ABSTRACT BOOK



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Modernizing a large fungal strain collection for high-throughput screening workflows and the identification of future fungal biosolutions

Jacob Blæsbjerg

Fungi have an indispensable role and impact in environments, for example through interactions with living systems as well as organic and inorganic matter. The relation with other organisms may be displayed as pathogenic and destructive, or beneficial mutualism through symbionts such as mycorrhizae and endophytes. The production of fungal metabolites and enzymes are often pivotal for the success of fungal adaptation. At DTU Bioengineering, we aim at tapping into our fungal bioresource in the IBT Culture Collection, to identify the fungi and products for novel sustainable solutions. We have established a high-throughput screening workflow for a diverse array of filamentous fungi and have initiated a larger characterization effort to enable fungal solutions and contribute to a larger repertoire of species for future industrial applications.

Adapting the GTDB workflow for Fungi

Masha Chuvochina

The Genome Taxonomy Database (GTDB; https://gtdb.ecogenomic.org), an essential taxonomic resource for microbiologists worldwide, marked its tenth release (R10-RS226) in 2025 and is now expanding to the kingdom Fungi. This milestone marks a significant step in broadening GTDB's scope, with the fungal expansion currently in the investigation phase and the first public release anticipated in 2027. Here we present the results of initial quality control (QC) for genomes, evaluations of average nucleotide identity (ANI) thresholds for species delimitation, and marker gene selection for reference phylogeny inference. Of 22,432 fungal genomes downloaded from the NCBI Assembly database in September 2025, 17,522 genomes passed our QC for subsequent analyses. Application of the widely used 95% ANI threshold for operationally defining bacterial species resulted in 4,838 fungal species clusters, with 17.1% of named species being merged, reflecting the need for reclassification but also further investigation about the utility of ANI-based species delineation. We then evaluated 1,122 fungal-specific BUSCO markers from OrthoDBv12 for ubiquity and copy-number stability across the representative genome set. Based on these criteria and evaluations of individual marker gene trees, we selected 137 ubiquitous, single-copy markers to be tested for tree reconstruction using various sequence alignment filtering options and substitution models. We anticipate presenting the results of tree tests and our first reference tree for fungi in time for ISME20. The fungal GTDB will allow accurate classification of not only existing diversity but also the increasing number of fungal MAGs anticipated from environmental samples as long-read sequencing throughput continues to improve.

Expanding the molecular toolbox for Nannochloropsis oceanica a microalgae for biomanufacturing and elucidating evolutionary trajectories of photosynthetic eukaryotes.

Martina Dajak, Henrike Heinemann, Daniel Poveda Huertes, Danny Liu, Rocio Ochea Fernande, Jonna Pohankukka, Johan Andersen Renberg, Jannis Straube, Waranid Sutthacharoenthad, Alberte Thude, Laura Vimenet

Photosynthetic microalgae can drive biomanufacturing directly from CO_2 with an areal efficiency 10-20x higher than current agricultural systems (M. J. Barbosa, et al., Trends in Biotechnology 2023, 41, 452-471), so algal "crops" hold considerable promise for future food production.

Nannochloropsis oceanica is a eustigmatophyte alga, related to diatoms and brown algae, already cultivated industrially as a source of omega-3 oils. Realising its potential as a production host organism, and using it as a model for the evolution of marine eukaryotes, requires efficient tools for genetic engineering.

We have exploited the synchronised cell division that N. oceanica undergoes during its diurnal cycle to develop nuclear transformation protocols of substantially improved efficiency, and have established a robust selection marker based on sulfadiazine. Using these tools we engineered strains that produce the octaketide precursor of carminic acid and the ketocarotenoid canthaxanthin from CO_2, sunlight and seawater alone, showing that both polyketide and carotenoid pathways can be supported by eustigmatophyte metabolism.

Beyond their applied value, these tools makes it possible to study carotenoid biosynthetic genes in N. oceanica, and how they are conserved in photosynthesus across algae species underpining their evolutionary origin.

H5N1 is expanding it's host range: A step up the pandemic ladder?

Lars Erik Larsen[1]
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This presentation reviews the global emergence and zoonotic potential of highly pathogenic avian influenza viruses (HPAIV), with particular focus on the Goose/Guangdong (Gs/Gd) H5 lineage clade 2.3.4.4b viruses that currently circulate worldwide. The seminar introduces key concepts in influenza A virus biology, including viral evolution through antigenic drift, reassortment, and the generation of new subtypes and genotypes. It highlights the unprecedented expansion, geographic spread, and genetic diversification of H5 viruses since 2020, resulting in sustained outbreaks in wild birds, poultry and an increasing range of mammalian species including humans.

Special attention is given to species barriers, host adaptation, and the factors enabling avian influenza viruses to infect mammals and humans. Recent developments, including widespread H5N1 infections in dairy cattle in the United States and associated human cases, are discussed as examples of viral adaptation and emerging zoonotic risks. While human-to-human transmission remains very limited, the ongoing circulation of H5 viruses in birds and mammals creates opportunities for further adaptation and reassortment.

The presentation concludes by outlining priorities for surveillance, biosecurity, genomic data sharing, preparedness, vaccine development, and international One Health collaboration to mitigate future pandemic threats

Listeria monocytogenes – predictive models and control options

Paw Dalgaard
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Rates of listeriosis are high within the EU and these rates in Denmark, Finland, Iceland and Sweden are much higher than the EU average. Concerning listeriosis the growth of L. monocytogenes in chilled ready-to-eat foods is a major challenge. Predictive food microbiology models and software have been developed to predict L. monocytogenes growth depending on product characteristics and storage conditions. This presentation provides examples of how these tools can be used (i) to identify safe product recipes that prevent growth of L. monocytogenes and (ii) to determine safe shelf-life for ready-to-eat seafood and meat products. Listeriosis outbreak investigations, including the use of whole genome sequencing, is most important to identify responsible products, enable targeted recalls and limit the extent of an ongoing outbreak. To reduce future outbreaks is it suggested to evaluate characteristics of implicated products using predictive modes and determine the changes of recipes that are required to prevent unacceptable growth of L. monocytogenes.

Staphylococcus aureus – from commensal to “killer bug”

Andreas Peschel

As for most of the major bacterial human pathogens, Staphylococcus aureus uses two entirely different lifestyles, as a frequent member of nasal or skin microbiomes, or as an invasive cause of local or systemic infections that entail high mortality and morbidity. S. aureus uses sophisticated mechanisms to facilitate its fitness in competition with other microbiome members and to evade protective human immune responses.

Nevertheless, some beneficial microbiome members use effective ways to eradicate S. aureus from human body surfaces. Moreover, our innate immune system uses specific mechanisms for the detection and elimination of S. aureus. Elucidating and harnessing such mechanisms can lead to innovative, sustainable ways of preventing infections caused by facultative bacterial pathogens.

Location matters: Modeling bacterial infection in tissue contexts

Keira Melican

Summary: Understanding bacterial behaviour in the context of human tissues remains a major challenge in infection biology. In this talk, I will present advances in human skin infection models that enable the study of host-pathogen interactions in a physiologically relevant environment. These models reveal bacterial phenotypes that are not apparent in conventional culture systems, including changes in virulence, metabolism, and antimicrobial susceptibility. By bringing microbiology closer to the clinical setting, tissue-based models are providing new opportunities to study infection mechanisms and identify improved treatment strategies.

Key Refs:

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The gut microbiome: For better or worse

Andreas Munk Petersen

The human gut microbiome has emerged as a key factor in health and disease, influencing immunity, metabolism, and host resilience against infection. This lecture explores the growing therapeutic potential of microbiome-based interventions, with a focus on fecal microbiota transplantation (FMT) and probiotics.

Drawing on both current evidence and the speaker's own research, the presentation will highlight the established efficacy of microbiome restoration strategies in the treatment of Clostridioides difficile infection (C. difficile) and the eradication of multidrug-resistant organisms (MDROs), where the most convincing clinical benefits have been demonstrated. In addition, the lecture will review data regarding the application of microbiome-targeted therapies to conditions such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and other microbiome-associated disorders.

The dilemma of Scale-up and the promise of Scale-out in biomanufacturing

Andreas Worberg

Using microbiome analysis to rediscover traditional forage preservation

Ida Hindrichsen

Silage is one of the world's largest fermentation systems, preserving approximately 200 million tons of forage dry matter annually and playing a critical role in sustainable livestock production (Weinberg and Ashbell, 2003). Despite decades of research on forage preservation, the microbial ecology underlying silage fermentation, aerobic stability, and spoilage remain incompletely understood. Recent advances in microbiome analysis now provide new opportunities to investigate these microbial processes and revisit traditional forage preservation through a modern microbiological perspective.

Current knowledge of the silage microbiome discusses how microbiome analysis is reshaping our understanding of forage preservation. A literature survey identified more than 6,000 publications related to forage preservation over the last four decades, but only around 120 publications specifically address silage microbiomes, highlighting the relatively recent emergence of this research field.

Published studies demonstrate that microbial communities associated with forage vary substantially between fields, environments, and crop varieties. During ensiling, these diverse epiphytic communities undergo strong ecological selection, resulting in fermentation-adapted communities typically dominated by lactic acid bacteria. However, silage is a dynamic ecosystem, and major microbial shifts continue during aerobic exposure at feed-out, where spoilage organisms can become active and compromise silage quality. These findings emphasize that successful preservation depends not only on fermentation but also on the microbiological processes governing aerobic stability (Sun et al. (2021), Dunière et al. (2017), Ridwan et al. (2023)).

The presentation further reviews recent evidence showing that microbial inoculants can consistently influence silage microbiome composition and fermentation outcomes. Meta-analysis data indicates increases in beneficial lactic acid bacteria and reductions in undesirable microbial groups, accompanied by improved fermentation parameters. However, significant knowledge gaps remain regarding the mechanisms by which specific microbial species and strain combinations shape microbial succession and silage performance.

Overall, microbiome research is moving silage science beyond traditional culture-based approaches and providing a more comprehensive understanding of the microbial ecosystems responsible for forage preservation. By integrating microbiome insights with classical silage science, new opportunities emerge to improve preservation efficiency, reduce nutrient losses, and develop more predictive approaches to silage management. The presentation is primarily based on published literature and is supplemented with selected examples from ongoing microbiome investigations within Novonesis.

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Transforming Industrial CO_2 Emissions into Renewable Fuel via Microbial Power-to-Gas Conversion

Michael Vedel Wegener Kofoed

Mitigation of industrial CO_2 emissions represent a major challenge – however, the emitted CO2 also constitute a substantial and underutilized carbon resource for production of renewable fuels and chemicals. In particular, CO2 from combustion processes constitutes a challenge due to the dilute nature and the scale of these emissions.

The novel concept of Bio-Integrated Carbon Capture and Utilization (BICCU) combines chemical carbon capture with microbial conversion, enabling microorganisms to directly transform captured CO2 into renewable methane while reducing the energy demand associated with conventional capture agent regeneration. By integrating CO2 capture agents with hydrogenotrophic methanogens, BICCU can facilitate production of synthetic natural gas, which can substitute fossil natural gas as fuel.

However, conventional CO2 capture agents are not designed for application with microorganisms and will strongly influence microbial activity and survival. Here, we present the results from an interdisciplinary initiative, aimed at identifying and selecting robust methanogenic microorganisms, in parallel with the design of biocompatible CO2 capture agents.

Beyond infection: the hidden lives of virus–cell interactions

Ramunas Stepanauskas

Viral infections are widely assumed to be a dominant source of microbial mortality and lateral gene transfer in the ocean and other environments, yet the specific interactions between viruses and their hosts in nature have remained largely inaccessible, hindered by the challenges of cultivation and metagenomic assembly. My group has pioneered single-cell genomics approaches that resolve these interactions at the level of individual cells and viral particles, enabling robust, quantitative recovery of marine phage genomes directly linked to their hosts, host defenses, and viral counter-defenses — including the use of non-canonical DNA chemistry. Beyond canonical infective and lysogenic relationships, we discovered that several abundant lineages of marine protists graze directly on free viral particles, forming a “viral link” that counteracts the classical “viral shunt” in carbon flux through the microbial loop. We also find that non-infective associations are widespread between bacteria and phages. Together, these findings reveal a far richer and more diverse landscape of virus–cell interactions than the infection-centric paradigm has assumed, with implications for microbial mortality, gene exchange, and carbon cycling in the ocean.

Characterisation and impact of interspecies interactions in biofilms

Mette Burmølle

Most bacteria in natural environments live in multispecies biofilms, featuring high diversity, chemical heterogeneity and spatial organisation. This leads to interspecies interactions, of which many relate to bacterial metabolism. We have studied how different carbon substrates impact metabolic interactions, bacterial abundances and spatial organisation in a four species model biofilm. By 3D bioprinting, confocal imaging and metabolic network analysis we show that the community members organise differently, in some cases to enhance proximity to the strain possessing the highest metabolite donor potential. In addition, digital PCR and transcriptomics uncovered distinct community dynamics, interactions and activities, when grown in xylose vs. cellobiose. Combined, our findings reveal strong impacts of substrate type on community dynamics and functionality.

Ecosystem impacts from farming termite symbioses

Michael Poulsen

The more than 3000 termite species that inhabit Earth amount to ca. 100 million tons of global biomass, or about the same as the entire human population. This makes termites one of the most important detritivores in terrestrial ecosystems. In addition to increasing mineralisation, termite mound building, water collection, and nutrient leakage to the surroundings, make termites important ecosystem engineers. Cooperation and division of labour are the root to termite success: millions of worker and soldier offspring of a queen and king build and work within meter-tall colonies. The majority of these colony members are sterile workers that secure colony homeostasis and support the colony germline (the queen and king) – the only individuals that reproduce – and natural selection works at the colony "superorganism" level. This termite superorganism then relies on thousands of gut symbionts to convert nutrient-poor plant matter to termite nutrition. In African savannah ecosystems, one of most important groups of termites are the fungus farmers (subfamily Macrotermitinae) that cultivate co-evolved fungi in the genus Termitomyces on dead plant biomass. In this presentation, I will summarise our current understanding of how farming termite symbioses allow ecosystem impacts by generating islands of fertility that support a rich biodiversity of plants, animals, and microbes.

Zooming in on bacterial warfare: From molecular weapons to cellular dynamics

Lin Lin

Polymicrobial infections are highly prevalent in clinical settings and often lead to increased disease severity and treatment failure. During these infections, interspecies interactions among pathogens are the main driving force for emergent virulence, immune modulation and drug tolerance. Furthermore, these interactions can promote horizontal gene transfer and thereby contribute to the spread of antibiotic resistance. In this talk, I will present work investigating the molecular basis and regulation of weapons deployed by opportunistic pathogen Acinetobacter baumannii duringinteractions with other bacteria, as well as the cellular consequences of these encounters. I will show that these interactions are not static but dynamic and highly context-dependent, involving coordinated changes at the molecular, cellular, and population levels.

Quorum sensing controls antiphage defense and prophage induction in Vibrio

Sine Lo Svenningsen

Bacteriophages are ubiquitous predators of bacteria, shaping microbial diversity and evolution. While phage-receptor-based resistance is well characterized, it is poorly understood how surface and intracellular defense mechanisms are integrated with bacterial physiology. Using several phage–host pairs of marine Vibrio isolates, we show that bacterial quorum sensing (QS) is a key regulator of phage-bacterial interactions in this genus.

In the marine pathogen Vibrio alginolyticus, we uncover a dual-layered phage defense strategy in which QS controls both the phage receptor and a novel intracellular defense system. The intracellular system is activated by the combined signals of QS and phage infection. Through comparative genomics and functional assays, we show that it is carried by a conserved two-gene module that occurs across the genus Vibrio in association with variable defense-gene cargo, and that excises from the chromosome as an extrachromosomal element.

Another example concerns a widespread family of prophages found in isolates of the marine fish pathogen Vibrio anguillarum. We show that QS strongly represses induction of the prophage from lysogenic cells, via direct binding of the master QS regulator VanT to multiple sites in the prophage genome. Specifically, in the QS state, VanT represses an operon necessary for prophage induction that includes a TorI-like recombination directionality factor, preventing excision of the prophage from the host chromosome.

Our findings demonstrate that quorum sensing not only modulates surface-level phage susceptibility but also governs intracellular defense systems and prophage induction, offering new insight into how QS coordinates microbial survival strategies in marine environments.

5:2 molecular motors: from bacterial motility to anti-phage defense

Nicholas Taylor

Bacteria move through the rotation of large filaments know as flagella. Flagellar rotary motion is powered by a flagellar motor, driven by stator units (MotAB). The MotAB proteins convert the ion motive force across the bacterial inner membrane into rotation of the filament, but it was not understood how this occurred.

Using cryo-EM we have determined structures of the MotAB complex, which we show has a 5:2 stoichiometry shared across different species. By visualizing MotAB in its plugged, inactive state, as well as mimics of its active state, we come up with models for how torque is generated in the flagellar motors, as well as how direction switching in the flagellar motor occurs. We also reveal our recent progress on how ion specificity is obtained and propose a mechanism for how stator units become active upon motor incorporation.

I will also present results on a newly discovered bacteriophage defense system, Zorya, that uses a 5:2 motor complex to sense bacteriophage infection. Using a combination of structural biology, functional assays, light microscopy and mass spectrometry, we provide novel insight into the unique Zorya mechanism of action. We provide data indicating that Zorya detects phage infection by monitoring integrity of the peptidoglycan layer. Upon phage infection, the ZorAB motor proteins get activated and through a 700 Å long tail locally recruit and activate ZorD nuclease that can degrade the phage genome, halting the infection.

Eco-evolutionary processes of microbiome assembly in the wild

Jennifer Martiny

The assembly of microbial communities is likely influenced by both ecological and evolutionary processes. At one end of the spectrum, their composition (assessed at broad taxonomic levels) dramatically shifts in response to environmental change. At the other end, laboratory studies demonstrate the potential for rapid evolution in response to the types of environmental change. In between these two extremes, closely related microbial strains coexist in natural communities, and this standing variation results in allele frequency shifts within microbial taxa. New advances in microbial population genomics allow us to investigate these blurry eco-evo boundaries. I will present our recent approaches to investigate ecological and evolutionary processes simultaneously in soil communities in field setting. I will also briefly discuss potential applications of these ideas for microbiome engineering.

[O1] An Ontology-Augmented Graph Framework for Exploratory Microbial Functional Analysis

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Fungi possess substantial genomic and functional diversity, enabling adaptation to diverse environments and offering potential applications in industrial biotechnology. However, exploring fungal genomes remains challenging because phylogenetic relationships, gene-associated functions, and ontology hierarchies are often analyzed separately, limiting an integrated understanding of taxa, genes, and biological functions. We develop an ontology-augmented graph analysis pipeline that integrates genome annotations, taxonomic metadata, phylogenetic trees, and Gene Ontology (GO) information into a unified graph, enabling joint analysis of functional and evolutionary relationships. The resulting graph supports three analytical approaches. First, node-level graph metrics can prioritize functions based on their importance and potential to distinguish across taxa. Second, topology-based node similarity can identify genes with comparable relational profiles without relying solely on sequence similarity. Third, community-detection algorithms can identify modules of taxa, genes, and functions that share graph characteristics. The framework generates ontology-informed subgraphs for targeted functional categories, including toxin-related and stress-response metabolic processes, and then performs separate clustering analyses for each category using the corresponding subgraph to support experimental investigation for biotechnological applications. By incorporating hierarchical biological relationships into comparative genome analyses, it provides a systematic approach to exploring functional diversity. While fungi constitute the primary use case, the workflow could potentially be extended to other microorganisms, including bacteria.

[O2] Conservation and Diversity of an NRPS in Aspergillus fumigatus and Aspergillus spp.

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Non-ribosomal peptide synthetases (NRPSs) are important drivers of fungal secondary metabolism and produce structurally diverse bioactive peptides. The conservation and distribution of individual NRPS biosynthetic systems and their associated peptide products across the genus Aspergillus remain incompletely characterized. Here, we investigate the conservation, distribution, and peptide products associated with an NRPS biosynthetic gene cluster across Aspergillus. Using HPLC-HRMS, we first characterized the associated peptide profile in A. fumigates and investigated its conservation across A. fumigatus isolates. We then expanded the analysis to a diverse collection of Aspergillus species and isolates to assess variation in peptide production. Finally, genome mining and phylogenetic analyses were used to investigate the distribution and evolutionary conservation of the underlying biosynthetic gene cluster. Peptide production was detected across several Aspergillus subgenera, with a conserved peptide architecture but variation in the abundance and occurrence of individual peptide variants. Genome analysis revealed conservation of the underlying biosynthetic gene cluster across the lineages in which peptide production was detected, suggesting that this biosynthetic system has been maintained across substantial evolutionary divergence. In contrast, the cluster was absent from the subgenus Aspergillus, representing a notable exception to its broad distribution. Together, these findings reveal a highly conserved NRPS biosynthetic system and associated peptide family across diverse Aspergillus lineages, while highlighting lineage-specific differences in peptide production. This provides insight into the evolutionary conservation of fungal biosynthetic systems and the relationship between biosynthetic gene clusters and their chemical products across the genus.

[O3] Assessment of phenotypic carbapenem susceptibility and genomic epidemiology of Campylobacter from animal, food and human domains – "CarbaCamp"

Elisabeth Chang[1], Athina Andrea, Joana Mourão, Ayperi Akkas, Freya Helena Mølnitz, Birthe Susanne Rosenqvist Lund, Tatjana Kristensen, Gunhild Larsen, Erika Matuschek, Gunnar Kahlmeter, René S. Hendriksen

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Emerging evidence of carbapenem non-susceptibility led to the EFSA recommendation to include ertapenem in the resistance monitoring of Campylobacter from 2021. The CarbaCamp project investigated carbapenem susceptibility in C. coli and C. jejuni populations from food animal surveillance contexts as well as of human origin. A total of 2,315 isolates from 16 European countries were requested, of which 1,889 were tested by disc diffusion (DD). From these, 600 isolates were selected for minimum inhibitory concentration (MIC) determination and WGS, and 596 passed sequencing Quality Control (QC) for downstream analyses. Overall, DD and MIC data showed no major differences in wild-type distributions between animal hosts (cattle, pigs, broilers and turkeys), but clear species-specific differences were observed between Campylobacter species. Ertapenem had broad and bimodal DD and MIC distributions, with substantial overlap between suspected WT and non-WT populations. A similar but less pronounced pattern was observed for meropenem. For imipenem narrow, unimodal distributions were observed. Correlation analysis demonstrated the strongest association between ertapenem and meropenem zone diameters, whereas correlations with imipenem were weaker, supporting a distinct phenotypic behaviour among the carbapenems included in this study. DD and MIC epidemiological cut-off values (ECOFFs) could not be established for ertapenem and meropenem due to the bimodal distributions. Imipenem distribution behaved as expected and tentative ECOFFs were established for imipenem in both species (DD 35 mm; MIC 0.25 mg/L). Parallel broth microdilution using EUCAST and CLSI recommended media showed no systematic MIC differences. No clear genetic determinants were identified that could explain the observed carbapenem susceptibility patterns but the chemical structure of the carbapenem likely plays an important role. No definitive recommendation on which carbapenem should be used for monitoring can currently be given, due to the unresolved issues with the ertapenem and meropenem data, although imipenem appears to be the most suitable for interim monitoring.

[O4] Gut microbiota responses to environmental UV-light exposure in piglets: a shotgun metagenomic study

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Ultraviolet (UV) radiation is considered a strategy to reduce environmental pathogens, while UVB stimulates skin synthesis of vitamin D3. In pig production, vitamin D3 is essential for suckling piglets due to low vitamin D3 in sow milk. Reduced environmental pathogen load and improved vitamin D3 status may influence gut microbial ecology. However, the direct relationship between UV light and the porcine gut microbiome remains poorly understood. Therefore, this study investigated the effect of early-life UV exposure on gut microbial community composition in weaned piglets.

For the present study, 40 piglets, 10 per treatment group, were selected from a large 2 × 2 factorial study investigating the effects of UV exposure on immunocompetence and microbial ecology, with tetanus toxoid vaccination included as an immune challenge. Sows and piglets received standard feed with 200 IU/kg vitamin D. UV lamps (UVB and UVC) were operated for 12 h/day until day 7 after farrowing, and 24 h/day thereafter. Three weeks after weaning, digesta were collected from three intestinal segments: SI1 (proximal half small intestine), SI2 (last 1 m of the distal small intestine), and Co50 (50 cm at mid-colon), followed by shotgun metagenomic sequencing (150bp paired-end reads, 20M). Read-based taxonomic profiling was performed using MetaPhlAn4.2.5. Microbial diversity was assessed using complementary alpha diversity indices corrected for read depth differences. Differences in community composition were assessed based on Bray-Curtis dissimilarity. Differential abundance was analyzed using MaAsLin3.

Lactobacillus amylovorus was the dominant species in SI1 and SI2. UV exposure increased observed SGB richness in SI1 compared to pigs not exposed to UV ($p = 0.010$), but no effect on richness was observed in SI2 and Co50. UV exposure increased Shannon index in all three segments ($p < 0.05$). Simpson's Index of Diversity was increased by UV exposure in SI1 and Co50 ($p < 0.01$), and in SI2 in vaccinated pigs (padj = 0.008), indicating reduced dominance by a few taxa and a more even community structure. Faith's PD was higher in UV-exposed pigs in both small intestinal segments ($p < 0.05$) but not in the colon. Beta diversity analysis revealed significant differences in microbial community composition associated with UV exposure in all three segments (PERMANOVA $p < 0.05$). Two SGBs were reduced by UV exposure in SI2: Lactobacillus amylovorus ($q = 0.025$) and Limosilactobacillus SGB7095 ($q = 0.088$).

These findings suggest that UV exposure in the barn environment can influence piglet gut microbiota composition.

[O5] Antimicrobial Resistance as a Context-Dependent Phenotype in Complex Bacterial Communities

Saria Otani[1]

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Antimicrobial resistance (AMR) is commonly treated as an intrinsic property of a bacterial isolate, defined by static testing under highly standardized conditions and one bacterium at a time. In vivo, however, bacteria experience fluctuating physicochemical conditions, host-associated gradients and complex microbial communities. In our research, we asked how strongly such environmental context can reshape the expression of resistance.

Using isogenic Escherichia coli carrying 78 clinically important β-lactamase genes, we measured susceptibility to 10 β-lactam antibiotics across different pH conditions and under aerobic and anaerobic growth. Environmental changes produced substantial and highly gene- and drug-specific effects, with MIC shifts of up to 512-fold in a subset of the AMR genes. Anaerobiosis generally increased resistance, although both the magnitude and direction of the effect depended on the resistance determinant and antibiotic. Closely related resistance enzymes could respond differently to the same environmental change, and complementary experiments linked these shifts to changes in enzyme activity and bacterial fitness.

Together, these findings indicate that resistance is not a fixed genotype-to-phenotype property, but a context-dependent “resistance state” shaped by the interaction between resistance mechanism, bacterial physiology, enzymology and environment. Antimicrobial standard testing may therefore capture only one point within a broader resistance landscape. We are now extending this framework beyond static, isolate-based measurements towards physiologically relevant systems, including gut-on-chip models, to examine how host-like gradients, microbial interactions and antimicrobial exposure jointly shape resistance. Such approaches may help bridge susceptibility testing with the conditions under which resistance is actually expressed in vivo and in infection sites.

[O6] Membrane vesicle-bound quorum sensing molecule persists in phage lysates inducing virulence and confounding phage therapy studies

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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 defenses, 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 defense 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.

[O7] Single-cell measurements of transcriptional regulation under growth arrest reveal design challenges for engineered bacterial biosolutions

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A central challenge in developing effective microbial biosolutions is that their performance can differ between the laboratory and the application context, whether it be a bioreactor, human gut, or crop rhizosphere. This effect is driven by the fact that these environments push the microbe into physiological states that differ from those that they experience in well-controlled laboratory conditions. In particular, microbes in both natural and industrial contexts are often found in non-growing states like growth arrest and stationary phase, and yet the impact of these states on the performance of engineered genetic systems like biosensors, kill switches, and modified metabolic pathways remains underexplored.

In this work, we developed a single-cell fluorescence-based assay to quantitatively measure transcriptional dose-response curves under both active growth and growth arrest, and characterized a library of 23 response curves from both synthetic and natural repressors in E. coli. Unexpectedly, we found that the impact of growth arrest on the response curves was dominated by just a single effect: a >100-fold reduction in global expression levels. In contrast, the sensitivity, sharpness, and noise of the response curves were only minimally affected.

Our results predict a general failure mode for complex engineered genetic systems under growth arrest, where the drop in expression levels breaks down the interaction between successive layered transcriptional modules. This effect is consistent with observed, and heretofore unexplained, performance failures in an engineered biosensor in a barley rhizosphere commensal.

The fact that the transcriptional response curves in the two conditions differ only along a single parameter, however, also suggests that resolving this failure mode may be more feasible than expected. We discuss new potential design paradigms that could mitigate this reduction in global expression levels, thereby enabling the development of microbial biosolutions whose performance in application environments can be reliably predicted from their performance in the laboratory.

