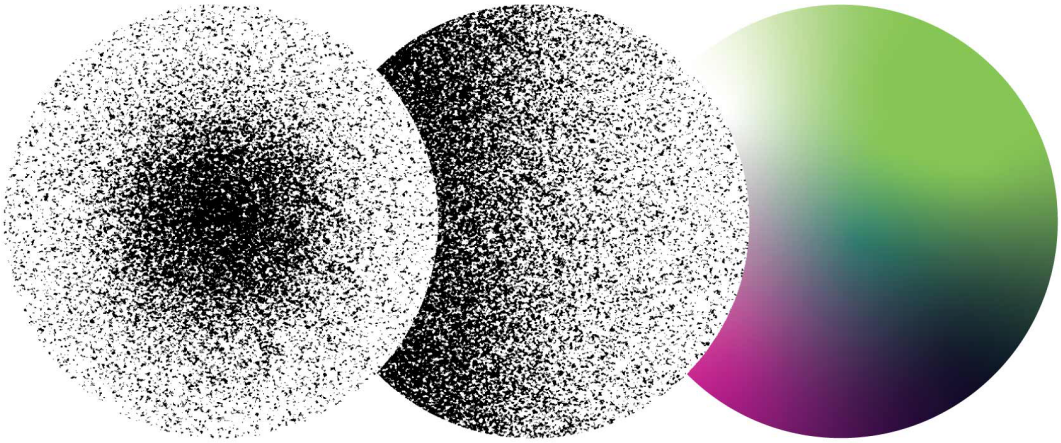
'25 RIGA CBM

6th Congress of Baltic Microbiologists



book of abstracts

University of Latvia, Riga
October 1 - 3, 2025

Welcome to CBM 2025

Dear CBM 2025 participants!

The Organizing Committee is honored to welcome you to CBM 2025, the 6th Congress of Baltic Microbiologists, taking place in Riga at the University of Latvia. The tradition of regular meetings of the community of microbiologists of Baltic States was started in 2012 in Riga. With a gap created by Covid-19 pandemic the meetings were rotated among the Baltic States in a regular, biannual periodicity, meanwhile seeking for broadening the outreach, involving the researchers and practitioners from broader Balic region and beyond. The 6th meeting back again in Riga will create a venue for ca. 100 researchers from 6 countries, Germany, Italy, US and the Netherlands extending the range of Baltic participants.

Across the three days the scientific program of CMB will create cohesive, timely narrative of microbiological research and application. We aim to initiate transdisciplinary dialogues and foster integrative insight. The scope of the program reflects this: from medical and antimicrobial microbiology, to virology, environmental microbiology, microbial diversity and preservation, microbial communities and interactions, waste valorization, to systems biology and metabolic engineering. These themes, taken together, capture both the challenges and opportunities at the frontier of microbiological science and biotechnology today.

The program supports cross-talk, allowing you to see how tools, concepts, and challenges overlap across domains. Multiple scales at which microbiology operates cover molecular investigations — enzymes, proteases, genes — alongside with community-level studies and system models. Topics like antimicrobial resistance, waste valorization, microbial interactions in plant systems, and bioeconomy engineering all respond to real-world needs for sustainable solutions. Advanced methods — omics, synthetic communities, modeling, imaging, electrochemistry — provide new insights into the studied topics.

We hope that flash talks, poster sessions, and younger presenters will ensure fresh ideas and productive discussions. The CBM 2025 Abstract Book will provide summaries of oral presentations followed by the abstracts of poster presentations.

The organizers invite you to CMB 2025, a platform to engage and share, challenge assumptions, and leave inspired by new connections, questions and new cooperation ideas!

On behalf of the Organizing Committee

Dr. Indriķis Muižnieks

Professor, University of Latvia

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Session: Medical and antimicrobial microbiology

Chair Asoc. Prof. Triinu Visnapuu

Advancing One Health microbiology research: integrating genomic and environmental surveillance

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Keywords: One Health, environmental surveillance, zoonoses, antimicrobial resistance (AMR)

Institute of Food Safety, Animal Health and Environment (BIOR) advances One Health by linking laboratory diagnostics, research, and risk assessment across human, animal, food, and environmental microbiology. As national reference laboratory (NRL) in several domains, BIOR is a member of EU surveillance and NRL's networks, contributing harmonized data that inform national and EU risk management authorities. Current BIOR's One Health platform mostly combines (i) whole-genome sequencing (WGS) for pathogen characterization in clinical, veterinary, food and water matrices; (ii) wastewater-based epidemiology (WBE) for population-level respiratory pathogen trends; and (iii) participation in EU-harmonized antimicrobial resistance (AMR) monitoring in zoonotic and indicator bacteria from animals and food chain.

WGS capacity supports rapid outbreak investigation and high-resolution typing, exemplified by sequencing of clinically important bacterial isolates and routine food/water microbiology. WBE provided early-warning signals for SARS-CoV-2 transmission and enabled detection of emerging variants in municipal wastewater in Latvia, demonstrating the value of environmental surveillance for timely public-health action. Alongside general environmental surveillance, specific research is carried out in wildlife (carnivores, wild boars, birds, bats etc.) to develop resilient and comprehensive disease surveillance systems and to better understand infectious agent reservoirs and transmission routes between animals, humans and environmental compartments. Recent research findings have strengthen also understanding of more advanced control strategies for important infectious diseases such as African Swine Fever (ASF) or Highly Pathogenic Avian Flu (HPAI).

BIOR's contributions to EU AMR datasets also strengthen trend analyses on food- and waterborne pathogens such as *Salmonella*, *Campylobacter*, *Escherichia coli* and enterococci across the food chain. By participating in EU4Health program activities such as JAMRAI-2 and the EARS-Vet networks BIOR developing One Health AMR surveillance systems at national and international level.

By coupling genomics, environmental monitoring, and harmonized AMR surveillance within an institutional One Health framework, BIOR delivers actionable evidence that links animal, food, environmental and human health. Priorities for the next phase include routine metagenomics for complex matrices, interoperable/FAIR data flows into EU and other global platforms, and quantitative integration of WBE/WGS/AMR signals to enhance forecasting and source attribution. Recent national engagement underscores the strategic role of BIOR in safeguarding public health and biosecurity.

Acknowledgements

Recent research activities on One Health AMR surveillance have been supported by funding of Project No. VPP-ZM-VRIIILA-2024/1-0002 "Scientifically Grounded Solutions for a Sustainable Food System to Achieve the Goals of the European Green Deal (GreenAgroRes)", EU4Health programme projects EU-WISH and JAMRAI-2.

Silver nanoparticles obtained using *Geobacillus sp.* bacteria in biocontrol of pathogenic skin microbiota

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Keywords: antimicrobials, silver nanoparticles

Scientists are searching for novel antimicrobial agents for the biocontrol of pathogenic bacteria and yeasts because of the growing resistance to current antimicrobials and the adverse effects of various medications. Silver has long been recognized as a superior antimicrobial substance, and silver nanoparticles (AgNPs) are a promising antimicrobial alternative. Although AgNPs can be produced chemically or physically, the environmental damage and increased expense of both methods have increased interest in the biological synthesis of AgNPs using microorganisms or plant extracts.

The main advantage of AgNPs compared to other widely used antimicrobial agents is their complex antimicrobial effect. AgNPs affect different parts of the cell at the same time, starting with the outside parts, like cell walls and membranes, and later internal cell structures. Therefore, AgNPs disrupt various functions of the cell, for example, DNA and protein synthesis, cause oxidative stress, etc.

