskal–Wallis, $p < 0.05$). Of 89 bacterial species identified, 18 occurred in both indoor and personal samples, of which 11 were risk class 2 pathogens. Among 24 fungal species, 11 appeared in both environments, including 3 classified as risk class 2 pathogens. Dust levels paralleled microbial patterns: The GM concentrations were 0.55 mg/m³ in personal samples, 0.41 mg/m³ indoors, and 0.21 mg/m³ outdoors, indicating indoor exposure may be influenced not only by outdoor air but also by activities associated with plants.

Conclusion: Biofuel workers experience substantially higher exposures to airborne bacteria, fungi, and dust than outdoor reference levels. Elevated exposures during straw handling and cleaning indicate the need for further task-specific investigation.

Acknowledgments: This work was supported by funding from AMFF (Grant number: 02-2023-09 20235100787).

[P081] Functional Characterisation of the Gut Microbiome in Type 1 and Type 2 Diabetes through Multi-Omics Approaches

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The human gastrointestinal tract contains a complex microbial community that plays a central role in immune regulation, metabolic homeostasis, and maintenance of gut integrity. Alterations in the gut microbiome have been associated with both type 1 diabetes (T1D) and type 2 diabetes (T2D). Diabetes is defined as a set of autoimmune, metabolic and genetic disorders characterised by insufficient insulin production, cellular insulin resistance or both. While previous studies have described differences in microbial composition, findings are often inconsistent across the literature, suggesting that functional capacity, rather than taxonomy alone, may be key to understanding microbiome–host interactions in diabetes.

This study aims to investigate the functional potential of the gut microbiome in individuals with T1D and T2D using a multi-omics approach by integrating taxonomic, functional, and metabolic data. A collection of approximately 100 faecal samples from individuals with T2D and 60 from individuals with T1D has been collected, and analysed using full length 16S rRNA gene sequencing. In addition, detailed clinical metadata from blood samples are available. The current stage of the project focuses on qNMR to generate metabolic profiles. These metabolomic data will allow us to identify metabolites that are either produced or depleted in T1D and T2D and to explore their associations with clinical parameters. Furthermore, the metabolomic data are integrated with the 16S rRNA gene sequencing data to investigate the link between microbial taxonomy associated with T1D and T2D and metabolites produced by these microorganisms.

Future work will integrate these findings with shotgun metagenomic data to uncover functional pathways and mechanistic insights into microbiome–host interactions in diabetes.

[P082] Beyond Photosynthesis: Exploring Dark Oxygen Production Across The Microbial Tree Of Life

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While most of Earth's dioxygen (O_2) is generated through oxygenic photosynthesis, it can also be produced by processes that are light independent. Biological dark oxygen production (DOP) has been reported in certain prokaryotes in laboratory cultures. Those microorganisms produce O_2 via dismutation reactions. So far, nitric oxide dismutation (NOD; 2NO --> N_2 + O_2 or 4NO --> 2N_2O + O_2) and chlorite dismutation (CLD; ClO_2- --> Cl- + O_2) are known, but DOP microbes and pathways might have been overlooked in many environments. If ubiquitous and abundant, these microbes may contribute to O_2 cycling in ecosystems on Earth where no photosynthesis can occur. For example, microbial DOP has recently been proposed to be an important process in groundwater ecosystems. Here, we investigate candidate microorganisms which possess nod genes encoding NO dismutases for their capability to perform DOP as identifying the true diversity of DOP microbes will help us to assess the global ecological significance of microbial DOP. While nod genes are found in phylogenetically diverse groups of microbes, physiological evidence for DOP is lacking in all but few lineages. To elucidate the capability to perform DOP by candidate microorganisms, we conduct incubation experiments while monitoring oxygen concentrations with O_2-sensing optodes, which can quantify oxygen down to the nanomolar range. In incubations with stable-isotope labelled nitrite, isotopic compositions of produced N_2 and N_2O are quantified with isotope ratio mass spectrometry to constrain DOP pathways and their intermediates. As O_2 production and consumption may be tightly coupled, O_2 may not accumulate. Therefore, we use inhibitors such as azide, cyanide, and acetylene during these incubations to inhibit O_2 consumption.
The novel experimental setup is uniquely suited to understanding DOP at very high resolution across a broad range of conditions and species.

[P083] Engineering of methylotrophic Bacillus methanolicus MGA3 for lactic acid production

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Bacillus methanolicus MGA3 is a fast-growing thermophilic bacterium with strong potential as a workhorse for methanol-based bioproduction. It naturally overproduces L-glutamate at high titers and has been engineered for various value-added compounds. Lactic acid (LA) is a highly versatile compound with widespread industrial applications, particularly in the food and beverage sector. It is primarily generated through microbial fermentation in the reaction of pyruvate reduction catalysed by LDH using NADH as electron donor. Here, we explored the possibilility to redirect the reducing power generated from methanol oxidation in form of NADH toward LA synthesis via heterologous expression of lactate dehydrogenases (LDHs).

Eight candidate LDHs were selected from the BRENDA database for the thermostability testing. The LDH from Thermodesulfatator indicus retained activity at 50 °C when expressed in B. methanolicus, while enzymes from Heyndrickxia coagulans and Thermoanaerobacter ethanolicus were active at 37 °C but inactive at 50 °C. None of the engineered strains produced LA, suggesting LDH activity is limiting under thermophilic conditions.

To overcome this bottleneck, we screened thermophilic lactic acid bacteria for LDH activity at elevated temperatures. Eleven strains were tested for their growth at 45 °C and 50 °C, resulting in five candidates with promising LDH activity at 50 °C. These LDHs are now being used to construct recombinant B. methanolicus strains to test their ability to produce LA from methanol at thermophilic conditions.

Together, our study provides a novel strategy to screen for thermostable enzymes feasible for heterologous expression in a thermophilic microbial cell factory B. methanolicus and establishment of production of value added-products such as LA.

[P084] Exploring the potential of brown juice as a substratefor anaerobic short-chain carboxylic acids formation

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Brown juice is a by-product in large quantities in green biorefinery from grass protein extraction containg proteins, water-soluble carbohydrates and minerals (Feng, 2021). These can be substrates for microbes to produce short-chain carboxyl acids (SCCAs), which have an antimicrobial effect and activity against biofilm formation (Ng, 2024). Our aim is to produce SCCAs such as caproic acid and valeric acid for food preservation through cross-feeding of anaerobic bacteria (lactate producer and obligate anaerobic lactate utilizer) using brown juice as the substrate. We hypothesis that glycolytic and fermentative microbes will be able to produce SCCAs. The brown juice, which was incubated in the absence of oxygen, was analyzed using UHPLC-RI for sugars and acids. Before fermentation, the pH was 5.64 and it contained (61.3 ± 0.3 mM) glucose, (38.1 ± 0.3 mM) fructose and (8.7 ± 0.02 mM) arabinose, of which (23.8 ± 2.3 mM) glucose, (11.1 ± 0.9 mM) fructose and (2.6 ± 0.1 mM) arabinose were consumed during fermentation. The UPLC-RI measurements revealed (36.7 ± 1.7 mM) lactate formed as the main fermentation product while other products were (2.5 ± 0.4 mM) acetate and (0.08 ± 0.33 mM) succinate. Suprisingly, the cell counts were only 5.3 × 103 CFU/ml indicating a highly effective turnover. The results indicate that brown juice is a promising substrate for SCCA production using anaerobic bacteria strains cross-feeding with added lactate producer or the naturally occuring microbes in the brown juice, which can already ferment mostly to lactate according to the results.

Feng, L. W.-J. et al. (2021). Pilot-scale anaerobic digestion of by-product liquid (brown juice) from grass protein extraction using an un-heated anaerobic filter. Process Safety and Environmental Protection, 146, 886-892. https://www.sciencedirect.com/science/article/pii/S0957582020319534

Ng, K. S. et al. (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. https://journals.asm.org/doi/full/10.1128/spectrum.01658-24

[P085] Antimicrobial activity of yoghurt cultures beyond lactic acid

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Introduction: Lactobacillaceae, which mainly produces lactic acid, is the most widely used stater culture for dairy yoghurt. Lactic acid has contributed to the lowering of pH and inhibiting the growth of spoilage microorganisms. However, microbial metabolism of Lactobacillaceae is rather diverse and lactic acid is not the only short-chain carboxylic acids (SCCA) that can be found in fermented dairy products. Megasphaera elsdenii is capable of converting lactate into valerate through fermentation and chain elongation activity. This study aims to investigate the SCCA profile and antimicrobial activity of commercial yoghurts, and the potential of Megasphaera jensenii as adjunct culture to produce valeric acid during yoghurt fermentation for biopreservation purposes.

Methods: A total of 11 yoghurts were collected from Danish supermarkets and tested for carbohydrate and SCCA concentration using UPLC-RI. Samples were serial diluted and plated on MRS and M17 agar to enumerate Lactobacillaceae and Lactococcus count, respectively. DNA was extracted from isolates and sent for 16S rRNA sequencing. The supernatant was collected for minimal bactericidal concentration (MBC) assay. For fermentation, M. elsdenii (2%, v/v) was inoculated into the fresh whole fat milk that spiked with 50 mM lactate and incubated at 30°C for 48 h under anaerobic and aerobic conditions.

Results: Lactose was the major sugar detected (64-160 mM) in the commercial yoghurts, followed by galactose and glucose. A near-equimolar ratio of approximately 10:1 between lactate and acetate was observed in 55% (6 out of 11) of tested yoghurts, indicating the presence of heterofermentative Lactobacillaceau in these samples; while a low amount of butyrate (< 1 mM) was detected in 2 samples. The viable cell counts of Lactobacillus (4.8–8.2 log CFUmL-1) and Lactococcus (6.7–9.4 log CFUmL-1) differed among the tested samples. Other than the classical yoghurt starter cultures Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus, the samples contained other species such as Leuconostoc mesenteroides, Lactococcus lactis, Lacticasebacillus rhamnosus and Lactobacillus acidophilus. A combination of lactate and acetate showed bactericidal effect against Staphylococcus aureus, but not when presented alone. During fermentation with M. elsdenii, we did not observe the production of valerate, but the lactate was increased 1.6 to 2.4–fold at both conditions, while propionate (< 1 mM) was detected when cultivating aerobically.