[O8] High-yield biosynthesis of synthetic proteins in next-generation genome-recoded organisms

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While expanding the chemical repertoire of proteins with non-standard amino acids (nsAAs) promises breakthroughs in biotechnology, medicine, and sustainability, significant challenges remain. Host constraints and inefficiencies in orthogonal translation systems currently limit efficient bioproduction. Here, we present a genomically recoded Escherichia coli strain that enables high-yield production of synthetic proteins with site-specific incorporation of two distinct nsAAs at up to six sites. Building on our recently published Ochre strain[1], which relies exclusively on UAA as the stop codon, we rationally engineered release factors 1 and 2 to facilitate efficient reassignment of UAG and UGA codons by orthogonal translation systems. Additionally, we genomically integrated T7 RNA polymerase to enhance transcriptional output, enabling high expression of synthetic proteins. Combining these optimizations with two orthogonal aminoacyl-tRNA synthetase/tRNA pairs, we demonstrate the unprecedented high production of a synthetic protein, ELP-3x(TGA-TAG)-GFP, containing two nsAAs at three distinct sites each in. We demonstrate 15 combinations of dual nsAA incorporation with high yields and achieve ~180 mg/L in shaking flask cultures with Nε-Boc-L-lysine and para-acetyl-L-phenylalanine, a significant advancement toward industrial scalability. In summary, our work establishes an efficient microbial chassis for the biosynthesis of chemically enhanced proteins, demonstrating potential for scalability for biotechnological applications.

1 Grome, M. W. et al. Engineering a genomically recoded organism with one stop codon. Nature 639, 512-521 (2025). https://doi.org:10.1038/s41586-024-08501-x

[O9] The overlooked role of the seminovaginal microbiota in infertility

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Infertility affects up to 17% of couples worldwide and remains unexplained in a substantial proportion of cases. While the vaginal microbiome has been widely linked to reproductive outcomes, including success of assisted reproductive technologies (ART), the contribution of the male genital microbiome is less well defined.

Emerging evidence suggests that microbial exchange between partners may influence vaginal microbiome composition, local immune responses, and ultimately fertility outcomes, highlighting the need for a couple-based perspective.

In this study, semen and vaginal swabs are collected from couples initiating fertility treatment in a clinical setting. Bacterial isolates are identified using MALDI-TOF. In addition, 16S rRNA gene sequencing (Illumina) is used to characterize the microbial communities and potential sharing of taxa between partners. To resolve strain-level relatedness and confirm microbial transmission, whole-genome sequencing (WGS) is performed on isolates shared within couples.

Preliminary culturing results demonstrate bacterial species sharing in 37% of couples. WGS analysis of shared isolates confirms identical strains in 50% of investigated isolates, suggesting microbial transmission between partners. 16S rRNA gene sequencing analyses further identify bacterial taxa shared between partners despite distinct overall microbial community structures in semen and vaginal samples.

By integrating culture-based and high-throughput sequencing approaches, this study aims to elucidate microbial interactions within couples and may identify novel biomarkers of treatment success and support development of targeted, couple-based interventions to improve reproductive outcomes.

[O10] Seasonal dynamics of gut microbiome composition and function in wild Tanzanian chimpanzees reflects microbial adaptation to potential dietary stress

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Unlike most chimpanzees [1], the wild chimpanzees in Issa valley in The Greater Mahale Ecosystem (GME) in western Tanzania live in a mosaic habitat dominated by miombo woodland and characterised by extreme seasonality. During the dry season, the Issa valley chimpanzees increase their daily foraging distances, concentrate feeding effort to woodland vegetation, and travel longer distances [2], and chimpanzees living in similar dry mosaic woodland habitats has been shown to rely on nutritionally poorer food [3].

Here we aimed to study how the gut microbiome composition and function of wild chimpanzees adapt to seasonal changes.

Using a combined strategy of metagenomic long- and short-read sequencing of 557 faecal microbiomes collected in GME during a period of 10 years, we constructed a Metagenome-Assembled Genome (MAG) catalogue, classified the de-replicated MAGs into bacterial taxa, and functionally annotated them using KEGG and CAZy databases.

Two distinct clusters emerged in our analysis of bacterial abundances: One containing samples primarily from June and July (mid dry season), and one containing samples from the rest of the year. This was explained by a high abundance of complex carbohydrate-degrading taxa such as Prevotella spp. in the latter group. These bacteria become less abundant during June and July, while certain host glycan degrading-bacteria increase in relative abundance together with spore-forming and stress-tolerant taxa.

The Issa valley chimpanzee gut microbiome undergoes restructuring, potentially in response to dietary stress like seasonal food scarcity or fluctuating dietary resources. During June and July, the microbiome shifts from a community dominated by complex carbohydrate-degrading taxa to one enriched in host glycan-degrading, spore-forming, and stress-tolerant bacteria. The gut microbiome adopts an alternative functional strategy, which later reverts, demonstrating the resilience and functional plasticity of the gut microbiome.

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[O11] PanRes: A structure-guided resource bridging acquired and latent antimicrobial resistance

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Antimicrobial resistance (AMR) is a leading cause of global mortality, directly contributing to 1.27 million deaths each year. AMR databases are central to genomic surveillance, yet resistance genes remain scattered across resources with different scopes, structures, and annotations. Here, we present PanRes, a curated resistome database of 11,717 genes that integrates acquired and latent resistance determinants, including antibiotic, biocide, and metal resistance genes, within a unified ontology. We predicted representative protein structures, clustered them by structural similarity, and used structure-guided multiple sequence alignments to build Hidden Markov Models (HMMs) for remote homology detection. The resulting 598 structural clusters showed strong within-cluster conservation despite substantial sequence divergence.

To evaluate the ability of PanRes to detect distant resistance determinants, we searched wastewater metagenomes from seven major European cities. On average, 35.2% of retained hits were detected exclusively by structure-guided HMMs and generally showed lower sequence similarity to known PanRes proteins than hits recovered by BLASTp. Focusing on beta-lactamases, we used catalytic-residue conservation to prioritise distant candidates with low similarity to known references. Selected candidates retained beta-lactamase-like folds and conserved catalytic geometry despite weak or absent BLASTp similarity.

PanRes is available through an interactive web platform (https://panres.rambio.dk/) for exploring the ontology, curated sequences, predicted structures, structural clusters, and HMMs. Together, PanRes provides a structure-informed framework for extending AMR detection beyond close sequence similarity and exploring the hidden resistome.

[O12] Extracellular proteolysis remodels the quorum sensing signals of Staphylococcus chromogenes

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Quorum sensing (QS) in staphylococci is controlled by the accessory gene regulator (agr) system, in which each strain is thought to produce a single autoinducing peptide (AIP) that activates its cognate receptor and mediates interbacterial competition. Here, we show that bovine-associated Staphylococcus chromogenes challenges this paradigm.

We analyzed 34 environmental S. chromogenes isolates obtained from bovine milk and teat apices and identified AIPs from two prevalent agr specificity groups (agr-II and agr-III). Unexpectedly, most isolates from both agr specificity groups produced multiple AIP variants derived from a single AgrD precursor peptide. Up to five distinct AIP variants were detected in agr-II isolates and up to three variants in agr-III isolates, differing only in N-terminal tail length.

Functional characterization revealed that all identified variants retained the ability to inhibit quorum sensing in competing staphylococci. However, the variants exhibited distinct activities toward their native signaling system. In a representative agr-III strain, the longer AIP variants functioned as potent autoinducers, whereas the shortest variant lacked autoinducing activity and instead showed weak inhibitory effects on endogenous agr signaling. These findings reveal that a single bacterial strain can simultaneously produce multiple functional AIPs with distinct biological activities.

To investigate the origin of this diversity, we incubated isotope-labeled precursor-like peptides in cell-free supernatants and detected cleavage products corresponding to the AIP variants identified in native cultures. Protease inhibition reduced this processing, supporting a model in which extracellular proteolysis remodels secreted quorum sensing signals. Transcriptomic analysis identified several agr-induced extracellular proteases as candidate contributors to this process.

Together, our findings reveal that quorum sensing signal production in staphylococci can be considerably more complex than previously appreciated. Rather than producing a single defined signal, S. chromogenes produces a mixture of AIPs with distinct signaling activities, adding a previously unrecognized layer of complexity to staphylococcal quorum sensing.

[P001] Targeted In Vitro Suppression of Parkinson's Disease-Associated Desulfovibrio Strains by Bioactive Metabolites of Probiotic Candidates

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Emerging evidence highlights a strong clinical association between colonic Desulfovibrio (DSV) overabundance and Parkinson's disease (PD) pathogenesis. DSV-derived H_2S degrades the intestinal mucosal barrier and promotes α-synuclein aggregation along the gut-brain axis. While antimicrobial peptides and probiotic metabolites offer alternatives to antibiotics, targeted strategies against neurodegeneration-associated pathobionts remain unexplored. Here, we evaluated the in vitro inhibitory potential of 32 probiotic strains against three PD-derived clinical DSV isolates (Desulfovibrio desulfuricans DES 1, D. fairfieldensis DES 4, D. piger DES 10), uncoupling non-acidic metabolite activity from organic acid acidification. Cell-free supernatants (CFS) were neutralized (pH 7.0) and treated with catalase (0.1 mg/mL). Growth inhibition (OD_{600}) and operational MIC_{90} based on crude dry matter were assessed via broth microdilution. Initial pH and biomass effects were statistically uncoupled using ANCOVA and Tukey's HSD. Primary screening showed broad-spectrum inhibition by LAB and selected Bifidobacterium strains. ANCOVA confirmed that strain-specific inhibition remained highly significant ($p < 0.005$) after controlling for pH, confirming functional non-acidic metabolite activity. Upon pH neutralization, Lacticaseibacillus rhamnosus GG (LGG) maintained robust anti-DSV efficacy against D. piger DES 10 (44.82% ± 8.31% inhibition). B. bifidum CUL-17 and B. longum E-96664 exhibited comparable non-acidic inhibition, whereas spore-forming Bacillus strains showed no direct inhibition. Gravimetric determination identified Lactobacillus acidophilus Hambi1448 and Lactiplantibacillus plantarum E98 as most potent (MIC_{90}: 25.2- 29.8 mg/mL). Specific LAB strains produce potent, non-acidic bioactive components (likely proteinaceous/peptidic) that suppress PD-derived DSV isolates at physiological pH, establishing a foundation for targeted formulations to mitigate gut-driven neuroinflammation.

[P002] Context-dependent quorum sensing dynamics in Staphylococcus aureus during early human skin colonization

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Staphylococcal skin infections remain a major healthcare burden due to prolonged treatment, recurrence, and antibiotic use rather than acute mortality. A key driver of Staphylococcus aureus pathogenicity is quorum sensing (QS), mediated by the accessory gene regulator (agr) system and its effector RNAIII, which coordinates virulence factor expression in a population-density–dependent manner.

Here, we investigated whether inhibition of QS can modulate S. aureus colonization and virulence using a human ex vivo skin explant model. We aimed to determine whether disrupting agr signaling can shift bacteria towards a less virulent state and alter early colonization dynamics.

To characterize QS activity on skin, we quantified RNAIII expression by qPCR from bacteria recovered from human skin explants over time. While bacterial load increased, RNAIII expression decreased, suggesting that agr activation on skin differs from canonical in vitro behavior. We next investigated QS inhibition using inhibitory autoinducing peptides (AIPs) and agr-deficient mutants. Both approaches reduced bacterial colonization at early time points (6h), although bacterial numbers recovered by 24 h. To confirm agr inhibition, we used P3 agr-GFP reporters and microscopy, demonstrating that AIP treatment suppressed GFP production and thus agr activation. Parallel broth experiments highlighted a contrasting response: RNAIII expression increased over time alongside CFU in wild-type cultures, while AIP treatment significantly reduced RNAIII expression without affecting bacterial growth.

Together, these findings reveal a disconnect between QS behavior in vitro and within human skin tissue. Our results suggest that QS dynamics are strongly influenced by the host microenvironment and that early skin colonization does not strictly follow density-dependent agr activation observed in broth. This highlights the importance of physiologically relevant human skin models for studying S. aureus behavior and evaluating QS-targeting strategies.

[P003] Challenging antimicrobial susceptibility testing and how microenvironmental factors influence β-Lactamase mediated resistance

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Antimicrobial Resistance (AMR) is identified as a significant threat globally according to WHO, this calls for new strategies to prevent resistance and enhance treatment outcomes.

Antimicrobial susceptibility testing (AST) is performed under highly standardized laboratory conditions, while bacteria encounter substantially different physicochemical environments in natural and host-associated settings. Such variation may influence the phenotypic expression of antimicrobial resistance genes and complicate interpretation of genotype-to-phenotype.

In order to improve the interpretation of resistance genes across ecological and infection-relevant contexts, we investigated how selected microenvironmental conditions influence the resistance phenotype conferred by selected clinically and One Health-relevant β-lactamase resistance genes. Isogenic bacterial systems carrying resistance determinants were assessed for antimicrobial susceptibility, growth and fitness across controlled environmental gradients, including acidic, neutral and alkaline pH, aerobic versus anaerobic growth, and changing temperature or nutrient conditions.

Variations in antimicrobial susceptibility were observed for several target resistance genes across different microenvironmental conditions. These observations indicate that the phenotypic contribution of a resistance gene depends on its surrounding microenvironment. Ongoing experiments are assessing the consistency of these effects across a broader panel of β-lactamases and environmental conditions and their connection to bacterial growth and fitness.

Understanding when and under which conditions individual resistance genes confer a measurable phenotype may improve interpretation of AMR genotypes across ecological and infection-relevant settings. More broadly, incorporating environmental context into genotype-to-phenotype studies may contribute to a more functional assessment of antimicrobial resistance beyond gene presence or absence alone.

[P004] Time- and concentration-dependent selection of β-lactamase resistance using Gut-on-Chip model

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As usage of antimicrobials increases, the prevalence of antimicrobial resistance genes have also increased. However, gut bacteria experience changing microenvironmental conditions that may induce antibiotic resistance differently compared to a more stable environment like a petri dish or antimicrobial susceptibility testing conditions. Here we investigated the dynamics of AMR gene response over time during antibiotic exposure in gut-on-chip model. Four E. coli populations were introduced into the gut-on-chip model, three of which carried β-lactam resistant genes namely blaTEM-52, blaSHV-12 and blaIMP-1, with an additional E. coli as a background native strain. All 4 strains were initially added equally to each chip model at Day 0, and subsequently treated with a 4th generation beta-lactam Cefepime at either 2.5 mg/L or 6 mg/L. Samples were collected over three days, sequenced and analysed with ONT. Reads were mapped to the AMR genes with resfinder and KMA. SHV showed the highest abundance throughtout the experiment, and was already dominant at Day 0. At 2.5 mg/L cefepime, IMP shows gradual increases from 16% to 24% by Day 3. At 6 mg/L, IMP displayed a much stronger initial effect: IMP was increased to roughly 32% at Day 1, however by Day 3 IMP had declined to 18%, while both SHV and TEM had recovered. These results indicate that the selective advantage associated with different β-lactamase-carrying E. coli populations can change with both cefepime concentration and time in a gut-on-chip environment. The findings highlight the potential of organ-on-chip models for investigating dynamic antimicrobial resistance ecology at infection sites beyond conventional endpoint susceptibility measurements.

[P005] Studying the role of gyrA and gyrB pathoadaptive mutations in Pseudomonas aeruginosa airway infections

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Mutations in gyrA and gyrB, encoding the two subunits of a type II topoisomerase, are frequently detected in clinical isolates of Pseudomonas aeruginosa. This study aims to explore how mutations in these genes contribute to bacterial adaptation during chronic lung infection.

We generated a panel of single gyrA and gyrB mutants using CRISPR-Cas9, representing pathoadaptive mutations commonly identified in clinical isolates from people with cystic fibrosis (pwCF). The ciprofloxacin (CIP) minimum inhibitory concentration (MIC) was determined in Mueller–Hinton medium using the broth microdilution method. The impact of these mutations during infection was investigated using air–liquid interface (ALI) models that closely mimic the airway environment, both in the absence and presence of antibiotic pressure.

Mutations in gyrA and gyrB are commonly associated with CIP resistance. Interestingly, we observed that not all mutations identified in clinical isolates from pwCF lead to an increased CIP MIC. This suggests that some of these mutations may play additional roles beyond antibiotic resistance, potentially contributing to host adaptation, affecting bacterial fitness, or acting as compensatory changes.

Overall, this work improves our understanding of the role of gyrA and gyrB mutations in the adaptation of P. aeruginosa during airway infection. These insights may have clinical relevance by helping to predict bacterial evolutionary trajectories and optimise treatment strategies, ultimately supporting more personalised therapeutic approaches.

[P006] Exploring Triple Beta-Lactam Synergy Against Multidrug-resistant Urinary Pathogens and Characterization of Virulence Factors

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Over the past 10–15 years, the increasing prevalence of multidrug-resistant (MDR) Enterobacterales has become a major challenge in treating urinary tract infections (UTIs).
β-lactamase-mediated resistance limits treatment options, highlighting the need for new therapeutic strategies. Although Enterobacterales are the main UTI pathogens, Gram-positive bacteria can also cause UTIs. Therefore, new antibiotic combinations with activity against resistant Gram-negative and Gram-positive uropathogens are needed.

This study aims to investigate the antimicrobial activity of mecillinam, ampicillin, and avibactam against clinical UTI isolates, and to explore the relationship between antimicrobial resistance and bacterial virulence.

Approximately 300 Gram-negative Enterobacterales and 50 Gram-positive uropathogens will be tested for susceptibility to mecillinam, ampicillin, and avibactam using MIC assays. Comparator antibiotic tests will then be performed to evaluate the activity and potential synergy of the combinations with newer antibiotics. Selected MDR and susceptible isolates will be further characterized for resistance and virulence factors using whole-genome sequencing. Finally, selected strains will be compared for their ability to adhere to and invade epithelial cells using adhesion and invasion assays, both with and without antibiotic exposure.

The study may provide insight into new treatment strategies for UTIs caused by both MDR Gram-negative and Gram-positive uropathogens. The findings could support further investigation of β-lactam combinations and help clarify the relationship between antimicrobial resistance and virulence.

[P007] Evaluating Synthetic Retinoids for their ability to mimic Retinoic Acid-Induced Expression of Gut-Homing molecules in human T-Cells (Temporary title)

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Traditional parenteral injection vaccines trigger strong systemic immune responses, but often fail to establish protective immunity at the gut mucosa. Homing of activated lymphocytes from lymph nodes to the gastrointestinal tissue is regulated by all-trans retinoic acid (ATRA), a vitamin A metabolite that induce immune cells to express the specific gut-homing surface receptors α4β7 and CCR9 Use of ATRA as a vaccine adjuvant is, however, limited by its instability, light sensitivity, and potential toxicity. The Blood2Gut project aims to solve these limitations by evaluating safe synthetic retinoids capable of mimicking ATRA-induced gut-homing in human T-cells. Building on recent in vivo models showing that retinoic acid and the synthetic retinoid AM80 successfully trigger mucosal IgA production after parenteral immunization, this project will screen a library of candidate retinoids. Human CD4+ and CD8+ T-cells procured from anonymous donors will be cultured with these synthetic compounds and the level of α4β7 and CCR9 expression will be evaluated by flow cytometry. Additional immune-activating molecules will be investigated for synergistic or antagonistic effects. Identifying synthetic retinoids with specific and optimal ability to induce gastrointestinal homing may help design new vaccine adjuvants, that reprogram systemic T-cells to target mucosal tissues, making it possible to develop effective human gut-targeted vaccines after standard injection vaccination.

[P008] Adaptive Evolution Reshapes Host Interactions During Chronic Airway Infection by Pseudomonas aeruginosa

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Chronic airway infections caused by Pseudomonas aeruginosa frequently persist despite prolonged antimicrobial treatment. Although antibiotic resistance contributes to treatment failure, increasing evidence suggests that long-term persistence is also driven by adaptive evolutionary processes that alter bacterial physiology and interactions with the host environment. However, how sequential adaptive mutations collectively influence these traits remains incompletely understood.

To investigate this, we examined a clinically observed evolutionary trajectory from the DK02 clone type, a prevalent P. aeruginosa lineage adapted to cystic fibrosis airways. Using a series of isogenic PAO1-derived mutants carrying sequential combinations of mutations in regulatory genes repeatedly observed during clinical within-host evolution, we assessed phenotypes associated with host interaction and persistence. Analyses included macrophage phagocytosis assays, airway epithelial infection models, inflammatory responses, biofilm- and virulence-associated traits, and antibiotic survival in broth or cell culture models.

Sequential acquisition of adaptive mutations resulted in extensive phenotypic remodeling across multiple experimental systems. Individual mutational steps were associated with distinct changes in host-cell interactions, virulence-associated characteristics, and survival during antimicrobial exposure. Notably, phenotypes observed in host-associated infection models did not consistently correspond with susceptibility measurements obtained in conventional broth-based assays, emphasizing the importance of studying bacterial phenotypes in biologically relevant contexts. Collectively, the data revealed a dynamic pattern of adaptation, with different stages of the evolutionary trajectory characterized by distinct phenotypic states rather than a simple progressive trend.

Our findings demonstrate that clinical evolutionary trajectories reshape bacterial phenotypes and physiology. The results suggest that adaptation during chronic infection involves complex physiological reorganization across multiple traits linked to persistence and host interaction. This highlights how evolutionary remodeling of host-microbe interactions contributes to the establishment of chronic infections.

[P009] Non-invasive lower respiratory tract infection diagnostics with untargeted metagenomics and host response profiling

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Lower respiratory tract infections (LRTI) represent one of the leading causes of morbidity and mortality. Despite extensive diagnostic testing, a substantial proportion of cases remain without an identified etiology, reflecting both limited assay sensitivity to known pathogens and an inability to detect atypical or previously uncharacterized ones. This diagnostic gap often results in continued empirical use of broad-spectrum antibiotics.

Current LRTI diagnostics rely primarily on sputum samples combined with targeted assays such as RT-PCR and multiplex panels. However, many LRTI patients cannot produce a sputum sample, and physicians resort to collecting bronchoalveolar lavage fluid (BAL).

BAL sampling is an invasive procedure requiring bronchoscopy and associated with patient discomfort, and non-invasive, untargeted approaches capable of detecting a broader range of pathogens remain largely unestablished in clinical practice. This is, in part, due to the extremely low biomass of non-invasively obtained samples and the resulting difficulty of downstream DNA/RNA handling.

This project aims to establish and optimize a workflow for pathogen and host DNA/RNA extraction and purification from low-biomass, non-invasive breath samples, incorporating untargeted pathogen and host response profiling using the Oxford Nanopore Technologies platform, to contextualize pathogen presence and inform the etiology of respiratory disease. By enabling detection of pathogens not covered by targeted panels, this approach could help reduce unnecessary antibiotic use in non-infectious suspected LRTI and optimize the treatment in cases where the causative agent would otherwise go undetected.

[P010] Klebsiella as a Potential Translational Biomarker of Inflammatory Bowel Disease Associated Dysbiosis: A Cross-Study Meta-Analysis

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Inflammatory bowel disease (IBD) is associated with intestinal dysbiosis, but interindividual and study-level variation complicates identification of reliable microbiome associated biomarkers. Such biomarkers may be useful to characterize IBD associated dysbiosis and assess microbiome targeted therapeutics during in vitro screenings. Klebsiella has been associated with IBD and may contribute to intestinal inflammation. A previous study reported 39.28% K. pneumoniae prevalence in IBD patients across four geographically distinct cohorts. To assess whether Klebsiella associated signals are reproducible and consistent across studies, we performed a cross-study meta-analysis of 2,113 samples from 23 publicly available microbiome datasets.

Following a systematic literature search, 16S rRNA gene sequencing data underwent uniform bioinformatic processing and statistical analysis. The dataset included 673 healthy and 1,440 IBD samples. Klebsiella prevalence was 37.29% in IBD and 18.87% in healthy individuals. The IBD prevalence closely corresponded to the previously reported 39.28% despite methodological differences, supporting reproducibility of the overall prevalence in a large cross-study dataset. However, no significant association was observed after adjustment for sequencing depth and study-level variation (OR=1.29, 95% CI 0.94–1.78; p=0.119). Ulcerative colitis showed the highest prevalence (39.50%) and a trend towards increased detection (OR=1.44, 95% CI 0.97–2.14; p=0.069).

Although Klebsiella alone appears insufficient as a robust biomarker of IBD-associated dysbiosis, it may contribute to multi-marker signatures and remain a potential target for microbiome directed interventions, including phage therapy.

Keywords: Klebsiella; inflammatory bowel disease; gut microbiome; dysbiosis; microbiome biomarkers; cross-study meta-analysis; reproducibility; 16S rRNA gene sequencing

[P011] Building functional marine biofilms: from community assembly to potential biosolutions

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Marine biofouling imposes major economic and environmental costs, while conventional antifouling coatings often rely on toxic or persistent compounds. We explored an alternative strategy: converting naturally occurring marine biofilms into protective “living coatings” through the rational assembly of bacterial communities.

Using isolates recovered from fouled marine surfaces, we constructed defined communities comprising one to nine members and tested whether interspecies interactions enhanced biofilm formation, community stability and resistance to invasion. Biofilms were challenged with Pseudoalteromonas tunicata, a competitive marine colonizer, and selected isolates and consortia were also screened for their ability to inhibit settlement of cyprid larvae of the model barnacle Amphibalanus improvisus.

Community composition, rather than species richness alone, determined functional performance. Several multispecies combinations produced more biofilm than expected from their individual members while maintaining stable composition. Specific consortia also restricted colonization by P. tunicata more effectively than single-isolate biofilms, with communities containing Pseudoalteromonas spp. showing particularly strong invasion resistance. In parallel, individual isolates and a defined multispecies community markedly inhibited barnacle larval settlement, demonstrating that engineered biofilms can combine ecological robustness with an antifouling function.

Our results show that microbial interactions can be harnessed to design stable, functional marine consortia. Rather than attempting to maintain sterile surfaces, this approach uses beneficial microorganisms to occupy them and resist undesirable colonizers, providing a foundation for environmentally friendly, self-renewing living coatings for marine infrastructure.

[P012] Bacterial partners expand the carbon niche of microalgal hosts

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Microalgal mixotrophic cultivation offers a cost-effective route to sustainable biomass production but is constrained by the narrow range of organic carbon sources microalgae can utilize. Bacteria possess broader metabolic capacities and may act as intermediaries that expand substrate accessibility for algal hosts. Here, we investigated whether fermentation-derived bacterial consortium broadens the range of carbon sources accessible to the model microalga Chlamydomonas reinhardtii.

Axenic and co-cultured algae were grown under acetate, glycerol, or carbon-free conditions and assessed via growth measurements, bacterial community profiling, FTIR-based macromolecular analysis, and ^{1}H-NMR metabolomics. The consortium enhanced chlorophyl synthesis on glycerol, a substrate that does not support mixotrophic growth in axenic C. reinhardtii. The same consortium did not confer any benefits on acetate, which the alga readily utilizes alone, or under carbon-free conditions. This substrate-dependent pattern indicates that bacterial partners expand access to specific carbon resources rather than promoting growth through a general mechanism.

Community profiling revealed strong carbon source-dependent selection of bacterial taxa, with growth-promoting conditions enriched in specific taxa. Despite marked differences in algal productivity, the relative abundance of proteins, carbohydrates, and lipids remained stable across treatments, indicating that community-assisted growth did not compromise algal macromolecular composition. Metabolomic analyses further showed that bacterial presence reshaped intracellular and extracellular metabolite pools, consistent with microbial modification of the extracellular metabolic environment.

Together, these findings show that microbial communities can expand the realized carbon niche of microalgal hosts, enabling use of resources beyond their intrinsic metabolic repertoire while preserving physiological stability. Our results position community-assisted metabolism as a promising strategy for circular microalgal production systems that leverage ecological interactions rather than genetic modification.

[P013] A first step toward characterizing Danish groundwater microbiomes as a baseline in bioremediation studies

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Bioremediation using specialized microorganisms is a promising strategy for removing environmental contaminants. For example, chlorinated ethenes, a group of groundwater contaminants, can be degraded by the microbial consortium KB-1®. However, introducing these microorganisms and modifying environmental conditions may affect native microbial communities. To evaluate their ecological impact, baseline knowledge of natural groundwater microbiomes is needed. This project aims to characterize groundwater microbial communities across Denmark.

Groundwater samples were partly collected in collaboration with the Danish Agency for Green Land Use and Aquatic Environment (SGAV). In total, 21 wells in Zealand and Jutland during May and June 2026 were sampled. In general, three 1 L samples were collected, kept cool, filtered within 24 h, and filters were stored at −80°C. DNA and RNA were co-extracted from the filters using the Macherey-Nagel "RNA and DNA from Soil" protocol. RNA quantity and quality were assessed using Qubit and TapeStation, and DNA contamination was evaluated by qPCR before and after DNase treatment. Metatranscriptomic libraries were prepared using the xGen™ Broad-Range RNA Library Prep Kit on a subset of samples.

Preliminary results show that RNA can be recovered from 1 L groundwater samples despite low biomass. RNA concentrations were below Qubit detection limits but were detected by TapeStation. Libraries were successfully generated from RNA inputs below the manufacturer's recommended threshold.

This workflow enables RNA library generation from low-biomass groundwater. While 1 L seems sufficient, larger volumes may improve RNA yield. Once libraries are prepared for all samples, sequencing and community analyses will be conducted. This work represents a first step toward establishing a baseline for Danish groundwater microbiomes. It will support future comparisons with contaminated and bioremediated sites.

[P014] Utilisation of dissimilation-deficient Bacillus methanolicus for the production of value-added compounds

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During methanol-based growth, Bacillus methanolicus MGA3 oxidises methanol to formaldehyde (FA), which is subsequently assimilated through the ribulose monophosphate (RuMP) pathway. As FA is highly toxic, efficient assimilation is essential to prevent its intracellular accumulation. When the capacity for FA assimilation is insufficient, excess FA is instead channelled into dissimilatory pathways that generate reducing equivalents and energy but result in carbon loss and CO_2 formation. To reduce carbon loss associated with FA dissimilation, the folD gene, encoding a key enzyme of tetrahydrofolate-dependent formaldehyde oxidation pathway, was deleted, resulting in the MGA3 ΔfolD strain.