In this study, *Geobacillus* sp. strain 612 was used to obtain AgNPs. The antimicrobial effect was tested against pathogenic bacteria and yeast (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Candida guilliermondii*). It was evaluated that these AgNPs disrupt the cell membrane of microorganisms, cause oxidative stress, and activate caspases in yeast cells. Moreover, it was evaluated whether these AgNPs are safe to use for biocontrol of skin pathogenic microbiota on keratinocyte cells.

Acknowledgements

The authors would like to thank Indrė Dalgėdienė for performing flow cytometry measurements.

Fueling the shield: the role of F_1F_0-ATPase in AMP resistance of *Zymomonas mobilis*

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Keywords: stress resistance, *Zymomonas mobilis*, antimicrobial peptides, resistance, uncoupled growth; H+dependent ATPase

Zymomonas mobilis is a facultatively anaerobic alphaproteobacterium known for its efficient ethanol production and its notable inhibitory activity against a broad spectrum of microorganisms. Our research demonstrates that its antimicrobial effects are primarily driven by the production of organic acids. Remarkably, *Z. mobilis* exhibits an exceptional resistance to a variety of antimicrobial peptides (AMPs), with minimal inhibitory concentrations exceeding those of most other bacterial species by up to two orders of magnitude. This phenomenon of AMP super-resistance suggests the presence of previously unrecognized defense mechanisms, possibly linked to the bacterium's uncoupled energy metabolism. We hypothesize that *Z. mobilis* repurposes its transmembrane proton motive force (PMF)—typically used for ATP synthesis—to counteract the membrane-disruptive effects of AMPs. In this study, we investigate the role of uncoupled energetics and the H^+-dependent F_1F_0-type ATPase in this resistance. Our early findings reveal that inactivation of either the F_1 or F_0 subunits reduces both ATP hydrolytic activity and resistance to AMPs, underscoring the importance of ATPase function in this defense strategy.

These findings not only expand the known spectrum of bacterial AMP resistance mechanisms but also offer a new framework for engineering peptide-producing strains with enhanced resistance capabilities.

Identification of microorganisms possessing biological threats using MALDI-TOF MS enhanced by Artificial Intelligence

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Keywords: Biological Warfare Agents, Artificial Intelligence, machine Learning, MALDI-TOF MS

The accurate and rapid identification of bacterial pathogens is critical in clinical diagnostics and medical biodefense. In the last two decades, the MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry) has become an effective and reliable methodology for the identification microbial species. Although MALDI-TOF has many advantages, it also has limitations that can compromise its accuracy and reliability in identifying certain pathogens: (i) the MS databases used to identify pathogens are often incomplete, particularly for rare and poorly characterized species, (ii) technique struggles to differentiate between phylogenetically closely related bacterial species and subspecies, (iii) viral identification remains limited due to the lack of comprehensive virus-specific databases, (iv) reliance on proprietary software restricts algorithmic transparency and customization. This limit scientists' access to the underlying algorithms and hinder their ability to adapt them to the specific needs. Consequently, scientists have turned to open-source machine and deep learning algorithms to develop more suitable pipelines for analysis of complex MALDI-TOF spectra data.

This study presents initial results from a comparative analysis of nine ML/DL models applied to a dataset of nearly 200 spectra from 18 bacterial species — including 12 Gram-positive and 6 Gram-negative species, five of which were Biological Warfare Agent (BWA) simulants. We applied a four-step preprocessing pipeline and evaluated model performance on two classification tasks: (i) Gram-status discrimination and (ii) identification of four BWA simulants. Among the tested models, the 1D Convolutional Neural Network (1D-CNN) achieved the highest performance, with accuracy and sensitivity ranging from 97% to 100%, confirming its suitability for spectral classification tasks. In addition to the classification tasks, we also present the results of the methodology for defining dominant peaks associated with different bacteria.

Acknowledgements

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Session: Virology

Chair Dr. Laura Kalinienė

Estonian phages going viral

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Keywords: bacteriophages, *Pseudomonas putida*, phage defence, stress responses

Pseudomonas putida is a metabolically highly versatile environmental bacterium with great promise to be used as cell factory in biotechnological production, and bioremediation approaches for degrading a variety of aromatic pollutants. Different metabolic pathways of *P. putida* have been extensively studied, but surprisingly nothing is known about its phage defence. Until recently almost no phages had been isolated for the common laboratory strain *P. putida* KT2440 (isogenic to PaW85) and no phage defence mechanisms have been characterized yet.

We isolated a collection of environmental *Pseudomonas putida* phages from Estonia (CEPEST). The collection currently consists of 22 species of lytic dsDNA phages that infect the common laboratory strain *P. putida* PaW85 and can be grouped into nine phage genus clusters. Most of the phages have a very narrow host range, are temperature sensitive and require intact lipopolysaccharides for efficient infection. CEPEST phages were isolated using a predictably weakened derivative of the bacterium. It lacks four cryptic prophages and 13 toxin-antitoxin systems (TAS) from the genome as these entities may increase phage resistance. We show that the four cryptic prophages in *P. putida* chromosome strongly protect against the infection of many phages, whereas the chromosomal TAS seem to have no positive effect for *P. putida* upon phage infection [1].

The CEPEST collection can now be employed to study different aspects in the phage-bacteria interactions, including the mechanisms behind temperature sensitivity and prophage-provided defence. It also opens up the studies of ecology and co-evolution of *P. putida* and its phages and the discovery and functional studies of yet unidentified phage defence systems of this biotechnology workhorse.

Acknowledgements

I am grateful to Andres and Rita and our group members with whom we tackle the problems of *P. putida* phage biology. The work is funded by Estonian Research Council: PRG1431 for RH, STP39 for AA, and Estonian Research Council with EMBO (IG5323) and ERC starting Grant PhaBacArms (101116205) for HT.

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A test system for exploring the substrates of the viral protease

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Keywords: one viral protease, cleavage specificity, *in vitro* cleavage assay

Viruses lack the translational machinery required for protein synthesis. Instead, they hijack the host´s ribosomes and depend on the cellular translation machinery. Upon entry into the cell, a polyprotein consisting of structural and non-structural proteins is synthesised from the viral genome. This polyprotein is further processed into functional proteins by the virus-encoded protease. Therefore, an active protease is essential for both the replication of the viral genome and the assembly of the viral particles.

Identifying the substrates of viral proteases helps us to understand the viral replication cycle. The substrates vary between viruses, depending on the type of the protease they encode. Our work focuses on the Cricket Paralysis Virus (CrPV) and its 3C cysteine protease [1]. To study the protease´s substrate specificity, a molecular tool consisting of a recombinant protease and a model substrate was constructed. Firstly, the recombinant GST-tagged CrPV 3C protease and its inactive form – in which the cysteine in the catalytic triad was replaced with alanine – were expressed and purified from *Escherichia coli* cells. The model substrate was constructed based on the heat shock protein DnaK from *Pseudomonas putida*. The native polyprotein cleavage site of the CrPV 3C protease was inserted into the unstructured linker region of DnaK. Additionally, a mutant substrate was constructed by changing the native polyprotein cleavage site sequence.