Conclusions: A limited number of bacterial species were isolated from commercial yoghurts and the SCCA profiles were lacking diversity since mainly contained lactate and acetate. Lactate and acetate worked synergistically to inhibit pathogen growth when presented in yoghurt samples. Since propionate is the product of acrylate pathway and can be converted to valerate, the presence of propionate in our results suggested the possibility of M. elsdenii as a valerate producer in yoghurt fermentation but further optimization on the fermentation conditions is needed.

[P086] Signatures of positive genomic selection in cyanobacteria for PFAS degradation

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants that pose significant challenges to microbial life due to their chemical stability and toxicity. While microbial defluorination has been observed, the ecological and evolutionary drivers behind this capability remain unclear. In this study, we conducted a comprehensive genomic survey of over 15,000 bacterial genomes to identify homologs of 43 candidate PFAS-degrading enzymes, with a particular focus on their mobilization and genetic context.

We identified a conserved fluoroacetate dehalogenase (FAcD) gene in 14 Microcystis cyanobacterial genomes, consistently flanked by genes involved in stress response, photosynthesis, and toxin-antitoxin systems. These genetic arrangements suggest an adaptive response to PFAS-induced physiological stress, including membrane disruption and photosynthetic inhibition, which have recently been documented in cyanobacteria. The presence of insertion sequences and mobile genetic elements further supports the hypothesis of positive selection and potential horizontal gene transfer.

Our findings propose that in Microcystis, PFAS degradation may have evolved not for metabolic gain, but as a detoxification strategy to preserve photosynthetic function and cellular integrity. This highlights cyanobacteria as promising candidates for PFAS bioremediation and underscores the importance of ecological context in the search for effective degraders.

[P087] Mode of action studies of Ticagrelor and its derivatives

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Keywords:
Multidrug-resistant (MDR) pathogens, Antimicrobial activity, Mode of action, Drug repurposing, Ticagrelor, Derivative, MRSA (methicillin-resistant Staphylococcus aureus), MRSE (methicillin-resistant Staphylococcus epidermidis).

Abstract:
Multidrug-resistant (MDR) bacterial infections represent a growing global health threat, emphasizing the urgent need for innovative therapeutic strategies (Ranjbar and Alam., 2023). Some of the potential solutions include the discovery of novel natural products, synthetic or semi-synthetic antibiotics, drug repurposing. Among these, drug repurposing, which is basically finding new uses for already-approved drugs, offers a cost-effective and time-efficient approach (Pushpakom et al., 2018).

Ticagrelor, a reversible P2Y12 receptor inhibitor widely prescribed as an antiplatelet agent in patients with acute coronary syndrome or prior myocardial infarction (Wallentin et al., 2009), has recently attracted attention as a candidate antimicrobial. A 2019 clinical study, supported by preclinical data, showed that patients treated with ticagrelor had a lower risk of infection-related mortality, particularly from Staphylococcus aureus, compared with those receiving clopidogrel (Lancellotti et al., 2019). This observation prompted investigations into ticagrelor's antimicrobial activity.

In vitro studies confirmed its effectiveness against Gram-positive and MDR Gram-positive pathogens, including methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant Staphylococcus epidermidis (MRSE), with MICs of 20 µg/mL. Luciferase-based reporter assays in Bacillus subtilis demonstrated activation of the YpuA and liaI promoters, indicating cell envelope and lipid II cycle stress. Ticagrelor was also found to dissipate bacterial membrane potential, even at sub-MIC concentrations and it also induces pore formation on the bacterial membrane (Leeten et al., 2024).

Building on these findings, our current work provides deeper insight into ticagrelor's mode of action. We identified an additional antimicrobial effect directed at the bacterial cell wall and peptidoglycan layer, revealing a secondary mode of action. Importantly, in collaboration with an industrial partner, we now have access to ticagrelor derivatives that lack antiplatelet activity but exhibit improved antibacterial potency. These compounds not only share the mechanisms described for ticagrelor but also represent promising scaffolds for the development of next-generation antimicrobials.

[P088] CRISPR interference as a tool for targeted gene repression in lytic Campylobacter phages during host infection

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While CRISPR-Cas genome editing is widely used to study gene functions in lytic phages, few studies have investigated using CRISPR interference (CRISPRi), which would allow targeting and studying essential phage genes. Here, we assess the applicability of CRISPRi for functional gene studies in lytic Fletchervirus phages infecting the major zoonotic pathogen Campylobacter jejuni. As a proof of concept, we targeted the non-essential receptor binding protein gene rpb2 of Fletchervirus phage F358 during infection of its host C. jejuni NCTC12662, using a constitutively expressed CRISPRi system integrated into the bacterial chromosome. RBP2 is phase-variably expressed and associated with a clear plaque phenotype that varies in frequency among phage stocks. In this study, we used two stocks of F358, one expressing RBP2 at a low frequency (F358) and the other expressing RBP2 at a high frequency (F358cp). Our results showed that CRISPRi targeting of the terminal region of rbp2 in F358 resulted in a 5-log reduction in clear PFU/ml, reflecting a repression efficiency of 99.99%. However, in F358cp, expressing RBP2 at a high frequency, no overall significant reduction in clear PFU/ml was observed, but variability within replicates significantly increased. These findings demonstrate that CRISPR interference can be used to target and repress genes in lytic C. jejuni phages during host infection, but the efficiency depends on target gene expression levels, which may impact its use for gene studies in lytic Campylobacter phages.

[P089] Early-life PFAS-exposure and gut microbial diversity across infancy

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Purpose: To investigate whether exposure to Perfluorooctane Sulfonic Acid (PFOS) and Perfluorooctanoic Acid (PFOA) affects gut microbial diversity across multiple timepoints in infancy

Background: Per- and polyfluoroalkyl substances (PFAS) exposure occurs in unborn children through transplacental transfer and in infants via breastfeeding postnatally. Recent studies have shown that PFAS exposure is associated with changes in the gut microbiota composition in infants. However, the findings across studies are inconsistent, and thus further studies are needed to determine how PFAS exposure may affect infant gut microbiota. The longitudinal design of our study contributes new evidence on the impact of PFAS exposure on infant gut microbiota.

Methods: Among 215 infants from the Norwegian Microbiota Cohort, we collected fecal samples at six timepoints from four days to two years. PFOS and PFOA concentrations were measured in breastmilk at approximately 31 days. The association between PFAS-exposure and gut microbiome α-diveristy was assessed in linear mixed effects model with random intercepts while adjusting for relevant covariates identified in a Directed Acyclic Graph.

Results: PFOS-exposure was significantly associated with reduced Shannon diversity ($\beta = -0.81$ $p = 0.036$). However, we did not observe changes in Shannon diversity associated with PFOA-exposure or in Faith's phylogenetic diversity associated with PFOA or PFOS exposure.

Conclusion: Our results, in combination with findings from previous studies, indicate that certain PFAS compounds may influence infant gut microbiota diversity, though effects appear modest and compound-specific.

[P090] Establishing a thermostable T7 Expression System in Bacillus methanolicus

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Methanol is a promising alternative to traditional raw materials used in bioprocesses for industrial biotechnology. The thermophilic methylotroph Bacillus methanolicus is an attractive production host because it grows optimally at 50 °C and secretes high concentrations of amino acids and valuable compounds. However, its efficient exploitation requires a tightly controlled expression system. This project aims to establish a two-plasmid system based on the bacteriophage T7 promoter to enable regulated gene expression in B. methanolicus. The plasmid pTH1mpT7-sfGFP, which carries an sfGFP reporter gene under the control of the T7 promoter, was successfully constructed and verified in Escherichia coli. In parallel, the plasmid pBV2xp-mpol encoding a thermostable variant of the T7 RNA polymerase, was designed and constructed. Next, both plasmids will be co-transformed into B. methanolicus, followed by fluorescence analysis under selective pressure. Demonstrating inducible sfGFP expression will confirm the functionality of the thermostable T7 RNA polymerase in B. methanolicus thereby establishing a novel tool for controlled gene expression. Ultimately, this system could facilitate the efficient production of industrially relevant compounds, such as cadaverine and γ-aminobutyric acid, directly from methanol.

[P091] Vancomycin induced surface modification affects Staphylococcal phages

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Vancomycin intermediate Staphylococcus aureus (VISA) is often associated with reoccurring infections and reduced treatment success due to its fast nature of readaptation. With limited treatment strategies, the possibility of phage therapy is being explored as an alternative option. VISA manages to maintain its resistance phenotype by suppressing resistance genes through acquisition of second site mutations. These mutations have been found to impact the metabolism, gene expression, cell envelope and virulence, which begs the question how the susceptibility to phages are affected. Recently, a JE2-derived VISA strain demonstrated a significant loss of phage susceptibility when exposed to five different phages. We therefore wanted to explore how susceptibility to phages is impacted once they revert back to being vancomycin sensitive and assess if reduced susceptibility is caused by surface modulations. Here we show that reverting the same VISA strain, phage susceptibility was restored to wildtype levels. Furthermore, the increased resistance to phages is maintained when exposing phages to readapted VISA strains. We also show via transmission electron microscopy a difference in thickness and septum formation in VISA strains compared to wildtype. However, we could not find evidence for increase phage resistance being tied to the surface as phages adsorb faster to both VISA and revertant strains compared to wildtype. We are therefore currently exploring the role of a point mutation found in rpoB to identify if this mutation may prevent the expression of phage genes. These findings would hopefully give us new insights into alternative therapy approaches when treating VISA and provide a next step of understanding the interplay between phages and its host.