This study investigates whether increased expression of genes involved in FA assimilation can improve the growth and fitness of MGA3 ΔfolD. Genes encoding 3-hexulose-6-phosphate synthase (hps), 6-phospho-3-hexuloisomerase (phi), transketolase (tkt), phosphofructokinase (pfk), and fructose-bisphosphate aldolase (fba) will be overexpressed individually or in combination, and the resulting strains will be evaluated for growth and physiological performance during methanol-based cultivation. In addition, the effect of reduced FA dissimilation on the formation of value-added products, including acetoin and riboflavin, will be assessed. These experiments will provide insight into the relationship between FA assimilation capacity, carbon conservation, and bioproduction in B. methanolicus, and may contribute to the development of improved methanol-based production strains.

[P015] Kefir Consortia for Hybrid Milk Fermentation

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Kefir milk is widely consumed and considered natural and healthy, but kefir is also associated with a climatic burden due to animal production. To offer plant-based dairy alternatives, investigating the fermentation capacity of kefir cultures in plant substrates is essential. The aim of this study was to construct a kefir consortium and determine its fermentation performance in cow and hybrid oat milk. For isolation of kefir microorganisms, kefir grain and its fermented milk were serially diluted and plated on modified MRS, M17 and YPD agar for cultivation at 30°C (mMRS and M17) and 25°C (YPD) for 3 to 7 days. Colonies were selected based on morphology and were further identified with 16S rRNA gene sequencing and Maldi-TOF Biotyper. Seven species from the Lactobacillaceae, Acetobacteraceae, and Saccharomyceta-ceae were used for fermentation in 100% cow milk (four batches) and two subsequent batches in 70% cow and 30% oat milk. Acidification was monitored, and changes in levels of mono- and disaccharides and fermentation metabolites were monitored with UPLC-RI.

Cow milk fermented with the reconstructed kefir consortium acidified to a final pH of 4.3, which was comparable to kefir grains. Acetate levels of the consortium were six-fold higher than those of kefir grains. When oat milk was introduced, lactate production by the consortium decreased from 73.7 ± 2.7 mM to 64.8 ± 1.3 mM, while the acidification rate increased from 0.12 to 0.16 **Δ**pH/h probably because the consortium metabolised glucose available in the oat matrix more efficiently than lactose in cow milk. Ethanol (10.5 ± 3.2 mM) and acetaldehyde (5.1 ± 0.1 mM) were also detected in hybrid milk. No separation of phases was observed across all fermentation batches.

In conclusion, this study provides promising results for the usage of hybrid milk in kefir fermentation. Further investigations on individual and combined strains' performance will aid in optimising the metabolic profiles and sensory properties of the final product.

[P016] Uncovering Microbial Drivers of Groundwater Pollutant Biodegradation via Total RNA

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Chlorinated solvents are persistent groundwater contaminants, and their biodegradation depends strongly on local microbial activity and geochemical conditions. Bioaugmentation with specialized dechlorinating cultures can enhance contaminant removal, yet evaluating treatment performance remains challenging in heterogeneous aquifers, where chemical and DNA-based measurements alone may not fully reflect active microbial processes.

This study evaluated RNA-based approaches for monitoring bioaugmentation with the commercial mixed culture KB-1® in a highly contaminated Danish aquifer. Transcriptional responses were assessed using RT-qPCR and whole-transcriptome sequencing, complemented by comparison with the KB-1® metagenome.

RT-qPCR revealed that expression of the reductive dehalogenase genes vcrA and bvcA increased following bioaugmentation and continued to rise over time, indicating an enhanced dechlorination-related transcriptional response. At the whole-transcriptome level, differential expression analysis revealed a clear functional shift in treated groundwater, with multiple genes significantly up- or downregulated relative to untreated conditions.

To investigate whether part of this response could be linked to the introduced culture, the KB-1® metagenome was used as a reference for identifying environmental transcripts with high similarity to KB-1® genes. Several KB-1-like genes were detected among the transcripts, allowing functions potentially associated with the introduced culture to be distinguished within the broader groundwater transcriptome.

Taken together, these results demonstrate that RNA-based monitoring can capture both targeted dechlorination activity and broader functional responses to bioaugmentation. Combining RT-qPCR, whole-transcriptome analysis, and culture-specific metagenomic information provides a complementary framework for evaluating bioaugmentation performance in heterogeneous contaminated aquifers.

[P017] Enhancement of lactic acid production in Bacillus methanolicus

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The thermophilic methylotroph Bacillus methanolicus is a promising platform for the methanol-based bioproduction of various compounds, including lactic acid (LA). LA is primarily produced through microbial fermentation, although it can also be obtained through chemical synthesis. Chemical synthesis relies on petroleum-derived feedstocks and typically results in a racemic mixture consisting of equimolar amounts of the L- and D-lactic acid isomers. LA is used in a wide range of industrial applications. In the food and beverage industry, it is used as a preservative, acidulant, flavour enhancer, antioxidant, prebiotic activity enhancer, and viscosifier. In the pharmaceutical and chemical industries, LA is primarily used as a building block for polymers and as a platform chemical. To establish LA production in B. methanolicus, overexpression of a gene encoding lactate dehydrogenase (LDH) is required. Heterologous expression of ldh from Lactobacillus delbrueckii ATCC 10705 enables methanol-based LA production in B. methanolicus, although only at low concentrations. In this project, the effects of overexpressing genes involved in the glyoxylate shunt and malic enzyme, in combination with reduced pyruvate carboxylase activity, will be investigated with respect to LA accumulation. These modifications are expected to redirect carbon flux towards pyruvate and subsequently increase the availability of precursor for LA biosynthesis.

[P018] Long-term phage stability for biocontrol: Investigating pre-production storage and applied perspectives

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The practical implications of using phages as biocontrol agents in food production, goes beyond safety and efficacy concerns. Research, development, certification, biobanking, distribution and application; all requires stable and reliable methods for (mixed-) phage stock conservation. Here we are comparing long-term (two year) stability of 50 diverse phages as cryo-stocks versus freeze-dried. Furthermore, did we use a subset of eight diverse mixed phages which were monitored in an aqueous solution for 20 months, on potato tubers for six months and in soil for one week. Freeze-drying proved equally efficient at preserving viable virions as cryo-storage, but with considerably lower cost and environmental impact. The subset of eight mixed phages proved stable in the aqueous solution for 20 months and detectable on potato tubers for up to six months. In soil, phages proved stable for the test period of one week and did not affect the overall soil microbiome. While freeze-drying proves a more sustainable alternative to cryo-stocks for pre-production long-term storage, broadly applicable across diverse phage types, the results also re-enforce the need to tailor pre-production storage methods to the specific phage. Furthermore, our stability test with a mixed subset of eight phages demonstrates that even without stabilizing agents' these phages are stable for at least 20 months in an aqueous solution, can persist for up to six months on tubers and are stable when added to soil for seven days without altering the indigenous soil bacterial community.

[P019] A modeling-to-bioreactor pipeline for improving production of a natural insecticide by Streptomyces

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Biological crop protection offers a promising route to reduce reliance on synthetic agrochemicals, but many microbial natural products remain challenging to manufacture economically at scale. This work focuses on a Streptomyces-derived polyketide insecticide, a bio-based alternative for sustainable pest management. Industrial production is complicated by the filamentous growth characteristics of Streptomyces, including pellet formation and sensitivity to bioprocess conditions, while little systematic data exists on how cultivation conditions influence scalable production. We are therefore developing a rational framework for fermentation optimization that links medium design and bioreactor operation to product yield, and that compares performance between mini- and bench-scale bioreactors.

Genome-scale metabolic modeling is used to identify promising nutrient supplements and metabolic precursors for enhanced production. These hypotheses are evaluated through high-throughput screening campaigns, followed by structured design of experiments in mini-bioreactors to quantify factor effects and interactions and define improved cultivation conditions. Leading conditions are validated in bench-scale bioreactors with cascading dissolved oxygen control, pH regulation, and off-gas analysis, linking medium composition to oxygen demand and productivity. In parallel, fed-batch cultivation strategies are being implemented to sustain precursor availability and prolong product formation. Initial results suggest that several medium supplements enhance polyketide formation up to 2-fold.

By integrating metabolic modeling, systematic screening, experimental design and scale-down bioreactor validation, this study establishes a data-driven approach to accelerating the development of microbial biosolutions. Beyond the insecticide investigated here, the workflow aims to provide a generalizable strategy for improving natural product fermentations and supporting the cost-effective manufacture of biological crop protection products.

[P020] Auxotrophy-driven interactions determine community composition in independent microbial cultures

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Chain elongation allows anaerobic microbial communities to convert ethanol and acetate into caproate, a medium chain fatty acid of industrial interest. It remains unclear whether the interactions between community members determine which species persist during enrichment. The aim of this study was to examine whether the same species recur in independently enriched lineages.

There enrichment cultures were established from the same environmental inoculum and maintained as separate lineages through successive transfers on ethanol and acetate. Despite their independent histories, all three lineages converged on a stable caproate-producing community composed of the same bacterial species.

To explore what holds this community together, the genomes of the members were recovered and used to reconstruct five individual genome-scale metabolic models, one for each species, which were used to construct the community model. Several members were found unable to synthesize their essential amino acids. On this basis, a role was proposed for each species. Two of them are are predicted to carry out caproate production, whereas the other three support the community by supplying intermediates and taking part in the exchange of amino acids.

These results suggest that the recurrence of the same species across independent lineages is not coincidental but reflects the metabolic interdependence between them, with each species occupying a distinct role. This project shows how genome-scale metabolic modeling can link the reproducible composition of a chain-elongation community to the interactions that sustain it.

[P021] Targeted isolation of ammonifying spore-forming Bacilli for improved soil nitrogen management

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Nitrogen losses from agricultural soils reduce fertilizer use efficiency and contribute to environmental nitrogen pollution. Dissimilatory nitrate reduction to ammonium (DNRA) represents an alternative to denitrification by retaining nitrate-derived nitrogen as ammonium rather than releasing it as gaseous nitrogen. Despite its potential importance for soil nitrogen retention, the diversity and functional characteristics of DNRA-performing bacteria in agricultural soils remain poorly understood.
Here, we present an ongoing effort to isolate and characterize endospore-forming Bacilli capable of DNRA. Agricultural soils spanning a range of pH conditions were used as sources for targeted isolation. Heat selection and enrichment strategies were applied to favor endospore-forming bacteria, followed by screening of isolates for nitrate reduction and ammonium production. Endpoint analysis was used to distinguish ammonifying phenotypes from alternative nitrate-reduction pathways.

Initial screening confirms the presence of diverse Bacilli across the investigated soils, including acidic environments, and has yielded endospore-forming isolates with contrasting nitrogen transformations. Candidate isolates showing ammonium production during nitrate reduction are currently being characterized in greater detail.

This targeted isolation approach aims to establish a collection of physiologically robust DNRA-performing Bacilli and improve our understanding of their functional diversity. The resulting strain collection will provide a basis for subsequent assessment of additional nitrogen-transforming traits, including N_2O reduction, and for evaluating the potential of spore-forming DNRA bacteria as biological tools for improved nitrogen retention and nutrient use efficiency in agricultural soils.

[P022] From Fungal Biodiversity to Biosolutions: A High-Throughput Platform for Discovery of Glycoalkaloid-Degrading Activities

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Microbial diversity represents a vast reservoir of metabolic innovation with potential to address challenges across food production, biotechnology, and sustainable manufacturing. Among microorganisms, filamentous fungi are particularly attractive sources of novel enzymes due to their remarkable ability to adapt to and transform complex natural compounds. However, the scale of fungal biodiversity presents a significant challenge, as identifying valuable activities within large strain collections exceeds the capacity of traditional screening approaches.

Potato glycoalkaloids, including α-chaconine and α-solanine, are natural defense compounds that confer bitterness to potato-derived protein food ingredients. Although microbial degradation of these compounds has been reported, the underlying enzymatic mechanisms remain to be revealed. While glycosidase-mediated removal of sugar moieties has been described, the subsequent degradation of the aglycone solanidine remains largely unresolved, indicating the existence of novel fungal enzymes.

In this study, we investigate a diverse collection of filamentous fungi as a source of novel glycoalkaloid-degrading enzyme activities. Potato-associated fungi have evolved strategies to tolerate and overcome glycoalkaloid-based plant defenses, indicating that they may harbor specialized enzymatic systems with potential biotechnological value. To systematically explore this diversity, we are developing a robotics-assisted high-throughput screening platform that enables rapid and standardized assessment of fungal enzyme activities across the fungal strains in the IBT culture collection hosted by DTU.

By integrating fungal biodiversity screening, automation, and enzymology, we aim to identify candidate enzymes and uncover previously undescribed metabolic pathways involved in glycoalkaloid transformation and degradation.

This study is part of the REFINES project that is funded by Plant2Food (https://projects.au.dk/plant2food/funded-projects/refines).

[P023] Host and context-dependent modulation of microalgal biomass by bacterial co-cultivation

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Bacterial co-cultivation is increasingly explored as a strategy to enhance microalgal cultivation, yet most studies focus primarily on growth responses. Here, we investigated how two phylogenetically distinct bacterial species, Halomonas aquamarina and Lactiplantibacillus plantarum, influence three industrially relevant microalgae i.e. Chlorella vulgaris, Chlamydomonas reinhardtii, and Nannochloropsis oceanica. Growth, chlorophyll content, bacterial persistence, and biomass composition were assessed over 15 days of co-cultivation. No bacterial species affected microalgal growth, indicating stable coexistence across all host species. Instead, L. plantarum consistently increased chlorophyll content at stationary phase with increases of approximately 24% in C. vulgaris, 23-28% in C. reinhardtii, and 28% in N. oceanica relative to axenic controls. Further, macromolecular biomass composition responses were host specific as seen in N. oceanica and L. plantarum co-culturing with increased lipid content from ~8% to ~16%. Under specific nutrient conditions, L. plantarum also increased protein content in C. vulgaris from ~32% to ~42%. In contrast, H. aquamarina showed no detectable compositional effects in C. vulgaris or N. oceanica but increased protein content in C. reinhardtii from ~67% to ~83% of biomass (~24% relative increase).
Overall, our findings demonstrate that bacterial partners can alter microalgal biomass composition without affecting growth, and that such effects depend on both the host species and cultivation environment. This highlights the importance of evaluating biomass composition alongside productivity when selecting bacterial partners for microalgal biotechnology. Moreover, these results suggest that bacterial partners may be used in tailoring microalgal biomass composition through biological interactions rather than genetic modification.

[P024] Electrifying Microbial Nitrogen Fixation

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The Haber-Bosch process is essential for global food production but remains highly energy intensive. Bioelectrochemical nitrogen fixation (e-BNF) offers a potential alternative by coupling microbial nitrogen fixation with electrical energy under mild conditions.

Here, nitrogen-fixing microbial communities were enriched from the rhizosphere of nodulated plants and cultivated in single-chamber, membrane-less bioelectrochemical systems operated at a constant current of 5 mA. The growth medium strongly influenced how microbial communities interacted with the cathode. While Burk's medium favored predominantly planktonic growth, Norris' medium promoted dense colonization of the carbon-felt cathode and stronger electrode-associated activity.

Bioelectrochemical reactors accumulated substantially more biomass-associated nitrogen than electrode-free controls, while extracellular ammonia remained negligible, indicating predominantly assimilatory nitrogen fixation. Microsensor measurements revealed that the carbon-felt cathode generated localized anoxic zones (<1 µM O_2) even when the surrounding medium remained highly oxygenated. Sequencing further identified Clostridium and Sporolactobacillus among the dominant cathode-associated microorganisms.

Together, these results demonstrate that cathodic electron supply can substantially enhance microbial nitrogen fixation. This proof-of-concept highlights e-BNF as a promising route toward electrically driven, low-energy nitrogen fixation for sustainable fertilizer production.

[P025] Real time monitoring of RNA-interference effects in Aspergillus fumigatus

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RNA interference (RNAi) is a conserved gene regulatory mechanism in eukaryotes in which double-stranded RNA (dsRNA) mediates sequence-specific silencing of target genes. In xerophilic filamentous fungi, RNAi pathways can be harnessed to selectively suppress essential genes, enabling highly targeted growth inhibition without the use of conventional antifungals. This positions RNAi as a promising strategy for sustainable fungal control in agriculture, food systems, and material preservation. Here, we aim to induce RNAi in the filamentous fungus Aspergillus fumigatus and assess the dose-dependent efficacy of dsRNA treatment. Effects on fungal spore germination and hyphal development are monitored and quantified in real time using live-cell imaging technology (oCelloScope, BioSense Solutions). Double-stranded RNA is designed to target essential genes in A. fumigatus, including beta-tubulin A and B, putative cytochrome P450 pisanthin demethylase, cytochrome P450 bifunctional NADPH reductase, and NADPH cytochrome P450 reductase. A. fumigatus conidia are suspended in liquid Sabouraud medium and treated with increasing concentrations of target-specific dsRNA. Spore germination and hyphal development are monitored by live-cell imaging over 24 hours of incubation. We hypothesise that dsRNA exposure will trigger RNAi-mediated gene silencing, resulting in measurable inhibition of spore germination and hyphal development in A. fumigatus. This work is expected to provide new mechanistic insights into RNAi-mediated regulation of filamentous fungal growth and establish a validated, quantitative, and scalable workflow for RNAi-based growth inhibition. We employ small RNA-sequencing to investigate dsRNA uptake and processing into small interfering RNAs (siRNAs) and study the stability and potential transgenerational persistence of siRNAs across successive fungal generations.

[P026] geneML: Gene annotation across diverse fungal species using deep learning

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Accurate ab initio gene prediction is a major bottleneck in fungal genomics. We present geneML, a new gene prediction tool based on deep learning that is tailored to fungal genomes. Across nine reference genomes spanning diverse fungal taxa, geneML improved gene-level F1 score from 64.9 to 67.1 compared to BRAKER3 with protein-based hints, driven by substantially higher recall (69.0 vs. 64.1) at equivalent precision. geneML is very fast, averaging around 6 minutes per genome on a standard 8-core CPU, a 32 x speedup over BRAKER3. A key feature of geneML is its ability to predict alternative transcripts. Compared to Fusarium graminearum Iso-Seq control data, it achieves 41.1% transcript recall and 71.1% precision, outperforming AUGUSTUS (33.8% recall, 48.9% precision), one of the few tools that support isoform prediction. Reannotation of the curated training dataset further suggests improved biological completeness, with geneML recovering 15.3% more genes containing complete PFAM domains than the reference annotation. These results demonstrate that geneML enables faster, more sensitive, and more biologically informative fungal genome annotation. geneML is available as an open-source command-line tool at https://github.com/hexagonbio/geneML.

[P027] Climate and soil chemistry shape compartment-specific fungal communities of Salicornia europaea across Europe

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Halophyte-associated fungal communities may contribute to plant performance under saline conditions, yet the extent to which climate, soil chemistry and plant compartment jointly structure these communities across broad geographic gradients remains poorly understood. The edible halophyte Salicornia europaea, which is increasingly relevant to saline agriculture and food production, provides a suitable model for investigating these relationships.

Fungal communities were characterised by ITS amplicon sequencing in bulk soil, rhizosphere soil, roots and shoots of S. europaea collected from ten sites in Norway, Poland, France, Croatia and Spain, spanning four Köppen–Geiger climate types. The microbiome data were integrated with regional climatic records and 15 soil physicochemical parameters. Relationships between environmental variables, fungal alpha diversity, dominant fungal genera and overall community composition were evaluated using correlation-based and multivariate ordination approaches, including Spearman rank correlations and distance-based redundancy analysis.

The analyses revealed pronounced differentiation of fungal communities along the soil–plant continuum and substantial variation among geographic locations. Associations between fungal diversity and climatic conditions differed among compartments, while soil physicochemical gradients were linked to both taxon-specific distributions and broader patterns of community composition. The strength and direction of these environmental relationships varied across bulk soil, rhizosphere, root and shoot communities, demonstrating that environmental associations within the S. europaea mycobiome are strongly compartment-dependent.

This pan-European study highlights the need to consider climate, soil properties and plant compartments jointly when interpreting fungal community assembly in halophytes. The findings provide a basis for future functional studies of fungal taxa potentially relevant to plant performance and microbiome-based strategies for saline agriculture.
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[P028] Progress update on the development of a fungal GTDB

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The Genome Taxonomy Database (GTDB; https://gtdb.ecogenomic.org), an essential taxonomic resource for microbiologists worldwide, marked its tenth release (R10-RS226) in 2025 and is now expanding to the kingdom Fungi. This milestone marks a significant step in broadening GTDB's scope, with the fungal expansion currently in the investigation phase and the first public release anticipated in 2027. Here we present the results of initial quality control (QC) for genomes, evaluations of average nucleotide identity (ANI) thresholds for species delimitation, and marker gene selection for inference of a reference phylogeny. Of 22,432 fungal genomes downloaded from the NCBI Assembly database in September 2025, we delineated 4,838 genomically defined species clusters using a 95% ANI threshold, a criterion widely applied for operationally defining bacterial species. This clustering showed that 7% of named species were merged and 6% were split, reflecting the need for reclassification of existing fungal species and further investigation into the utility of ANI-based species delineation in fungi. In parallel, we are developing a quality control metrics that explicitly distinguishes true genomic duplication, well-documented in fungi, from contamination, a distinction not resolved by currently available tools, which will refine the dataset used in subsequent steps. We then evaluated 1,122 fungal-specific BUSCO markers from OrthoDBv12 for ubiquity and copy-number stability across the representative genome set. Based on these criteria and evaluations of individual marker gene trees, we selected 77 ubiquitous, single-copy markers to be tested for tree reconstruction using various sequence alignment filtering options and substitution models. The first reference tree revealed a highly skewed representation toward the major fungal phyla, Ascomycota and Basidiomycota, with 15% of species clusters lacking names in the tree. To guide tree curation and provide connectivity between genome-based and ITS-based taxonomies, we extracted and classified genomic ITS regions using the UNITE database. Where possible, we plan to reuse UNITE Species Hypothesis for unnamed taxa. Manual curation will also rely on information about types and synonyms extracted from both Mycobank and Index Fungorum. We are currently in the process of curating and rank-normalising the reference tree. The fungal GTDB will allow accurate classification of not only existing diversity but also the increasing number of fungal MAGs anticipated from environmental samples as long-read sequencing throughput continues to improve.

[P029] Evidence of the zoonotic potential of ape Plasmodium vivax-like

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While infection with malaria causing Plasmodium vivax is common in humans in Asia, there has only been one reported case of human infection with ape P. vivax-like, from Africa, more than a decade ago. An unusually divergent Plasmodium parasite was found in a Swedish patient with malaria symptoms. The patient had recent travels to Asia and Africa. Using selective whole genome amplification (SWGA), we enriched the parasite genome ~6000 fold over the background of human DNA, allowing whole genome sequencing. We show that the infection was caused by an ape P. vivax-like parasite, a species infecting gorillas and chimpanzees in Africa. The evidence of the other report of human infection with ape P. vivax-like from 2013 consisted of mitochondrial and two short nuclear fragments, limiting accurate placement. Thus, the case presented here is the first comprehensively documented naturally acquired human infection with ape P. vivax-like malaria parasite. This case highlights the value of two complementary sequencing approaches for understanding parasite infection. Firstly, amplicon-based NGS results from the sample were instrumental in flagging it as divergent from other P. vivax parasites. Secondly, SWGA enabled us to generate genome-wide coverage to determine the position of this sample within the overall diversity of ape P. vivax-like parasites.. This represents evidence that ape P. vivax-like parasites have zoonotic potential. The finding raises questions about the frequency of such spillover infections and the extent to which they may remain unrecognized by routine malaria diagnostics

[P030] Paired-omics-based exploration and characterization of biosynthetic diversity in lichenized fungi

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The increasing demand for novel drug leads requires bioprospecting non-model taxa. Comparative genomics and correlative omics are a fast and efficient method for linking bioactive but genetically orphan natural products to their biosynthetic gene clusters (BGCs) and identifying potentially novel drug leads. Here we implement these approaches for the first systematic comparison of the BGC diversity in lichen-forming fungi (LFF) (comprising 20% of known fungi), prolific but underutilized producers of bioactive natural products. We first identified BGCs from all publicly available LFF genomes (111), encompassing 71 fungal genera and 23 families, and generated BGC similarity networks of each class. We recovered 5,541 BGCs grouped into 4,464 gene cluster families. We used mass spectrometry (MS) and correlative metabolomics to link five MS-identified metabolites – alectoronic acid, alpha-collatolic acid, evernic acid, stenosporic acid and perlatolic acid – to their putative BGCs. We subsequently used MS on an additional 80 species to explore the taxonomic breadth of common lichen compounds, uncovering a strong pattern between specific families and secondary metabolites. We found that (1) ~98% of the BGCs in LFF are putatively novel (uncharacterized to date), (2) lichen metabolic profiles contain a plethora of unidentified metabolites and (3) ribosomal peptide-related BGCs constitute about 20% of the LFF BGC landscape. Our study provides comprehensive insights into the BGC landscape of LFFs, highlighting unique, widespread and previously uncharacterized BGCs.

[P031] Food-driven metabolic specialization drive phenotypic diversity of emetic Bacillus cereus

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Food safety assessments largely rely on microbial growth as a proxy for risk. However, pathogens can exhibit similar growth while expressing remarkably different hazard-related traits. As plant-based foods become increasingly important, they introduce distinct habitats that alter pathogen behavior beyond growth. Although Bacillus cereus is a well-known foodborne pathogen, its adaption to plant-based food environments remains poorly understood.

In this study, seven emetic (cereulide-producing) B. cereus strains were characterized using whole-genome sequencing and their behavior in oat- and pea-based substrates compared with whole milk. Growth, sporulation, post-germination metabolism, biofilm formation, and cereulide production were assessed.

Despite comparable growth kinetics, significant variation was observed in metabolism-associated phenotypes. Plant-based substrates enhanced biofilm formation and metabolic activity, while cereulide production varied by several orders of magnitude depending on the strain and substrate. Statistical analysis revealed significant effects of strain and strain x substrate interaction on cereulide levels, highlighting the strong context dependence of toxin production. Comparative genomic analyses identified strain-specific variation in genes associated with replication and cellular processes, while the prevalence of genes with unknown functions suggests that important drivers of strain-specific virulence remain unresolved.

Together, these findings reveal a decoupling of growth and hazard expression, whereby similar population dynamics mask great differences in metabolism, biofilm formation, and cereulide level. We propose that growth-metabolism trade-offs contribute to phenotypic diversity in emetic B. cereus across food systems and highlight the limitations of growth-based risk assessments. Incorporating strain diversity and functional phenotypes into challenge testing may improve evaluation of B. cereus risks in diverse food systems.

[P032] Utilization of dietary lactose by the intestinal microbiota – can lactose be considered a prebiotic?

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Globally, 65~70% of the adult population is lactase nonpersistent. Dairy consumption can trigger symptoms of lactose intolerance in lactase nonpersistent individuals, e.g. bloating, diarrhea and flatulence, but there is a possibility that regular dairy consumption can lead to colonic microbiota adaption, allowing for the consumption of high amounts of lactose. The adapted colonic bacteria may hydrolyze lactose to glucose and galactose and ferment them to lactate and short-chain fatty acids (SCFA)--important contributors to intestinal homeostasis, reducing H2 and CO2 production. However, little was known whether and how the ingested lactose has an impact on intestinal microbiota fermentation activity in vivo.

In this study, we aim at gaining insight into the response of complex intestinal microbiota upon exposure to lactose. We used mouse models and investigated the impact of lactose after different treatment strategies in C57BL/6NTac mice after single consumption and regular uptake (1 and 3 weeks). We systematically determined the impact of lactose supplementation on microbiota composition, microbial β-galactosidase activity, metabolite cross-feeding, and fermentation metabolite profile in different segments of gastrointestinal tract (GI). We also assayed the host intestinal gene regulation and immune response to lactose.

Lactose consumption of 3 weeks increased microbial β-galactosidase activity and propionate levels in cecum. Both diversity and β -diversity analysis showed lactose treatment led to changes of microbiota compositions in distal small intestinal (SI) and cecum. Deseq analysis also revealed a difference ($p<0.05$) in species level from the family Lachnospiraceae between control and lactose treatment. Three weeks of lactose feeding upregulated duodenum lactase gene expression but downregulated mucin Muc2, cytokine TSLP and ion tranporter Slc9a gene expressions in duodenum and proximal SI. More activated T-cells/immune system mLN were detected in lactose treatment than control. Single consumption study showed a clear time effect on lactose digestion: lactose was detected in distal SI only after 1 and 2 h, particularly in groups without lactose adaption, while microbial β-galactosidase activity in cecum increased with increasing digestion time (< 6 h). It additionally verified that lactose adaption improved cecum butyrate concentrations.

Taken together, regular lactose uptake not only affected host intestinal gene expression and immune responses but also enhanced microbial β-galactosidase activity in the lower GI and altered the intestinal microbial composition and fermentation activity, promoting the production of propionate and butyrate. These findings provide new insights into the use of lactose adaption strategy to relieve lactose intolerance and the potential of lactose to be a prebiotic.