The functionality of the test system was analysed using an *in vitro* cleavage assay. The recombinant viral protease was considered active and specific based on the accumulation of cleavage products. The protease was indeed active on the native and not on the mutant cleavage site sequence, indicating that the design of the test system was successful. The constructed molecular tool can be exploited to determine specific substrates in future studies.

Acknowledgements

This research was funded by the Estonian Research Council grants (grant number PRG1741 to T.T.).

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From leaves to lab: tracking plant viruses with NGS

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Keywords: plant virus, next generation sequencing, RNA-Seq, *Marafivirus*, Cryo-EM

Next-generation sequencing (NGS) technologies have significantly expanded our capacity to investigate plant virus diversity, enabling comprehensive characterization of viral genomes, structures, and host associations. Unlike classical diagnostic methods, NGS allows for unbiased detection of novel viruses, particularly those involved in asymptomatic or mixed infections.

In this study, virome analysis was performed on sea buckthorn (SBT) and several berry crops grown in Latvia using rRNA-depleted total RNA sequencing. Prior to our investigation, no viral agents had been identified in SBT. From symptomatic leaves, two novel RNA viruses were discovered and fully sequenced: sea buckthorn marafivirus (SBuMV), a new species within the genus *Marafivirus*, and sea buckthorn-associated picorna-like virus (SBTaPLV), an unclassified virus with distant homology to *Picornavirales*. SBuMV capsid protein was heterologously expressed and self-assembled into VLPs, allowing 3D structure determination via Cryo-EM, revealing close structural similarity to tymoviruses. SBTaPLV virion architecture and replicative proteins were modelled using AlphaFold and compared to existing viral structures in the PDB.

Additionally, virome profiling of asymptomatic and symptomatic samples from blackcurrant, redcurrant, raspberry, blackberry, and grapevine revealed multiple unreported or poorly characterized viruses in Latvia. Notable findings include new tombus-like and polero-like viruses in grapevine and redcurrants, and rhabdoviruses in blackcurrant, as well as complex mixed infections in grapevine and *Rubus* plats involving grapevine emaravirus A, Vitis cryptic virus and others, and Rubus yellow net virus together with and raspberry bushy dwarf virus, respectively.

These results expand the known diversity of plant-infecting RNA viruses in the Baltic region, contribute to viral taxonomy, and demonstrate the utility of structural and genomic virology in identifying and characterizing emerging plant pathogens.

First steps towards the Latvian *Klebsiella spp.* bacteriophage biobank

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Keywords: bacteriophages, phage diversity, comparative genomics, *Klebsiella spp.*, biobank, antimicrobial resistance, Whole Genome Sequencing

Klebsiella spp. are non-motile Gram-negative encapsulated rods from the family *Enterobacteriaceae* that are widely distributed in various terrestrial, aquatic, and host-associated environments. *Klebsiella spp.* are also normally found on healthy human mucosal surfaces, where they commonly colonize (e.g., nose, throat, intestinal tract). At the same time, genus *Klebsiella* representatives are recognized as opportunistic, mainly nosocomial pathogens (known for high mortality rates and limited treatment options). Emergence and spread of antibiotic-resistant *K. pneumoniae* strains is especially concerning and alternative treatment and control options are necessary as we enter the «post-antibiotic» era.

Deliberate use of bacteriophages (phages) to treat bacterial infections - phage therapy seems a promising alternative/complementary strategy to treat extensively drug-resistant bacteria. To increase the prospects for phage therapy in Latvia, we are accumulating a collection of phages infecting relevant *Klebsiella spp.* strains prevalent in Latvia.

Bacterial strains isolated from patients infected by drug-resistant *Klebsiella spp.* or "healthcare-environment" strains from hospital sewage are used as the isolation hosts for tailed phages. These phages are propagated and subjected to WGS. Strictly lytic phages not encoding any known products precluding their practical use are characterized in terms of their host range, lifecycle features, and proceed to long-term storage as potential candidates for therapeutic use.

The initial workflow using randomly requested drug-resistant bacterial strains as hosts has resulted in a frequent recovery of very similar phages with little value added to the effective phage diversity of our emergent collection. This has prompted a greater focus on the informed diversification of the isolation host panel (selecting relevant isolation hosts based on their WGS data). Strategic host selection has resulted in a wider diversity of relevant phages isolated.

Acknowledgements

Iekšējā RSU un ārējā RSU ar LSPA konsolidācija", Nr.5.2.1.1.i.0/2/24/I/CFLA/005 grants Nr. RSU-PAG-2024/1-0023 Inovatīvas stratēģijas Klebsiella spp. antibakteriālās rezistences ierobežošanai: Fāgu un antibiotiku sinerģijas izmantošana (ReScUe-Kleb)

Session: Environmental microbiology

Chair Asoc. Prof. Linda Mežule

Is the concept of water biostability still valid?

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Keywords: biostability, viability, low and high RNA bacteria

The concept of water biostability—the notion that treated drinking water should contain minimal biodegradable organic carbon to prevent microbial regrowth—has historically guided the design and regulation of drinking water distribution systems [1, 2]. However, emerging evidence suggests that this substrate-focused paradigm may be insufficient in addressing the microbial dynamics of increasingly complex and climate-sensitive water networks [2, 3, 4].

This study critically re-evaluates the biostability concept using field and pilot-scale experiments, highlighting the role of biofilms, microbial diversity, and the unintended effects of nutrient limitation. Results demonstrate that low biodegradable carbon does not guarantee microbial stability. Instead, factors such as microbial community structure, residual disinfectant efficacy, pipe material, and environmental variables (e.g., temperature) play a more significant role in shaping microbial resilience and regrowth potential.

Advanced molecular tools, including 16S rRNA sequencing, flow cytometry, and cell viability assays, were employed to monitor microbial population shifts under varying operational stresses. Pilot-scale studies incorporating real-time monitoring and growth potential measurements reveal that interspecies competition among bacteria is influenced by substrate availability and temperature—challenging the classical assumptions of passive microbial control.

The findings underscore the need for a more holistic framework for managing drinking water microbiology, shifting from a purely substrate-limiting approach toward one that integrates ecological interactions, system design, and operational dynamics. This has critical implications for water utilities, regulators, and the application of biotechnology in safeguarding public health through resilient and adaptive drinking water systems.

Acknowledgements

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Label-free droplet image analysis with CellProfiler and CellProfiler Analyst

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Keywords: microfluidics, microplastics, microbiology, image analysis, open-source software

Droplet microfluidic methods used for microbiological experiments are fast, cost-effective, and provide high-throughput data. However, analysis of such image data can be difficult, and detection of molecular labels is limited by microscope parameters.

Currently, there is lack of user-friendly methods to analyse a large volume of label-free droplet images without the need for trained personnel, or expensive, proprietary software. Such methods would make droplet microfluidic technology more widely accessible for a larger range of biological applications, especially microbiology.

In this paper we demonstrate an image analysis pipeline designed using Cellprofiler™, a free, open-source software, and image classification using its sister software, Cellprofiler Analyst™. The pipeline identifies water-in-oil microfluidic droplets, microplastic particles, and bacterial growth without using fluorescent or other labels, while image classification sorts identified droplets into 3 classes based on their degree of bacterial growth.