[P092] Citizen science approach reveals clonal spread of soft rot Pectobacterium in Danish potato production and the possibility to implement bacteriophage-based biocontrol strategies at early stages of infection

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Soft rot Enterobacteriacea (SRE) are among the most economically important plant pathogenic bacteria and are especially known to be problematic in potato production. The epidemiology of disease transmission has been investigated for almost a century, and several aspects have been highlighted as plausible infection routes. However, it is generally accepted that the major source of disease is the latently infected mother tuber, but several parameters are still influencing disease prevalence including contaminated equipment, soil water status as well as temperature. Management of the disease is limited to hygiene practices, dry storage and seed certification systems but several studies have also proven biocontrol agents such as bacteriophages as promising tools. Despite the severity of SRE on potato production, little is known about the genetic diversity of SREs in Denmark, and few isolates are available limiting the possibility to design a broadly effective phage cocktail. Here we describe a three-year field study utilizing a citizen science approach where Danish farmers provided symptomatic potato plants or tubers, together with metadata such as date, location, potato variety and origin. By using whole genome sequencing (Illumina and Nanopore) together with metadata we were able to investigate and monitor the epidemiological disease spread across the country using 110 complete genomes sampled across all three years. Our results revealed several clonal clades across diverse locations (SNPs < 15) which strongly indicate common origin. We also isolated a total of 16 bacteriophages on several Pectobacterium species, targeting one third of all Pectobacterium isolates. Based on the clonality across the soft rot isolates we propose the possibility to set in early on using bacteriophages targeting strains relevant for softrot development, with the possibility of a surveillance program together with customizing the bacteriophage preference.

[P093] High-quality pangenome of cable bacteria provides insight into species differentiation

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In aquatic sediments all around the world lives a unique type of sulfide-oxidizing bacteria called cable bacteria. These bacteria grow as multicellular filaments several centimeters long, and they have the capacity to spatially separate the half-reactions of sulfide oxidation and oxygen reduction. They do this by conducting electrons harvested from sulfidic sediments through periplasmic conductive fibers to oxygen several centimeters away. 16 different species of cable bacteria have been identified across two different genera within the Desulfobulbaceae, but functional differences between most of these species have yet to be described. So far only a difference in salinity tolerance has been described with the presence/absence of a Na+/H+ antiporter gene (nhaA) and other ion transporters. While cable bacteria have not been cultivated in axenic cultures yet, a method has been developed to grow them as single-strain enrichment cultures in sediment. This allows us to couple a metagenome assembled genome (MAG) to the physical and morphological observations on each single strain cultured. And with long-read sequencing options becoming more available, the collection of high-quality cable bacteria genomes has expanded. This allowed us to produce a high-quality pangenome from 13 genomes that cover the two genera of cable bacteria. This very reliable pangenome contained 9526 gene clusters in total and had a stable core genome of 1117 gene clusters. With this pangenome, we studied the different metabolic capabilities encoded by individual genomes and used this information to create hypotheses on the impact of environmental factors on niche differentiation of cable bacteria species. We plan to test these hypotheses in the future both in situ and in laboratory studies with single-strain enrichments of cable bacteria.

[P094] Developing a method to use live-cell imaging to quantify the time till germination and the hyphal growth rate of filamentous fungi

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Live-cell imaging can be used as a tool for studying community interactions, morphological development, and population variability in microbial research. Here, we aim to develop a new approach to monitor the time till germination and the hyphal development rate of filamentous fungi using live-cell imaging (oCelloScope, BioSense Solutions). Seventeen xerophilic Aspergillus conidia were suspended in liquid Sabouraud media, and conidia were tracked and imaged for 96 hours of incubation. Secondary analysis was done to categorize the conidia into germinated or non-germinated pools using decreasing circularity and increasing area as determining factors in a sorting algorithm. A decrease below 0.92 was used as the circularity parameter. Critical area fold change was found using a segmented linear regression to determine the inflection point of area increase, which was taken as a proxy for the transition from isotropic growth to polarized growth. Post-germination identification, we derived the hyphal growth rate until termination of the experiment or until growth caused the conidia to leave the imaging frame. In an extended study, the sorting algorithm was used to assess growth differences in 16 xerophilic fungi grown in 4 media with varying water activities to determine the effect of water activity on germination of xerophilic fungi. Lower water activity leads to a statistically significant decrease in isotropic swelling and hyphal growth rate; additionally, there is a significant delay in time to germination in xerophiles. Using live-cell imaging to monitor fungal germination and hyphal development provides essential information about fungal establishment in various environments and conditions. Methods to analyze fungal germination and establishment can aid in fungicide/antifungal drug research and provide in-depth answers about fungal community interactions.

Funding for this research was provided by the Augustinus Fonden.

[P095] Ghost Genomes of the Past: Separating and Characterizing Relic DNA

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With the advent and improvement of long-read sequencing technologies, it has become possible to sequence complex environmental samples, revealing many previously unknown microbial genomes. Despite the fidelity and capacity of these new methods, there continues to be an inherent obstacle with metagenomic studies. In environmental samples, relic DNA from dead microorganisms can persist for long periods of time, while retaining sequencing capability. Sequencing the total DNA pool extracted from an environmental sample, therefore risks conflating the past and the present composition of a microbial community. Upon cell death, DNA repair mechanisms cease to be active, relic DNA therefore starts to accumulate lesions. We hypothesize that these lesions can be exploited to distinguish relic DNA from the DNA of viable cells.

Here we aim to develop a Nanopore sequencing based method to detect relic DNA based on damage signatures. We focus on the oxidative base modification 8-oxo-deoxyguanine, a well-studied lesion that may accumulate in DNA after cell death. We outline an experimental strategy to extract Relic DNA and characterize the 8-oxo-deoxyguanine content, which could uncover the potential for using this specific lesion as a molecular marker of relic DNA.

[P096] The impact of bacterial phage defense systems in a biofilm setting

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Phages are the most abundant biological entities on the planet; they have a tremendous impact on both bacterial diversity and their ecological roles across ecosystems.
Phages impact diversity by imparting selective pressure through lysing bacteria in the environment, in turn, bacteria adapt to the pressure by changing their environment to make infection harder, while exhibiting properties that make bacteria resistant to phages.

Two key adaptations bacteria utilize to protect themselves against phages are anti phage defense systems (APDS), which directly impact the phage infection, and biofilm formation that has the emergent property of enhanced phage tolerance. Much work has been done to describe the mechanisms of the many novel APDSs discovered in recent years, but knowledge of their ecological role in phage-bacteria interactions is limited, particularly outside of planktonic settings. The dynamics surrounding the use of APDSs in biofilm settings is therefore largely unknown.

These dynamics will thus be investigated using Vibrio alginolyticus, a marine fish pathogen with biofilm producing capabilities. The first phase of this project is to knock-out in silico predicted APDS in V. alginolyticus, using two-step allelic exchange. The end goal is to obtain an APDS null-mutant, a strain that doesn't have any of the innate APDSs found in the wild type strain. The second phase is to isolate a diverse collection of phages, that can target the V. alginolyticus null mutant. The third phase is then to test the effects of different APDSs using the knock-out mutants and the phage collection, both in a biofilm- and planktonic-setting. The fourth phase will be to track the importance of spatial and temporal gradients in biofilm settings on the expression of APDS using fluorescent reporter systems.

Understanding the dynamics surrounding APDSs in biofilms could lead to a greater understanding of bacterial immunity to phages and their ecological context in what is thought to be the predominant mode of microbial life.

[P097] Antimicrobial resistance patterns in Staphylococcus epidermidis isolates from acne-affected versus healthy skin: A comparative pangenome analysis

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Acne vulgaris affects approximately 10% of the global population and significantly impacts quality of life. Multiple factors contribute to acne pathogenesis, including increased sebum production, follicular hyperkeratinization, inflammatory cascades, and skin microbiome dysbiosis. In relation to this dysbiosis, most research has focused on Cutibacterium acnes as a key contributor to disease development, whereas Staphylococcus epidermidis, a ubiquitous colonizer of both healthy and acne-affected skin, remains understudied.

To explore its potential role in acne, we analyzed whole-genome sequencing data from 123 Staphylococcus epidermidis isolates: 32 from acne-affected skin and 91 from healthy controls. Pangenome analysis identified genes differentially enriched between groups, particularly those related to metabolism, stress response, mobile genetic elements, and antimicrobial resistance. Regarding antimicrobial resistance, the fusidic acid resistance gene fusB, which confers resistance to fusidic acid, was more prevalent in acne-associated isolates (31%) than in healthy-skin isolates (11%). In vitro susceptibility testing confirmed high-level fusidic acid resistance (MIC >1 µg/mL) in all fusB-positive strains, 70% of which also displayed penicillin resistance. In addition, the VraH family peptide resistance protein was also enriched in acne-associated strains, potentially contributing to resistance to cationic antimicrobial peptides such as gallidermin and daptomycin.

These results underscore that antibiotic resistance is not restricted to C. acnes but also extends to species like S. epidermidis, which may influence treatment outcomes. This emphasizes the need for careful antibiotic stewardship in acne management and the development of alternative therapeutic approaches.

[P098] BIOCUE: Nature Based Solutions and carbon stabilization in Danish soils

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The BIOCUE consortium is an international collaboration involving Denmark, Switzerland, Spain and South Africa. We aim to determine the impact of Nature Based Solutions (land management techniques that aim to restore ecosystem diversity and function) on soil microbial Carbon Use Efficiency, enzyme activity, microbial diversity, and other indicators of soil health. Each team has selected different sites in their respective countries; in Denmark, we sampled areas around Zealand undergoing urban rewilding, regenerative agriculture, cattle grazing, restorative wetland management, and afforestation . We compared them to control sites, such as traditional agricultural fields, from the same area. Here, we present the project and selected preliminary results from the Danish sites.

[P099] Emergent properties of bacterial consortia in plant-based yogurt fermentation

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Plant-based yoghurts, particularly those derived from soy milk, often fail to meet consumer demands because of undesirable aroma attributes such as grassy odours. To enhance the flavour profile of fermented soy yoghurt, we investigated the use of multi-strain microbial consortia with emergent properties, in comparison to conventional single-strain inoculants.

We characterised a diverse set of lactic acid bacteria for their potential to grow and acidify plant-based milks. We noticed that Fructolactobacillus sanfranciscensis (Ls), a lactic acid bacterium commonly found in sourdough, effectively coagulated soy milk and reduced its pH to 5.1± 0,34 within 20 hours. Propionibacterium freudenreichii (Pf), frequently used to biofortify plant-based cheeses with vitamin B12, was co-inoculated with Ls. To improve aroma formation, we designed a three-strain consortium incorporating Ls, Pf, and one of ten additional candidate strains selected for their odour profiles.