[P033] Multi-Omic Characterization of Microbial Diversity and Nutritional Changes in Traditional Ethiopian Enset (Ensete ventricosum) Based Fermented Food

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Enset (Ensete ventricosum) plays an important role in the food security of about 25 million people in Ethiopia. Kocho is a traditional food made by fermenting the enset plant. Research on the proteome and microbial changes across different fermentation stages and methods of kocho remains limited. In this study, we used full-length 16S rRNA and ITS gene sequencing by nanopore technology to examine the microbial community and bottom-up proteomics to analyze protein features. We found that Leuconostoc mesenteroides, Enterococcus italicus, Bifidobacterium minimum, and Liquorilactobacillus sicerae were the main microbes at the initial, middle, and late fermentation stages. We identified thirteen protein groups, which included 655 unique peptides. NDP-glucose-starch glucosyltransferase and chitinase were the most common proteins, showing their important roles in starch changes and microbial activity during fermentation. Our study offers new insights into the microbial and protein changes in kocho. These results can help develop starter cultures to make fermentation more consistent and improve the product's nutritional and functional protein content.

Keywords: Traditional fermentation, proteomic analysis, microbial community, nutritional value, starter culture

[P034] Unveiling Alternative Hydrogen Pathways in the Cattle Rumen

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Unveiling Alternative Hydrogen Pathways in the Cattle Rumen
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Methanogenesis is a major sink for metabolic hydrogen generated during rumen fermentation, while alternative hydrogen-consuming pathways may provide opportunities to redirect hydrogen away from methane formation, but the microorganisms and pathways involved remain incompletely characterized. We developed a long-read, genome-resolved metagenomic workflow using 16 rumen-fluid samples from four cows in a 4 × 4 Latin-square experiment with treatments comprising of control, nitrate, 3-nitrooxypropanol (3-NOP), and nitrate + 3-NOP t. Oxford Nanopore reads were quality filtered, depleted of Bos taurus DNA, assembled individually and by co-assembly, and binned using four complementary algorithms. Bins were refined with DAS Tool, evaluated with CheckM2, and taxonomically classified using GTDB-Tk. The workflow was first benchmarked using a ZymoBIOMICS mock community. Application to the rumen samples recovered 12,292 refined genome bins, including 6,026 metagenome-assembled genomes with ≥80% completeness and ≤10% contamination. Dereplication with Galah yielded 2,462 species-level and 3,669 strain-level representative clusters. Species representatives were integrated with published rumen genome collections to construct a non-redundant rumen genome superset and assess taxonomic novelty, identifying 1,035 putatively novel species, 54 genera, and three families relative to the reference collections. Ongoing analyses use a factorial model incorporating nitrate, 3-NOP, their interaction, period, and cow to identify treatment-responsive MAGs at strain level. A non-redundant gene catalogue and read-based functional profiles will identify treatment-responsive genes and pathways. Linking these functions to MAGs will nominate microorganisms involved in alternative hydrogen-utilization pathways, while read-based profiling will capture functional changes arising from low-abundance taxa and unbinned DNA. This integrated framework combines genome-resolved and community-wide evidence to investigate how nitrate and 3-NOP reshape rumen hydrogen metabolism.

[P035] Fresh produce revealed as a novel reservoir of microbial diversity and shared plasmidomes

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Plasmids are key drivers of horizontal gene transfer which facilitate the dissemination of adaptive traits across microbial communities and ecological niches. Although plasmid dissemination has been extensively studied in established transfer hotspots such as healthcare facilities and wastewater treatment plants, its role in food-associated microbiomes remains poorly understood. This gap is especially consequential for fresh produce, which represents a critical interface between agricultural environments and consumers as it is typically consumed minimally processed.

Using long-read metagenomics, we characterized microbiomes and plasmidomes associated with alfalfa sprouts, bean sprouts, arugula, spinach, and potatoes. We recovered 130 metagenome-assembled genomes (MAGs), uncovering extensive, previously unexplored diversity, with more than one-third representing previously undescribed or poorly characterized bacterial species. We also identified a diverse plasmidome spanning multiple bacterial lineages and observed related plasmids across vegetable types, suggesting a partially shared fresh produce plasmidome. Integrating plasmid identification with methylation-based host assignment allowed us to predict host-plasmid associations at various taxonomic ranks directly from metagenomic data, revealing widespread connectivity between plasmids and diverse bacterial lineages.

Together, these findings position fresh produce as a source of microbial dark matter sitting at the consumer interface with an overlooked reservoir of both uncharacterized bacterial diversity and mobile genetic elements. The sharing of plasmids across vegetable types, and their association with diverse hosts, suggests fresh produce functions as a nexus for plasmid-mediated connectivity within the One Health microbiome. These results extend the ecological landscape of plasmid biology beyond traditional transfer hotspots and underscore the need to incorporate plasmid ecology into food microbiology and surveillance frameworks going forward.

[P036] Increasing water salinity improves fish survival in Flavobacterium psychrophilum-challenged rainbow trout: Effects on fish health, pathogen load, and microbiome changes

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Altering water parameters is sometimes used as a preventive method to reduce infection of certain bacterial pathogens in fish production systems. Recent experiments have shown the potential of increasing water salinity (1%) to delay and partially prevent infection caused by the bacterial pathogen Flavobacterium psychrophilum in rainbow trout fry (Donati et al. 2025).

In this study, we wanted to further evaluate the robustness and efficacy of increasing water salinity in fish challenged with a diverse range of F. psychrophilum isolates differing in serotype, presence of antimicrobial resistance genes, virulence, and epidemiological background. Animal testing was conducted in which fish were maintained either in freshwater or in water with increased salinity (0.5%), a level selected to meet the operational capacities of commercial farms. Rainbow trout fry (1–2 g) were challenged by co-habitation, monitored and sampled over time to: (1) assess health and survival; (2) characterize pathogen presence within the fish and in the surrounding environment using classical microbiological and molecular approaches, including absolute quantification; and (3) determine the effects of bacterial infection and/or increased water salinity on the gut and gill microbiota. Water samples were also collected weekly to characterize pathogen dynamics. Negative controls for the infection were included and monitored/sampled over time.

The experiment showed that increasing water salinity improved fish survival for all four tested isolates. Pathogen detection was also lower in salinity tanks. Fish growth was not affected by the increased salinity. Analyses of bacterial quantification in internal/external fish organs as well as Nanopore-generated microbiome data will be presented.

These initial results support previous findings: Increasing salt percentage in tank water might decrease infection pressure by the bacterial pathogen F. psychrophilum.

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[P037] Defined Microbial Consortia to transform Agricultural Waste streams into Bioactive Vinegar

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Agricultural waste streams provide a matrix rich in carbon and nitrogen that have potential as growth substrates for microbial biotransformations. With fermentation, such waste streams can be converted into value-added products through the action of microbes and their enzymes. The aim of this study was to design and employ microbial consortia to upcycle the agricultural waste streams of sweet whey, apple pomace, and brown juice into vinegars with antimicrobial properties.
Sweet whey and apple pomace powders were reconstituted in ultrapure water to final concentrations of 5% and 8.6% (w/v), respectively, whereas biorefinery-derived brown juice was utilized without modification. Lactose-fermenting Kluyveromyces lactis and glucose-, arabinose-, and fructose utilizing K. marxianus were co-cultured with Lacticaseibacillus paracasei to enable concurrent alcoholic fermentation and acidification. On day three ethanol and lactate were detected in whey (258 and 51 mM), apple pomace (113 and 12 mM), and brown juice (28 and 39 mM). On day five, Komagataeibacter saccharivorans, previously isolated from artisanal vinegars, was introduced to oxidize the accumulated ethanol. By day seven, 150-161 mM acetate were detected in whey and apple pomace. Levels were lower in brown juice (11 mM acetate), which also contained lactate (37 mM). During fermentation of apple pomace, phenolic compounds were deglycosylated and caffeic acid, phloretin, and quercetin were released. The vinegars exhibited antimicrobial activity against the spore forming Bacillus spp. and other food spoilers and pathogens. Together, this study highlights the potential of defined multi-species consortia to build a reproducible framework for upcycling agricultural waste into functional biosolutions. The resulting vinegar can find application in food, personal and home care, positioning our approach as a significant contribution to sustainable innovation.

[P038] Novel fermentation for improved extraction yield and quality of microalgae proteins

Grith Lachenmeier[1], Villads Oliver Nielsen[1], Nina Aagaard Poulsen[2], Clarissa Schwab[1], Jin Xie[1]

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Green microalgae Chlorella, which contains 50 – 60% proteins and provides all essential amino acids, is considered a high-quality food protein source, but the application in foods is limited due to the lack of sustainable approaches for upscaling of Chlorella protein extraction and purification. Bacillus spp. that secrete extracellular amylolytic and proteolytic enzymes, exhibit many beneficial technological traits in traditional food fermentations. In this study, we investigate the potential of designed consortia of Bacillus species and lactic acid bacteria to improve the extraction yield, flavor and nutritional value of single cell proteins from microalgae Chlorella. We screened Bacillus strains using an in-silico genome analysis focusing on the potential for expression of carbohydrate-active enzymes (CAZymes) and proteases. B. sublitis FUA2114, B. velezensis FUA2155, and B. amyloliquefaciens FUA2008 were selected as promising candidates. We will select homofermentative and heterofermentative Lactobacillaceae strains that acidfy and express enzymes to metabolize the (poly)phenolic compounds andreduce lipid oxidation aldehydes Experiments will be performed to verify the ability of bacilli to enhance mechanical lysis of Chlorella and to release proteins from the cell and cell wall debris. Microalgae cell structure will be visualized using microscopy. Subsequently, we will employ LAB to acidify the Chlorella extracts and precipitate proteins, replacing precipitation operations such as heating and chemical acidification. The concentration and size distribution of proteins will be analyzed. We will monitor all starter/adjunct cultures during fermentation and determine profiles of fatty acids and phenolic compounds in all samples. Take together, this study will develop microbe-assisted sustainable bioprocesses to improve Chlorella protein extraction and facilitate application as food ingredients.

[P039] Gut microbiota modulation by resistant starch

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Early-life microbiota contributes to the immune system development through direct microbiota-host interaction and the production of short-chain carboxylic acids (SCCA). High levels of butyrate and propionate in early life were associated with protection against atopic dermatitis (AD). There is a consensus that dietary fiber intake stimulates the growth of utilizers of complex carbohydrates and SCCA producing bacteria for the benefit of gut health. This study aims to investigate the impact of resistant starch (RS) modulation on composition and fermentation capacity in a host. We used a murine oxazolone-induced model of atopic dermatitis and introduced a diet with low or high content of RS after weaning at four weeks of age. Mice were sacrificed at seven weeks and the impact of RS supplementation on microbiota composition, microbial α-amylase activity, and fermentation metabolite profiles in different segments of gastrointestinal tract (GI) were determined. Samples collected from the cecum exhibited higher α-amylase activity and contained higher concentrations of reducing sugars than those from the distal small intestinal. There was no significant difference between treatments. Next, we will quantify fermentation metabolites in different GI segments. The compositions of intestinal microbiota in different treatments and segments will be analyzed using full-length 16S rRNA gene sequencing by Nanopore technology. We will integrate microbiome and metabolome data to uncover the relationships between intestinal microbiota, metabolites, and RS interventions. This study will provide key information on the effect of nutritional interventions on intestinal microbiota and host health in early life.

[P040] Fermentation of Chenopodium Album Flour: An Overlooked High-Nutritional Food Source

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Chenopodium album seeds have a high nutritional value and contain up to 22% protein with all essential amino acids, fatty acids, vitamin A and several minerals. The plant contains bitter-tasting saponins and is considered a weed in Europe. The overall goal of this study is to establish C. album as a food source; the aim is to characterize the microbiota of fermented C. album flour. Spontaneous and starter-culture fermentations were conducted, and pH, metabolite formation, and microbial cell counts were determined. The dominant strains were isolated and characterized with Maldi-TOF MS and 16S rRNA gene sequencing.

Four seed flours with varying saponin and protein content were spontaneously fermented and propagated through backslopping. The culturable indigenous bacteria were soil and/or plant-associated such as Bacillus spp., Pantoea spp., and Stenotrophomonas nematodicola. Acidification (final pH 3.8) occurred after two backslopping steps in 4/16 doughs. Up to 129 mM lactate was produced, and eleven strains of Enterococcus faecalis, Lacticaseibacillus rhamnosus, and Levilactobacillus brevis were isolated. In the presence of saponins, three strain-specific growth patterns were observed indicating differences in saponin tolerance among the isolates. Starter-culture fermentation with co-cultures of L. rhamnosus and L. brevis resulted in greater acidification (pH4.7) than of single-strains (pH 4.8-5.4). Although the indigenous microbiota was not completely outcompeted, its growth was suppressed by the co-culture (5.1 to 6.6 log CFU/mL) compared to the single-cultures (5.1 to 8.9 logCFU/mL). Fermentation altered saponin profiles based on LC-MS analysis.

Overall, our data suggests that C. album flours harbored a low abundant population of Lactobacillaceae that initiated spontaneous fermentation and can be used as starter-cultures. The application of C. album derived starter cultures showed potential to perform a more consistent fermentation to improve food safety.

[P041] In vivo experiment to compare bath, feed, and bath + feed phage treatments against Flavobacterium psychrophilum in rainbow trout fry

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Flavobacterium psychrophilum, the causative agent of Rainbow Trout Fry Syndrome (RTFS), is an important bacterial pathogen in rainbow trout aquaculture, causing mortality in hatcheries worldwide. The potential of bacteriophage (phage) therapy against this pathogen has been previously demonstrated, but further understanding of phage persistence, distribution and potential effects on fish health is needed.

A phage-based prophylactic treatment targeting F. psychrophilum was evaluated in an in vivo experiment, where rainbow trout fry (Oncorhynchus mykiss, ~1 g) were challenged via immersion (bath challenge). Phages were administered either by bath, phage-sprayed feed, or a combination of bath and phage-sprayed feed, starting five days before the infection and for a total of six weeks. While phage-sprayed feed (1,4 x 10^8 PFU/g) was administered daily, bath treatments (1 x 10^6 PFU/ml) were performed three times per week. Negative controls groups for phages as well as for the infection were included. To assess bacterial/phage persistence in water and fish organs, water samples were collected once a week from all aquaria immediately before and after the bath treatment, and five fish from designated sampling tanks, one for each treatment and control group, were sampled (external and internal organs). Phage treatment effects on the host were evaluated through survival analyses.

Overall, the initial data analysis supports the beneficial effect of prophylactic phage treatment: increased fish survival was observed in phage-bath and phage-feed groups (79.8% and 77.4%, respectively) compared to the control (67%). However, the combination of phage-sprayed feed and bath did not show any positive effect (66.7%).

Phage replication was observed in the water during bacterial infection. In phage bath and combination groups, phage concentration in the water ranged between ~10^3-10^5 PFU/ml, and during infection phages were consistently present even before administering the phage baths. Phages were also detected in water of phage-feed groups: with an overall lower and less consistent concentration, higher detection was observed during the infection (~10^2-10^3 PFU/ml). No phages were detected in untreated tanks. Bacterial/phage dynamics in fish as well as evaluation of phage resistance development will be presented.

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[P042] Pre-weaning Supplementation Accelerates Fecal Microbiota Maturation and Functional Readiness for Weaning in Piglets

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The gut microbiota plays a central role in piglet adaptation to weaning, and early-life nutritional strategies may provide opportunities to modulate microbiome development. We hypothesized that supplemental feeding during lactation would accelerate microbial and functional maturation, thereby promoting a smoother transition to weaning.

Piglets from 12 hyperprolific sow litters standardized to 15 piglets per litter were assigned to either a control treatment (CON; n = 6 litters) or a supplemented treatment (SUP; n = 6 litters). Supplemented piglets received milk replacer from day 3 of life, which was replaced by a cereal-based liquid weaner feed from day 12 until weaning at day 24. Fecal samples from 2 piglets per litter were collected longitudinally before and after weaning (11, 18, 24, 27, 31, 38, 49 days of age) and analyzed using shotgun metagenomic sequencing (Illumina NovaSeq X Plus, 2 × 150 bp, ~ 20 M paired-end reads). Taxonomic and functional profiling were performed using the bioBakery framework and CAYMAN. Alpha diversity increased markedly with age, indicating progressive microbiome maturation, whereas supplementation had limited effects on overall diversity. Likewise, age and weaning were the primary drivers of microbial community structure, accounting for most variation in community composition. Despite this dominant temporal effect, supplementation modulated the developmental trajectory of the microbiome around weaning. Species-level analyses showed that supplemented piglets established several post-weaning-associated taxa earlier than control piglets, indicating an accelerated microbial succession. Functional analyses via CAYMAN further showed that supplementation accelerated the acquisition of genes involved in complex carbohydrate degradation before weaning. Supplemented piglets exhibited an increased abundance of carbohydrate-active enzymes (CAZymes) associated with utilization of plant-derived substrates, reflecting enhanced functional preparedness for the dietary transition from milk to solid feed. These functional shifts were consistent with the observed acceleration in colonization by post-weaning microbial species. In conclusion, age and weaning remain the dominant determinants of gut microbiome development in piglets. However, pre-weaning nutritional supplementation accelerated the establishment of post-weaning microbial species and enhanced the pre-weaning functional capacity for complex carbohydrate degradation. These findings suggest that lactational supplementation may provide a strategic window to promote microbiome maturation and increase readiness for the weaning transition. PIG-PARADIGM project, funded by the Novo Nordisk Foundation (NNFSA210073688).

[P043] Reduction of Aflatoxin B1, Ochratoxin A, and Zearalenone by Microbial Fermentation in Faba Bean-Based Systems

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Faba bean (Vicia faba) is a promising raw material for the development of plant-based foods due to its high protein and dietary fiber content. However, during cultivation and storage, it may become contaminated with mycotoxin-producing filamentous fungi; therefore, the presence of aflatoxin B1 (AFB1), ochratoxin A (OTA), and zearalenone (ZEA) may pose a potential food safety risk. Fermentation may offer a sustainable approach to reducing the concentrations of these compounds.

The aim of this study was to investigate the mycotoxin-removal capacity of microorganisms suitable for food applications in faba bean-based fermentation systems. The filamentous fungi Aspergillus oryzae, Rhizopus oryzae, and Neurospora intermedia, as well as two yeast strains, were tested in artificially mycotoxin-contaminated liquid faba bean medium. In addition, the filamentous fungi were also evaluated in faba bean dough. Fermentation was carried out at 30 °C for seven days, and the residual concentrations of AFB1, OTA, and ZEA were determined using HPLC-HRMS.

In liquid medium, ZEA concentrations decreased below the detection or quantification limits following fermentation with R. oryzae and N. intermedia, while AFB1 concentrations fell below these limits for all filamentous fungi tested. In contrast, OTA remained detectable in all treatments. In faba bean dough, ZEA was not detectable after fermentation with R. oryzae and A. oryzae, and OTA concentrations were reduced, whereas AFB1 removal was less consistent. During yeast fermentation, the recovered AFB1 concentration decreased to 62.87–87.84 ppb compared with 259.29 ppb in the control, while OTA reduction remained limited.

Our results demonstrate that the efficiency of microbial fermentation is strongly dependent on the mycotoxin, microorganism, and fermentation matrix. In particular, the reduction of AFB1 and ZEA showed promising results, providing a basis for the development of safer fermented faba bean-based foods.

[P044] FROM WHOLE TO REFINED FLOURS: EFFECTS ON MICROBIAL SELECTION IN SPONTANEOUS SOURDOUGH ECOSYSTEMS

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Sourdough is a flour-water ecosystem in which lactic acid bacteria (LAB) and yeasts drive fermentation, progressively establishing acidification and leavening capacity through repeated propagations. Flour serves both as a nutrient source and a reservoir of autochthonous microbiota; however, its role in shaping microbial selection during sourdough development remains insufficiently explored. In this study, the effect of flour type and composition on fermentation performance and microbial selection during spontaneous type I sourdough preparation and propagation is investigated.

Spontaneous sourdoughs are prepared using type 00 and type 1 wheat flours, wholewheat, and wholegrain rye flour, and are propagated through daily propagations (20% inoculum) at 30°C. Fermentation is monitored by acidification and cell densities of LAB and yeasts. Both raw flours and mature sourdoughs are subjected to microbiological (culture-dependent) and biochemical characterisation, including analysis of sugars, organic acids, free amino acids, peptides, and fermentation quotient.

A total of 72 presumptive LAB and 30 yeasts are isolated from the four flours. In preliminary sourdough propagation experiments, rapid acidification is observed after two consecutive 24 h propagations , with pH decreasing from 5.94 ± 0.04 to 3.86 ± 0.05 in type 00 sourdough and from 5.97 ± 0.12 to 3.88 ± 0.06 in type 1 sourdough. During subsequent propagation, pH values stabilise between 3.85 and 3.90.

Future work aims to apply culture-independent approaches, including shotgun metagenomics, to raw flours and mature sourdoughs to characterise microbial community structure and functional potential, providing insight into how flour composition shapes the transition from flour microbiota to mature sourdough communities.

Keywords: type 1 sourdough; wheat flour; wholegrain rye flour; sourdough microbiota; flour composition

[P045] Biochemical transformation and functional enhancement of rapeseed through lactic acid bacteria fermentation (poster)

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This study aims to investigate how lactic acid fermentation modifies the physicochemical, biochemical, antioxidant, and antifungal properties of rapeseed (Brassica napus).

Rapeseed paste was inoculated separately with Lactiplantibacillus plantarum AVEF17 or Weissella cibaria PEP23 and fermented at 30 °C for 48 h. Raw, uninoculated, and chemically acidified pastes were included as controls. Microbial growth and acidification were monitored throughout fermentation, together with sugar consumption and organic acid production. Protein content was measured using an NDA 702 Dumas analyser. Peptides, phenolic compounds, glucosinolates, fatty acids, and hydroxy fatty acid derivatives were profiled using Ultimate 3000 UHPLC systems coupled to Q Exactive Plus Orbitrap mass spectrometers. Antioxidant activity was assessed using ABTS and DPPH assays, while lipid peroxidation by the TBARS assay. Antifungal activity was determined against Aspergillus niger, Penicillium aethiopicum, and Penicillium roqueforti.

Starting from an initial population of 7 log CFU/g, AVEF17 and PEP23 reached 9.25 and 8.77 log CFU/g, respectively, in rapeseed paste. AVEF17 showed stronger acidification (pH 3.56) and produced more lactic acid (14.97 mg/g fresh weight) than PEP23 (pH 4.28; 6.88 mg/g). While AVEF17 showed hydrolytic activity toward most of the detected glucosinolates, PEP23 significantly increased the content of glucoiberin, glucoraphanin, and glucoerucin. Both strains markedly depleted sinapic and ferulic acids, while L. plantarum AVEF17 was distinguished by the formation of catechol (4.87 µg/g dry weight) and the distinctive accumulation of hydroxy fatty acids. Its water-soluble extract (WSE) showed the highest ABTS activity, while no significant differences were detected in DPPH assay. AVEF17 also resulted in the lowest lipid peroxidation (2.3 µg malondialdehyde/g dry weight versus 4.2–4.4 µg/g in the incubated controls and PEP23). Antifungal effects were strain-, extract-, and fungal-species dependent. Notable responses included approximately 65% inhibition of P. roqueforti by the AVEF17 (WSE) and 78% by the PEP23 (lipid extract).

Selected lactic acid bacteria metabolically remodel rapeseed paste during fermentation. Lb. plantarum AVEF17 particularly enhanced antioxidant activity and limited lipid oxidation, whereas W. cibaria PEP23 promoted a distinct glucosinolate and antifungal profile, highlighting the potential of fermentation for developing value-added rapeseed ingredients.

[P046] Uterine bacterial communities and antimicrobial resistance profiles in Danish breeding mares

Karl Pedersen[1], Frederik Damsgaard, Freia Lærke Madsen, Maja Lund Olsen, Sinne Ross Sørensen, Sally Inger Svensson, Sarah Olivia Arscott Tvilum Sønnichsen, Jenny Katrine Boye

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Bacterial endometritis is an important cause of reduced fertility in mares and antimicrobial treatment is frequently used in equine reproductive practice. Knowledge of the bacterial populations present in the equine uterus and their antimicrobial susceptibility profiles is essential for evidence-based therapy. This study aimed to characterize bacteria isolated from uterine lavage samples collected from Danish mares prior to breeding and to evaluate antimicrobial resistance patterns among the most frequently recovered species.

Uterine lavage samples collected as part of routine pre-breeding examinations on Danish stud farms were cultured. Bacterial isolates were identified by MALDI-TOF M/S. Selected clinically relevant isolates, Streptococcus equi subsp. zooepidemicus, Streptococcus dysgalactiae subsp. equisimilis, coagulase-negative staphylococci, and Escherichia coli, were subjected to antimicrobial susceptibility testing by minimum inhibitory concentration determination.

A total of 192 bacterial isolates representing 53 species were recovered from 123 culture-positive uterine samples. The most prevalent species were S. equi subsp. zooepidemicus (18.8%), E. coli (16.7%), and S. dysgalactiae subsp. equisimilis (5.2%), all known pathogens. Streptococci constituted the dominant bacterial group overall, followed by staphylococci and Enterobacterales. Several infrequently reported eq-uine uterine bacteria were also identified, including Corynebacterium uterequi and Arcanobacterium hip-pocoleae, which have not previously been found in Denmark and whose pathogenic properties are un-certain. All streptococci were susceptible to penicillin, whereas 66% of the staphylococci were resistant. Some resistance to macrolides and tetracyclines was recorded. Among E. coli, 53% were resistant to aminopenicillins, followed by tetracyclines and trimethoprim-sulphonamide.

In conclusion: The uterine microbiota of breeding mares is highly diverse and includes both well-recognized opportunistic pathogens and several rarely described bacterial species. Antimicrobial resistance patterns differed substantially emphasizing the continued use of culture and susceptibility testing to guide treatment decisions and promote prudent antimicrobial use in equine reproduction.

[P047] Anti-methanogenic potential of Asparagopsis taxiformis and Asparagopsis armata extracts on rumen methanogen pure cultures

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Meeting an increasing demand for dairy products while minimizing the environmental cost of dairy farming has led to an increase in the research and development of additives with anti-methanogenic properties. Bromoform, a halomethane found in some seaweeds, has been shown to inhibit methane production in the rumen. There is little research on the differences in the anti-methanogenic properties of seaweeds processed using different methods. It is unclear how effective these different preparations are relative to pure bromoform, as preparations from seaweeds may also contain other compounds that could contribute to the products' methane-inhibiting properties. Differences in susceptibility to these products among rumen methanogen species are also poorly understood. This study compared the anti-methanogenic potential of two red algae species, Asparagopsis taxiformis (AT) and Asparagopsis armata (AA), when added to pure cultures of rumen methanogens. Water- and rapeseed oil-based extractions were prepared from freeze dried biomass of the two seaweeds. The bromoform content was measured, and the extracts were added to actively growing cultures of Methanobrevibacter ruminantium, Methanobrevibacter millerae, and Methanosphaera stadtmanae at two final concentrations of 0.9 µM and 1.8 µM of bromoform. Pure bromoform (BMF) mixed in water and oil were also used at the same rates to act as controls. Cell counts and headspace gas samples were collected every 2 days for 8 days to assess the impact of treatment on cell growth and methane production. We observed a near total reduction in methane production with most of the treatments ($p < 0.05$). M. stadtmanae, when treated with the AT, AA, and BMF oil extracts, did not exhibit as large a reduction in methane production as with the H2O extracts. We also observed a significant inhibition of cell growth under all treatments ($p < 0.05$), but again, except for M. stadtmanae when given the oil-based treatments. The results of this study show the H2O-based preparations of AT, AA, and BMF inhibited both the growth and the methanogenic capabilities of the three tested methanogens. Oil-based preparations, however, even when at the same concentration as H2O-based ones, were not always as effective, which presents a hurdle for the usage of oil-based extracts in research and commercial settings.

[P048] Novel fermentation for improved extraction yield and quality of microalgae proteins

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Novel fermentation for improved extraction yield and quality of microalgae proteins

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Green microalgae Chlorella, which contains 50 – 60% proteins and provides all essential amino acids, is considered a high-quality food protein source, but the application in foods is limited due to the lack of sustainable approaches for upscaling of Chlorella protein extraction and purification. Bacillus spp. that secrete extracellular amylolytic and proteolytic enzymes, exhibit many beneficial technological traits in traditional food fermentations. In this study, we investigate the potential of designed consortia of Bacillus species and lactic acid bacteria to improve the extraction yield, flavor and nutritional value of single cell proteins from microalgae Chlorella. We screened Bacillus strains using an in-silico genome analysis focusing on the potential for expression of carbohydrate-active enzymes (CAZymes) and proteases. B. sublitis FUA2114, B. velezensis FUA2155, and B. amyloliquefaciens FUA2008 were selected as promising candidates. We will select homofermentative and heterofermentative Lactobacillaceae strains that acidfy and express enzymes to metabolize the (poly)phenolic compounds andreduce lipid oxidation aldehydes Experiments will be performed to verify the ability of bacilli to enhance mechanical lysis of Chlorella and to release proteins from the cell and cell wall debris. Microalgae cell structure will be visualized using microscopy. Subsequently, we will employ LAB to acidify the Chlorella extracts and precipitate proteins, replacing precipitation operations such as heating and chemical acidification. The concentration and size distribution of proteins will be analyzed. We will monitor all starter/adjunct cultures during fermentation and determine profiles of fatty acids and phenolic compounds in all samples. Take together, this study will develop microbe-assisted sustainable bioprocesses to improve Chlorella protein extraction and facilitate application as food ingredients.