Acknowledgements

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Microplastic influence on antibiotic susceptibility and bacterial aggregation

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Keywords: microplastic, antibiotics, droplet microfluidics, antimicrobial susceptibility, aggregation, biofilm

Antibiotic resistance in bacteria is a growing problem in healthcare [1]. One mechanism contributing to resistance is bacterial aggregation, which can lead to biofilm formation and decreased antibiotic susceptibility [2]. Aggregation can occur between one (auto-aggregation) or multiple (co-aggregation) bacteria species [4]. Microplastics, now ubiquitous in the environment, can promote bacterial aggregation by providing surfaces for microbial adhesion and biofilm development [3]. It is important to understand the influence microplastic has on bacterial aggregation and susceptibility to antibiotics, however, interactions between bacteria and microplastic are difficult to study [5]. We consider droplet microfluidics to be an effective platform for isolating bacterial cells and microplastics into individual microenvironments, enabling more precise investigation of their interactions.

The susceptibility and auto-aggregation were studied using droplet microfluidics' methods – *Escherichia coli* and *Staphylococcus aureus* were first individually encapsulated into nanolitre water-in-oil droplets with varying antibiotic concentrations (cefotaxime, ciprofloxacin, doxycycline) in two parallels (with and without 10 µm polystyrene microplastic beads). Droplets were incubated at 37°C overnight and imaged with confocal microscope. Images were analysed with open-source software CellProfiler™ using specialized pipelines [6,7].

Experiments with *S. aureus* showed increase of autoaggregation in samples with microplastic for ciprofloxacin. However, no difference in antibiotic susceptibility was observed for samples with and without microplastic. Preliminary results for *E. coli* indicate varying bacterial growth and aggregation tendencies.

The next step is to cultivate both bacterial strains together to observe possible co-aggregation between strains. This could improve understanding of microplastic and aggregation effects on bacterial susceptibility.

Acknowledgements

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Diversity of magnetotactic bacteria and magnetosome biomineralization

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Keywords: magnetotactic bacteria, magnetosomes, biomineralization

Magnetotactic bacteria are a particular type of microorganisms sharing the abilities of intracellularly mineralizing nanoparticles called magnetosomes. The magnetosomes are magnetic nanoparticles, often aligned in chains within the bacterial body, and which passively orient the bacteria along the magnetic field lines of the Earth's geomagnetic field, thereby directing the motility of the prokaryotes. These bacteria, however, represent a morphologically, phylogenetically, and physiologically diverse group of bacteria.

The magnetosomes are composed of a magnetic core made out of the mineral magnetite (the iron oxide Fe_3O_4) or greigite (the iron sulfide Fe_3S_4) embedded in a phospholipidic membrane. The particles exhibit a variety of morphologies, varying from classical cuboctahedron to bullet- or tooth shapes defying structural principles. Genes responsible for magnetosomes biomineralization are clustered within the so-called magnetosome genetic island.

In this presentation, I will focus on two cultured species *Magnetospirillum magneticum* AMB-1 and *Desulfovibrio magneticus* RS-1. These cells indeed form magnetosomes of different morphologies. I will highlight the associated physiological / chemical as well as the genetic backgrounds. In particular, I will show how physiological differences impact the chemical reaction pathway leading to the formation of magnetite [1,2].

Acknowledgements

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Session:
Microbial diversity and preservation

Chair Dr. Daiva Burokienė

Isolation and identification of novel non-dairy starter culture candidates from plant matrix using backslopping propagation

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Keywords: plant-based food; backslopping technique; lactic acid bacteria; metagenetic analysis; non-dairy starter cultures; MALDI-TOF

The majority of non-dairy starter cultures on the market are originally isolated from milk and therefore do not provide the most optimal fermentation for plant matrices. Developing plant-derived starter cultures is essential for creating high-quality, tasty dairy alternatives.

This study aims to isolate and characterize bacterial strains with the potential to be used as non-dairy starters from plant sources via backslopping evolution. A natural consortium of macerated plants was inoculated into two oat and two pea commercial drinks and backslopped for seventeen cycles to evolve the bacterial consortium at 25 °C, 34 °C, and 42 °C.

The results showed that the initial natural consortium contained less than 1% lactic acid bacteria, and after the seventeenth cycle, lactic acid bacteria dominated in all investigated consortia. Oat Od1-25 and Od2-42 and pea Pd1-34 and Pd1-42 samples were selected for strain isolation based on amplicon-based metagenetic analysis of 16S rRNA gene sequencing and sensory properties. The strain isolation was performed using an out-plating technique, and colonies were identified by MALDI-TOF mass spectrometry. Altogether, eleven lactic acid bacteria species of plant origin were obtained. The strains belonged to the *Leuconostoc*, *Enterococcus*, *Lactobacillus*, and *Lactococcus* genera.

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The richness of the wild yeast population in Estonia – examples from two case studies

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Keywords: Estonian Yeast Stock Collection, yeast species, biodiversity, fungicide susceptibility

The habitat of a microbial community is a diverse ecological niche that includes soil, plant phyllosphere, fruits, etc. This microbiome comprises bacteria and fungi, as well as archaea, protists and viruses. Yeasts, unicellular fungi, are also part of this community. The Estonian Yeast Stock Collection was initiated as a project to popularise science and teach modern molecular microbiology methods to secondary school students. Over the years, an impressive collection of wild yeasts from natural habitats in Estonia has been established. The collection includes over 3,500 strains belonging to 160 yeast species.

Our first study [1] aimed to characterise the culturable epiphytic yeasts associated with apple fruits. A total of 230 isolates were collected and their species identity was determined by sequencing the rDNA gene. Of the 33 species identified, most belonged to the phylum Basidiomycota. Members of genera *Vishniacozyma, Filobasidium,* and *Rhodotorula* were the most frequently isolated. Several isolates were considerably divergent from their closest relatives and may represent new, distinct species requiring further characterisation. The study revealed the high diversity of yeast species on apple fruits.

The second study [2] focused on yeasts colonising the leaves of winter wheat. Samples were taken twice during the growing season and cultivable yeasts were then isolated. A total of 454 isolates belonging to 34 species were collected. The three most frequently isolated species - *Sporobolomyces roseus*, *Vishniacozyma aff. tephrensis* and *Vishniacozyma victoriae* - formed a "core" yeast community. These species accounted for almost half of all isolates. The presence of pure yeast cultures enabled testing of their susceptibility to the active substances in commonly used fungicides for controlling septoria tritici blotch, one of the most common wheat diseases worldwide. Evaluating the *in vitro* susceptibility of the yeast species allowed us to estimate the effect of the widely used fungicides on the microbial community of wheat leaves.

Acknowledgements

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[2] manuscript in preparation

Microbial diversity and presence of opportunistic pathogens in ready-to-eat plant-based meat alternatives in Estonia

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Keywords: plant-based meat alternatives, ready-to-eat products, opportunistic pathogens, food safety

The growing popularity of plant-based diets has significantly increased the demand for plant-based meat alternatives, especially ready-to-eat (RTE) products. Many of these products are classified as ultra-processed and often undergo high-temperature and high-pressure treatments, such as ultra-heat treatment (UHT), to reduce microbial load and extend shelf life. However, concerns remain regarding microbial contamination during post-processing stages, such as slicing, handling, and packaging. This study aimed to assess the microbial diversity, focusing on opportunistic pathogens, in commercially available RTE plant-based meat alternatives across Estonia. The investigation concentrated on pea-, mushroom-, and soybean-based products with either minced or fibrous textures.