Several three-strain combinations demonstrated accelerated acidification, achieving a pH below 5 within 8 hours of fermentation. Proton-transfer-reaction mass spectrometry (PTR-MS) analysis revealed that these consortia produced a more complex and desirable volatile profile, including sweet (2-butanone), buttery, (2,3-butanedione) and caramel-like compounds (2,3-pentanedione), while reducing sharp and off-flavour volatiles (acetone). In contrast, single-strain fermentations yielded aroma profiles that were markedly different from both commercial soy and dairy yoghurts, and generally less favourable.

Use of these three-strain consortia for fermentation generated aroma profiles more closely resembling those of commercial dairy yoghurts, suggesting that emergent metabolic interactions among strains contribute to improved acidification and flavour development. These findings support the use of rationally designed microbial consortia as a promising strategy for enhancing the sensory quality of plant-based fermented products.

[P100] Environmental DNA and environmental RNA-based insights into soil microbiome responses to nitrification inhibitors

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Nitrification inhibitors (NI) are applied with fertilizers or manures to suppress the microbial oxidation of ammonium (NH4+) to nitrate (NO3-), thereby increasing nitrogen retention in soil, and reducing emissions of nitrous oxide (N2O), a potent greenhouse gas. While NIs are increasingly seen as tools for low-emission agriculture, their broad effects on overall soil microbiome remains poorly understood. In this study, we combined environmental DNA (eDNA) and RNA (eRNA) metabarcoding with qPCR of functional genes to study the effect of commonly used NIs: nitrapyrin and 3,4-dimethylpyrazole phosphate (DMPP) on soil microbial communities in a cultivated field across two cropping seasons. Sampling was carried out in spring - two weeks after NI application, and in fall, after crop harvest. Both eDNA and eRNA profiling recovered a broad diversity and overlapping taxa of prokaryotic and fungal communities, while also showing clear distinctions between the eDNA and eRNA methods. Seasonal variation and fertilization regimes significantly affected the community structure and showed stronger effects than NI alone. eRNA based data showed lower alpha diversity under nitrapyrin compared to DMPP in spring samples, whereas non-significant effect was observed in the fall. qPCR analysis showed that DMPP significantly suppressed the bacterial amoA gene in slurry-amended soils, whereas archaeal amoA and NIR genes were unaffected. Our findings, thus, show that eDNA and eRNA metabarcoding provide complementary insights into soil microbial diversity and activity, and that NI effects, although detectable, are minor compared to seasonal and agronomical management-driven dynamics.

[P101] Sustainable production of biodegradable plastics from bioethanol

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Abstract
Plastic waste remains one of the world's biggest environmental challenges, with non-biodegradable plastics contributing heavily to greenhouse gas emissions. In 2019, the plastics industry emitted 1.8 billion metric tons of CO_2-equivalent gases, representing 3.4% of global emissions. Without intervention, this share could rise to 15% by 2050. Global initiatives such as the Paris Agreement and the UN's Sustainable Development Goals are therefore driving the search for sustainable alternatives, including recyclable and biodegradable plastics.

Polyhydroxyalkanoates (PHAs), biodegradable plastics produced from renewable resources, are seen as one of the most promising solutions. However, PHA production from biomass still faces challenges, including high costs, low yields, and poor scalability. Bioethanol, derived from biomass, offers an attractive alternative feedstock thanks to its availability and established industrial infrastructure.

In this project, commercial ethanol at 60 g/L was efficiently oxidized into acetic acid using resting cells of Gluconobacter oxydans, achieving a 74% yield with 100% selectivity. Ethanol (45%) from Lantmännen was also tested with G. oxydans DSM 50049 in a lab-scale bioreactor. At a higher concentration of 41 g/L ethanol, cell activity dropped, resulting in only a 62% yield despite full selectivity. The reduced yield was linked to ethanol evaporation caused by the higher aeration requirements and extended reaction time. This limitation was overcome by lowering the ethanol concentration to 27.5 g/L, applying 40% aeration, condensing vapors at +4 °C, and shortening the reaction time. Hence, the final acetic acid yield was improved to reach 100% after 31h of the reaction.

For PHA production, sodium acetate was used as a carbon source by Cupriavidus necator and Cobetia marina in a lab-scale bioreactor. The results showed that C. marina displayed much higher tolerance, growing and accumulating PHA at 20 g/L acetate, compared with C. necator, which managed only 10 g/L. Under controlled conditions, C. marina further demonstrated the ability to consume 35 g/L acetate in fed-batch mode, reaching an OD of 15.

Together, these findings show a renewable pathway to PHA production that reduces dependence on fossil fuels while supporting the global push for sustainable plastics.

Keywords:
Bioethanol, Acetic acid, Polyhydroxyalkanoates (PHA), Acetic acid bacteria, Gluconobacter oxydans, Cupriavidus necator, Cobetia marina,

[P102] Improving flow in the blood culture laboratory: Direct full length 16S rRNA gene sequencing from positive blood culture flasks

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Background:
Rapid identification (ID) and susceptibility testing of organisms from positive blood culture flasks (+BCF) is critical for patient management. Direct MALDI-TOF ID and susceptibility testing usually provide results within 1-2 days, but slow/fastidious/anaerobic organisms often delay results to 7-14 days with disproportionate workload. To streamline workflows, we evaluated direct full-length 16S rRNA gene sequencing (d16Sseq) from 1) selected +BCF and 2) isolates not reliably identified (NRI) by MALDI-TOF, using Oxford Nanopore Technology (ONT).

Method:
Since September 2024, eligible +BCF were processed with Qiagen DNeasy PowerSoil Pro or benzylalcohol/guanidine pretreatment. Libraries were prepared with ONT 16S Barcoding Kit 24 v14 and sequenced on GridION (R10.4.1 flowcells). Data analysis used EPI2ME and EMU.

Results:
28 NRI isolates were identified to species level (e.g., Erysipelatoclostridium ramosum, Fusobacterium watanabei).

Of 17 monomicrobial +BCF, 13 reached species-level ID, while 4 remained at genus level (e.g., Campylobacter sp., Curtobacterium sp.).

Of 16 polymicrobial +BCF, 15 yielded all cultured isolates plus up to 4 additional genera/species; most were linked to complicated intra-abdominal infections.

Species resolution was limited for some genera (e.g., Aeromonas, Listeria, Salmonella).

Conclusion:
Direct 16S sequencing of selected +BCF shows promise in reducing workload and improving ID, though full impact on turnaround time remains under evaluation. The method also highlights the underestimated frequency of polymicrobial bloodstream infections.

[P103] Impact of nanoplastics exposure on Coccolithophore calcification

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Micro- and nano-plastics (MNPs) are some of the most pervasive contaminants in the marine environment, posing threats to fundamental biogeochemical processes such as calcium carbonate (CaCO3) biomineralization. Coccolithophores are unicellular algae and the major producers of biogenic CaCO3 on Earth[1]. Through the process of calcification, coccolithophores fix dissolved CO2 and Ca ions from seawater to form CaCO3 scales called coccoliths[2]. These carbonate biominerals eventually sink and become buried in the sediment, creating one of the major long-term sinks of atmospheric CO2. Given the importance of coccolithosphores as primary producers and as major control in global carbon turnover, disruptions to coccolithosphore health and calcification process could have cascading consequences for marine biogeochemical cycles. Still, the potential influence of plastic pollution on their fitness, coccolith integrity and ultimately the strength of coccolithophores as a carbon sink remains unexplored.

Here, we address this gap by investigating the physiological impacts of fluorescent 25 nm polystyrene nanoparticles (NPs) on model coccolithophore Emiliana huxleyi. Cultures were exposed to NP suspensions (0.002% w/v) under controlled laboratory conditions and monitored for cell growth, chlorophyll a, biomass, and pH dynamics. Internalization of NPs in coccolithophores was identified using confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM), while scanning electron microscopy (SEM) and atomic force microscopy (AFM) were applied to evaluate coccolith morphology and mechanical stability. Our findings show NPs were internalized within coccolithophores, as evident from CLSM and TEM. However, no adverse impact on bulk physiological parameters could be detected. Preliminary analysis of coccoliths using AFM suggested potential perturbation of the coccoliths' mechanical strength when exposed to NP.

Here, we present our recent results on the impact of nanoplastics on coccolithosphore calcification and discuss our findings in the context of the carbon cycle. Furthermore, we emphasize the role of advanced microscale approaches to study coccolithosphore-nanoplastic interactions.

[1]Brownlee 2021, https://doi.org/10.1016/j.actbio.2020.07.050
[2]Flores 2025, https://doi.org/10.1016/B978-0-444-53643-3

[P104] Rewriting the Rules: Non-Canonical 5’ End Modifications in Archaeal mRNA

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Eukaryotic mRNAs are typically modified with an m^7GpppN capping structure at the 5’ end, a canonical cap essential for mRNA stability and efficient translation. In contrast, archaeal mRNAs have long been considered uncapped, bearing a 5’ triphosphate (5’ppp) at their 5’ ends. Here, we report an exciting discovery: a substantial fraction of mRNAs in the archaeon Sulfolobus islandicus (Sis) are chemically modified at the 5’ end with a metabolite. Global mapping of RNA 5’ ends reveals widespread 5’ metabolite capping of Sis mRNAs that begin with a 5’ adenosine. For a subset of these transcripts, dephospho-Coenzyme A (dpCoA) was identified as the capping molecule. Furthermore, we demonstrate that Sis expresses a 5’ decapping enzyme that specifically cleaves dpCoA-capped RNAs. These findings challenge the classical view of prokaryotic RNA structure and suggest that metabolite capping may have been present in the archaeal ancestors of eukaryotes.