[P049] Targeted fermentation of side streams for optimizing their use as feed components

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The European Union imports around 40 million tons of feed annually, which is a direct challenge for sus-tainability and resilience of EU food supply. Further, various side streams, such as potato pulp, apple pom-ace, rapeseed cake, cheese whey and brewer's spent grain (BSG) represent underutilized resources that can be considered sustainable alternatives to partially replace imported feedstuff. However, impaired nutrient availability, high content of lignin and/or anti-nutritional factors (ANF) such as glucosinolates, phytic acid, fiber, glycoalkaloids, pectins, tannins and arabinoxylan, limit their direct use as feed components in particu-lar for monogastric animals like pigs. To meet this challenge, targeted fermentation, specifically solid-state fermentation, is a low-cost strategy with the potential to reduce ANF content and increase digestibility, nu-trient availability, and protein contents.

The present project, as part of the larger Horizon Europe project "NUTRIFEEDS", aims to design a sustain-able and reproducible fermentation strategy for selected side streams. The project focuses on screening lac-tic acid bacteria (LAB) and yeasts from local (Aarhus University and Freie Universität Berlin) strain collec-tions. The microorganisms will be characterized individually with respect to optimal growth conditions, or-ganic acid production, and ANF-degrading enzymatic capacities like expression of phytase, tannase, β-glucosidase and β-glucuronidase (to degrade phytic acid, tannins and glucosinolates in e.g., rapeseed cake), pectinase and B-glucosidase (to degrade pectin and polyphenols in e.g., apple pomace), xylanase and cellu-lase (to degrade arabinoxylan and fibre in e.g., BSG). Further, the nutritional cross-feeding mechanisms be-tween different LAB and yeast as well as their enzymatic complementary properties will be used to develop functional fermentation starter consortia. Two to five strains with complementary properties will be com-bined and tested for stability and will be applied to ferment selected side streams to test reproducibly across a range of process conditions. Finally, fermented products will be selected for an animal (pig) trial to inves-tigate impact on parameters like feed acceptance, performance, gut morphology and microbial ecology, and inflammatory markers

[P050] Antibiotic-resistance plasmids encode Evangelion, a widespread mobile antiphage defence system

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Plasmids are major drivers of antibiotic resistance, but whether they also shape bacterial susceptibility to phages remains unexplored. Here, we identify Evangelion, a single-gene anti-phage defence system encoded by a naturally occurring plasmid in methicillin-resistant Staphylococcus aureus (MRSA), where it protects against lytic Silviavirus phages. Evangelion is frequently associated with antimicrobial-resistance plasmids, many of which carry multiple resistance genes and signatures of horizontal transfer, linking plasmid-associated resistance with bacterial immunity. Evangelion proteins combine a conserved DUF4062/TIR-like core with diversified auxiliary regions, revealing an evolutionarily conserved defence architecture adapted to distinct phage environments. Genetic, structural and evolutionary analyses support a model in which Eva01 responds to intracellular phage replication-associated processes and couples activation of its DUF4062 effector to NAD^+ depletion and abortive infection. Distinct Evangelion lineages protect against different phage groups, suggesting that diversification of their auxiliary regions contributes to adaptation to distinct host-phage environments. Together, our findings define Evangelion as a family of mobile anti-phage systems and reveal that antimicrobial-resistance plasmids can couple antibiotic resistance with anti-phage immunity, potentially shaping bacterial susceptibility to both antibiotics and therapeutic phages.

[P051] Linking ecological interactions and community function in methane-fed microbiomes for water treatment

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Despite being fueled solely by methane, methane-oxidizing microbiomes (MOM) comprise diverse and interconnected microbial guilds whose ecological interactions remain poorly understood, limiting our ability to harness their potential for environmental applications. We combined multi-omics, ecological network analysis, and genome-scale metabolic modeling to investigate MOM during sulfamethoxazole (SMX) biodegradation under four environmental conditions.

A total of 91 high-quality metagenome-assembled genomes were recovered, revealing communities composed of methanotrophs, methylotrophs, and heterotrophic bacteria. SPIEC-EASI network analysis revealed distinct community interaction networks in SMX-exposed and unexposed microbiomes. Several taxa occupied central network positions, exhibiting high connectivity (mean degree 20-30) and elevated normalized centrality metric (0.3-0.7). Notably, distinct Hyphomicrobium species consistently exhibited the highest keystone scores despite low abundance, with SMX exposure selecting for a different dominant Hyphomicrobium species.

WGCNA analysis of 60 metatranscriptomes linked ecological interactions to functional activity. Methane oxidation and SMX degradation were associated with distinct transcriptional modules showing opposing correlation patterns, indicating functional specialization within the community. The module most strongly correlated with SMX removal contained genes involved in sulfonate transport (ssuA), RND efflux systems (acrA/mexA), and highly expressed SadA/B-like flavin-dependent monooxygenases. Integration of metabolomic and transcriptomic data linked the transformation product Ac-OH-SMX to the expression of these monooxygenases in Hyphomicrobium, providing a mechanistic connection between community structure and pollutant degradation.

Community metabolic models predicted extensive cross-feeding involving acetate, methanol, and fumarate exchange, with Hyphomicrobium emerging as a major consumer of metabolites supplied by methanotrophic taxa.

Together, these results identify low-abundance Hyphomicrobium species as recurrent keystone taxa linking methane-derived carbon flow to broader community function. Despite shifts in community structure following SMX exposure, key metabolic dependencies were maintained, indicating that pollutant transformation is supported by metabolically interconnected microbial guilds rather than individual taxa.

[P052] Sulfate and temperature shape denitrifying communities in cold bioelectrochemical systems

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Groundwater and agricultural drainage waters are major pathways for nitrate losses from intensive farming, yet their low organic-carbon content and low temperatures challenge conventional biological treatment. Hydrogenotrophic bioelectrochemical systems offer an attractive alternative by producing H_2 directly at the cathode to sustain autotrophic denitrification. However, sulfate, innocuous at typical levels, is also metabolized. Here, we show that sulfate is not a passive background ion, but a major ecological driver that selects the dominant denitrifiers and reshapes nitrogen metabolism in cold bioelectrochemical systems.

We investigated hydrogenotrophic denitrifying biofilms operated with and without sulfate at 10 and 4 °C and characterized their microbial communities using whole-metagenome sequencing. Fifty-six medium- to high-quality metagenome-assembled genomes were reconstructed, revealing distinct microbial niches driven primarily by sulfate availability and temperature.
At 10 °C, sulfate-rich conditions consistently selected for Sulfuricurvum sp. DTU53, which reached up to 32% relative abundance. Its genome encoded sulfur oxidation, assimilatory sulfate reduction, H_2 oxidation, nitrate reduction and rTCA-based carbon fixation, revealing a highly competitive chemolithoautotrophic strategy linking the sulfur and nitrogen cycles. Removing sulfate produced a strikingly different community. Janthinobacterium tructae DTU63 and Acidovorax sp. DTU11 became dominant, reaching 19.5% and 18.8% relative abundance, respectively. These taxa relied primarily on H_2 oxidation, nitrate respiration and Calvin-cycle carbon fixation. Sulfate exposure therefore shifted the system from nitrate respiration coupled primarily to H_2 oxidation toward a mixed sulfur- and hydrogen-based metabolism. Interestingly, in mature biofilms this ecological advantage came with a process-level cost: sulfate promoted H_2S accumulation, hindering H_2 uptake and denitrification. Total nitrogen removal rates declined from 0.069 to 0.041 kg-N·m^{-3}·d^{-1} at 10 °C and from 0.095 to 0.025 kg-N·m^{-3}·d^{-1} at 4 °C. Direct H_2S exposure also enhanced N_2O accumulation, reaching 3.3 mg-N·L^{-1} at 4 °C, although prior sulfate adaptation alleviated this effect.

Temperature imposed an additional selection pressure. At 4 °C, microbial diversity decreased markedly and Burkholderiaceae sp. DTU59 dominated the community at 35.4% relative abundance. Unlike the sulfur-specialized populations favored at 10 °C, this organism encoded complete denitrification together with broad carbon and energy metabolisms, suggesting that metabolic versatility becomes advantageous under cold, energy-limited conditions.

Together, these results reveal that sulfate availability and temperature do more than affect denitrification rates: they determine which organisms dominate, which electron-donor pathways are exploited, and how nitrogen and sulfur cycling become coupled. Understanding these ecological selection pressures is therefore critical for designing robust bioelectrochemical systems for nitrate removal from cold groundwater and agricultural drainage waters.

[P053] Temperate bacteriophage populations in the human gut: culturomics and experimental insights

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While often overlooked, the most abundant entity within the gut are the viruses that infect bacteria, called bacteriophages (phages). Large-scale metagenomic and data mining efforts have uncovered an expansive diversity of phages within the human gut. However, the functional understanding of phage-host interactions and their impacts within this complex ecosystem have been limited due to a lack of cultured isolates for experimental validation. In recent work, we characterise 182 active prophages originating from 252 diverse human gut bacterial isolates using diverse induction conditions, including chemical cues, metabolites, starvation, and human cell derivatives. We found that active Bacteroidota prophages were among the most prevalent members of the gut virome, with extensive use of diversity generating retroelements and exhibiting broad host ranges. Moreover, active polylysogeny was present in 58% of studied gut lysogens and led to coordinated prophage induction. Transcriptomic analysis of selected isolates revealed fundamental information on life cycles of gut phages, including the constitutive expression of defence and counter-defence genes across both polylysogenic phages and their hosts. In this presentation, I will summarise our recent and unpublished work into the study and use of experimentally validated gut prophages, providing key insights into their diversity, biology, genetics, and function within these complex microbial communities. More broadly, this presentation will highlight the importance of experimental validation alongside genomic based computational prediction to enable further functional understanding of these commensal viruses within the human gut.

[P054] Adaptive laboratory evolution of methanogenic cocultures in bioelectrochemical reactors

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Anaerobic digestion (AD) is widely used to convert organic waste into biogas, but the process is often limited by slow degradation of complex substrates, accumulation of volatile fatty acids, and low resilience to organic overloading. Microbial electrolysis cells (MEC) have been proposed to improve AD performance, by providing electrodes that may facilitate extracellular electron transfer coupled with more rapid organic matter degradation ultimately supporting downstream methanogenic degradation. However, the mechanisms underlying these improvements remain poorly understood.

In this project, we will use adaptive laboratory evolution (ALE) to investigate how defined methanogenic cocultures adapt to MEC-AD conditions. We expect that combining ALE with MEC technology will generate methanogenic communities with improved adaptation to electrode-assisted digestion and provide insight into the biological mechanisms underlying enhanced methane production.

[P055] Variation in tick biting frequency in humans and the role of skin associated microorganisms

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Ticks are important vectors of pathogens that can cause diseases such as Lyme borreliosis and tick-borne encephalitis in humans. Tick biting frequency varies considerably between humans despite similar exposure, suggesting that host characteristics may influence tick attraction and biting. Ticks use sensory cues to locate suitable hosts, including heat, carbon dioxide, movement, and odor. One potential factor influencing host attractiveness is the human skin microbiome. Skin associated microorganisms metabolize sweat and sebum and produce volatile organic compounds that contribute to individual odor profiles. While volatile organic compounds influence host attractiveness in other arthropod vectors, particularly mosquitoes, their role in tick host selection remains poorly understood.

This study investigates the potential role of skin associated microorganisms and their volatile organic compounds in tick host selection. Selected skin bacterial isolates will be investigated to characterize differences in their volatile organic compound profiles using gas chromatography-mass spectrometry. The bacteria will be cultured under conditions designed to mimic the skin environment. The resulting bacterial odor profiles will be investigated in relation to behavioral responses of nymphal Ixodes ricinus using olfactometer-based assays. Field collected ticks may additionally be screened for Borrelia burgdorferi to investigate whether pathogen status is associated with variation in behavioral responses.

By linking skin associated bacteria’s chemical profiles and tick behavior, this study aims to investigate differences in tick host selection and biting frequency.

[P056] Linking greenhouse gas emissions to bacterial communities in arctic and temperate wetlands

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Wetlands are among the largest natural sources and sinks of greenhouse gases (GHGs), yet the microbial drivers of CO_2, CH_4, and N_2O fluxes remain poorly understood across contrasting climatic and human disturbance settings.

In this project, we will combine in situ GHG flux measurements with bacterial community and functional profiling to investigate how microbial composition and function shape emissions along two distinct wetland system gradients. The first is a degradation gradient in Lille Vildmose, Denmark, which includes the largest intact raised bog in northwestern Europe. The second is an elevation and moisture gradient across pristine wetlands in Narsarsuaq, Greenland. Together, these two wetland systems provide a strong contrast in both climate and human influence, allowing us to investigate how the bacterial communities and GHG emissions respond to different environmental and disturbance conditions

GHG fluxes are measured using LI-COR portable gas analysers, paired with abiotic soil properties (pH, organic matter, water table depth, nutrient content) and continuous microclimate logging of surface and belowground temperatures. Bacterial community composition will be assessed using metagenomic sequencing, enabling taxonomic and functional profiling to be linked with the in situ fluxes of the measured GHGs.

By combining flux and community data across two climatically distinct gradients, we aim to identify whether shared or divergent microbial species compositions underlie GHG variation in arctic versus temperate wetlands, thereby contributing to an overall better understanding of wetland-climate feedbacks and disentangling the effects of human influence on the wetland microbial communities.

[P057] Baiting Microbes with Metal-Organic Frameworks (MOFs): Can MOFs enhance methanogenesis in Anaerobic Digestion?

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Anaerobic digestion (AD) is an important bioprocess for energy recovery through methane production from organic waste sources. AD is dependent on tightly coupled communities of methanogens and syntrophic microorganisms. However, these thermodynamically driven bioprocesses can be limited by inadequate interspecies electron transfer (IET), leading to metabolite accumulation and reduced substrate conversion. Conductive materials are promising strategies to overcome energetic bottlenecks in complex microbial consortia by facilitating direct interspecies electron transfer (DIET), thus accelerating this type of syntrophic metabolism and consequently organic matter degradation. Metal-organic frameworks (MOFs) offer redox-active Fe2+/Fe3+ sites, tunable porous structures that may promote microbial attachment while facilitating electron transfer. When functionalized with magnetite (MOF@Fe), these materials can be magnetically captured, enabling selective recovery and enrichment of AD-beneficial syntrophs from complex communities. Here, we studied whether these functionalized MOFs could enhance metabolism and attachment of the DIET consortium: Geobacter metallireducens - Methanosarcina barkeri. MOF@Fe was synthesized solvothermally and characterized using spectroscopic and microscopic methods. Addition of MOF@Fe (0.5 g/L) to the co-culture showed an increase in methane production rate (0.04 ± 0.01 mM/day; n=3), compared to 0.0071 ± 0.001 and 0.0010 ± 0.0008 mM/day with magnetite and control incubations without MOF, respectively. After only 24 hours, microbial attachment to MOF@Fe was 2.6-fold higher (0.616 ± 0.044 mg/mL) than on magnetite alone (0.233 ± 0.056 mg/mL, $p<0.001$), as determined by protein quantification. Furthermore, MOF@Fe's (0.5 g/L) performance was tested on digestate communities from AD treating agricultural waste. Methane production from digestate organic matter increased 3-fold ($p < 0.001$) (4.6 mM ± 0.3; n=4), as compared to magnetite (1.6 mM ± 0.16) and control incubations without MOFs (1.5 mM ± 0.6) in 10 days. These results demonstrate that MOF@Fe enhances cell attachment while promoting methanogenesis. The effects persist in a complex digestate system, highlighting the application of MOFs as a functional material to enrich functionally relevant microorganisms from AD systems.

[P058] Linking Methanogen Community Composition to Substrate-Specific Activity in Pig Slurry Using ^{13}C Tracing

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Methane emissions from stored pig slurry are produced by methanogenic archaea using a restricted range of substrates, with acetate and CO_2/H_2 representing major precursors. Measurements of methanogen community composition can identify which methanogens are present but provide limited information about their contribution to methane production. We combined stable-isotope tracing with mcrA amplicon sequencing and absolute mcrA quantification to relate methanogenic community structure to substrate-derived methane formation in pig slurry.

Pig slurry from eight Danish farms was incubated under controlled conditions and amended with either ^{13}C-labelled or natural-abundance acetate and bicarbonate. Methane production and isotopic composition ($^{12}CH_4$ and $^{13}CH_4$) were monitored repeatedly using a Picarro G2201-i analyzer, and $^{13}CH_4$ enrichment was used to trace methane formation from acetate-derived carbon and dissolved inorganic carbon. Methanogenic communities were characterized by mcrA amplicon sequencing, while total mcrA abundance was quantified by ddPCR.

Methanogenic activity differed markedly among farms, with mean methane production rates ranging from approximately 6.86 to 283 nmol CH_4 min^{-1} for equal incubation volumes. Initial mcrA abundance varied by approximately 20-fold, and the relative abundance of major methanogenic taxa differed among slurry samples. The $^{13}CH_4$ responses to ^{13}C-acetate and ^{13}C-bicarbonate also differed among farms, indicating differences in substrate-specific methane formation.

Combining methane production, ^{13}C tracing and quantitative microbial analyses provides a framework for relating methanogenic community composition to the processes contributing to methane production. Following isotope mass-balance correction, the approach may enable estimation of the relative contributions of major methanogenic pathways in pig slurry.

[P059] Importance of Adhesion in interaction with host and environment: identification of molecular determinants of attachment in O. anthropi ATCC49188

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Adhesion, surface colonization, and biofilm formation are key determinants of bacterial interactions with the environment and host organisms. In the environment, attachment enhances survival in diverse niches by protecting from desiccation, mechanical damage, and harmful chemical agents, including antibiotics. Simultaneously, adhesion is the initial stage of bacterial association with a host, whether for symbiotic, commensal or pathogenic bacteria. During infection, subsequent attachment and biofilm formation within the host further enhance bacterial persistence by promoting evasion of host immune responses.

Our study aims to identify the molecular factors underlying bacterial attachment to diverse surface types. Specifically, we aim to investigate the similarities and differences in the mechanisms underlying colonization of biotic surfaces, such as plant tissues, and abiotic surfaces, including artificial materials.

As a research model, we selected Ochrobactrum anthropi ATCC 49188, a bacterium that naturally occurs in soil but can also act as an opportunistic pathogen associated with nosocomial infections. Even though it is considered low virulent, O. anthropi is capable of colonizing medical devices, including catheters or infusion tubing. Our preliminary data indicate that O. anthropi can also efficiently attach to plant tissue, particularly tomato plants' roots. These characteristics make this species a suitable model for investigating the molecular mechanisms underlying bacterial adhesion to diverse surface types.

Our study aims to contribute to a better understanding of the mechanisms underlying bacterial adhesion in both clinical and environmental contexts.

To identify genes associated with bacterial adhesion, we employed two complementary approaches. Targeted mutagenesis was used to generate a deletion mutant in a gene predicted to be involved in polysaccharide biosynthesis. We assessed the ability of the mutant to attach to polystyrene plates, glass, infusion tubing, and plant roots under different experimental conditions. In parallel, a transposon mutant library was generated and is currently being screened to identify novel genes involved in bacterial adhesion.

[P060] Can HGT lower mutational thresholds for environmental adaptation?

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Previous work has shown that mutualistic cooperation between two auxotrophic strains is beneficial in stable environments, however, this cooperation collapses when exposed to environmental stress such as antibiotics. This suggests a penalty associated with cooperation. In a microbial community, a strain that is dependent on another strain e.g. via auxotrophy, must both, adapt to the stressor itself, but also wait for the cooperating strain(s) to adapt, compared to if the strain was independent, it would only have to adapt once, and it would not be reliant on others. When this adaptation requires genetic mutation, the time for adaptation would be significantly longer. Our hypothesis is that if the genes for adaptation are carried on an MGE, then this could be shared within the cooperation network, compared to each network member needing to adapt independently, reducing the time required for the network to adapt. This could remove the penalty associated with cooperation under stress.

To study this, we are using a non-functional antibiotic resistance gene whose function can be restored via random genetic mutation and placing it on the chromosome, an immobile, or mobile plasmid. We grow the cells in sublethal concentrations of antibiotic creating a selective pressure for them to mutate. We use the time until the emergence of functional resistance as a metric for adaptation, and we compare how the location of the non-functional resistance gene affects the ability of mutually cooperating strains to adapt, compared to independent strains. We are currently still developing this assay and trying to get resistance to emerge in a predicable manner. Once developed, we would expect resistance to develop faster on a plasmid, and for the cooperating strains to outperform the non-cooperating strains when the resistance is on an MGE, similar to the observations in a stable environment. This could support the notion that MGEs, such as plasmids, play a critical role in stabilising mutually beneficial interactions between species in bacterial communities.

[P061] Lactose enhances N-acyl amino acids formation by gut microbiota

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Could lactose be more than a carbon source for gut microbiota, potentially contributing to the formation of metabolites involved in host appetite suppression? Recent studies identified N-lactoyl phenylalanine (Lac-Phe), a conjugate of the short-chain carboxylic acid (SCCA) lactate and the amino acid (AA) phenylalanine, as a bioactive metabolite associated with appetite regulation and energy balance. Whether dietary carbohydrates such as lactose enable gut microbiota to form Lac-Phe and related N-acyl amino acids (N-acyl AA) from fermentation-derived SCCA and AA remains poorly understood. Fecal batch fermentations were supplemented with unlabeled or 13C-labeled lactose. We traced the integration of lactose-derived 13C into SCCA and microbial biomass using UPLC-QTOF-MS and RNA stable-isotope probing.

N-lactoyl, N-acetyl, and N-butyryl AA were detected in fermentations with or without lactose with temporal changes in N-acyl AA pattern during incubation, highlighting the general capacity of gut microbes to form these metabolites. Lactose supplementation increased overall N-acyl AA levels. Lactose-derived 13C was incorporated into lactate at 12 h, and the signal disappeared by 24 h. Similarly, M+3 isotopologues of L-lactoyl valine, L-lactoyl leucine, and L-lactoyl phenylalanine were mainly detected at 12 h. At 24 h, 13C was recovered in acetate and butyrate, and in N-acetyl leucine and N-butyryl leucine. RNA-SIP demonstrated incorporation of lactose-derived carbon into microbial biomass. 13C-labeled community members included lactose- and lactate-utilizing taxa such as Bifidobacterium, Collinsella, and Anaerostipes.

Together, this study shows that lactose could be metabolized into N-acyl AA. Temporal shifts in N-acyl AA pattern suggested that formation followed the production of SCCA precursors. Lactose fermentation supported the formation of N-lactoyl AA, pointing to a previously unexplored aspect of lactose metabolism in the gut.

[P062] Interactions between gut microbiome and immune system markers during aging in mice.

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The gut microbiota comprises a diverse community of microorganisms residing in the gastrointestinal tract that play important roles in host metabolism and immune regulation. The composition of gut microbiota and immune system biomarkers both change with age, yet the contribution of gut microbes to systemic immune ageing remains incompletely understood.

We longitudinally followed germ-free (GF) and conventionally colonized (CONV) mice from birth to 15 months of age. Gut microbial community dynamics were assessed using full length 16S rRNA gene sequencing and circulating immune proteins were quantified in serum using Olink Target Mouse 48 cytokine panel. Associations between age-related microbial composition alterations and changes in immune markers were evaluated using longitudinal statistical modelling.

Age was associated with substantial restructuring of the gut microbiome, with numerous microbial taxa showing significant age-dependent dynamics, such as a decline in Desulfovibrio and A. municiphila Immune ageing trajectories differed markedly between colonization groups: age-related changes in circulating cytokines were generally more pronounced in CONV mice, whereas GF mice displayed flatter immune profiles. This was especially evident for IL-17a and IL-17f, as well as IFNγ, INF-λ2 and IFN-l2, exhibiting minimal expression in GF mice. Several immune markers showed strong associations with microbial community structure, with IFN-λ2 emerging as a prominent microbiome-associated cytokine.

Together, these findings demonstrate that microbial colonization is a major determinant of systemic immune ageing and reveal coordinated changes between gut microbial communities and circulating immune signals across the lifespan. The identified microbiome-immune associations provide candidate targets for future mechanistic studies.

[P063] Expression of Cytochromes in Geobacter metallireducens Change in Response to Shifting Electrode Potential

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Since its discovery in the late 20th century, the bacteria Geobacter metallireducens has been investigated for its remarkable ability to produce electrical currents and respire on insoluble minerals and metals in a process known as extracellular electron transfer. Yet, many aspects of how this ability is performed remain unexplored.

In this project, we aim to investigate the proteomic change occurring when G. metallireducens grows on different electrode redox potentials in order to explore which proteins are important under which potentials.

After growing G. metallireducens on four different electrode redox potentials (+300 mV, +100 mV, -100 mV, and un-poised), we analyzed the G. metallireducens biofilm using voltametric methods to determine any change occurring at the cell surface redox potential. Afterwards, the proteins from attached cells were harvested and subsequently analyzed using mass spectrometry. We decided to focus our analysis primarily on the differential expression between cytochromes since these are the main proteins known to be involved in extracellular electron transfer.

We observed that the largest change in cytochrome expression occurred at the periplasmic level. Additionally, we observed that some inner membrane cytochromes showed a similar pattern of regulation as previously observed in the more well studied G. sulfurreducens. Finally, we found several cytochromes unique to G. metallireducens, which were differentially regulated in response to changing electrode potential.

Together, these results indicated that G. metallireducens had a similar response to changing extracellular redox potential compared G. sulfurreducens while also possessing some unique adaptations. Additionally, the results indicated that the main cytochromes regulated by extracellular electron acceptor potential were located in the periplasm.

[P064] Development of a quorum sensing reporter strain in an industrial anaerobic digestion biofilm community

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Bacteria coordinate their collective behavior by communicating with each other through quorum sensing (QS). In anaerobic digestion (AD), where thousands of species interact in an industrial setting, QS plays a role in microbial aggregation into biofilms or granules. However, it is still poorly understood which microorganisms drive aggregation, what triggers their activity, and when during biofilm formation QS-mediated interactions are the most important. Understanding the underlying mechanisms that drive aggregation will allow improved stability and efficiency of anaerobic digestion and other industrial processes that rely on microbial biofilm formation.

We present our progress towards developing QS reporter strains from native anaerobic sludge derived bacterial isolates. This procedure includes characterizing oxygen-independent alternatives to GFP that can be used in situ in the reactor biofilm community, as well as characterizing community interactions between isolated strains to identify promising candidates to serve as the reporter chassis. Immersing the reporter strain in the community allows for in situ measurements in anaerobic biofilms that lets us gain knowledge about the dynamics and importance of QS in AD. The ultimate aim is to then use this knowledge to speed up or enhance microbial aggregation. By understanding when and where QS activity occurs during biofilm formation, we aim to identify the role of QS in microbial aggregation and determine whether it can be leveraged to promote biofilm formation in AD.

[P065] Soil contaminants as drivers of emerging antibiotic resistance in natural environments

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Antibiotic resistance is increasingly recognised as an environmental as well as a clinical challenge. Soil microbial communities contain diverse antibiotic resistance genes and represent potential reservoirs from which resistance determinants may persist, spread, or enter human-associated environments. However, the environmental factors shaping the soil resistome remain incompletely understood.

This project investigates whether anthropogenic contaminants and agricultural management practices influence antibiotic resistance in soil microbial communities. Soils representing different environmental conditions and management histories are examined using complementary microbiological and multi-omics approaches. These include long-read metagenomic sequencing to characterise resistance genes, their microbial hosts and genomic context, metaproteomics to investigate expressed microbial functions, and measurements of microbial activity and biomass. Selected experimental systems are additionally used to examine microbial responses to defined environmental pressures.

By integrating information across genomic, proteomic and community-level measurements, the project aims to distinguish changes in the genetic resistance potential of soil communities from broader effects on microbial activity and function. Particular attention is given to identifying environmental conditions that may favour the persistence, enrichment or expression of resistance determinants and to understanding how these responses relate to the surrounding microbial community.

[P066] Genomic Potential versus In Vivo Valerate Production by Gut Megasphaera

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Keywords: Megasphaera; valerate; gut microbiome; reverse β-oxidation; metagenome-assembled genomes; short-chain fatty acids; odd-chain elongation

Valerate is a short-chain fatty acid (SCFA) with potential health-promoting effects. However, its microbial production and pathway in the human gut remains poorly understood. This study investigated Megasphaera, a known valerate producer, and its contribution to valerate production through odd-chain elongation via the reverse β-oxidation pathway (r-BOP).

We integrated 16S rRNA gene amplicon sequencing data, SCFA measurements, and donor-derived Megasphaera metagenome-assembled genomes (MAGs) from faecal samples collected from 19 healthy donors across seven sampling days. Two representative MAGs (rMAGs) were obtained and screened for genes associated with r-BOP. Associations between microbial relative abundance and valerate concentrations were evaluated using Spearman correlations in 102 samples.

Complete r-BOP gene sets were found in both rMAGs, indicating genomic potential for odd-chain elongation and valerate production. Amplicon sequence variant (ASV) level analysis showed donor specific dominance of M. elsdenii-like or M. massiliensis-like lineages. However, Megasphaera abundance was not significantly positively correlated with valerate concentrations at either genus or ASV level. In contrast, Coprococcus showed a significant positive association with valerate concentrations (ρ=0.49, p=1.3·10^(-7))

These findings demonstrate that genomic potential alone does not predict in vivo metabolite production. Although donor-derived Megasphaera populations showed genomic potential for valerate production, they were not identified as primary predictors of valerate concentrations. Instead, valerate production might rely on environmental conditions, microbial interactions, or greater community-level processes. Coprococcus is a promising candidate for further genome-resolved and experimental investigation.

[P067] Can Metagenomes Predict Peatland Restoration Success?

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Can Metagenomes Predict Peatland Restoration Success?