Between April 30, 2024, and June 9, 2025, a total of 195 samples were collected from retail outlets. All samples were transported under chilled conditions and stored at 4 ± 2 °C prior to analysis. For microbial examination, 10 g of each sample was homogenized with 90 ml of sterile phosphate-buffered saline (PBS) using Stomacher bags. Bacterial isolates were cultivated on selective and non-selective media following ISO-standard protocols for the enumeration of *Bacillus* spp., *Clostridium* spp., *Listeria* spp., and aerobic mesophilic microorganisms. The isolates were incubated under appropriate conditions, and species identification was performed using MALDI-TOF MS at the National Centre for Laboratory Research and Risk Assessment (LABRIS).

The results demonstrated notable microbial diversity, including several opportunistic and commensal bacteria. Detected species included *Micrococcus luteus* (2.6%) and *Leuconostoc mesenteroides* (2.6%), followed by *Enterococcus faecium* (2.1%) and other *Staphylococcus spp. (2.1%)*. Additionally, *Micrococcus endophyticus (1.5%), Staphylococcus hominis (1.5%), and Staphylococcus epidermidis* (1.5%) were identified. Less frequently detected species included *Staphylococcus warneri* (0.5%), *Macrococcus caseolyticus* (0.5%), *Staphylococcus simulans* (0.5%), *Staphylococcus pasteuri* (0.5%), and *Streptococcus spp.* (0.5%). These microorganisms, while often harmless in healthy individuals, may pose risks to immunocompromised populations.

Despite the application of UHT, the presence of these bacteria suggests possible survival through processing or post-process contamination. The findings emphasize the need for stringent hygiene measures during post-treatment handling and packaging. Improved food safety protocols and quality control are crucial for protecting public health, particularly for vulnerable consumers.

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Session: Microbial communities and interactions

Chair Prof. Ott Scheler

Dissecting mechanisms of root microbiota establishment using synthetic communities

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Keywords: plant microbiome, synthetic communities, SynComs, functions, machine learning

Plants harbour dynamic complex microbial communities in and around their roots. These communities can inhibit pathogens, prime or activate the plant's immune system, aid in nutrient uptake, stimulate plant-growth, and induce resilience against a variety of abiotic stresses. To leverage these plant-beneficial microbial traits in sustainable agriculture, a thorough understanding of root microbiome assembly and functioning is essential. However, these processes are shaped by the plant and vary across environments and hosts, challenging microbiome prediction and engineering. To uncover principles of bacterial selection, we applied a systems-level approach using reconstitution studies with communities of hundreds of isolates from Arabidopsis, Barley, and Lotus roots grown in soils. Functional divergence among their microbiota reflected distinct strategies: in Arabidopsis and Barley, recruitment was primarily shaped by inoculum, while the Lotus root environment favoured fewer, functionally diverse isolates, akin to a 'Swiss army knife' strategy. Despite taxonomic variability, root microbiomes encoded largely overlapping functions. Across major taxa, isolates with broad but distinct functional repertoires within their families were consistently more abundant. Using a genome-to-function framework that is function-centric, taxonomically inclusive, and host-context aware, we identified 266 functions enriched across all root microbiomes. This functional backbone emerged as a core signature of plant-associated bacteria, providing a solid foundation for microbiome engineering in agriculture.

Probiotic potential of yellow mealworm (*Tenebrio molitor*) larvae: an experimental evaluation

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Keywords: *Tenebrio molitor* larvae, probiotics, *Bacillus coagulans*, *Lactobacillus acidophilus*

The aim of this study was to determine the effect of *Bacillus coagulans* and *Lactobacillus acidophilus* on *Tenebrio molitor* larval growth and their natural microbiota, to assess the changes in the quantity of these probiotic bacteria in the mealworm gut, and their survival during larval processing.

T. molitor larvae were fed with three concentrations of probiotic suspensions: 0.15×10^9, 0.75×10^9, and 1.35×10^9 CFU in a millilitre. The larvae were reared for seven or fourteen days, and their weight was measured during the rearing period. At the end of the experiment, larval samples were collected, a part of them frozen to evaluate the effect of probiotics on the natural gut microbiota using culturing and MALDI-TOF MS identification. The remaining larvae were subjected to industry used processing to assess changes in *B. coagulans* counts after treatment. Processing included blanching for 1 minute at 92–95 °C, followed by drying for 10 minutes at 120 °C and 95 minutes at 75 °C. The cultured samples from different time points (frozen and processed) were used to determine changes in *B. coagulans* and *L. acidophilus* levels in the larval gut, depending on probiotic concentration and exposure time.

Based on the collected data, it was found that *B. coagulans* and *L. acidophilus* had no significant effect on larval weight or native microbiota over the 14-day period. *L. acidophilus* levels in larvae were not dependent on the concentration of probiotic suspension provided, and its growth in the mealworm gut appears questionable. *B. coagulans* levels in larvae depended on the administered probiotic concentration but declined over time. After processing, the levels also decreased but remained sufficient to enrich *T. molitor* products with probiotic bacteria. Linear dependency equations were also generated reflecting the amount of *B. coagulans* CFU in the larvae and could be used to predict the quantity of the probiotic bacteria in frozen and processed yellow mealworm larvae.

The microbiome of *Sambucus nigra*: uncovering its role in modulating phytochemistry and antiviral activity

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Keywords: *Sambucus nigra*, microbiome, anti-viral properties, SARS-CoV2

The microbiome of medicinal plants has emerged as a subject of growing scientific interest due to its potential to influence the production and bioactivity of plant-derived compounds. In recent years, most studies have taken an observational approach—correlating microbial community composition with the phytochemical profile and biological activity of medicinal plants. However, such studies are inherently limited in establishing causative relationships between the plant-associated microbiota and the plant's medicinal properties.

In this study, we investigated the microbiome of *Sambucus nigra* (elderberry), a medicinal plant traditionally used to alleviate symptoms of viral infections. Our aim was to elucidate potential links between the microbial communities associated with *S. nigra*, its chemical composition, and its antiviral activity. We employed both observational and experimental approaches: characterizing the microbiome of wild-growing elderberry populations using high-throughput amplicon sequencing of fragments of ITS1 and 16S rRNA genes and performing microbiota transfer experiments to assess functional effects.

The general structure of the *S. nigra* microbiome aligns with patterns commonly observed in other plant species. Specifically, root-associated microbial communities exhibited higher diversity than those of aboveground tissues. Microbiome composition varied across years and among geographically distinct populations. Importantly, while overall microbial diversity and community structure did not show significant correlation with the general phytochemical profile of elderberries, specific microbial taxa were associated with the presence or abundance of individual secondary metabolites.

Our findings suggest that while components of the *S. nigra* microbiome may influence the plant's chemical composition and antiviral properties, the complexity and variability of these communities present significant challenges for their direct application in enhancing the medicinal efficacy of *S. nigra*. Further studies employing reductionist and synthetic community approaches may be necessary to identify and harness functionally relevant microbial taxa for biotechnological or therapeutic applications.

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Fructan-metabolizing bacteria from strawberry as potential biocontrol agents

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Keywords: levan, levanase, glycoside hydrolase family GH32, defence priming, *Paenarthrobacter* sp., *Streptomyces* sp., *Botrytis cinerea*

The garden strawberry is an important crop which yield and quality are reduced by fungal pathogens such as *Botrytis cinerea*. To promote sustainable agriculture and reduce reliance on chemical fungicides, there is a growing demand of biological control agents (BCAs). Recent studies on several agricultural plants have demonstrated that fructans, especially bacterial levan and levan oligosaccharides (LOS), induce plant immune response and suppress pathogen infection. Moreover, the synergistic effect of levan and levan-metabolizing bacteria of *Bacillaceae* family have been recorded in protection of tomato plants against *B. cinerea* infection [1,2].