[P105] Exploring the Danish nitrifiers in MicroFlora Danica, Denmark's microbial atlas

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Flora Danica, an atlas of Denmark's native plants compiled over 122 years, contains more than 3,000 botanical engravings. Initially inspired by this work, we introduce Microflora Danica, the first microbial atlas of Denmark, built from 10,689 samples spanning Denmark's diverse habitats and dominant land uses. From these, we generated 10,683 short-read metagenomes, near full-length 16S rRNA gene datasets from 412 samples, 16S/18S rRNA operon sequences from 450 samples, and assembled 19,253 microbial genomes (MAGs). Agriculture covers 63% of Denmark, and while disturbed soils (e.g. farmland, road sides, and urban parks) showed high within-sample richness (alpha diversity), their overall habitat diversity (gamma diversity) was lower than in natural areas, reflecting species homogenisation in managed landscapes. As agriculture depends on nitrogen cycling, we focused on the nitrifiers, characterising their distribution and diversity. We built and refined new HMM search models for the key nitrifier marker genes amoA and nxrA, adding sequences from MAGs we recovered, and improving resolution to separate canonical nitrifiers from homologous enzymes and putative new nitrifier candidates. MAG-based analyses, using single copy marker gene searches (SingleM) and genome quantification (sylph), confirmed and extended our gene-based findings. Linking to high-quality MAGs from long-read sequencing further allowed metabolic and functional inference through the analysis of gene synteny. These analyses uncovered novel taxa, including a widespread potential new nitrite oxidizer previously misclassified as Nitrobacter, and revealed that comammox Nitrospira clade B, currently lacking any cultured representative, is the most widely distributed and abundant nitrifier in natural Danish habitats. In contrast, poorly characterised archaeal ammonia oxidisers (e.g. TA-21 NS-δ) dominate disturbed soils. We reassembled genomes to represent the type material of these lineages, recovering a closed circular genome for "Ca. Nitronatura plena" (comammox clade B) and an eight contig MAG of "Ca. Nitrososappho danica" (TA-21). Altogether, Microflora Danica establishes a comprehensive national resource for investigating functional microbial groups from both gene- and genome-level perspectives. Linking the species-level resolution of our work to in situ activity and greenhouse gas emissions is the next step towards microbially informed agricultural management, ecosystem monitoring and restoration.

[P106] Food Matrix Ecology Shapes Bacillus cereus Emetic Potential across Plant- and Dairy-Based Systems

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Keywords: B. cereus, microbial physiology, virulence factors, biofilm, spores, cereulide, food safety, food microbiology

Plant- and dairy-based foods constitute distinct ecological niches that modulate the physiology and virulence of Bacillus cereus, a ubiquitous opportunistic pathogen capable of causing emetic and diarrheal foodborne illnesses. The emetic syndrome is triggered by ingestion of cereulide, a heat- and acid-stable toxin that withstands conventional food processing. Understanding how food matrices influence cereulide-producing (emetic) B. cereus is essential for microbial risk assessment and for elucidating trait plasticity in food-associated environments.

This study characterizes seven emetic B. cereus strains isolated from diverse environments, examining growth dynamics, sporulation, germination, biofilm formation, and cereulide production in plant- and dairy-based matrices. Strains were cultivated at 30 °C in oat drink, milk, and liquid pea media. Growth was modeled using the Gompertz equation to estimate lag time, maximum growth rate, and carrying capacity. Sporulation was assessed at 24, 48, and 72 h, while spore germination was monitored via isothermal calorimetry. Biofilm formation was quantified using crystal violet staining, and cereulide levels were measured by LC-MS/MS in selected samples.

Although growth kinetics were comparable across matrices, secondary traits - including cereulide synthesis, biofilm formation, and post-germination activity - were matrix- and strain-dependent. Biofilm formation was consistently higher in plant-based matrices, particularly oat drink, which also supported the highest levels of cereulide production. These findings reveal the ecological versatility of emetic B. cereus and underscore the role of environmental context in shaping virulence expression, particularly as plant-based food systems expand on the global market.

[P107] Using genomic approaches for better understanding anaerobic digester microbial community and investigating ramp-up dynamics to enhance biogas production

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Sustainable energy production contributes towards a more circular economy and helps mitigate GHG emissions and is thus more relevant than ever. Anaerobic digestion is a highly complex process driven by highly diversified microbial communities that transform organic waste into energy. However, the immense diversity of the microbial consortia effectively makes this process a biological black box, making process optimisation challenging. As Europe's market leader, Shell Biogas A/S owns and operates 15 large-scale anaerobic digesters that produce biogas and is pursuing strategies to improve process performance through microbial insight. Our work focuses on two main approaches: (1) to perform community composition analysis of lab-scale reactor experiments with Oxford NanoPore 16S rRNA gene amplicon sequencing, and (2) by using long-read Oxford NanoPore sequencing to obtain high-quality metagenomic assembled genomes (MAGs) to gain deeper insight into the microbial composition. We aim to assign functional roles to microbes, define microbial interactions between taxonomies, and connect this knowledge to conditions parameters, thereby obtaining essential insight into process optimisation strategies.

[P108] The sediments of a meromictic lake provide diverse niches to electroactive microbes linking iron, manganese and sulphur cycling.

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In meromictic lakes, the water column is stratified into oxic, hypoxic, and anoxic, often sulphidic layers. Due to global warming and increase in nutrient input, water stratification in lakes and consequent expansion of euxinia are expected with detrimental effects on the lake's functioning. Despite their importance in redox ecosystems, electromicrobial processes in the sediments of meromictic lakes remain poorly described. Our study aims to investigate niches of electroactive microbes in the sediments of meromictic Lake Idro (Italy) by identifying key taxa, their potential electroactive metabolisms, and their impact on nutrient cycling.

We describe three distinctive electro-habitats: iron cycling in sediments underlying oxic waters; cable bacteria dominating the transitional chemocline; and potential manganese-fuelled sulphur oxidation in anoxic layers. Using microprofiling, we identify characteristic pH signatures of iron redox processes in the oxic layer sediments, potentially enhanced by bioturbation, as reflected in 18S rRNA data. Despite low DNA-based relative abundances in 16S rRNA gene amplicon data, electro-active iron bacteria, including Crenothrix, Ferritrophicum, Leptothrix and Sideroxydans, were enriched in RNA-based 16S datasets, indicating high potential activity. Similarly, in chemocline sediments, cable bacteria rank among the most active in microbial communities, with 10 times higher RNA to DNA-based relative abundance. The influence of cable bacteria on sediment biogeochemistry was further evident through the formation of a suboxic zone and a distinct pH signature. In sediments exposed to transient anoxia, cable bacteria may prevent toxic sulphide release through their unique metabolism, which couples deep sulphide oxidation to oxygen or nitrate reduction near the sediment–water interface and by favoring iron-oxides formation at the sediment surface. Their activity may thus mitigate euxinia in meromictic lakes and enhance ecosystem resilience under ongoing environmental change. Finally, using metagenomic sequencing, we identified potentially novel metabolism linking manganese oxide reduction and sulphur oxidation in Sulfuricurvum, which dominates anoxic layers of Lake Idro and is commonly present in many meromictic lakes.

[P109] Reliable Computational Discovery of Antimicrobial Peptides: A Machine Learning Perspective

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Aim: Multidrug-resistant bacteria, such as the ESKAPE pathogens, pose a critical threat to public health due to their limited susceptibility to conventional antibiotics. There is a high need for development of new antimicrobial agents, such antimicrobial peptides (AMPs) which are a promising alternative to antibiotics. AMPs often have broad-spectrum activity with a reduced risk of inducing resistance. However, discovery of AMPs is labor-intensive and costly. Computational approaches, like machine learning (ML), offer an opportunity to accelerate peptide screening, but their accuracy and precision is highly dependent on large, high-quality datasets.

Method: We have developed an ML-based prediction model trained on experimentally validated 9-residue peptides with half-maximal inhibitory concentrations (IC50) relative to the reference peptide Bac2A. AMPs were defined as active when having an IC50 below 0.5 compared. Ensemble voting was used to strengthen the precision of the model, and predicted peptides were considered active if they had 80% of the votes.

Results: We demonstrate that the predictive capacity of our model trained on our own datasets or online databases, only containing 9-residue peptides, are limited by data inconsistencies. Peptide libraries such as APD6 only contain 56 AMPs, and while the larger library, DBAASP, have over 800 AMPs containing 9-residues. Many of the AMPs that are active against both Gram-positive and gram-negative strains, have minimum inhibitory concentrations, far above what this study would consider active. Due to heterogeneous antimicrobial activity assays, there is a lack of direct comparability.

Conclusion: This study highlights the potential of using ML for AMP discovery. It also demonstrates the need for more standardized activity definitions and experimental protocols. Improving data reliability would allow for the development of robust and accurate AMP prediction tools, but standardization is essential.

[P110] Adjusted PCR for Low DNA Input in Diagnostic 16S rRNA Sequencing Using Oxford Nanopore Technologies (ONT)

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Introduction
Sequencing with the Oxford Nanopore Technologies (ONT) 16S Barcoding Kit 24 enables rapid, culture-independent bacterial identification in clinical samples. However, low bacterial load in some samples poses a significant challenge for generating reliable results for clinical diagnostics. Optimizing PCR conditions and workflow efficiency is essential to ensure sensitive detection while maintaining practical turnaround times in a clinical laboratory setting. SQK-16S114 is set to replace the current used 16S V3-V4 PCR (Molzym)/SQK-RBK114 setup.

Methods
Samples were selected based on findings with our routine 16S sequencing setup. DNA was extracted from four clinical samples and six external quality assessment samples (B16SrRNA25, QCMD) using the Micro-Dx system automated on the SelectNA plus platform (Molzym). PCR amplification and barcoding were performed with the SQK-16S114 kit (ONT) using the official protocol with the following adjustments (Figure 1). To assess optimal performance with low DNA-input samples, we compared results obtained by PCR-cycles (25, 35, 40, or 45), annealing temperatures (52°C or 55°C), and PCR elongation times of (60, 80, 100, or 120 sec). Negative controls were included. Sequencing read- depth per sample was quantified to assess amplification efficiency and specificity. ONT data were processed using an in-house pipeline using the k-mer alignment (KMA) tool, which maps reads to a database built from the NCBI RefSeq and SILVA 138.2 databases.

Results
Target sequencing read-depth increased substantially with the number of PCR-cycles and lower annealing temperature. Shortening PCR elongation time does not seem to reduce assay sensitivity.

Conclusion
The adjusted SQK-16S114 protocol enables detection of bacterial DNA while remaining compatible with routine laboratory workflows. Importantly, these adjustments support the development of a rapid, same-day protocol capable of delivering precise clinical results, facilitating integration of culture-independent 16S sequencing into diagnostic practice. Increasing PCR cycle count does, expectedly, also amplify contamination DNA increasing false positive risk. Further work is needed to establish a clearer distinction between true low level positive samples and background noise.