Drained peatlands are a disproportionately large source of greenhouse gas emissions, when considering the area they cover. Peatlands restoration is therefore an important strategy for reducing greenhouse gas emissions and recovering degraded ecosystems, but restoration outcomes remain highly variable and difficult to predict. Microbial communities play a central role in peatland carbon cycling through processes such as methane production, methane oxidation, and organic matter decomposition (peat), making them a potential source of information about ecosystem recovery.

This project investigates whether metagenomic data can be used to predict peatland restoration success. Using metagenomes from Danish peatlands eg. Store- and Lille Vildmose, we will characterise the microbial community compositions and functional potential across sites in different states of restoration. Particular attention will be given to microbial taxa and metabolic pathways involved in carbon and methane cycling. These microbial features will be integrated with actual environmental and greenhouse gas measurements using statistical modelling to identify signatures associated with successful restoration.

A central aim is to determine whether restored sites increasingly resemble high-quality reference peatlands at the microbial level, and whether this similarity can provide a simplified indicator of restoration. The project will provide a preliminary framework for linking peatland microbiomes to ecosystem function and may contribute to future tools for evaluating and predicting restoration outcomes from metagenomic data.

[P068] Low-abundance microbiome members support essential catabolic functions in syngas biomethanation

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Mixed microbial consortia have been widely applied in waste-to-energy, thanks to their numerous advantages. It is commonly observed that many members of these communities lose certain biosynthetic capabilities and become auxotrophic during long-term evolution in environments where essential metabolites are readily available. As a result, these auxotrophs typically depend on metabolic partners for the supply of metabolites they can no longer synthesize. We investigated auxotrophy and the resulting metabolic exchange in a microbial consortium performing syngas biomethanation. Reconstructing the metagenome assembled genomes (MAGs) of the community showed functional redundancy among the main microbial guilds involved in syngas biomethanation, a feature commonly associated with community resilience against environmental disturbances. Evaluating the completeness of amino acid and vitamin synthesis pathways revealed predicted auxotrophy for multiple vital compounds among these guilds. Looking at the broader community, we identified several MAGs that, despite their low relative abundance, retained substantially more complete amino acid and vitamin biosynthetic pathways than dominant catabolic guild members, suggesting that rare taxa may contribute disproportionately to essential biosynthetic functions. These findings indicate the presence of a mutually beneficial metabolic interdependence. Dominant carbon-catabolizing community members are predicted to rely on low-abundance members for biosynthesis of essential amino acids and vitamins while supporting them by driving the carbon flow through the community. Further, considering that all low abundance community members are also auxotrophic to some extent, these findings suggest that the interaction space within these anaerobic communities is exceptionally vast and likely contributes to their metabolic versatility and resilience.

[P069] Extracellular G-quadruplex RNA promotes the formation of electroactive syntrophies

Chiara Di Mattia[1], Rhitu Kotoky[1], Konstantinos Anestis[1], Amelia-Elena Rotaru[1]

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Direct interspecies electron transfer (DIET) enables syntrophic microorganisms to exchange electrons without diffusible carriers, yet the factors governing the establishment of electroactive partnerships remain poorly understood. Recently non-canonical RNA structures (G-quadruplexes) were shown to increase electron transfer in monoculture biofilms(1), and in the methanogen Methanosarcina barkeri when grown on cathodes(2). In this work we investigated whether extracellular G-quadruplex-forming RNA (G4-RNA) influences DIET between Geobacter metallireducens and Methanosarcina barkeri. Cocultures were supplemented with synthetic G4-RNA at different concentrations and evaluated using methane-production kinetics, microscopy, and transcriptomic analysis. G4-RNA accelerated early coculture establishment in a concentration-dependent manner, increasing methane-production rates by up to 25-fold and shortening the apparent lag phase. In contrast, only modest effects were observed in monoculture controls, indicating that the strongest response was associated with syntrophic growth. RNA-treated cocultures formed larger and more abundant aggregates. Transcriptomic analysis further revealed extensive physiological responses in both partners: G. metallireducens altered expression of genes related to extracellular electron transfer, membrane transport, and redox metabolism, while M. barkeri strongly upregulated proteins involved in surface rearrangements and methane metabolism. These findings show that extracellular G4-RNA promotes the early establishment of DIET-based syntrophy and suggest that extracellular RNA can influence microbial aggregation, electron transfer, and methane production.

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[P070] Temperature-dependent host–parasitoid interactions in Rhopalosiphum padi: the role of the defensive endosymbiont Hamiltonella defensa

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Aphids are cosmopolitan economically important agricultural pests, which frequently maintain mutualistic associations with endosymbiotic bacteria. Control of these pests is becoming increasingly challenging due to withdrawal of, and development of resistance towards, several synthetic insecticides. The cereal pest Rhopalosiphum padi can be controlled by parasitoid wasps such as Aphidius colemani. However, aphid-associated defensive symbionts, including Hamiltonella defensa, may alter susceptibility to parasitoid attack. Temperature and other environmental variables may further influence this host–symbiont–parasitoid interaction. This project aims to investigate how H. defensa responds to a parasitoid challenge at different temperatures, and whether symbiont dynamics are associated with host susceptibility to parasitism. R. padi will be exposed to A. colemani at different temperatures, and parasitism outcome and post-exposure fecundity will be monitored. Quantitative PCR will be used to assess changes in H. defensa abundance following parasitoid exposure. The study provides a model for studying host–microbe–parasite interactions, and how environmental conditions shape symbiont-mediated protection. By combining molecular measurements with parasitism and reproductive fitness outcomes, the project may contribute to a broader understanding of microbial protection, host susceptibility and host-associated microbiome dynamics.

[P071] Electrochemical stimulation of digestate microbiomes to enhance organic matter degradation and methane recovery

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Anaerobic digestion (AD) is a microbially driven process that converts organic matter from waste streams into biogas, typically containing 50–70% methane (CH_4). In addition to biogas, AD produces a residual liquid/solid fraction known as digestate, which can retain a substantial fraction of the original organic carbon (30-55%) undigested as less readily degradable compounds. This remaining organic carbon represents unrecovered biomethane potential and may contribute to greenhouse gases and eutrophication if further disposed on agricultural fields as fertilizer.

For a long time, digestate has primarily been regarded as a residual product and fertilizer, but emerging technologies offer opportunities to recover additional energy and value from its remaining organic matter. Microbial electrolysis cells (MECs) have emerged as a promising green technology for enhancing the degradation of organic compounds while enabling production of hydrogen, CH_4 or other value-added products[1].The integration of MECs with AD (MEC-AD) can enhance CH_4 production and organic carbon removal compared with conventional AD[2]. These improvements are often associated with the enrichment and activity of electroactive microorganisms on electrode surfaces.

In my project, I aim to investigate how electrochemical stimulation affects various digestate microbiomes from different anaerobic digesters in Denmark, with the goal of increasing CH_4 recovery from digestate by stimulating electroactive microorganisms to oxidize residual organic matter.

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[P072] Antimicrobial Resistance and Resistome Profiling in Wild Brown Bears (Ursus arctos) from Greece

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Background and Objectives:
The brown bear (Ursus arctos) can serve as a valuable marker of antimicrobial resistance (AMR) spread given its wide-ranging behavior and opportunistic feeding. The aim of this study was to evaluate AMR in brown bear fecal samples from Greece, using both phenotypic and genotypic approaches.

Material and Methods:
Between 2022 and 2023, 24 fecal samples were non-invasively collected from three well-known brown bear habitats in Greece. Samples were cultured on chromogenic media targeting ESBL-producing Enterobacteriaceae, CRE and VRE. Samples were also inoculated onto MacConkey agar and one colony per sample presenting E. coli-like morphology was selected for identification and antimicrobial susceptibility testing. A subset of 11 samples underwent metagenomic analysis. Sequencing was conducted on an Illumina® NextSeq 2000 platform and assembly and annotation were performed using SqueezeMeta. Antibiotic resistance genes (ARGs) were identified using Resistance Gene Identifier against the CARD database.

Results:
Chromogenic media resulted in the isolation of one E. coli and one Klebsiella oxytoca isolate, both demonstrating intermediate resistance to imipenem. Additionally, three VRE isolates (E. gallinarum and E. faecalis) were identified. All 24 E. coli isolates exhibited resistance to cefalexin; however, no ESBL phenotype was detected. Additional resistance was observed to ampicillin, enrofloxacin and tetracycline. ARGs were identified in all samples tested. The total number of ARGs ranged from 60 to 310, while the number of unique ARGs varied from 45 to 80. The most prevalent genes were vanY, CRP, emrR, acrA, and mdtE. Efflux genes and genes conferring resistance to beta-lactams, tetracyclines and quinolones were also detected.

Conclusion:
Phenotypic profiles confirmed AMR beyond first-line antimicrobials, while resistome profiling revealed a broader latent resistance potential. Variability in ARG abundance and diversity suggests that environmental factors influence resistome composition. Our study highlights the importance of a One Health approach in addressing antimicrobial resistance.

[P073] Vagina-on-a-chip: The effects of Lactobacillus crispatus and Gardnerella vaginalis on ectocervical and vaginal cells

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The vaginal microbiome is important for reproductive health, and disruptions in its balance have been linked to infertility. Vaginal dysbiosis, characterised by reduced Lactobacillus species and increased pathogenic bacteria have been shown to be suggested to contribute to adverse reproductive outcomes.

Lactobacilli help maintain vaginal health through the production of lactic acid, which lowers vaginal pH and inhibits the growth of pathogens such as Gardnerella vaginalis (G. vaginalis), a key contributor to the development of bacterial vaginosis. To investigate host-microbe interactions within the female reproductive tract, two models will be established. Both models will consist of a PET membrane flanked by vaginal and ectocervical cells. The first model will be a vagina-on-a-chip platform, with continuous flow, whereas the second will be a conventional static transwell model. The aim of this project is to evaluate the effects of live bacteria (G. vaginalis and L. crispatus) and Lactobacillus-derived cell-free supernatants on epithelial colonisation, host-response and metabolite production in both models. Bacterial adhesion and colonisation will be quantified using colony-forming unit (CFU) enumeration and quantitative PCR (qPCR). Then, transcriptomics on the host epithelial cells will be conducted to identify genes and pathways that are upregulated or down regulated following bacterial exposure. Finally, 1H NMR will be used to detect and quantify metabolites produced by Lactobacillus spp. including lactic acid and hydrogen peroxide but also metabolites produced by G. vaginalis. The NMR data will also be used to identify other potential biomarkers using untargeted metabolomic procedures.

Through this project, we aim to provide mechanistic insights into host-microbiome interactions and contribute to developing targeted microbiome-based therapies for vaginal dysbiosis as alternatives or complements to antibiotics.

[P074] The investigation of syntrophic partnerships between electrogenic bacteria and methanogens in bioelectrochemical system for methane production

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Conventional anaerobic digestion (AD) often leaves a large amount of high-organic-content in the digestate, resulting in lost biogas potential and an increased risk of greenhouse gas (GHG) emissions into the atmosphere. Microbial electrochemical cell – Anaerobic digestion (MEC-AD) systems enhance methane production and high-organic-content degradation by driving the microbial activity. In such systems, the presence of electrodes may reshape syntrophic partnerships between electrogens and methanogens and enable more methane production from organic substrates. Electrogenic bacteria can convert more effectively complex organic substrates to volatile fatty acid (VFAs), carbon dioxide, hydrogen or electrons, which serve as materials for biomethane production by methanogens. This study investigates the syntrophic partnerships of different electrogenic bacteria and methanogens in single-chamber MEC reactors, poised at different anode potentials. The performance of the co-cultures is assessed through gas production, total organic carbon removal, VFA profiles, and current generation. The study is expected to explore how syntrophic partnerships respond to anodic potentials and MEC-AD operating conditions, contributing to the optimization of the system performance.

[P075] In vitro comparison of water and oil extracts of Asparagopsis taxiformis and A. armata with synthetic bromoform for methane mitigation

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During the last decade, anti-methanogenic feed additives have been extensively investigated to reduce enteric methane emissions from dairy cows. The red seaweeds Asparagopsis taxiformis (AT) and Asparagopsis armata (AA) contain bromoform, a potent methane-mitigating compound. However, seaweed harvesting, cultivation, and processing are labour intensive. This study investigated whether bromoform could be extracted from AT and AA and whether water- or oil-based extracts differed in methane-mitigating efficacy. Freeze-dried biomass of AT and AA was extracted in water or rapeseed oil at a 1:20 ratio, and bromoform concentrations were quantified by GC-MS. The extracts were diluted, and 1 mL of each extract was added to Durun bottles containing 0.5 g of freeze-dried corn silage and 90 mL of buffered rumen fluid to achieve final bromoform concentrations of 0.9 mM (low) or 1.8 mM (high), resulting in eight seaweed extract treatments. In addition, synthetic bromoform was dissolved in water and rapeseed oil to achieve the same low and high bromoform concentrations and added to corn silage and buffered rumen fluid, resulting in four synthetic bromoform treatments. An ANKOM pressure sensor module (ANKOM Technology, Macedon, NY, USA) was mounted on each bottle to monitor gas production, with gas collected in air-tight gas bags during the 24-h incubation. Pure corn silage supplemented with 1 mL of either water or rapeseed oil served as negative control treatments. Data were analyzed separately for water and oil extracts. For the water extracts, total gas production was affected by treatment ($P = 0.03$), with values ranging from 103 to 124 mL/g DM. Methane production was significantly reduced by treatment ($P < 0.001$), with the greatest reductions observed for high AT and both syn-thetic bromoform treatments, whereas high AA resulted was intermediate. High bromoform al-most completely inhibited methane production. For the oil extracts, total gas production tended to be affected by treatment ($P = 0.08$), whereas methane production was significantly reduced ($P < 0.001$). High AA and high bromoform resulted in the lowest methane production, with synthetic bromoform showing the greatest inhibition. In conclusion, bromoform extracts from AT inhibited methanogenesis more effectively than those from AA, whereas synthetic bromoform was more effective than extracts from either seaweed species.

[P076] Brown juice as cultivation medium for lactic acid bacteria

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Green biorefineries extract food-grade proteins from green biomass such as grass and clover. The carbohydrate-rich by-product of green biorefinery, brown juice, has so far had limited use beyond that of fertiliser or biogas feedstock. With the overall goal to establish the suitability of brown juice as cultivation medium for lactic acid bacteria, this study investigates the production of short-chain carboxylic acids (SCCAs) by single strains and synthetic consortia. SCCAs confer antimicrobial activity and prevent biofilm formation in food and other biotechnological systems LAB were grown in unprocessed brown juice in Hungate tubes and lab scale bioreactors. The used brown juice had a pH 5.5 and contained around 60-80 mM glucose, 40 mM fructose and 10 mM arabinose. The native microbiota consists of spore-forming Bacillus spp. such as Bacillus licheniformis, Niallia circulans, Heyndrickxia coagulans, Cytobacillus kochii. Added strains of Liquorilactobacillus, Latilactobacillus, and Lactococcus utilized the present sugars and produced mainly 110-180 mM lactate, 20-50 mM acetate and 5-95 mM ethanol in unprocessed brown juice. Propionate levels did not exceed 1 mM. Bacillus spp. growth was inhibited due to the decrease of pH in the early stage of fermentation. This study contributes to the development of sustainable bioprocesses for agricultural side-stream valorisation, aligning with circular bioeconomy principles. The results indicate that brown juice is a promising substrate for SCCA production using lactic acid bacteria alone or as co-cultures.

[P077] Unmasking the Missing Methanogens: High-Quality MAG Recovery from Danish Peatland Restoration Sites

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Peatlands store a large share of the Earth's soil carbon, but decades of drainage for agriculture have shifted these ecosystems from carbon sinks to net greenhouse gas (GHG) sources. To reduce GHG emissions, the Danish Green Tripartite aims to restore 140,000 ha of drained peatlands by rewetting. Rewetting could significantly reduce CO2 emissions but must be approached carefully due to the risk of unwanted methane emissions during restoration – a greenhouse gas that is 28x more potent than CO2. To effectively implement restoration by rewetting and to track its progress, it is important to characterise the microbial communities driving carbon cycling in natural and drained peatlands at the species level. As such, building a peatland-specific and curated microbial high-quality genome database is fundamental for long-term restoration monitoring, especially for those microbes generating methane (i.e., methanogens). This additionally allows for linking microbial community composition to restoration outcomes. This project targets the recovery of taxa central to carbon cycling in peatlands by applying long-read sequencing and bioinformatic approaches to generate high-quality metagenome-assembled genomes (MAGs) from Danish peatland restoration sites.

[P078] Exploring the relationship between tick bite and the skin microbiome diversity

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The tick population of Ixodes ricinus is growing in the northern hemisphere, which is expected to lead to an increase in tick-borne diseases (TBD). Lyme borreliosis is the most common TBD, caused by the pathogen Borrelia burgdorferi sensu lato. It is unknown why individuals with mutual tick exposure experience different amounts of tick bites.

Research has shown that the skin microbiome influences the production of compounds that attract arthropods. This suggests that the skin microbiome could contribute to variation in tick attraction.

This study aims to investigate the role of the skin microbiome in the risk of tick bites as well as compare the potential effect the skin microbiome has on Borrelia ssp. disease development. Skin swabs will be sampled from the knee hock and the elbow joint from individuals who experience frequent versus few tick bites, and the samples will be compared. As well as samples from individuals with an active tick bite.

The samples will be analyzed using 16S rRNA gene sequencing from the V1-V3 region and the samples will be cultured on blood agar and anaerobic blood agar to investigate which bacteria are viable and growth pattern.

We expect to see a difference in the skin microbiome diversity in individuals who experience many tick bites compared to ones who experience few to no tick bites when exposed to the same environment. Suggesting that the skin associated microorganisms contribute to the tick attraction of the host. We also expect to see a change in the skin microbiome following tick attachment and/or Borrelia spp. compared to no tick attachment. '

[P079] Quorum-Sensing-mediated biofilm formation in anaerobic microbial communities

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Biofilms are now known to dominate all natural habitats of the Earth, accounting for 40%-80% of all bacterial and archaeal cells. Whether composed by multi- or single-species populations, biofilm communities emerge from complex interaction networks largely dependent on cell-to-cell communication facilitated by Quorum Sensing (QS) signalling. QS has been found to modulate a broad range of phenotypes across microbial domains, including biofilm formation. Nevertheless, understanding the regulation of biofilm assembly in anaerobic microbiomes poses a major challenge due to the diverse and complex interplay between QS mechanisms, microbial interactions and population dynamics prevailing in biofilm communities.

This study investigates the role of QS molecules, specifically N-acyl homoserine lactones (AHLs), in modulating the dynamics of biofilm formation in methanogenic microbial communities. A series of high-throughput experimental methods and flow-cell systems were run to study the impact of different AHLs on the growth of anaerobic biofilms. Initial screening experiments in microtiter plates with anaerobic cultures provided preliminary insights on the effects of AHLs on biofilm formation rates. Further experimentation using flow-cell systems and confocal laser scanning microscopy on a subset of AHLs allowed to accurately quantify the biofilm formation and visualize its structure, as well as to study changes in population dynamics and presence of QS genes within the microbial community.

The results of the initial screening indicated that most of the AHLs had a positive impact on the biofilm growth rate during the initial growth phase. Further experimenting with C10-HSL using flow-cells confirmed prior observations and revealed a much higher biofilm thickness and complex structure compared to the control after 7 days. Overall, this work provides valuable insights on the role of AHLs in governing biofilm formation and population dynamics in anaerobic microbial communities.

[P080] The effect of fat type and inclusion level on the rumen microbiome and methane in dairy cows

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Dietary fat inclusion is a way to reduce enteric methane (CH4) emission in dairy cows. Fat sources differ in fatty acid composition, but their effects on the rumen microbiome remain poorly understood. The aim of this study was to test the effect on rumen microbiota when cows were fed diets including either rapeseed (RS) at 3 inclusion levels (low=LR, mid=MR and high=HR) or palm kernel oil (PK) at 2 inclusion levels (low=LP and mid=MP) or no fat (control=CO). Twenty-four multiparous Holstein cows were assigned to six dietary treatments in a replicated 6×6 Latin square design. Amplicon sequencing targeting bacteria, archaea, protozoa and fungi using specific primer sets was performed to assess community composition, diversity and differential abundance.
Mid- and high-RS and both PK diets reduced CH_4 yield (CH4/DMI) and intensity (CH4/ECM). The largest reduction in CH_4 production occurred with MP, but after accounting for reduced ECM, CH_4 intensity reduction was similar between MP (13%) and HR (14%).

RS caused minor changes in rumen microbial diversity, whereas PK, particularly MP, induced larger shifts. Bacterial and fungal communities were most affected, protozoa communities changed moderately, and archaea changed the least. RUG420 (Acutalibacteraceae) increased with both fat sources and was the only bacterial genus differentially abundant across all RS levels. In contrast, 14 and 41 additional genera differed under LP and MP, respectively. Archaeal responses were limited, with only a few genera differing across diets (1, 4, 1, and 6 genera differentially abundant in MR, HR, LP and MP diets, respectively). Fungal communities responded strongly, particularly under PK diets, with marked shifts in several dominant genera. Protozoal communities were less responsive to RS than PK diets (1 RS vs 4 PK taxa). Correlations between microbial taxa and dietary FA intake clustered into PK and RS associated FA groups (PK has predominately C12 and C14 FA and RS had predominately C18:1n9 and C18:2n6 FA).

In conclusion, PK resulted in a greater disruption of the microbiome and the largest drop in CH4 production but was accompanied by a drop in DMI and ECM. Low RS minimally affected microbiota, performance and CH4 emissions. Mid and high levels of RS resulted in less changes to the rumen microbiome than PK and reduced CH4 measures linearly.

[P081] Microbial manipulation of sex ratios: using endosymbionts to improve insect protein production

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Endosymbionts are maternally inherited bacteria that can manipulate host reproduction. Male-killing endosymbionts is a group of sex-distorting microbes known from several dipteran host species such as Wolbachia in Drosophila pseudotakahashii and Spiroplasma in D. melanogaster. In these hosts, the endosymbiont leads to highly female-biased offspring and can also affect other important life-history traits such as development time and body size.

We are investigating the transfer and establishment of endosymbionts from Drosophila sp. to black soldier flies (Hermetia illucens) using microinjection, with the aim to induce male-killing and thereby produce female-biased populations. Black soldier flies are considered ideal candidates for sustainable food and feed production, and insect protein has been proposed as a novel alternative to conventional animal production with the potential to reduce the environmental impact of our food systems.

Our research has shown that female black soldier fly larvae attain greater body mass and utilize resources more efficiently than males. Further, males are capable of successfully inseminating multiple females. Combined, this suggests that female-biased populations could increase production efficiency by increasing larval biomass production without substantially compromising reproduction. The use of endosymbionts to achieve female-bias have several advantages including low labor once the endosymbiont-host system is achieved. Further, the endosymbiont-approach will enable female-biased populations already at the larval stage. If successful, this approach could provide a novel tool for improving the efficiency and sustainability of insect protein production.

[P082] Covering the uncovered – increasing the understanding of soil microbes by partially killing them

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The worlds highest diversity among living organisms is found in a soil sample with an estimated diversity of more than 10.000 different microbial species in just one gram of soil (Torsvik et al 1990). This basic observation was done almost 40 years ago using DNA-DNA re-association kinetics, and since then microbial ecologist has been searching for the true picture of the microbial community in soil – in resent year by performing metagenomics and metatranscriptomic studies in soil. We present data in this paper that indicate that this search is not in anyway close to be finalized. We show that a much higher percentage of the soil metagenomics data that actually can match genebank are found in sporeforming R-strategist rather than the average soil microbe. We hypothesize that the reason for this is that the current ways to culture microorganisms favoring fast growing bacteria – and this is not representative for the average soil microbiome. The direct implication of this is that at the moment the metagenomics and metatranscriptomic analysis are only showing a small part of the truth.

We added fresh ash from a wood combusting heater plant in Denmark to the O-layer of a spruce forest soil from Gedhus plantation, DK (56°16’38”N 09°05’12”E), in a concentration of 4.5% w/w. This caused the pH to increase to 8.50 from only 4.10. In a former experiment (Bang Andreasen et al 2017) due to the high pH this drop in pH resulted in a 4 log units drop in culturable bacteria making the spore formers make up just as many as the total numbers of culturable bacteria on diluted Tryptic Soy Broth, suggesting that only sporeforming bacteria survived the pH change (Bang-Andreasen et al 2017). We extracted total RNA using MOBIO PowerSoil RNA kit and after transformation to cDNA with hexamer primers the cDNA was sequenced using 8 lanes of HiSeq.

The main finding is that using our bioinformatics TotalRNA pipeline we find a much higher "perfect fits" of full length mRNA sequences (around 25%) from the high pH soil compared to the outcome analyzing the natural forest soil (around 5%).

This observation is explained by the relative absence of slow growing microorganisms in the pH disturbed soil - i.e. the rRNA show a massive dominance of fastgrowing bacteria.

[P083] Uniting One Health with Geobiology: Do biofilms on Aerosol particles enhance Infections with Antimicrobial Resistant Microorganisms? (GAIA)

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Antimicrobial resistance (AMR) is a critical global health threat requiring a One Health approach that recognizes the interconnected risks across humans, animals, and the environment.

Environmental drivers of AMR remain poorly understood. In particular, fine particulate matter (PM2.5) has received limited attention despite its potential to act as a vector for resistant bacteria and antibiotic resistance genes, impose selective pressures that may promote resistance, and alter host susceptibility, thereby influencing infection dynamics.

GAIA consortium was established by seed funding of the SUND collaborates programme to address this knowledge gap by integrating molecular geobiology, medical microbiology, environmental epidemiology, and geochemistry.

We hypothesize that bacterial exposure to air pollutants in the environment induces adaptive mechanisms that may promote biofilm tolerance and facilitate the development of AMR upon reaching the human airways.

To investigate bacterial adaptation to air pollution, Pseudomonas aeruginosa (PAO1) was subjected to experimental evolution for ten passages in a colony biofilm model in the presence or absence of nanosized carbon black (CB) and with or without a sub-inhibitory concentration of ciprofloxacin (CIP; 0.1 mg/L) as a selective pressure. Whole-genome sequencing of the evolved populations identified soot-associated adaptive mutations in genes involved in environmental and membrane-associated sensing, cellular physiology and redox processes, small-molecule metabolism, and biofilm formation. CIP-exposed lineages harbored mutations in the established CIP resistance determinants gyrA and gyrB. Phenotypically, increased CIP MICs were observed in passage 10 populations exposed to CIP (0.38–0.50 mg/L) compared with the wild type PAO1 strain (0.094 mg/L). Notably, one population exposed to both CB and CIP showed the highest CIP MIC of 1 mg/L.

In conclusion, our findings demonstrate that soot exposure can drive adaptive evolution in P. aeruginosa biofilms and suggest that particulate air pollution may contribute to bacterial trajectories relevant to antimicrobial resistance. These findings highlight the importance of considering environmental exposures as potential drivers of bacterial adaptation within a One Health framework.

[P084] Pyrroloquinoline quinone biosynthesis and exchange is com-mon in root-associated Pseudomonas and involved in solubilization of P and microbial interactions

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Phosphate solubilizing bacteria (PSB) have received much attention for their potential benefit in agriculture to increase soil phosphate (P) availability for plants. However, the underlying microbial ecology is not clearly understood, specifically in a community context. Here we utilized a bacterial synthetic community (SynCom) to investigate how the bacterial cofactor pyrroloquinoline quinone (PQQ) may be exchanged in soil bacteria to facilitate P solubilization via PQQ-dependent glucose dehydrogenase.

The SynCom Pseudomonas C1 strain produced PQQ and utilized it for P solubilization to facilitate growth under limited P availability, as verified via CRISPR-Cas3 knockout of pqqC and gcd genes. On solid Ca3PO4 media, PQQ could be acquired through an unknown mechanism by Agrobacterium, Ensifer and Pararhizobium strains from a PQQ producing Pseudomonas strain to induce P-solubilization. We extended these findings to a panel of rhizosphere Pseudomonas strains and found that most strains could similarly induce P solubilization in Agrobacterium. Within the panel, P solubilization and Agrobacterium induction was dependent on the structural differences in the PQQ oper-on between strains. We identified a specific group of Pseudomonas strains that were potent inducers of Agrobacterium, without solubilizing P themselves. This study demonstrates how P solubilization may be influenced by PQQ exchange in root-associated microbiomes.

[P085] An exploration of conflict-associated systems of Ralstonia solanacearum reveals prophage encoded immunity mechanisms

Zexin Li

Ralstonia solanacearum causes severe bacterial wilt disease in hundreds of plant species, posing a constant threat to agricultural produc on. Despite recent advances in phage therapy against this pathogen, its underexplored an phage immunity limits the use of phage-based biocontrol. Here, we demonstrate that an phage defence proteins are the most widespread and diverse class of biological conflict-associated proteins in the Ralstonia solanacearum species complex (RSSC), compared with other classes associated with virulence, toxin–an toxin, an-defence, and anmicrobial resistance. Our computa onal analysis revealed heterogeneity in defence system repertoires across different genospecies beyond the overall composion. To understand whether RSSC, which plays a vital role in the rhizosphere, harbours previously uncharacterised defence systems, we examined variable regions within prophage hotspots, combined with a machine learning-based approach that exploits sequence proper es. The model priori ses puta ve defence proteins characterised by low GC content, a high propor on of basic amino acids, and a high degree of predicted in vitro instability. As indicated by the predic on, we discovered a previously unknown two gene immune system, which we named Hemulen, comprising a gene that encodes a HEPN domain, with an upstream gene encoding the sensor homologous to a known defence system. Hemulen confers protec on against mul ple phages infec ng RSSC in plaque assays, as well as alters the plaque morphology of two phage families under heterogeneous expression in Escherichia coli. Structural modelling further suggests that Hemulen may perform its RNase ac vity through a tetrameric assembly. Together, this work provides a comprehensive overview of an phage immunity across RSSC, expanding our understanding of the known repertoire of bacterial an phage defence systems.