The aim of the study was to evaluate the ability of strawberry-isolated bacteria from wild and cultivated strawberries from Estonia to utilize different sugars, incl. levan, and to detect their antagonistic activity against *B. cinerea*. In addition, novel GH32 enzymes from levan-degrading strain were characterized to be connected with the mechanism of action and survival of the bacterium in plant context. Levan-degrading and LOS-producing capabilities of BCOs might be highly relevant to their protective and synergistic effect.

Several bacterial strains were identified, incl. *Paenibacillus* sp., *Perbacillus* sp., *Paenarthrobacter* sp., *Exiguobacterium* sp., *Curtobacterium* sp., *Pantoeae* sp. Sugar utilization pattern of the strains was evaluated in M9-based minimal medium supplemented with 5 g/L of various mono-, di- and polysaccharides. The competition assay revealed that *Streptomyces* spp. from wild strawberry are directly inhibiting *B. cinerea*.

Levan-utilizing *Paenarthrobacter* sp. S10 harbours three GH32 enzymes which were heterologously expressed, purified and biochemically characterized. One of the enzymes was confirmed as temperature-active LOS-producing levanase and the other as sucrose- and raffinose-acting fructosidase, both most probably involved in the plant-microbe interactions in the context of levan-based defence priming.

Acknowledgements

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Underground biological Internet: common mycelial networks in inter-plant signalling and resistance to pathogens

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Keywords: -

Arbuscular mycorrhizal fungi (AMF) like *Rhizophagus irregularis* form common mycelial networks (CMN) that interconnect multiple plant hosts, potentially serving as a conduit for inter-plant signal exchange (communication) that may influence stress responses in receivers. We investigate the impact of known plant defence elicitors on pathogen tolerance of plants receiving signals via CMN by analysing leaf metabolites, emitted volatiles, and transcriptome data. We found that the integrity of the CMN significantly shapes the responses of signal- receiving plants, with distinct changes in defence-related transcripts and plant isoprenoids, including volatile monoterpenes and triterpene saponins. Additionally, plants receiving signals through intact CMN from stressed donor plants exhibit increased resistance to *Fusarium sporotrichoides* and heightened susceptibility to *Botrytis cinerea*. Our findings emphasize the role of CMN in shaping plant responses to pathogens and suggest that the mechanisms of inter-plant signalling may influence genetic regulation of defence priming, offering new insights into plant-pathogen interactions. Our research utilizes not only model species *Medicago truncatula* and *Daucus carota* but extends to characterisation of inter-tree responses in hybrid aspen and silver birch. We further aim to explore the regional endemic diversity of AMF towards prospective agricultural applications for enhancement of community level resistance in different crops and tree species.

Session:
Waste and byproduct valorisation

Chair Asoc. Prof. Jānis Liepiņš

Waste or resource? Role of microbes in by-product valorisation

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Keywords: waste, resource, circular economy, recycling, biobased materials, composting, biofilter, bacteria

Industrial production inevitably leads to accumulation of by-products, generally regarded as a "waste". However, in the recent years, the concept of circular economy emphasises the transition from "take-make-dispose" linear economy towards reuse, repair, remanufacturing and recycling of such "waste" materials. On the top of waste hierarchy pyramid, the prevention and minimisation of waste accumulation are the most preferable options, whereas the disposal and incineration are the least preferred ones. The reuse, recycling, and energy recovery lies in the middle of this classification and will be discussed in this presentation.

Biobased materials like food, agricultural, forestry, or textile waste, sewage and eutrophication products are susceptible to the microbial action and may be turned into the valuable products. In this talk, the experiments describing the composting using textile waste, and also ones describing the production of biogas using macroalgae, will be presented. In addition, role of the bacteria in removal of ammonia gas using the plastic recycling waste as a biofilter, will be discussed. Microbial action on bio-composite materials such as thermal insulation boards or unfired clay bricks with sapropel will be discussed.

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Genome-scale modelling of *Galdieria sulphuraria* for bioconversion of agricultural residues into valuable bioproducts under heterotrophic growth conditions

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Keywords: *Galdieria sulphuraria*, genome-scale metabolic modelling, agricultural residues, flux balance analysis.

In 2023, the EU agricultural sector generated approximately €537.1 billion, with crop production contributing 51.3% (€275.8 billion) (Eurostat, 2024, accessed 06.10.2024). Agricultural residues, comprising both field residues (e.g., stalks, leaves, stubble) and process residues (e.g., husks, bagasse, molasses), represent ~46% of the EU's 956 Mt/year agricultural biomass [1]. While agricultural residues are predominantly directed toward feed and food (60%), bioenergy (19.1%), and biomaterials (18.8%) [1], a significant fraction remains underutilised or discarded [2],[3],[4], despite their proven potential as a sustainable, non-food, second-generation feedstock [5].

Redirecting agricultural residues streams from low-value applications toward controlled microbial bioconversion offers a viable strategy for value-added chemical production. *Galdieria sulphuraria*, a thermo-acidophilic red microalga, emerges as a robust candidate for such transformation. Thriving in extreme conditions—low pH (0.05–4), high temperatures (up to 56 °C), and heavy metal-rich environments—*G. sulphuraria* demonstrates remarkable metabolic versatility [6], [7], [8]. It can utilise over 50 carbon substrates in autotrophic, heterotrophic, and mixotrophic modes [9], making it particularly suited for valorising complex and variable agricultural residue streams.

Moreover, *G. sulphuraria* naturally synthesises key bio compounds of industrial relevance, including **floridoside**, **storage polysaccharides like starch**, and **lipids such as triacylglycerols (TAG) and polyunsaturated fatty acids (PUFAs)**. Coupled with its proven roles in bioremediation and metal recovery [10], [11], this extremophilic alga is well-positioned for biotechnological integration into sustainable agricultural residues-based biorefineries.

Metabolic model of *G. sulphuraria* shows innovative potential of predicting substrates such as glucose as one of the main sugars after agricultural residues pre-treatment and glutamine as one of the potential nitrogen sources for bioconversion to high-value products. This is the first red microalgae metabolic model and includes basic compartment-based eukaryotic properties for better prediction potential. Like photorespiration, the electron transport chain and TAG and PUFAs biosynthesis properties.

The genome-scale metabolic model (GEM) of *G. sulphuraria* exhibits robust predictive capacity, especially for simulating substrate utilisation following the pre-treatment of agricultural residues. The model optimisations are based on glucose as an efficient fermentable carbon source and glutamine as a viable nitrogen donor, both supporting the bioconversion of complex feedstocks into high-value biochemicals. As the first comprehensive GEM for a red microalga, it integrates eukaryotic compartmentalisation, enabling more accurate flux predictions across cellular organelles. Notable features include the representation of photorespiration, the mitochondrial electron transport chain, and detailed lipid biosynthetic pathways, including triacylglycerol (TAG) and polyunsaturated fatty acid (PUFA) synthesis. This reconstruction thus provides a valuable platform for *in silico* metabolic engineering, supporting sustainable biorefinery strategies utilising extremophilic microalgae for the valorisation of agro-industrial residues.