[P111] Uncovering the Plasmidome of Plant Microbiota Across Crops and Models

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Plant health depends not only on the genes of the plant itself, but also on the genes of its microbial partners. Plants and their microbiomes are intertwined through processes of co-adaptation. By shaping microbial recruitment via root exudates and immune filtering, plants assemble communities that enhance growth, nutrient uptake, and stress resilience. In turn, microbes adapt rapidly to host conditions, with horizontal gene transfer (HGT) enabling evolutionary change on ecological timescales.

Mobile genetic elements (MGEs), especially plasmids, are key drivers of this rapid adaptation, yet their diversity and ecological roles in plant microbiota remain largely uncharted. To address this gap, we are mapping the plasmidome of 4,918 bacterial isolate genomes from two model plants and four major crops (Lotus, Arabidopsis, Oryza, Triticum, Zea, Medicago). Using comparative genomics, clustering, and functional annotation, we aim to reveal lineage-specific plasmid distributions, transferability networks, and adaptive traits.

By linking plasmid function to microbial adaptation, this work will shed new light on how MGEs shape plant–microbiome co-evolution and pave the way for microbiome-based strategies to improve crop sustainability.

[P112] Phages for therapy against Pseudomonas aeruginosa

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Infections caused by multidrug-resistant bacteria are an increasing problem worldwide, and new therapies are urgently needed. One such bacterium is Pseudomonas aeruginosa, which is becoming increasingly resistant to antibiotics. One potential solution is the use of bacteriophage (phage) viruses for therapy. A major biological challenge for phage therapy is bacterial phage defenses and the acquisition of mutations in genes encoding phage receptors, which render the bacteria resistant to phage killing.

In this project, we will investigate the therapeutic potential of novel P. aeruginosa phages by characterizing their host range, receptor usage, and synergy with antibiotics. More specifically, we will use mutants deficient in the two major P. aeruginosa phage receptors, type IV and O-antigen, to determine if the phages require either one of these for infection. Phages that rely on alternative receptors will be further analyzed through sequencing of phage-resistant mutants and genetic complementation. We will also investigate the ability of selected phages to infect a panel of clinical P. aeruginosa isolates under different physiological conditions and test their synergy with antibiotics.

This project contributes to identifying promising phage cocktail candidates with antibiotic synergy for future therapeutic applications against P. aeruginosa.

[P113] Improving crop heat stress adaptation through 82T bacterial interaction

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Modern agriculture is facing harsh challenges, including rising temperatures, unpredictable weather patterns, and more frequent extreme events. These adverse conditions disrupt farming ecosystems, leading to reduced crop yields, increased pest and disease outbreaks, and soil degradation, ultimately threatening food security. To tackle these issues, strategies that improve agricultural systems resilience are vital. The plant-soil microbiota, consisting of diverse microbial communities, plays a key role in mitigating such stresses. Plant growth-promoting bacteria (PGPB) offer a sustainable solution by enhancing growth, nutrient uptake, and stress tolerance. Thus, PGPB are eco-friendly alternatives to chemical fertilizers and pesticides. Therefore, understanding plant-microbiota interactions and mechanisms by which PGPB confer stress tolerance is crucial for improving these interactions and advancing sustainable agriculture.

To study extreme temperature effects on plant growth, we developed TGRooZ (Temperature Gradient in the Root Zone), which generates a decreasing temperature gradient in the root ecosystem, mimicking natural soil conditions. Bacterial collections were isolated from tomato plants grown under heat stress in order to enrich in microbes that might help the plants. In a screening of bacteria endophytes that confer thermotolerance and promote plant growth, we identified a PGPB named 82T.

We evaluated 82T's effect on Arabidopsis in vitro, finding enhanced growth at high temperature. To unravel how this bacterium improves thermotolerance, an RNA-seq experiment was conducted during the interaction under different temperatures. At 22ºC, 82T changes gene expression of a few genes, but at 32TGRooZ, a large set of genes had their expression modified. It should be highlighted that many up-regulated genes, which are involved in heat stress response, reduced their expression level in plants co-cultivated with 82T, suggesting that the interaction of the bacteria with the root system alleviates the plant stress. These and further analyses will be presented at the meeting. This study characterizes the Arabidopsis–82T interaction, identifying candidate genes and pathways involved. It also highlights TGRooZ as a valuable tool for discovering and testing microbes to enhance plant heat tolerance in field-relevant conditions.

Keywords: Plant growth-promoting bacteria (PGPB), Heat stress tolerance, Sustainable agriculture

This work has been funded by grants: PID2020-113479RB-I00 grant by MCIN/AEI/10.13039/501100011033/ to J.C.P and by the "Severo Ochoa Program for Centres of Excellence in R&D" from the AEI of Spain (grant SEV-2016-0672 (2017-2021) to the CBGP. ITGM is supported by a predoctoral fellowship from Programa Propio I+D+I UPM-Santander Universidades 2022.

[P114] Spatial Arrangement Influences Competitive Outcomes in Three-Way Fungal Biocontrol Interactions

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Biological control using fungi holds significant potential for managing pests and pathogens. Co-application of biocontrol agents might enhance this and studies are developing compatible fungal pairs. While spatial and temporal dynamics profoundly influence co-infection outcomes and competitive exclusion in host ecosystems, dissecting multi-way interactions in such intricate environments remains challenging. To address this, I utilized a simplified, controlled 2D Petri dish system to investigate the fundamental impact of initial spatial positioning on interactions among key fungal biocontrol agents (Trichoderma, Metarhizium, and Beauveria), an area yet to be thoroughly investigated in a tractable experimental setup. Different fungal isolates were line-streaked on potato dextrose agar in various combinations and arrangements. Each fungal growth area in Petri dish was calculated by ImageJ after the incubation period and the interactions between them were assessed. Our findings show that in pairwise interactions, T. afroharzianum, T. guizhouense and M. brunneum each alone presented antagonistic effects, consistently outcompeting and dominating against the plant pathogen Fusarium oxysporum. Both Trichoderma isolates presented nearly equal effects with M. brunneum. Beauveria bassiana was consistently the less competitive fungus in its pairwise interactions with Trichoderma isolates and M. brunneum; and was particularly weak in antagonizing F. oxysporum. Also, the initial spatial positioning of fungal inoculations significantly influenced the competitive dynamics between the fungi in three-way interactions. The outcome of these interactions depended on the initial arrangement of the fungi, with Metarhizium and Beauveria influencing the antagonistic effects of Trichoderma.

[P115] A method to map cable bacteria activity in sediment by applied electric inhibition

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Microbial respiration accounts for the majority of oxygen consumption in marine and freshwater sediments, shaping redox gradients and driving biogeochemical cycles. Cable bacteria can account for up to 90% of total sediment oxygen consumption, making them dominant regulators of benthic oxygen budgets. These filamentous Desulfobulbaceae bacteria couple sulphide oxidation in anoxic layers to oxygen reduction at the sediment surface via long-distance electron transport (LDET). This activity can have a profound impact on sediment biogeochemistry, producing characteristic oxygen, pH, sulfide, and electric potential (EP) gradients, while also protecting benthic fauna and flora from sulfide toxicity during oxygen depletion. Despite their importance, the spatial and temporal variation in cable bacteria respiration remains unresolved. In this study, we developed a non-destructive method to measure cable bacteria respiration in sediment at µm and seconds resolution, inspired by the light–dark shift technique of Revsbech and Jorgensen (1983) for measuring rates of photosynthesis. Just as light–dark shifts remove photosynthetic oxygen production to isolate consumption, OCEM applies external potential differences to selectively block LDET and separate cable bacteria respiration from other oxygen consumption processes. By combining this perturbation with oxygen microsensor measurements, OCEM enables investigation of vertical, horizontal, and temporal variation in cable bacteria oxygen consumption. This approach can be used to refine our understanding of how cable bacteria respiration shapes benthic oxygen dynamics, providing a bridge between microscale activity and ecosystem-level impact.

[P116] The role of the global stress regulator Spx in resistance of Staphylococcus aureus to the anti-platelet drug ticagrelor

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Antimicrobial resistance (AMR) has become one of the major challenges for global health. Repurposing of existing drugs is one of the strategies that are currently investigated to combat the antibiotic crisis. The anti-platelet drug ticagrelor was demonstrated to have antimicrobial properties against various Gram-positive bacteria, including the high-priority pathogen methicillin-resistant Staphylococcus aureus (MRSA).

Ticagrelor-resistant S. aureus isolates were previously shown to have mutations in genes encoding proteins that modify the levels of Spx, a global stress regulator. In this project we are investigating whether accumulation of Spx is modulating resistance to ticagrelor in the same manner as it is causing resistance to beta-lactams and other envelope-targeting antibiotics. By investigation of the relationship between Spx-levels, ticagrelor resistance, and the mutational profile of ticagrelor-resistant isolates, this study will contribute to a broader understanding of ticagrelor resistance and the Spx regulon in bacterial responses to antimicrobial agents.

[P117] Disrupting Biofilm Formation and Scaling in RO Membranes through Controlled Salinity Oscillations

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Reverse osmosis (RO) is a widely adopted technology for water purification, yet its efficiency is significantly affected by scaling and biofouling, reducing membrane lifespan and elevating operational costs. The BE-SALT project is investigating a batch RO system designed to address both scaling and biofouling issues through the application of salinity oscillations. We developed a suite of experimental models to study how repeated exposure to varying salinity affects both planktonic and sessile microbial populations.

In planktonic cultures prepared from tap water and pre-adapted to optimal salinity, three cycles of salinity oscillation significantly reduced microbial growth but did not kill the bacteria. Using a membrane fouling simulator, we show that mature biofilms exposed to three oscillation cycles required more time to resume metabolic activity, as measured by oxygen consumption, compared to control biofilms. Furthermore, salinity oscillation caused partial detachment of the biofilm from the membrane. We then addressed whether salinity oscillation could prevent biofilm formation using a flowcell model. Short (1 min) salinity oscillations (0.5-3%) did not prevent biofilm formation, but slightly longer (3 min) and higher (0.5-5%) oscillations did inhibit biofilm formation during the 48 h observation period. These findings suggest that controlled salinity oscillations in batch RO systems, such as proposed by BE-SALT, can mitigate biofouling, offering a promising strategy for enhancing membrane performance. Future work will focus on optimising oscillation parameters and scaling up the approach for real-world applications.