[P086] Longitudinal stability and in vitro reproduction of the human gut core virome

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Background:
Bacteriophages are viruses that infect bacteria and shape gut microbial communities. The human gut virome is highly individual but contains a stable fraction detectable for at least one year. As this core may contribute to gut homeostasis, it provides a biologically relevant reference for assessing the reproducibility of chemostat-propagated phage communities.

Aim:
To quantify donor-specific longitudinal core viromes and determine their recovery during continuous in vitro cultivation.

Methods:
Faecal samples collected one year apart from three healthy donors were analysed by Oxford Nanopore viral metagenomics and 16S rRNA gene sequencing. vOTUs detected at both time points were defined as each donor's longitudinal core virome. Samples from the second time point were cultivated in triplicate anaerobic chemostats, and core recovery was assessed after five days of continuous cultivation.

Results:
Longitudinal vOTU overlap was 59%, 58%, and 51% for donors 1, 2, and 3, respectively, whereas only 29 of 436 combined core vOTUs were shared across donors. After five days of cultivation, 49.3%, 32.5%, and 61.7% of the donor cores were recovered, giving a mean recovery of 48%. Viral richness initially increased but subsequently declined, and viral and bacterial communities became more similar across donors.

Conclusions:
Healthy donors had substantial, predominantly donor-specific longitudinal core viromes. Nearly half of this core was recovered after continuous cultivation, supporting its use as a biologically relevant reference for evaluating the reproducibility of complex phage communities propagated in chemostats. However, convergence across donors showed that cultivation imposed selection and did not fully preserve donor-specific community structures.

[P087] Hypoxia as the key to unlocking hidden potential

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Humanity is in the midst of an antimicrobial resistance crisis. To combat this, the discovery of novel antibiotics is crucial. Bacteria from the phylum Actinomycetota produce most of the antibiotics used in the clinic today, but they still hold enormous untapped biosynthetic potential. Screening these bacteria under standard laboratory conditions often leads to rediscovery of known compounds, obscuring novel compounds with potential as future antibiotics.

The key is to unlock the silent and cryptic biosynthetic gene clusters hidden in plain sight. The production of the BE-43547 compounds in the natural host is silent under laboratory conditions and is only activated when the bacteria are systematically exposed to hypoxic stress. The BE-43547 compounds show excellent hypoxia selectivity, leading us to believe that the link between hypoxia-associated regulation, production, and selectivity is a general phenomenon in natural products.

We investigate natural products that are connected to hypoxia through either regulation, production, or selectivity. If all three aspects are linked to hypoxia, we will use the regulatory mechanisms as a tool to discover novel, hypoxia-selective compounds.

[P088] Nested Regulation of Biosynthesis in Streptomyces

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Bacteria produce a variety of small molecules (SMs) to mediate interactions with other organisms. In Streptomyces, the biosynthesis of small molecules is encoded by biosynthetic gene clusters (BGCs) that typically encode cis- acting transcription factors (TFs) [1,2]. These regulators are often manipulated for biotechnological purposes, for example, to increase the titre of desired molecules. The advent of DNA-affinity purification sequencing (DAP-seq) has enabled TF binding sites to be identified in high throughput [3]. In this study, we used DAP-sequencing to explore the regulatory networks of the herbicidal strain, Streptomyces viridochromogenes [4] and twenty related strains – with a specific focus on BGCs. As one of the first studies to apply DAP-seq in Streptomyces, our results provide new insights into the interconnectivity of biosynthetic pathways and other cellular functions. We show that 80% of cis-acting, so-called 'pathway-specific,' regulators demonstrate pleotropic functions. By comparing TF binding sites across multiple species, we also show how regulators and their pathways have been refunctionalised throughout the evolutionary process. Finally, we combine DAP-seq with RNA-seq [5] to validate the proposed biological functions of the TFs we tested. We show that DAP-seq is a powerful method for characterising TF functions and show how it can be used to rapidly generate hypotheses for engineering SM biosynthesis and elucidating their ecological roles.

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[P089] Genomic and Phenotypic Tracking of Cheater Emergence in P. aeruginosa PAO1 Evolving With and Without Antibiotics

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Microbial cooperation often relies on the production of shared extracellular factors, creating public goods systems that can be exploited by mutants that do not contribute to their production. In Pseudomonas aeruginosa, quorum sensing–regulated behaviors such as protease secretion and virulence factor production generate opportunities for cheating, particularly in mutants defective in lasR, a key quorum sensing regulator. In cystic fibrosis (CF), P. aeruginosa frequently acquires lasR mutations, and patients undergo prolonged, repeated antibiotic treatments. This clinical reality motivated our central question: how does antibiotic exposure influence the formation, suppression, or redirection of cheating during microbial evolution?

We evolved replicate P. aeruginosa PAO1 populations with and without subinhibitory concentrations of clinically relevant antibiotics and monitored population dynamics over 30 days. CFU counts, pyocyanin production, and cheater frequency were tracked throughout the experiment. Cheaters were identified using 2% milk plates, where reduced protease activity produces smaller or absent zones of clearance. Minimum inhibitory concentrations (MICs) were measured at selected time points to assess changes in antibiotic susceptibility. Whole genome sequencing will be performed to confirm lasR mutations and identify additional genomic changes associated with cheating or antibiotic adaptation. These genomic data will be integrated into mathematical models to capture how antibiotics reshape social interactions and evolutionary trajectories.

Preliminary results show that antibiotic stress can reshape social evolution, with some antibiotics limiting cheater formation and invasion while others accelerate it. Ongoing whole genome sequencing and quantification of cheater frequencies will further explain these patterns, allowing us to confirm the presence of lasR mutations, identify additional adaptive changes, and validate our hypothesis about how clinically relevant antibiotics affect cheater formation in P. aeruginosa.

Together, these emerging findings suggest that antibiotic exposure modulates both genomic evolution and social dynamics.

[P090] Visualising diverse extracellular nucleic acids in biofilms with DNA-binding dyes

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Extracellular nucleic acids (eNA) are central components of bacterial biofilms, contributing to structural integrity, antibiotic tolerance, and emerging functions such as extracellular electron transfer and peroxidase-like catalysis. While extracellular DNA has traditionally been assumed to adopt the canonical B-DNA conformation, biofilms are now known to contain non-canonical structures, including Z-DNA/RNA (Z-NA), G-quadruplex DNA/RNA (G4-NA), and substantial amounts of extracellular RNA. Conventional nucleic acid-binding dyes are widely used for rapid eNA detection, yet their specificity for these diverse structures has not been systematically evaluated. In this study, we compare the fluorescence properties of eleven membrane-impermeant dyes (TOTO™, BOBO™, YOYO™, and POPO™ series, SYTOX™ Green, SYTOX™ Red, and propidium iodide) against synthetic B-DNA, Z-DNA, G4-DNA, A-RNA, Z-RNA, and G4-RNA oligonucleotides, with Z-NA stabilised through brominated guanosine analogues synthesised in-house. A clear pattern emerged: green-fluorescent dyes preferentially bound canonical B-DNA, whereas red-fluorescent counterparts displayed broader specificity that extended to non-canonical structures. TOTO™-3 and SYTOX™ Red bound G4-NA with higher fluorescence than B-DNA, and propidium iodide showed an unexpected preference for A-RNA over B-DNA. These observations were validated in Staphylococcus aureus biofilms by parallel immunolabelling with structure-specific antibodies. TOTO™-3, YOYO™-3, BOBO™-3, POPO™-3, and propidium iodide reproduced the eNA distribution at the bacterial cell surface. Finally, we introduce poly-A tailing with fluorescently labelled ATP as a stringent, RNA-specific imaging method for biofilms. Together, these results provide practical guidelines for visualising the structural diversity of eNA in biofilms.

[P091] Global change drives antibiotic resistance gene mobilization in European rivers

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Background and objective
Freshwater ecosystems are reservoirs of antibiotic resistance and recipients of anthropogenic pollution, creating interfaces between environmental and human microbiomes. Climate change and pollution may promote the selection, mobilization, and dissemination of antibiotic resistance genes (ARGs). We investigated how aquatic pollutants and climate-related physical stressors influence ARG mobilization into mobile genetic elements (MGEs), including plasmids and bacteriophages.

Methods
River water was sampled from nine rivers across eight European countries in winter and summer, covering rural and urban systems with varying environmental pressures. Fifty-four samples underwent deep metagenomic sequencing (140 million paired reads on average), genome and MGE assembly, environmental characterization, and pollutant quantification by mass spectrometry. We assessed associations and dose-response relationships between global change stressors, riverine microbiomes, and mobile ARGs.

Results and conclusion
Our results demonstrate that environmentally relevant levels of global change stressors are sufficient to shape the composition of the river water antibiotic resistome and alter patterns of ARG selection and mobilization. These findings show that co-occurring global change stressors can promote the maintenance, mobilization, and dissemination of antibiotic resistance in aquatic ecosystems, potentially increasing risks to human health.

[P092] 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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Microbial communities drive essential biogeochemical processes that underpin soil health and agricultural productivity. Despite their fundamental importance, traditional ecotoxicological assessments still focus primarily on higher organisms, largely overlooking the effects of chemical contaminants on soil microbial communities. Total RNA sequencing offers a comprehensive approach to address this gap by simultaneously capturing rRNA-based changes in active community structure and mRNA-based functional responses.

In the GENEPEASE II project, we investigated the effects of the fungicide Prosaro on soil microbial communities across a concentration gradient in microcosms incubated for six months. Total RNA sequencing revealed distinct taxonomic and functional responses to fungicide exposure. Active community composition shifted toward increasing dominance of prokaryotes, particularly Proteobacteria and Actinobacteriota, while fungi declined. In contrast, mRNA profiles revealed dynamic functional responses, with functional diversity and gene expression recovering toward control conditions over time at concentrations up to 15 ppm despite persistent changes in community structure. At higher concentrations, both taxonomic and functional responses remained pronounced throughout the experiment. Co-expression network analysis further revealed coordinated responses of both annotated and currently unknown microbial functions that would be missed by conventional annotation-based approaches.

Together, these results demonstrate that microbial community structure and function can respond differently to chemical perturbation, suggesting that functional resilience may be maintained through shifts toward more tolerant microbial taxa. By simultaneously resolving taxonomic restructuring and functional responses, total RNA sequencing provides a powerful approach for incorporating microbial ecosystem responses into environmental risk assessment of agricultural chemicals.

[P093] No End in Sight: Telomeres Are Frequently Missing from Streptomycetaceae Genome Assemblies

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Unlike most bacteria, members of Streptomycetaceae have linear chromosomes and often carry linear plasmids. These linear replicon ends are capped by telomeres that form secondary structures to which telomere maintenance proteins are recruited [1,2]. We searched for the most common telomere-class in 762 complete Streptomycetaceae genomes from the G1034 collection [3] and found that none of the genomes contained it in full. Inspection of Oxford Nanopore (ONT) reads showed that they did not capture the complete telomere, leading to truncated telomeres in genome assemblies. We found that the complete telomere is captured by Illumina data, and we developed the tool Telomore to reconstitute the missing telomeric sequence. This allowed detection of complete telomeres in the G1034 collection. By combining the reconstituted G1034 genomes with all complete RefSeq Streptomycetaceae genomes, and previously identified telomeres, we created a compendium of over 2000 telomeres split into 129 clusters. Using this compendium, we observed that only 13% of complete RefSeq chromosomes contained two telomeres, compared to 76% of reconstituted G1034 chromosomes. This indicates that missing telomeres are common in Streptomycetaceae assemblies and that most assemblies are not telomore-to-telomere complete. Finally, genome mining of complete genomes identified a putative maintenance system for SG2247-class telomeres.

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[P094] 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. 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 (A), the RiPP recognition element (B1) and the leader peptidase (B2). Moreover, the isopeptide-forming lasso cyclase (C) 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 (triT) was critical for achieving triculamin production. In vitro reconstitution of TriT demonstrates that N-acetylation is a self-resistance mechanism.

Intriguingly, we found both 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 (gelatinamin and palmamin).

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.

[P095] Batch and fed-batch optimization for enhanced production of triculamin-like lasso peptide in Photorhabdus laumondii TT01

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Lasso peptides are ribosomally synthesized and post-translationally modified peptides (RiPPs) that exhibit high structural stability and have significant antimicrobial potential. Nonetheless, their discovery and characterization are hindered by low yields and silent biosynthetic gene clusters (BGCs).

Our previous research on triculamin, a potent antimicrobial lasso peptide, involved identifying its BGCs by genomic searches and elucidating its biosynthesis.1 This led to the identification of multiple triculamin-like lasso peptides with core sequences similar or nearly identical to triculamin across various bacterial phyla. The present work exploited bacteria from the Photorhabdus genus as a potential source of triculamin-like lasso peptides. Relevant BGCs from Photorhabdus spp. were expressed in a well-characterized host, Photorhabdus laumondii TT01. The production of the resulting peptides was evaluated first in shake flasks, and later in a controlled bioreactor fermentation. Very few lasso peptides have been reported to date to be produced in titers higher than 10 mg/L. Here we present P. laumondii TT01 as an ideal host for the enhanced production of triculamin-like lasso peptides, providing a basis for further exploration and optimization of RiPP production.
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[P096] Evaluation of SigH regulation and riboflavin metabolism on formaldehyde stress response

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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 MGA3 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. It is believed that much of the CO2 production is attributed to dissimilation of the toxic intermediate formaldehyde. To better understand natural methylotrophic gene regulation during formaldehyde accumulation, a comparative transcriptomic analysis of B. methanolicus was conducted in three conditions: no formaldehyde, a non-growth inhibiting concentration of formaldehyde and a concentration that induced cell stress. Noticeably, the transcriptome data during formaldehyde stress displayed an upregulation of riboflavin synthesis genes in B. methanolicus. Additionally, formaldehyde supplementation upregulated the sigma factor H (sigH), a gene whose native overexpression is known to boost riboflavin production in Corynebacterium glutamicum.

This study aims to investigate role of riboflavin in formaldehyde stress response in a natural methylotroph. This will be done through cultivation of B. methanolicus in media with varying formaldehyde and riboflavin concentrations. Furthermore, the effect of sigH overexpression on riboflavin biosynthesis and formaldehyde stress response will be examined. This study will contribute to better understanding of regulation of formaldehyde stress response in methylotrophic B. methanolicus.

[P097] Establishment of a thermostable T7 RNA polymerase-based expression system in Bacillus methanolicus

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The thermophilic methylotroph Bacillus methanolicus is a promising platform for methanol-based bioproduction, but the development of efficient production strains requires tightly regulated gene expression systems. Here, a two-plasmid expression system based on the bacteriophage T7 RNA polymerase (RNAP) was developed for controlled gene expression in B. methanolicus. The reporter plasmid pTH1mpT7-sfGFP, carrying an sfGFP gene under the control of the T7 promoter, was successfully constructed and verified in Escherichia coli. In parallel, expression plasmids pBV2xp-pol and pBV2xp-pol(T+), carrying wild-type and thermostable variants of T7 RNA polymerase genes, respectively, were constructed. Functional activity of the wild-type T7 RNA polymerase was demonstrated in B. methanolicus, with reporter expression detected at temperatures below 40 °C. The thermostable T7 RNA polymerase variant is being evaluated to extend RNAP T7-drven expression to the thermophilic temperatures suitable for growth and bioproduction with B. methanolicus. Establishment of a functional T7 RNAP-based expression system would provide a novel tool for precise and inducible gene expression in this thermophilic methylotroph and could enable improved production of industrially relevant compounds from methanol.

[P098] Association Between Urinary Microbiota Composition and Fertility Status

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Infertility is a critical public health issue affecting 1 in 6 people globally and is recognized by the WHO as a disease of the reproductive system. Traditionally, infertility research has focused on hormonal imbalances, anatomical defects, genetic factors, and semen quality. However, recent research highlights the importance of the microbiome in numerous physiological processes, including reproduction.

The female reproductive tract harbors a complex microbial community that may influence fertilization, while semen also contains a diverse microbiome that varies between individuals. Recent evidence suggests that, in addition to vaginal and seminal microbiota, the urinary microbiome may also play a role in fertility. The female urinary microbiome is a dynamic, individual-specific bacterial community influenced by age, hormonal status, and urogenital health. It shares several bacterial taxa with the vaginal microbiome, including Lactobacillus species. Alterations in urinary microbiota composition have been associated with urogenital disorders, chronic inflammation, and reproductive dysfunction.

Despite increasing interest in the reproductive microbiome, the relationship between urinary microbiota profiles and fertility remains insufficiently understood. Furthermore, most studies focus on either males or females rather than investigating fertility at the couple level, despite the potential for microbiome exchange between partners. Therefore, this project aims to investigate urinary microbiota composition in healthy and infertile individuals and evaluate whether urinary microbiota profiles may serve as potential microbial indicators of reproductive health and infertility.

This will be investigated using 16S rRNA gene sequencing of DNA extracted from urine samples collected from infertile patients and healthy controls. Furthermore, bacteria will be cultivated and sequenced bacteria isolated from urine samples will be compared with bacterial species identified in vaginal swabs and semen samples.

[P099] Antimicrobial Peptides and Peptoids Designed to Differentially Influence Post-Therapeutic Endotoxin Release

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Conventional antibiotics may trigger post-therapeutic endotoxin release (LPS/LOS) in patients with Gram-negative infections. It can lead to prolonged inflammations in the body, compromising the efficacy of treatment or post-surgical recovery. While many antimicrobial peptides (AMPs) are told to bind and neutralize bacterial endotoxins, it is unknown if antimicrobial peptoids possess similar properties.

This study investigates post-therapeutic release of LPS and LOS from Escherichia coli and Campylobacter jejuni as model organisms. Using rationally designed AMPs and corresponding peptoid analogues with identical side-chain composition and length of lipid tails, the biological influence of distinct backbone architecture can be determined.

Rationally designed AMPs and peptoids were selected through in silico screening, with further application of solid-phase peptide synthesis (SPPS), RP-HPLC, and LC-MS for their synthesis, purification and validation. Structural characterization was performed using circular dichroism (CD) spectroscopy, followed by biological testing with MIC/MBC assays. Ongoing work includes critical micellar concentration (CMC) measurements and growth curve kinetics analysis, for a better characterization of the antimicrobial designs, completed with limulus amebocyte lysate (LAL) assay for post-treatment endotoxin release quantification. The findings are expected to reveal whether natural AMPs or their mimetics could contribute to the limitation of post-therapeutic endotoxin release and the associated inflammatory consequences.

[P100] Exploring the Environmental Mobilome: Recovery and Classification of Jumbo Viral Sequences from Long-Read Metagenomic Data

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Mobile genetic elements (MGEs) are key mediators of horizontal gene transfer, driving rapid genomic change and microbial evolution . Current knowledge of MGEs is heavily biased toward elements linked to infectious disease and antibiotic resistance, leaving the broader environmental mobilome associated with the uncultivated microbiota largely uncharacterised. Among these are jumbo viruses – bacteriophages with genomes exceeding 200 kilobases, distinct from typical phages in both physical size and genomic complexity . Their large, complex genomes raise fundamental questions about their ecological roles, evolutionary origins, and interactions with microbial host communities.

This project aims to recover and classify jumbo viral sequences from environmental long-read metagenomic datasets generated through the Microflora Danica project, a comprehensive survey of Danish microbiota consisting of 10,683 samples spread across different habitats . Bioinformatic pipelines will be developed for the recovery of high-quality jumbo viral genomes from a subset of these samples. Recovered sequences will be characterised in terms of abundance, size distribution, genomic content, and taxonomic diversity providing an initial catalogue of jumbo viral diversity across Danish microbiota and laying the groundwork for a broader understanding of the environmental mobilome.

[P101] pH-dependent regulation of aromatic amino acid decarboxylase gene expression controls bacterial tyramine production in the human gut

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Tyramine is a biogenic amine produced by specific gut bacteria, which has been linked to adverse host effects. In the human gut, Enterococcus faecium and Enterococcus faecalis are the major tyramine producers, converting tyrosine to tyramine through a decarboxylation reaction catalyzed by the aadc gene-encoded aromatic amino acid decarboxylase (AADC). However, the physical and chemical factors in the gut that can influence tyramine production are not well understood. Here, using in vitro fermentations and human studies, we investigated the role of pH in regulating gut bacterial tyramine production. We performed pH-controlled in vitro fermentations of E. faecium and E. faecalis monocultures as well as human fecal microbiota cultures at pH 5.5 and 7.5, reflecting the proximal colonic pH range. In monocultures, pH 5.5 promoted tyrosine utilization and tyramine production through upregulation of aadc gene expression, despite lower growth compared to pH 7.5. Consistently, human fecal cultures revealed upregulation of aadc gene expression and higher tyramine production at pH 5.5 compared with pH 7.5. Interestingly, we found that under low pH conditions, which promotes expression of tyramine biosynthesis genes, tyramine production also depends on the abundance of the aadc gene. Furthermore, analysis of 150 fecal samples from two independent human cohorts consistently confirmed an inverse association between fecal pH and tyramine levels. Together, our findings reveal hierarchical control of tyramine synthesis by the gut microbiota, with pH regulating gene expression and gene abundance determining final tyramine levels only under permissive conditions.

[P102] Characterization of a putative formaldehyde dehydrogenase encoded by dhaS2 in B. methanolicus

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During methylotrophic growth, Bacillus methanolicus oxidizes methanol to formaldehyde (FA), which is subsequently assimilated through the ribulose monophosphate (RuMP) cycle. As FA is highly toxic, B. methanolicus activates dissimilatory FA oxidation pathways to prevent its intracellular accumulation. However, these pathways result in the loss of carbon as CO_2 rather than its assimilation into biomass and other metabolites. Transcriptomic analysis of the response of B. methanolicus to methylotrophic stress revealed the upregulation of a novel putative FA detoxification pathway.

In this project, the function of this putative FA detoxification pathway will be investigated using two complementary approaches: (1) deletion of the first gene in the pathway, dhaS2, encoding a putative FA dehydrogenase; and (2) in vitro characterization of the purified DhaS2 enzyme. The ΔdhaS2 strain will be characterized with respect to its response to FA exposure, with the hypothesis that deletion of dhaS2 will reduce FA resistance compared with the wild-type strain. In parallel, DhaS2 will be heterologously produced in Eschericha coli BL21(DE3), purified, and characterized with respect to its substrate specificity, cofactor requirements, optimal temperature and pH, preferred direction of the catalyzed reaction, substrate affinity, and catalytic efficiency. The overall aim of this project is to elucidate the role of dhaS2 in FA metabolism and detoxification in B. methanolicus.

[P103] Host-cell responses to HlyA at lytic and sublytic concentrations

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Ulcerative colitis (UC) and Crohn's disease (CD) are the two primary types of inflammatory bowel disease (IBD), which are characterized by chronic inflammation of the gastrointestinal tract (GI). The etiology of IBD is multifactorial and includes diet, gut microbiota, dysbiosis, and immune dysregulation. CD may affect any part of the GI tract, most commonly the terminal ileum and colon, and is characterized by transmural inflammation, whereas UC is limited to the colon and primarily affects the innermost mucosal layer. Common IBD symptoms include abdominal pain, diarrhea, rectal bleeding, and fatigue.

Increasing evidence suggests that alterations in gut microbiota composition contribute to IBD pathogenesis. In particular, the abundant presence of Enterobacteriaceae, specifically Escherichia coli (E. coli), has been reported in fecal samples from IBD patients, suggesting a potential role in disease progression.

Among the virulence-associated factors of E. coli, α-Hemolysin (HlyA) has attracted attention due to its potential effects on disease severity. HlyA can disrupt epithelial tight junctions, induce target-cell lysis through pore formation, and modulate cellular responses, thereby facilitating bacterial invasion and tissue damage.

This study focuses on functional analysis of an HlyA variant at lytic and sublytic concentrations. The variant investigated in this study was identified in an E. coli strain isolated from a patient with UC. To characterize its function, HlyA is expressed in an IPTG-induced transformant and purified using FPLC. Lytic and sublytic concentrations are determined using liquid hemolysis and cell-damage assays. The host-cell response is investigated in the Caco-2 cell line using LC-MS based proteomic analysis focusing on phosphoproteomics and surfaceomics. The results may contribute to a better understanding of HlyA-mediated effects, disease development and progression.

[P104] Bacterial non-canonical eNAs: Potential Drivers of Chronic Non-Communicable Diseases?

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Chronic non-communicable diseases (NCDs) such as neurodegenerative, cardiovascular, and auto-immune disorders severely impact our quality of life and longevity. However, the root causes and mechanisms driving these diseases remain incompletely understood. Recently, extracellular non-canonical nucleic acids (eNA) such as Z-NAs and G-quadruplexes (G4s) have been shown to be highly immunogenic and DNase I-resistant. These structures bind TLR2 and activate immune response pathways linked to flare-ups in chronic inflammatory NCDs.

Interestingly, Z-NAs and G4s are also highly abundant in bacterial biofilms. Together with DNA-binding proteins, they form strong DNase I-resistant eDNA networks crucial for resistance to environmental stressors, including the host immune system and antibiotic treatments. Recent studies demonstrated that clearing long-term bacterial infections reduced inflammation in the blood vessels of patients with atherosclerosis. This suggests that persistent infections may contribute to chronic inflammation in NCDs. Therefore, we hypothesize that during infection, non-canonical eNAs are released from the biofilm matrix and may accumulate systemically in the human body, triggering chronic inflammation.

To test our hypothesis, we screened a range of clinical samples for the occurrence, abundance, and spatial localization of eNAs. To specifically target and visualize Z-NAs and G4s in patient samples, we used fluorophore-labeled antibodies and confocal microscopy. Preliminary imaging confirmed the presence of Z-NAs and G4s in tissue and synovial fluid samples from NCDs as well as acute infections. This exploratory approach lays the ground work for identifying the most promising clinical samples for in-depth investigation of the potential link between bacterial infections and NCDs. In future experiments, we aim to determine the origin of the non-canonical eNA structures and further investigate their formation and therapeutic potential.

[P105] LACTIC ACID BACTERIA-PLANT CROSSTALK FOR IMPROVED CROP FUNCTIONALITY AND STRESS RESILIENCE

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The transition toward sustainable agriculture requires innovative strategies to mitigate the impacts of abiotic and biotic stresses on crops. Lactic acid bacteria (LAB), although well established in food systems, are emerging as underexplored members of plant-associated microbiomes. This study aims to identify and functionally characterize LAB isolated from tomato (Solanum lycopersicum), barley (Hordeum vulgare) and maize (Zea mays) plants, with a focus on leaves, roots and rhizosphere soils.

Functional screening includes the evaluation of enzymatic activities involving, phytase, protease, β-glucosidase and peptidase, colonization potential (biofilm formation and exopolysaccharide production), tolerance to abiotic stresses, production of bioactive metabolites, nutrient solubilization and antagonistic activity against fungal phytopathogens in plant systems. The best-performing strains are selected for further phenotypic and genomic profiling, with particular emphasis on quorum sensing-mediated communication mechanisms to elucidate how LAB regulate colonization, coordinate metabolite production, and interact with plant hosts under both optimal and stress conditions. Analyses are extended to plant tissue systems (in vitro and ex vivo) to capture biologically relevant interactions. Subsequently, selected LAB strains with distinct metabolic and communication traits are assembled into consortia, applied to plants (in vivo), and evaluated for their effects on growth performance and stress resilience under controlled greenhouse conditions.

Preliminary results reveal niche- and host-dependent LAB distribution, with Lactiplantibacillus spp. predominantly associated with aerial tissues and Leuconostoc spp. with root environments.

Overall, LAB represent promising multifunctional bioinoculants, and the design of optimized probiotic consortia may offer an innovative strategy to enhance crop functionality and resilience, supporting more sustainable agricultural systems.

[P106] Combating Antimicrobial Resistance by Signal Jamming Bacterial Two-Component Systems

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Bacterial cell envelope targeting antibiotics is the largest class of antibiotics in use, and the rise of resistance towards them is a concern for public health. One way to combat such antimicrobial resistance is to perturb conserved cell envelope homeostasis mechanisms, thereby potentiating or repurposing these antibiotics. Various bacterial two-component systems (TCS) that regulate cell envelope homeostasis have been described, and identifying inhibitors of these TCS is the premise of this study. ChvIG is one such TCS candidate from alphaproteobacteria consisting of sensor histidine kinase, ChvG and DNA binding response regulator, ChvI and regulates cell envelope homeostasis, virulence and motility. Hence, mutations in ChvIG reduce bacterial virulence and sensitize the bacteria to cell envelope targeting antibiotics and osmolytes such as NaCl or sucrose. We were able to complement ChvIG from plant pathogen Agrobacterium tumefaciens in free living Caulobacter crescentus, confirming its functional conservation in alphaproteobacteria. To identify inhibitors of ChvIG signaling in Caulobacter, chemical genetic screening of a phytochemical library was performed. We used both growth and reporter assays to optimize the hit selection. Firstly, we screened the library using the wild type (Wt), ΔchvIG, and Wt in the presence of 60mM NaCl to identify compounds that inhibit ChvIG and mimic the condition of its deletion. Secondly, we used GFP reporter driven by the promoter of ChvIG regulated sRNA, ChvR. We screened library using the GFP reporter assay with and without 60mM NaCl to identify activators and inhibitors of ChvIG signaling, respectively. Five hits retained inhibitor activity when the assay was upscaled. Additionally, two of these belong to the same class of compounds indicated that ChvIG could function as a mechano sensor. This was shown to be true using a membrane pressure perturbing agent. At present, we are analyzing the hits in the presence of cell wall targeting antibiotics.