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From by-product to benefit: bioconversion of whey into functional health-promoting compounds

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Keywords: whey, lactose, microalgae, prebiotic, lactic acid bacteria, yeast

In the dairy industry, the effective utilization of whey lactose remains a challenge, as it is a low-value by-product with negative environmental impact when discarded. Through microbial fermentation and enzymatic processes, whey components can be converted into functional food ingredients. Lactose can be transformed into galacto-oligosaccharides (GOS), which act as prebiotics by promoting the growth of beneficial gut microbiota. When combined with probiotic strains, GOS-based formulations can be developed into synbiotic products that enhance gut health.

The refinement of enzymatically produced GOS mixtures by removing residual sugars (lactose, glucose, galactose) via selective fermentation was investigated. Fermentation using non-conventional yeast *Kluyveromyces marxianus* not only eliminated unwanted sugars but also altered the GOS composition. In parallel, fermentation with probiotic lactic acid bacteria (LAB) demonstrated the potential to obtain synbiotic products containing both GOS and live probiotics.

The integration of microalgae cultivation into whey bioconversion is also emerging as a sustainable strategy for producing health-promoting compounds. Certain microalgae species can utilize lactose and other whey nutrients to generate biomass enriched with proteins, essential fatty acids, vitamins, and bioactive pigments. This biomass can be incorporated into functional foods or nutraceuticals, adding nutritional value while contributing to waste reduction.

Whey permeate was evaluated as a substrate for cultivating lactose-utilizing microalgae such as *Galdieria sulphuraria* and the newly isolated green alga *Graesiella emersonii* MSCL 1718 under heterotrophic and mixotrophic conditions. The effects of cultivation parameters and lactose concentration on biomass productivity and yield were assessed. Results indicate that *G. emersonii* MSCL 1718 is a promising candidate for whey lactose bioconversion into high-value algal biomass.

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Catabolic targets for engineering oxidative and temperature stress resistance in the ethanologen *Zymomonas mobilis*

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Keywords: stress resistance, *Zymomonas mobilis*, catalase, *ndh*, *adhB*

I Inhibitory compounds present in industrial substrates, e.g., furfurals in lignocellulose hydrolysate, induce oxidative stress in microbial cells, making oxidative stress resistance a valuable property of producer strains. Furthermore, resistance to the oxidative stress largely determines also thermal stress tolerance. The cellular responses to oxidative and temperature stress represent complex phenomena and depend on activity of multiple genes. Numerous studies have been performed to clarify their mechanisms in *E. coli*, *S. cerevisiae* and other biotechnological producer microorganisms, including *Zymomonas mobilis*. Transposon mutagenesis has revealed several tens of genes that are essential for stress tolerance, while adaptive laboratory evolution has highlighted a few typical traits acquired by the evolved strains. However, these studies have poorly covered the impact on stress resistance brought about by engineering of the central metabolic pathways.

In *Z. mobilis* catabolism, there are two prominent targets of metabolic engineering that are known also for their relevance to oxidative and thermal stress: Fe-containing alcohol dehydrogenase (*adhB*) and respiratory NADH dehydrogenase (*ndh*). AdhB is overexpressed under thermal shock conditions, while at the same time, it contains Fe^{2+} in its active center, representing a potential site of interaction with reactive oxygen species (ROS). The Ndh-negative mutants at elevated temperature, for some unexplained reason, grow and produce ethanol better that the wild type, showing improved stress resistance. To gain an insight in the underlying mechanisms, we have studied the resistance of *ndh* and *adhB* knock-out mutants to hydrogen peroxide and thermal shock. It appears that both mutations cause a strong increase of the peroxide and thermal stress resistance that is largely based on elevated catalase activity in these strains. The physiological meaning of these findings and their metabolic engineering applications are discussed.

Strategies for sewage sludge valorisation

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Keywords: biomolecules, sewage sludge, wastewater, hygienization

So far management of various waste streams has been one of the most difficult and challenging tasks in wastewater treatment plants (WWTP) due to their complex composition, poor dewaterability, potential harbour of pathogens and harmful chemicals, and subsequently strict regulations for sludge reuse or disposal.

Current assessment of WWTP waste show that the materials are rich in proteins, lipids and cellulose. The latter can be easily utilized for carbohydrate production via enzymatic hydrolysis that is regarded as a highly sustainable approach in degradation of various polymers. Numerous microorganisms possess the capability of enzymatically degrading lignocellulosic biomass, including the white-rot fungi, which are well-known producers of lignocellulolytic enzymes such as cellulases, hemicellulases and laccases. Application of this concept in in management of WWTP waste is still topical.

Within this study, WWTP waste treatment with a laboratory-made enzyme cocktail produced by *Irpex lacteus* was compared with a commercial enzyme preparations for saccharification. As a result we observed an increase in sugar levels for all sewage substrates. Furthermore, it was estimated that lipid and protein recovery from primary and secondary sludge prior to the hydrolysis was not advantageous in terms of sugar production. Alternatively the most common wastewater treatment process product – sludge was assessed for its potential application in agriculture as soil amender. Various combinations with soil were made to evaluate the plant growth potential and product microbiological quality. The results demonstrated the high potential of the use of sludge in growing plants.

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Session: Metabolic engineering and systems biology

Chair Prof. Uldis Kalnenieks

Systems-level understanding and engineering of metabolism limits at the thermodynamic edge of life

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Keywords: gas fermentation, acetogens, systems biology, metabolic engineering

Climate change and poor recycling of waste is threatening global biosustainability. Humankind is thus facing a pressing need for sustainable production of chemicals, fuels, and food and improved waste recycling. Acetogen bacteria have become attractive biocatalysts for converting inexpensive and abundant solid and gaseous waste feedstocks into high-value products using gas fermentation. However, our knowledge of limits of acetogen metabolism is scarce but this is required for both fundamental understanding of cellular behaviour and rational metabolic engineering. We thus challenged the model-acetogen *Clostridium autoethanogenum* to grow faster or at lower pH in autotrophic continuous cultures and used adaptive laboratory evolution (ALE) and reverse genetic engineering to improve growth limits. We were able to obtain steady-states at up to specific growth rates of ~2.8 day^{-1} (~0.12 h^{-1}) with faster growth supporting both higher yields and productivities of reduced by-products ethanol and 2,3-butanediol. Lowering pH led to significant changes in product and transcriptional profiles. Autotrophic ALE yielded superior strains that can grow faster, without complex nutrients, and are robust for operating continuous cultures. Reverse genetic engineering of mutations in genes potentially involved in regulatory networks recovered all three superior features of our ALE strains through triggering significant proteomic rearrangements. These results advance our understanding of limits of acetogen metabolism and offer engineering targets in an industrially relevant cell factory.

Establishing modular cloning toolboxs for non-conventional bacteria: *Zymomonas mobilis* to *Rhodobacter sphaeroides*

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Keywords: modular cloning, non-conventional microbes, genetic tools

Zymomonas mobilis is a promising organism for the production of bulk chemicals, as it exhibits a very high yield and specific productivity for ethanol, its native fermentation product. To make *Z. mobilis* a competitive chassis organism for products beyond ethanol several obstacles need to be overcome: (i) increasing tolerance against inhibitory substances, (ii) broadening the range of utilizable substrates, and (iii) efficient synthesis of heterologous products. One major limitation in achieving these goals is the lack of efficient and standardized genetic tools for *Z. mobilis*.