[P118] How to get long reads from soil?

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Soil hides an enormous diversity of microorganisms that drive key ecosystem processes, however, accessing this diversity at the genomic level remains a major challenge. A key limitation is the extraction of long DNA fragments, which are required for producing contiguous assemblies and reliable metagenomic insights. However, standard soil DNA extraction protocols often yield highly fragmented DNA due to the complex nature of soil matrices.

In this study, we systematically compared four extraction methods, including protocols combined with size selection and with or without bead beating, to evaluate their ability to recover long DNA fragments from soil.

Our results show that integrating DNA extraction with size selection dramatically improves read length. Moreover, we find that extraction protocols excluding bead beating yield longer DNA fragments compared to those using bead beating. These findings contribute to the development of more robust protocols for soil metagenomics. Optimized extraction strategies will enable more complete assemblies, improve taxonomic and functional resolution, and ultimately deepen our understanding of the structure and dynamics of soil microbial communities.

[P119] Recurrence and propagation of past functions through mineral-facilitated horizontal gene transfer

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Horizontal gene transfer (HGT) is one of the most important drivers of bacterial evolution, enabling the acquisition of new genetic traits beyond vertical inheritance. Natural transformation via uptake of extracellular DNA has traditionally been regarded as inefficient, as free DNA is thought to degrade rapidly in environments such as seawater and soil, persisting for only days1, 2. Recent discoveries have revealed that DNA can remain preserved in sediments for up to 2 million years (Ma)3, extending the temporal window of DNA availability for genetic uptake.

Here, we demonstrate that ancient environmental DNA adsorbed to common sedimentary minerals can serve as a reservoir for bacterial transformation. Using Acinetobacter baylyi as a model organism, we show that even short DNA fragments of ~60 bp, when bound to mineral surfaces, can be incorporated into bacterial genomes. Transformation frequencies vary with mineral surface properties, indicating that the DNA-mineral interactions directly modulate HGT potential.

Our findings challenge long-standing assumptions about the limitations of extracellular DNA in bacterial evolution and highlight mineral-facilitated transformation as a previously underappreciated pathway of HGT. With the recognition that sediments worldwide are vast reservoirs of ancient DNA, our results suggest that mineral-adsorbed, fragmented DNA can act as a persistent genetic archive, fueling the evolution of contemporary microbial communities. We propose that mineral-facilitated HGT substantially broadens both the spatial and temporal scales at which horizontal gene transfer shapes bacterial diversity and evolution.

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[P120] Defining the Spx regulon under normal growth conditions and during antibiotic stress

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Spx is a conserved global transcriptional regulator in Staphylococcus aureus, best known for its role in disulfide stress response. Recent work has shown that Spx confers homogeneous high-level resistance to β-lactams and other compounds targeting the cell envelope. Here, we investigated the Spx regulon under normal growth and antibiotic stress. Using S. aureus USA300 JE2 wild type and a yjbH-Tn mutant with CRISPRi-mediated Spx knockdown, we performed RNA-seq analysis.

Under normal conditions, Spx depletion upregulated fermentation-related genes, including those involved in formate, lactate, and ethanol fermentation, while downregulated genes were largely uncharacterised. Conversely, Spx accumulation repressed amino acid biosynthesis and nutrient uptake pathways, notably the ilv-leu operon, and downregulated genes involved in urea metabolism.

These results suggest that Spx balances metabolic processes by promoting fermentation when depleted, and repressing biosynthetic pathways when accumulated. Many uncharacterised proteins were also influenced by Spx levels. Together, this highlights Spx as a key regulator of global stress responses and resistance, and future work will focus on its role during antibiotic stress, particularly in modulating secondary regulatory networks.

[P121] Development of genetic tools functional across bacterial species

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Microbes have diverse metabolic capabilities and physiological traits that can be leveraged to valorize waste, minimize environmental impacts, and create high-value products. For example, some organisms naturally tolerate high temperatures or low pH, while others support valorizing and sustainable feedstocks, like CO2 and nonfood biomass. Microbes can be applied in numerous fields, such as in the sustainable production of bulk chemicals, the food industry, and bioremediation. Unfortunately, unconventional microbes with properties of interest are understudied and often have very few compatible genetic tools, required for strain study and engineering. Besides, the existing tools for commonly used organisms usually work only for closely related species.

This project focuses on developing tools functional across different bacterial species, both gram-positives and negatives, and mesophiles and thermophiles, and can be divided into three main parts. The first consists of the study of an inducible promoter system, which proved to be functional across phyla. The second part is a comparative study of gene expression regulation across species, performed by creating a promoter library with more than 65.000 variants to be analyzed in different organisms. The third part aims to develop broad-host range transformation vectors capable of evading the natural immune system of bacteria by removing the targets of the restriction-modification system. The project aims to create plasmid elements that are easily transferable to a wide range of bacteria to ease the establishment of genetic engineering protocols. Together, these efforts will enable the implementation of more advanced molecular tools and high-throughput screening approaches across several bacterial species. The ultimate goal is to accelerate the domestication of unconventional microbes for various sustainable microbial applications.

[P122] Effect of biochar on extracellular enzyme activity and microbiome dynamics across coarse sandy soil depths

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The aim was to assess the effect of straw biochar on microbiomes along the depth (30 to 80 cm) of two coarse sandy subsoils. We hypothesized that biochar modifies extracellular enzyme activities (EEA), and diversity and structure of microbiomes across the subsoil depths. Two subsoils were amended with straw biochar (0-4% wt/wt) and incubated for 16 months in a column experiment with two cultivations of spring barley. EEA were assessed using fluorogenic assays, while the prokaryotic and fungal communities were analyzed via 16S rRNA gene and ITS2 amplicon sequencing, respectively. Biochar significantly increased water holding capacity and pH. It also significantly decreased the phosphomonoesterase activity, suggesting enhanced soil phosphate bioavailability. In both subsoils, biochar significantly increased the prokaryotic α-diversity index. Biochar impacted prokaryotic community structures more than fungal community structures. Prokaryotic community structures were significantly different with increasing biochar content at deeper soil depths. Moreover, in both subsoils, biochar significantly increased the relative abundance of a prokaryotic consortium. We conclude that the biochar-induced improvements in physicochemical soil properties stimulate microbial diversity and functional activity across varying depths (30 to 80 cm) in coarse sandy subsoils. These findings are valuable for assessing the potential benefits of biochar application on agricultural subsoil health.

[P123] Optimizing sampling and extraction strategies for epithelial-associated gut microbiota in a pig colitis model

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The human gastrointestinal microbiome plays a critical role in host physiology, including digestion, metabolism, and immune regulation. Disruption of this ecosystem, termed dysbiosis, is linked to disorders such as inflammatory bowel disease (IBD). While fecal samples have been extensively studied, they mainly capture luminal stool communities and may overlook microbes adhering to the intestinal epithelium. Characterizing these epithelial-associated microbiota is particularly relevant due to their close interactions with host epithelial tissues and their potential role in shaping immune responses and barrier function.

Tissue-based microbiome research faces substantial challenges, including small sample sizes, dominance of host DNA, and difficulties in recovering high-quality microbial DNA. To address these issues, this study applies a veterinarian-controlled experiment using a cohort of 24 pigs, in which IBD is induced with DSS and monitored over time. Three sampling approaches (clinical biopsies, mucosal scrapings, and minimally invasive brush biopsies) are combined with different DNA extraction strategies to evaluate their performance for metagenomic sequencing. The workflow includes assessment of bacterial-to-host DNA ratios, reproducibility of extraction protocols, and feasibility for downstream bioinformatic analyses.

This work presents an experimental framework aimed at identifying suitable combinations of sampling and extraction methods for resolving epithelial-associated microbiota. By outlining methodological considerations, the study supports future efforts to improve metagenomic characterization of tissue-associated microbial communities and to strengthen translational links between animal models and human clinical research. At the DMS conference, we will present 16S rRNA gene amplicon sequencing data comparing community structures across methods, providing a foundation for optimized epithelial tissue-associated microbiota studies. These data will serve as an improved methodological approach to study intestinal inflammations in humans.

Keywords:
Gut microbiome, inflammatory bowel disease, Epithelial-associated microbiota, Metagenomics, DNA extraction methods, Sampling strategies, Microbial ecosystems, Pig model.

[P124] Multifaceted Transcriptional Regulation of Phosphonate Degradation in Escherichia coli: Potential Regulation Beyond the Promoter by PhnF

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Phosphate (Pi) is an essential nutrient for bacterial cell growth but is often scarce in natural environments. To overcome this, many organisms, including Escherichia coli can degrade phosphonates (Pn), a group of chemically stable compounds characterised by a carbon–phosphorus (C-P) bond.

The genes required for phosphonate uptake and breakdown are organised in the 14-cistron phn operon which under Pi limitation is upregulated by the transcriptional activator PhoB. The operon encodes an ABC transporter (phnCDE), the transcriptional regulator PhnF, and enzymes catalysing Pn degradation (phnGHIJKLMNOP). Of these, PhnF belongs to the HutC-subfamily of transcriptional regulators, which typically function as repressors that are relieved by pathway metabolites. However, the regulatory role of PhnF in E. coli Pn metabolism has not been investigated.

Here we show that PhnF binds within an intragenic region between phnE and phnF, rather than at the operon promoter, as revealed by ChIP-seq under Pi limitation.

RNA-seq further demonstrated that transcription of the full phn operon requires PhnF, since a ΔphnF strain failed to transcribe the operon under Pi limitation with Pn present. This strain also showed limited growth on Pn as the sole phosphorus source compared to wild type, suggesting that PhnF may not function as a classical repressor but is required for efficient utilisation of Pn. These findings point to an unexpected regulatory mechanism in which PhnF may influence transcription downstream of the transporter genes, phnCDE.

To test this hypothesis, we are developing promoter–reporter constructs to assess whether PhnF binding acts as a transcriptional roadblock. Defining the role of PhnF will broaden our understanding of how E. coli adapts to nutrient limitation and may reveal regulatory strategies more widespread among HutC-family proteins.