[P107] Combined Effects of Estradiol and Progesterone on Inflammatory Signaling and Hormone Metabolism in Human Vaginal Epithelial Cells

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The vaginal epithelium functions as a physical and immunological barrier and is involved in the maintenance of reproductive health. Epithelial function and local immune responses are affected by fluctuations in sex hormone levels throughout the menstrual cycle. However, the effects of combined estradiol and progesterone exposure on inflammatory signaling and hormone metabolism in vaginal epithelial cells remain insufficiently characterized. Previous studies have primarily investigated the effects of these hormones separately, resulting in limited knowledge of their combined effects.

The aim of this study is to investigate the effects of 17β-estradiol and progesterone, individually and in combination, on inflammatory signaling and steroid hormone metabolism in the human vaginal epithelial cell line VK-2/E6E7. VK-2/E6E7 cells will be exposed to estradiol, progesterone, or a combination of both hormones. The secretion of the pro-inflammatory cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8), as well as interferon-related responses involved in innate mucosal immunity, will be evaluated alongside cell viability.

To further characterize hormone-mediated cellular responses, the expression and activity of selected steroid-metabolizing enzymes, including cytochrome P450 (CYP) enzymes and catechol-O-methyltransferase (COMT), will be investigated. Hormone concentrations and metabolic profiles will be quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to assess changes in steroid metabolism following hormonal exposure.

This study will characterize the effects of combined estradiol and progesterone exposure on inflammatory signaling, cell viability, and steroid hormone metabolism in VK-2/E6E7 cells. The results may contribute to the characterization of hormonal regulation of immune responses and local steroid metabolism in the vaginal epithelium.

[P108] The two-component system ChvIG partially modulates the multilayered regulatory function of NtrYX in Caulobacter crescentus

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The two-component-system (TCS) ChvIG is essential for virulence and symbiosis in several pathogenic and symbiotic Alpha-proteobacteria such as Brucella abortus and Sinorhizobium meliloti. However, in the non-pathogenic Caulobacter crescentus, the system appears to mainly be important for viability under several conditions, primarily osmotic changes and cell envelope stress.

We investigated the phenotypes of ΔchvIG mutants in C. crescentus and found that not only do the mutants have reduced viability when exposed to osmotic stress, the motility of the strains was also severely impaired under osmotic stress and minimal media. To identify the cause of the motility loss, we sequenced spontaneous suppressor mutants that regained motility. We found that several of these mutations had occurred in another TCS, ntrYX, associated with iron homeostasis and acid stress response.

We found through following investigations the mutations in ntrYX were dominant, and able to rescue the detrimental growth and non-motile phenotypes of ΔchvIG mutants through overexpression. We sought to determine the nature of the possible dependency between ChvIG and NtrYX. We found through ChIP-seq that both regulators share some binding targets and NtrX is very dependent on ChvI to access its full regulon, but not vice versa. Following this, we validated through promoter assays a selection of shared and regulator specific targets such as PmalA and PtolQ, respectively. This confirmed the dependency of phosphorylated ChvI modulating the regulation of NtrX on target genes. To elucidate the type of regulatory control ChvI has over NtrX, we conducted tandem affinity pull-down of NtrX variants and found that ChvI was associated with the receiver domain of NtrX.

Based on our findings, we suggest a functional link between ChvIG and NtrYX where ChvI modulates the regulon of NtrX by directly interacting with the receiver domain of NtrX, likely altering the conformation and thereby also the regulon of NtrX.

[P109] HigX Links Membrane-Associated Transcriptional Regulation to Respiratory Energy Metabolism in Caulobacter crescentus

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Transmembrane transcription regulators (TTRs) were previously believed to regulate gene expression indirectly via classical signal transduction. Recent findings, however, suggests that this model is incomplete, as some TTRs can regulate gene expression directly from the cytoplasmic membrane. Why these transcription factors are within the membrane is still unclear, but their location may enable them to sense changes to the membrane status. HigX is a transmembrane regulator found across Alphaproteobacteria, including Caulobacter crescentus. We investigated the regulatory and phenotypic effects of HigX in C. crescentus, focusing on its regulation of energy metabolism.

We found through quantitative proteomics that HigX overexpression or deletion altered hundreds of proteins. Cross-referencing these data with a genomic HigX-binding profile from ChIP-seq identified potential direct targets, including genes associated with the four terminal oxidase complexes and the oxygen-responsive regulator FixK. This leads to a model where if HigX regulatory activity is lost, FixK level increases, and more high-affinity oxidase, complexes are made. Promoter assays supported these findings, showing HigX-dependent regulation of the qoxA promoter, the first gene in the cytochrome bo3 operon, and repression of fixK upon HigX overexpression.

Together, these findings suggest that HigX links membrane associated regulation to energy metabolism. Studying this regulator in C. crescentus, a well established model of tightly regulated cell cycle control gives insight into how membrane and metabolic signals are combined with gene regulation and may reveal shared regulatory mechanisms in Alphaproteobacteria.

[P110] Functional characterization of the autoinducing peptide in Clostridioides difficile - a chemical approach

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Background:
Quorum sensing (QS) is a density-dependent regulatory mechanism that enables bacteria to coordinate gene expression via secreted signaling molecules. In Gram-positive organisms, QS is typically mediated by autoinducing peptides (AIPs) that activate two-component regulatory systems, leading to transcriptional changes. In Clostridioides difficile, toxin production is regu-lated in part by the accessory gene regulator (agr) system, which also influences additional virulence traits. Although AIPs of other Clostridia, such as Clostridium acetobutylicum and Clostridium perfringens, have been characterized by extraction or bioinformatic predictions followed by functional assays, only a handful of studies address the AIP produced by C. dif-ficile. At present, the AIP produced by C. difficile is described as a peptide containing a thio-lactone with a mass >1000 Da, yet its full structure remains elusive.

This study aims to characterize the native AIP produced by C. difficile based on bioinformatic predictions. Furthermore, we investigate the stability of the thiolactone motif, as studies investi-gating QS in other Gram-positive pathogens suggest a rapid shift of the thiolactone motif in tailless AIPs resulting in a homodetic peptide at physiological pH.

Methods:
Based on reported agrD1 sequences across C. difficile strains, potential AIPs were synthesized by solid-phase peptide synthesis (SPPS) to obtain both the homodetic peptide and the cyclic thiolactone variants. The predicted S-to-N acyl shift of the cyclic thiolactone was assessed at pH 4–7, by monitoring the change in retention time in ultra high-performance liquid chroma-tography.

Results:
Cyclic thiolactone and homodetic peptides were successfully synthesized (purity >95%) using SPPS. The cyclic thiolactone undergoes a rapid S-to-N acyl shift at pH 7, resulting in homodet-ic peptide formation. At pH 7, the half-life of the thiolactone is 3.3 minutes, whereas it increas-es to 128 min at pH 4.

Conclusion:
The thiolactone motif of the predicted AIP produced in C. difficile readily undergoes an S-to-N acyl shift at pH 7, resulting in a homodetic peptide. This raises the question whether homodetic or thiolactone-containing peptide is the predominant AIP at physiological pH. The effects of the homodetic peptide as well as the thiolactone-containing peptide on toxin production, are cur-rently being examined by qPCR.

[P111] A novel type of DsrEFH sulfur transferase, first characterized from cable bacteria, indicates widespread sulfur oxidation capacity in sulfate reducers

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Microbial sulfur cycling is typically divided into an oxidative and a reductive branch, with microbes driving either sulfide oxidation or sulfate reduction distinguished by their genomic setup. Paradoxically, filamentous cable bacteria perform electrogenic sulfide oxidation but contain genes indicative of sulfate reduction, including the reductive type of dissimilatory sulfite reductase (DsrAB), while apparently lacking the canonical sulfur transferase DsrEFH essential for sulfur oxidation. AlphaFold2 structure prediction of conserved cable bacteria proteins with unknown functions identified a protein complex resembling canonical DsrEFH (hereafter termed DsrEFH type II). In vitro characterization of heterologously expressed DsrEFH type II confirmed its sulfur transferase function and, together with site-directed mutagenesis, verified that the conserved cysteine, Cys67, is the active sulfur transfer residue. Genes encoding the novel DsrEFH type II were found in 985 prokaryotic genomes. They typically co-occurred with genes for reductive DsrAB in microbes characterized as sulfate reducers or sulfur disproportionators. This indicates a widespread capacity for sulfur oxidation by reversal of the reductive DSR-pathway.

[P112] Guilt by Co-folding: Testing Computationally Predicted Anti-CRISPRs for CRISPR Type IV-A

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The on-going study of prokaryotic immunity has led to the characterization of numerous bacterial immune systems. This includes the now infamous CRISPR-Cas systems, of which 7 types have been classified to date[1]. The type IV CRISPR-Cas system has been found to differ from the remaining CRISPR-Cas systems, as most subtypes lack both an adaptation module and a dedicated nuclease. In the place of nuclease-activity, the CRISPR subtype IV-A systems contain a DinG effector helicase which mediates crRNA-dependent transcriptional repression of targeted genes[1–3].

In response to bacterial immune systems, some mobile genetic elements such as bacteriophages have developed anti-CRISPR proteins (Acrs)[4]. To our knowledge, no Acrs directed at CRISPR type IV have been discovered. In this work, we identified several candidate Acrs in silico by co-folding Csf1 (Cas8-like) of the type IV-A3 CRISPR-Cas system with small proteins encoded in putative plasmid anti-defense gene clusters. Using a dual plasmid assay, with one plasmid encoding a CRISPR type IV-A3 system targeting the λ phage genome and the other plasmid expressing one of two promising Acr candidates, we tested λ phage defense in *E. coli* by both phage spotting assays and liquid plate-reader experiments. Expression of the putative Acrs was not shown to abolish CRISPR type IV defensiveness against the λ phage, indicating no functional anti-CRISPR function under our testing conditions. Nonetheless, this work exemplifies an explorative workflow combining structure-based binding predictions with wet lab testing, which could be applied to identify and test other putative anti-defense proteins.

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[P113] BacPROTACs: A Novel Antibacterial Strategy

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Antibiotic resistance is one of the most urgent global health challenges, causing >6 million deaths worldwide in 2020, with projections of up to 10 million annual deaths by 2050 without effective intervention. The rapid spread of antibiotic resistance highlights the need for new antibiotics with novel modes of action that can circumvent existing resistance mechanisms. One emerging approach is targeted protein degradation, which eliminates proteins rather than transiently inhibiting their activity.

In eukaryotic systems, small-molecule degraders, termed PROteolysis-TArgeting Chimeras (PROTACs), have rapidly advanced toward clinical application, demonstrating that endogenous degradation machinery can be harnessed for therapeutic purposes. This concept has recently been adapted to bacteria through the development of bacterial PROTACs (BacPROTACs). In Gram-positive bacteria, the Clp protease system plays a central role in protein degradation and recognizes phosphorylated arginine (pArg) as a degradation signal (degron). By linking a pArg anchor to a ligand targeting a bacterial protein of interest (POI), BacPROTACs have the potential to hijack this bacterial endogenous degradation machinery to selectively target proteins for degradation.

We aim to establish a proof-of-concept toolkit for the development and evaluation of BacPROTAC-based targeted protein degradation in Staphylococcus aureus as a novel antibiotic strategy. For this study, we selected peptide deformylase (PDF), an essential bacterial enzyme, as our POI. To determine whether PDF can be targeted by the ClpCP system, we have established an in vitro degradation assay using recombinantly expressed, His-tagged ClpC, ClpP, and PDF, as well as the McsAB kinases. The McsAB system is used to phosphorylate surface-exposed arginine residues on PDF, enabling us to assess whether pArg-tagged PDF can be recognized and degraded by the ClpCP system. To overcome challenges in distinguishing PDF from ClpP due to their similar molecular size, a FLAG tag has been introduced into PDF, enabling efficient purification and reliable detection in degradation assays. In parallel, known PDF ligands have been tested, and the molecule actinonin has been selected as a promising candidate. Actinonin analogues have subsequently been chemically synthesized to optimize binding, and their IC50 and Kd values are currently being determined to evaluate their binding affinity for PDF. Preliminary data have shown promising results for one of the analogues, and our first PDF-targeting BacPROTAC has now been synthesized, marking an important milestone toward validating targeted protein degredation as a novel antibacterial strategy in S. aureus.

[P114] PorcuPINe is an Escherichia coli prophage-encoded PIN-domain-containing antiphage defence system responsive to phage tail assembly

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Prophages are reservoirs of accessory genes, including antiphage defence systems that protect bacteria from phage infections. Here, we discover and characterise PorcuPINe, a member of our 'zoo' of defence systems encoded by an Escherichia coli H311 prophage. PorcuPINe is a single-polypeptide system containing a predicted PIN ribonuclease domain.

PorcuPINe confers protection against diverse siphovirus dsDNA bacteriophages from the BASEL collection and common laboratory phages. Phages can escape PorcuPINe via mutations in the genes encoding the Tape Measure Protein (TMP) chaperone. The TMP chaperone is a helper protein associated with TMP, involved in phage tail assembly. We tested whether TMP and its chaperone, individually or together, could trigger PorcuPINe's RNase activity as assessed by toxicity. Co-expression of TMP and TMP chaperone with PorcuPINe resulted in smaller, translucent colonies, while individual phage proteins had no detectable effect. Additionally, affinity purification coupled to mass spectrometry identified other phage proteins enriched with tagged PorcuPINe, revealing additional candidates for functional validation.

We propose that untriggered PorcuPINe is maintained in an inactive state and is activated during the late stages of phage infection in response to the TMP/TMP chaperone complex, potentially triggering RNA degradation through its PIN domain. Our next steps are to identify additional triggers and targets of PorcuPINe's RNase activity and uncover the molecular mechanism of triggering.

[P115] eHAd is a prophage-encoded Hip kinase that partly rescues E. coli from phage infections

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Toxin–antitoxin (TA) systems have been assigned diverse roles in prokaryotic cells, including stabilization of mobile genetic elements, defense against bacteriophages, and antibiotic persistence. In Escherichia coli, high-persister (hip) kinases typically function as toxins that target tRNA synthetases and are encoded together with a cognate antitoxin. In contrast, HipH (also known as YjjJ) is a monocistronic kinase that targets the ribosome and central carbon metabolism. Its toxicity can be neutralized both by the HipB antitoxin from the hipBA operon and by cyclic di-GMP.

Using a pre-trained Hidden Markov Model, we searched available Escherichia genomes for prophage-encoded hip kinases and identified a HipH homolog (36.3% identity to E. coli K-12 MG1655 HipH) located in close prox-imity to a Thoeris anti-defense 2 (Tad2) gene and an anti-restriction DarA operon. We refer to this kinase as HipH anti-phage defense (HAd).

Ectopic overexpression of HAd in M9 minimal media showed an increased plaque turbidity against most of the phage families in the BASEL phage collection. A phenotype that was independent of kinase activity.

Because hip kinases are implicated in both phage defense and antibiotic persistence, further characterization of the HAd kinase is essential to understand the dynamics of prophage-encoded hip kinases in phage defense and antibiotic persistence.

[P116] Genome-Scale Screen for ncRNAs Enriched Near Defence Systems

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Bacteria and their viral predators — phages — are engaged in a longstanding evolutionary arms race. This has fuelled the diversification of anti-phage defence mechanisms. These defence systems confer immunity, protecting cells against foreign genetic elements. Non-coding RNAs (ncRNAs) are one part of this arsenal and have recently been identified as key elements in bacteria–phage conflicts. Unlike mRNAs, which primarily encode proteins, ncRNAs carry out diverse regulatory, structural, and catalytic roles. While many defence systems are well characterised at the protein level, ncRNAs in defence islands remain poorly understood. These ncRNAs often reside in intergenic regions (IGRs), making them difficult to identify and annotate systematically. Could more ncRNAs be linked to defence systems than currently known?

To answer this question, we computationally screen for ncRNA families near defence systems across bacterial genomes. Using precomputed defence system annotations from PADLOC across approximately 220,000 RefSeq genomes, we extract the IGRs flanking defence systems and search them against a set of bacterial Rfam RNA covariance models. As a background comparison, we apply an identical search to genome-wide IGRs. After reducing redundancy, we apply an enrichment test to identify ncRNA families that occur near defence systems significantly more often than expected by chance.

In summary, this work produces a genome-scale map of known ncRNA families enriched near bacterial defence islands. This extends our understanding of bacterial immunity beyond its protein-coding components. Additionally, a substantial fraction of defence-associated IGRs carry no match to any known Rfam family, pointing toward considerable undiscovered ncRNA diversity in these loci and motivating future de novo discovery.

[P117] Experimental Screening for Novel Anti-Phage Defence Systems

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The evolutionary arms race between bacteria and bacteriophages has led to the emergence of countless anti-phage defence systems. New defences are mainly discovered computationally using a guilt-by-association approach by interrogating unknown genes that are enriched next to known defence systems. To overcome this bias, we employ an experimental screening for the identification of novel defence systems. We isolated the DNA of over 280 phyllosphere bacterial isolates to create a fosmid library in E. coli, harboring random ~30 kb inserts. By challenging this library with E. coli phages from diverse families, we selected for surviving cells carrying potential defences. After sequencing the containing fragments, these were fragmented further, subcloned into E. coli and reselected for phage resistance to pinpoint individual anti-phage systems by deep sequencing. The molecular mechanisms behind the anti-phage activity of these candidates can then be characterized thoroughly by mutational analyses and biochemical assays. This work sheds light on the phage-host co-evolutionary dynamics and establishes the basis for the development of novel biotech tools.

[P118] ONT-cappable-seq enables transcriptome-wide characterization and detection of riboswitches in Priestia megaterium

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Riboswitches are cis-regulatory RNA elements typically located in the 5’ untranslated regions (5’ UTRs) of mRNAs. Upon ligand binding, they undergo conformational changes that modulate downstream gene expression at the transcriptional or translational level. Traditionally, their functional characterization has relied on in vitro assays or in silico predictions, leaving their in vivo regulatory dynamics relatively unexplored.
In this study, we used Oxford Nanopore Technologies (ONT) Cappable-seq to map the global landscape of transcriptionally-active riboswitches in Priestia megaterium and assess their activity in vivo. Condition-dependent premature termination of transcription produces a 3’ edge in bulk RNA-seq data, which we used to screen the transcriptome for putative intrinsic terminators. Using TERMITe, we characterized key features of intrinsic terminators, including those located within 5’ UTRs. Our results demonstrate that riboswitches can be assessed directly through their primary signal, transcription, using this method.

Using cobalamin (B12) to evaluate condition-dependent termination, we find that cobalamin riboswitches induce premature transcription termination even in an untreated group, with no added B12, likely because of the endogenous production of cobalamin derivatives. Nevertheless, several cobalamin riboswitches exhibit a condition-dependent increase in termination efficiency; although, interestingly, to different degrees.

Finally, we developed a pipeline for novel riboswitch discovery that uses Cappable-seq's full-length primary transcripts to define 5’ leaders and screen them for conserved RNA structure, scoring pairwise covariation across homologous leaders from related bacteria. The pipeline recovers known riboswitches without prior annotation and uncovers additional riboswitch-like structures, opening a route to discovering orphan riboswitches.

[P119] Discovery and characterisation of prophage-encoded ParA-associated antiphage defence systems

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Phages are viruses that infect bacteria, causing lytic infections that kill the host or establishing lysogeny as prophages. Prophages can benefit their hosts by encoding antiphage defences, often clustered in variable genomic regions. Using our iLund4u database of phage genomic diversity hotspots, we identified two related multicomponent defence candidates, provisionally named def-ParA1 and def-ParA2. Both encode a predicted ParA-like ATPase alongside one or two additional proteins, respectively. Given the role of canonical ParA in DNA partitioning, we have initially hypothesised that these ATPases act as DNA-binding phage sensors, while the extra proteins act as toxic effectors.

Testing against the BASEL coliphage collection confirmed that both systems confer antiphage defence but have distinct protection spectra. Def-ParA1 protects against Demerecviridae and Straboviridae, whereas def-ParA2 protects against phages from most BASEL groups, except Autotranscriptaviridae and Stephanstimvirinae, inactivating substitutions in the ParA ATPases abolish defence.

ParA1 ATPase – but not the associated effector or two complete def-ParA1 system – is toxic. A possible explanation is uncontrolled DNA binding by def-ParA1 which is suppressed in the full system. The DUF6988 component of def-ParA2 system is toxic only when co-expressed with ParA2; the third component suppresses the toxicity. Phage mutants escaping def-ParA2 carry mutations in genes encoding uncharacterised SH3-clan domain proteins. Co-expression of these phage proteins with def-ParA2 induces toxicity, suggesting that they trigger the defence system. Ongoing work aims to establish the molecular mechanisms underlying both systems.

[P120] Phage-encoded single-guide RNAs subvert CRISPR-Cas9 immunity

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Central to CRISPR technologies are single-guide (sg)RNAs, engineered fusions of a processed CRISPR RNA and tracrRNA that direct Cas nucleases to bind and cleave target DNA. Here, we report that phages naturally evolved their own compact sgRNAs to subvert CRISPR-Cas9 immunity. These viral (v)sgRNAs exert dual anti-CRISPR activities: they sequester Cas9 in a similar mechanism to all known RNA anti-CRISPRs but additionally use truncated guides to reprogram Cas9 to transcriptionally repress its own expression. vsgRNAs further evolved to avoid inhibition by co-encoded anti-CRISPR proteins, including one that inhibits DNA binding by standard sgRNAs. Tracing the evolution of vsgRNAs revealed multiple instances of phages hijacking host-encoded tracrRNAs and CRISPR-like RNAs responsible for CRISPR-Cas9 auto-repression. Finally, we reprogrammed vsgRNAs to mediate efficient genome editing in mammalian cells. These findings reveal a natural precedent for programming RNA-guided nucleases and expand the known RNA-based mechanisms by which phages subvert bacterial immunity.

[P121] Honoring the Past, Engineering the Future: Prophage-Driven Extracellular Replication Systems

Gabriel Antonio Minero[1], Line Mørkholdt Lund[1], Lasse Hyldgaard Klausen[1], Obinna Markraphael Ajunwa[1], Mingdong Dong[1], Victoria Birkedal[1], Rikke Louise Meyer[1]

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We report paradigm-shifting phenomenon of a programmable template-directed extracellular synthesis of DNA in bacterial cultures and biofilms. Bacteriophages (phages) invade bacteria and replicate enormously inside single bacterial cells bursting after the bacteriophage capsid assembly. The role of the prophages has been linked to production of extracellular DNA (eDNA), where a mutant strain lacking all prophages is particularly known to be poor in eDNA [1]. A superior phage replication machinery in most phages, including phages λSo in Shewanella oneidensis (S. oneidensis) and phi29 in Bacillus subtilis (B. subtilis), replicates via rolling circle replication. We suggest that deoxynucleoside triphosphates (dNTPs) and phage polymerase would be available after bursting the cell to continue replication of an exogeneous circular DNA in the extracellular space. In addition to the mutant ΔλSo ΔMuSo strain of S. oneidensis, we constructed and used reporter strains of S. oneidensis: wild type (tn7::CFP indicative of live cells, cro::Venus indicative of active λSo prophage replication) and ΔMuSo (SO_2963::sfGFP, indicative of phage λSo capsid assembly). The obtained trends indicated synthesis of G-quadruplex DNA from the exogeneous circular template following onset of starvation and prophage λSo active replication. This novel biosynthetic route resulted in large concentration of DNA that aggregated and phase-separated in the shapes of flowers and wires in 3-day S. oneidensis and B. subtilis biofilms, respectively. Read the full research article to find out more details and the implications of this research in the field of biochemistry [2].

[P122] Assessment of Dark Oxygen Production and Denitrification of Microbulbifer donghaiensis

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Photosynthesis has long been thought of as the only biological oxygen-producing process. However, it has been recently found that some microorganisms can produce oxygen in the dark (DOP; Dark Oxygen Production) via dismutation reactions. Moreover, the presence of obligate aerobic microorganisms in dark and anoxic subsurface aquifers implies that biotic and abiotic DOP may be an important process in subsurface environments or any other systems which are thought of as “anoxic” environments.

One of the biological DOP pathways is nitric oxide dismutation (NOD), in which nitric oxide (NO) is dismutated to O_2 and N_2 or N_2O. Microbes possessing putative nitric oxide dismutase genes may be able to perform NOD. Here, we investigate the potential DOP and denitrification processes in Microbulbifer donghaiensis, which is a NOD candidate bacterium isolated from ocean sediments. To measure these processes, NO_2 amended incubations were performed while monitoring O_2 concentration with high-sensitivity O_2 optodes and NO concentration with microsensors.

Furthermore, O_2 and NO consumption rates were measured at different pH by injecting oxic water or NO-flushed water. We also tested different potential inhibitors (cyanide, azide, and carbon monoxide) of aerobic respiration, but those do not affect aerobic respiration of M. donghaiensis. Our data show that M. donghaiensis may produce O_2 via NOD. A pH shift resulted in O_2 accumulation during incubation, suggesting that aerobic respiration was suppressed under low pH. Consumption rate measurements also show that the O_2 consumption rates decrease at low pH, whereas NO consumption rates can be high at low pH. This data complements the O_2 accumulation during the NO_2-amended incubation mentioned above.

In conclusion, DOP by M. donghaiensis may be one of the phenomena supporting that biological DOP has been overlooked in the “anoxic” environments. Aerobic microbial communities can thrive in more biospheres than we think.

[P123] Science Outreach Enables Antibiotic Discovery

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Tiny Earth, founded by Dr. Jo Handelsman, is a global crowdsourcing initiative addressing the challenge of antimicrobial resistance. The initiative combines science education with authentic research by engaging students in the search for novel antibiotic-producing bacteria. Tiny Earth Denmark adapts this framework into an outreach programme for Danish high school students (aged 16-19). The programme comprises four teaching modules that follow the scientific workflow from soil sampling and bacterial isolation through characterization and bioactivity screening to whole-genome sequencing and bioinformatic analysis of predicted biosynthetic gene clusters. The modules combine activities at local high schools, university-based lab work, and online teaching with university students serving as instructors.

Since its launch in 2020, more than 3,500 students across Denmark have participated, contributing more than 1,500 bioactive isolates to a growing microbial collection for further exploration. With the recent introduction of the fourth teaching module focusing on bioinformatics, these bioactive isolates are now systematically subjected to DNA extraction, Nanopore sequencing, and genome assembly with 600 genomes generated to date. All genomes are analysed for biosynthetic gene clusters using antiSMASH and linked with phenotypic data transforming student discoveries into a valuable genomic resource for antibiotic discovery.

[P124] Characterising Microbial Threats to Danish Cultural Heritage

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Danish cultural heritage is continuously exposed to the risk of biodeterioration caused by bacteria and fungi, yet the microorganisms involved remain poorly characterised, limiting evidence-based conservation. In this project, we isolated bacteria and fungi from Danish heritage sites and artifacts, including Den Gamle By and the Assistens Kirkegård, and identified them by DNA extraction, PCR, and amplicon sequencing, complemented by morphological characterisation of mycelium and spores by microscopy. Growth experiments at increasing temperatures determined optimal and sub-optimal growth conditions for each isolate, and biocide assays tested their susceptibility to chemical treatments used in conservation. Together, these data link species identity, growth behavior, and treatment resistance, helping to identify which organisms might threaten valuable Danish artifacts and heritage sites and provide insight into how to effectively discourage biodeterioration, supporting the long-term preservation of Danish cultural heritage.

[P125] Active Temperate Phages Linked to Core Bacterial Taxa Dominate the Wheat Phyllosphere Virome

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The wheat phyllosphere supports diverse bacterial communities, yet the phage life-history strategies that promote persistence in this dynamic habitat remain poorly understood. We analysed 36 non-amplified double-stranded DNA viromes, 35 with matched microbial metagenomes, spanning multiple wheat tissues, years, and locations in Denmark and the United States, to determine how temperate and virulent phages contribute to the active wheat phyllosphere virome and how phage prevalence relates to the distribution of their bacterial hosts.

We identified 7,958 viral operational taxonomic units (vOTUs), including 1,211 high-quality or complete genomes, highlighting extensive viral novelty. To distinguish active viral populations from residual cellular DNA, we defined active vOTUs as those enriched in the viral fraction relative to sample-specific microbial background. This yielded 726 active vOTUs. Temperate phages contributed disproportionately to the active virome: they showed significantly higher prevalence than virulent phages and represented a greater proportion of both detected vOTUs and viral relative abundance per sample.

The Danish virome was structured around a persistent core, with 290 active vOTUs detected in at least half of the samples and 13 present in all samples. Seven ubiquitous vOTUs predicted to infect Erwinia matched previously characterized inducible prophages, supporting in planta prophage induction as a source of extracellular viral particles. Two ubiquitous members belonged to the genus Sendosyvirus, including one detected across both Danish and United States samples. At the community level, viral presence-absence structure strongly tracked bacterial community composition, and active phages were more frequently predicted to infect prevalent bacterial genera.

Together, these results indicate that the wheat phyllosphere virome is organized around a recurrent set of active temperate phages associated with core bacterial taxa. Our findings support a model in which lysogeny and recurrent prophage induction promote phage persistence and couple viral community structure to the bacterial microbiome in this highly variable plant habitat.

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