Key words: Nutrient limitation, transcriptional regulation, repressor

[P125] Early-life microbial cross-feeding interactions and targeted modulation in vitro

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Infant gut microbial community profoundly influences the early and long-term human health development. The changing nutrient supply from liquid to solid diet is a critical force shaping the diversity and functionality of the human gut microbial community during the first year of life. During weaning period, the introduction of solid foods results in an increased abundance of complex carbohydrate degraders and propionate/butyrate-producing bacteria. Ruminococcus bromii and Bifidobacterium adolescentis are gut commensals with the ability to degrade resistance starch (RS) and to provide substrates for cross-feeding. Early colonization by R. bromii was linked to high levels of butyrate, which has been suggested to protect against infant atopic dermatitis, but the interdependence of resistant starch degradation, microbial cross-feeding and health at infant ages is not fully understood. This study aimed at investigating the occurrence of microbial cross-feeding of starch degradation at infant ages and the potential of a combination of resistant starch (RS) and R. bromii to modulate the cross-feeding activity of early-life gut microbiota.

I analyzed the longitudinal dynamic of starch utilizers R. bromii and B. adolescentis and butyrate-producer Anaerobutyricum hallii during the first year of life. Also, I conducted co-culture studies of R. bromii or B. adolescentis and the butyrate producer A. hallii and investigated the impact of microbial supplementation and RS addition on fermentation activity of fecal microbiota of 4-6 and 11-13 months old infants. R. bromii and B. adolescentis established in gut ecosystem at first few months, but A. hallii occurred at one year of age. R. bromii and A. hallii were positively associated with each other and fecal butyrate levels. In contrast, B. adolescentis was positively associated with fecal levels of acetate and propionate. In vitro, A. hallii crossfed on reducing sugars from RS degradation by R. bromii or lactate from RS fermentation by B. adolescentis to produce more butyrate than A. hallii alone. In vitro, fecal microbiota had higher fermentation activity if RS was supplied compared to controls without RS. A combination of RS with R. bromii increased the production of butyrate by 1.4 - 3.8-fold in infants at 4 - 6 months. Based on full-length 16S rRNA gene amplicon sequencing, we successfully modulated the fecal microbiota with the addition of RS, which increased the relative abundance of the family Veillonellaceae. The supplementation of R. bromii additionally led to a higher relative abundance of the family Bifidobacteriaceae and Lachnospiraceae in one-year-old infant fecal batch fermentation than controls

Taking together, microbial cross-feeding activity of starch degradation between primary degraders and secondary fermenter occurred during the first year of life, leading to the production of propionate and butyrate. Our results also suggest differences in the mode of cross-feeding depending on degradation and utilization pattern of primary degraders. The concurrent supplementation of RS with primary degraders modulated fecal microbiota fermentation activity highlighting the potential for targeted modulation of the gut microbiome.

[P126] Mapping Danish environmental resistome under the Microflora Danica campaign

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The environment plays an important role in the occurrence and spread of microbial antibiotic resistances (a collection of such genes are called “resistome”). However, at the Danish national scale, the level of environmental resistome and its role in the spread is unknown. Exploiting the available environmental microbiome data in the Microflora Danica project (MFD), where over 7,000 Danish environmental samples representing >23.7 Tbp of quality trimmed metagenomic data from 21 habitats of various soils, sediments, aquatic environments and wastewater treatment plants (WWTPs) were used, we showed that the environmental resistome in Danish natural habitats is of much lower relative abundance and transferability than human-associated habitats. Owing to the wide range of habitats involved, we observed that some antibiotic resistance genes (ARGs) formed the core resistome of a habitat, some ARGs could be indicative of a habitat, and some ARGs were found commonly abundant in several habitats. With the additional 110 deep long-read metagenomes in MFD representing >9 Tbp data to reveal the host and and genetic context of ARGs, those cross-habitat commonly abundant ARGs were suggested to have a wide host range and diverse mobile backgrounds. In soils and sediments, phage and plasmid had more limited contribution to the spread of ARGs than WWTPs. However, a few clinically relevant ARGs were suggestive of transferrable between the environmental and human pathogenic habitats indicated by the high ARG sequence similarities and presence of the same associated mobile genetic elements. We anticipate our results to support the implementation of environmental resistome surveillance by filling the knowledge gap of baseline levels, to aid in ARG pollution source tracking and cross-habitat ARG dissemination assessment, and to provide a resource for future search and exploitation of strongly needed new antibiotics.

[P127] Unravelling the regulatory network behind the production of antifungal cyclic lipopeptides by the plant-beneficial bacterium Pseudomonas nunensis In5

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Pseudomonas nunensis In5 is a plant-beneficial bacterium isolated from disease-suppressive potato fields in Greenland. Its antifungal capacity is mediated through the production of two cyclic lipopeptides (CLPs), called nunamycin and nunapeptin. These are bioactive specialized metabolites encoded by adjacent biosynthetic gene clusters (BGCs). The presence of CLP encoding BGCs has been positively linked to disease suppression in soil, making such producers promising candidates for controlling plant diseases in agriculture.

Although the production of lipopeptides and CLPs is widespread in the Pseudomonas fluorescens lineage, knowledge on signals inducing production remains sparse. While a few CLP regulators have been identified on the genetic level, knowledge of the molecular mechanisms controlling production and what specific environmental and microbial interkingdom signals dispersed in soil activate these switches is understudied.

This study uses Pseudomonas nunensis In5 as a model to explore the regulatory network controlling CLP production. Global regulation is mediated by the GacS/GacA two-component system, which responds to plant-associated signals. Local regulation is controlled by NunF, which responds to fungal signals. Disruption of either gacA or nunF abolishes the antifungal activity. In addition, the strain harbors a four-gene quorum-sensing (QS) system, consisting of pcoI an acyl homoserine lactone (AHL) synthase, two regulatory genes nupR1 and nupR2, as well as a transporter gene rhtb. Loss of QS regulator genes led to an impaired CLP production in similar Pseudomonas strains. In this work mutant strains for nupR1, nupR2 and pcoI were generated, to investigate the role of In5's QS system in the production of nunamycin and nunapeptin. Antifungal activity was abolished for the regulator mutant strains, while the pcoI mutant strain exhibited a greatly diminished antifungal activity. CLP production of the mutant strains is being further investigated using metabolomics as well as studying expression levels of core BGC genes. These preliminary findings support our hypothesis that the QS system plays an essential role in regulating the production of CLPs in In5. Observations on the QS genes for In5 differ from mechanisms reported in closely related strains, showing the complexity of regulatory systems behind specialized metabolite production. We expect this work to further our fundamental understanding of CLP regulation and their role in fungal disease suppression.

[P128] Who drives sand aggregation during succession following willow growth?

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Willows (Salix sp.) are commonly found on sand dunes and coastal areas, they are used as bioenergy crops, for phytoremediation and slope stabilization due to resilience to various stresses, ability to form root primordia on branches and high biomass production. These beneficial traits are to some extent supported by associated bacteria and mycorrhizal fungi.

We studied successional changes in bacterial and fungal communities following planting willow cuttings in clean sterile sand. Physical, chemical and microbiological parameters of root-affected sand were measured after 30, 60, 90, and 150 days of willow growth in an indoor pot experiment. Sequencing of gene libraries (bacterial 16S rRNA, fungal ITS2) and qPCR for bacterial and fungal marker genes was performed for bulk and aggregated sand samples.

As expected, organic carbon content, gene copy numbers and bacterial alpha-diversity increased with willow growth. Plant-associated Alpha- and Gammaproteobacteria were dominant in all samples, while some taxa became enriched at later stages. No clear patterns were observed for fungal communities.

An unexpected sand aggregation around roots was observed in pots sampled at day 150. Marker gene copy numbers were highest in aggregates, and they harbored distinct bacterial community. Concentrations of calcium and magnesium were 40-200fold higher in aggregates compared to bulk sand. SEM analysis shown that crystals were formed between sand grains. So presumably, calcium carbonate precipitated due to root respiration and local conditions, while biofilms on sand grains acted as crystallization points. Alternatively, oxalic acid could have been exuded by willows and either precipitated as calcium oxalate or converted by oxalotrophic bacteria. Bio-induced precipitation of carbonates around willow roots can be employed for enhancing slope stability and carbon sequestration.

This work is part of the project Soil Is Alive (SoIA) granted by the Carlsberg Foundation.

[P129] Unravelling Composition and Evolution of Pig Gut Microbiomes through Longitudinal Multi-Omics

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The pig gut microbiome underpins growth and health, making it a key target for optimizing livestock management [5]. However, it is also a reservoir of antimicrobial resistance (AMR) with potential transfer to environmental bacteria and human pathogens [4]. Much prior work relies on culturing or bulk metagenomics, which can miss unculturable microbes and uncoupled plasmid-borne AMR. Single-cell sequencing helps resolve these gaps but remains a less mature technology with known biases [2], [3]. To map the diversity and temporal dynamics of AMR in pigs, multiple techniques can be combined.

We present a multimodal, longitudinal study of individual Danish pig faecal microbiomes over 11 weeks during fattening. We queried the microbiome using (1) Illumina short-read shotgun sequencing, supplemented with (2) Nanopore long-read sequencing, and (3) single-cell DNA sequencing based on semi-permeable capsules [1]. We construct a bulk-based MAG catalogue to analyze taxonomic and functional composition and to track community interactions over time. For the single-cell data, we infer phylogenies to track potential within-farm strain sharing. We also map antibiotic resistance genes (ARGs) and mobile genetic elements to assess effects of tiamulin administration, applied to half the pens after Lawsonia-associated diarrhea was observed.

Our multimodal analysis reveals extensive diversity with >2,400 MAGs and >6,500 SAGs after filtering. Community structure follows expected pig gut trends, with many Bacillota and Bacteroidales taxa. Curiously, we find a clear separation with tiamulin-treated pigs seemingly getting lower richness of ARGs than untreated pigs. We will present further analyses testing these preliminary findings by quantifying ARG richness, diversity, abundance, and transmission. We leverage the synergy of having both bulk and single-cell sequencing available, and offer a template to study the potential threat of resistant microbes in agriculture and beyond.

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