As a major step forward in the direction, we have developed Zymo-Parts [1], a genetic toolbox of characterized regulatory elements (promoters, ribosome binding sites, and terminators) and replicons for *Z. mobilis*. The different elements of Zymo-Parts can be freely combined using a modular cloning (MoClo) Golden Gate-based system. Recently, we have added multiple shuttle vectors to the toolbox, allowing for a sophisticated choice of replication systems, which are compatible with each other [2]. The tools available lay the foundation to modularize the genetic engineering of *Z. mobilis* in order to reach the above-mentioned goals.

Furthermore, the assembly framework of Zymo-Parts was used for Rhodo-Box, a MoClo toolbox for applications in *Rhodobacter sphaeroides* [3]. Many of the genetic elements domesticated for Zymo-Parts are functional in other microbes like *R. sphaeroides* and only need to be characterized for the new host. The sophisticated assembly framework as well as the large parts collection offer reasons to adapt Zymo-Parts for other microbes in the future.

We present a overview of the toolbox and assembly framework and its benefits.

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Chemostats and omics: tools to improve lipid-producing yeasts

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Keywords: *Rhodotorula toruloides*, chemostat, transcriptomics, proteomics, lipidomics, glucose

Transition towards sustainable bioeconomy requires microbial cell factories capable of converting renewable feedstocks into high-value oleochemicals. Yeasts with a natural ability to accumulate high amounts of neutral lipids, called oleaginous yeasts, have emerged as promising candidates for this transition. However, improving their productivity requires a detailed understanding of the metabolic and regulatory mechanisms governing lipid biosynthesis.

Chemostat-based continuous cultivation coupled with the systems-levels omics analyses allow to investigate lipid production in yeasts under controlled environmental conditions. With this platform, it is possible to study growth-dependent phenomena, enabling the decoupling of nutrient and growth effects on metabolism. Integrating transcriptomic, proteomic, and lipidomic data allows to decipher key regulatory patterns, possible pathway bottlenecks, and resource allocation in central carbon metabolism.

We demonstrate how this combined approach reveals condition-specific resource distribution and coordination influencing the lipid biosynthesis in red nonconventional yeast *Rhodotorula toruloides*. Insights gained from this work are valuable for rational metabolic engineering to improve performance of lipid-producing yeasts.

Leveraging historical process data for recombinant *P. pastoris* fermentation hybrid deep modeling

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Keywords: *P. pastoris*, bioreactor, fermentations, hybrid modeling, deep learning, transfer learning

Mathematical models have long supported bioprocess monitoring, optimization, and control, but limited mechanistic knowledge constrains their accuracy. Machine learning offers data-driven alternatives, and hybrid neural networks (HNNs) combine prior knowledge with deep learning advances such as ADAM optimization [1]. With *P. pastoris* widely used for recombinant protein production, developing high-quality hybrid models is critical for bioprocess digitalization for Industry 4.0.

This study advances hybrid modeling of *P. pastoris* fermentations through systematic neural network architecture screening to identify robust, process-specific models. Transfer learning adapts pretrained hybrid models from historical data to smaller fermentation datasets, reducing retraining needs while preserving accuracy. The approach is integrated into a hybrid MPC framework and experimentally validated, demonstrating effective real-time control of *P. pastoris* fed-batch fermentations.

The optimal hybrid architecture (LSTM-2 + FC-8, ReLU) achieved strong accuracy (NRMSE 4.93%) and efficiency (AICc 998). Adapted to bacteriophage Qβ coat protein production via transfer learning, it maintained low validation (3.53%) and test (5.61%) losses. Integrated into an MPC system, the model enabled robust substrate feed control with predictive accuracies of 6.51% for biomass, 14.65% for product, and an average biomass tracking error of 10.64%. This work establishes a robust, adaptable hybrid modeling framework that combines automated architecture search, transfer learning, and MPC, enabling practical real-time control and supporting scalable digital twin deployment in industrial biotechnology.

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Engineering the Wood-Ljungdahl pathway to enhance carbon fixation for sustainable bioproduction

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Keywords: gas fermentation, acetogen, CRISPR, synthetic biology, protein engineering

Climate change is driving the urgent need for innovative technologies to convert carbon emissions into valuable products. Gas fermentation has emerged as a sustainable route to produce fuels and chemicals by recycling gaseous and solid one-carbon (C_1) waste feedstocks using microbes. Acetogens that use the Wood-Ljungdahl pathway (WLP) to fix carbon oxides (CO and CO_2) into metabolic products are the most advanced biocatalysts for gas fermentation. However, the function of genes in the C_1-fixing gene cluster and its closely linked genes is incomplete, and mutation studies of enzymes involved in the WLP are lacking.

In our study, we performed CRISPR/nCas9 deletion of two genes with unknown functions - hypothetical protein (*hp*; LABRINI_07945) and CooT nickel binding protein (*nbp*; LABRINI_07950) - adjacent to the WLP gene cluster in the gas-fermenting acetogen *Clostridium autoethanogenum*. Autotrophic phenotyping revealed significant growth defects and altered by-product profiles in both strains. These genes were also shown to influence complex nutrient utilisation and protein expression in the C_1-fixing gene cluster and ethanol synthesis pathways. Next, we are expanding our approach to investigate four genes in the WLP gene cluster with unclear functions. Preliminary characterisation suggests intriguing phenotypic changes worth further investigation.

Also, we are combining site-directed mutagenesis and recombination-based cloning to engineer a key WLP enzyme, generating a combinatorial mutant library with over 200,000 new mutant variants to improve the enzymes activity and carbon fixation efficiency.

Altogether, our work enhances the understanding of genotype-phenotype relationships in acetogens, advancing their potential as industrial chassis for gas fermentation, and brings us closer to transformative technologies for sustainable industrial bioproduction and climate change mitigation.

A new *Zymomonas mobilis* platform strain with tunable PDC expression

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Keywords: *Zymomonas mobilis*, platform strain, metabolic engineering, pyruvate decarboxylase

Zymomonas mobilis is well known for its outstanding ability to produce ethanol with high productivity and yield. To exploit its beneficial properties for producing other substances, the fluxes of the central metabolism must be redirected away from ethanol production and towards alternative products. The key enzyme in the ethanol production pathway is the pyruvate decarboxylase (PDC). Since PDC is considered to be essential, metabolic engineering strategies that aim to produce pyruvate- derived compounds must reduce PDC activity.

To facilitate genetic engineering in *Z. mobilis,* a modular cloning toolbox, Zymo-Parts, was developed [1,2]. Zymo-Parts contains a set of genetics elements such as promoters, ribosome binding sites and origins of repication which have been carefully characterized for their behavior in *Z. mobilis* and that can be easily combined with each other.

Using Zymo-Parts we have constructed a new *Z. mobilis* platform strain (sGB027), in which the native *pdc* promoter was replaced by PT7A1. The expression of *pdc* and the growth rate of sGB027 can be modulated by the amount of IPTG added. To achieve redox balance in the mutant, alternative NADH-consuming reactions must be provided. We introduced a plasmid expressing the lactate dehydrogenase from *E. coli* and demonstrated that the resulting strain produced high amounts of lactate. Similarly, we introduced a plasmid expressing the alanine dehydrogenase from *G. stearothermophilus*. The resulting strain produced significant amounts of alanine, though its growth was impaired [3].

Both strains still produce ethanol, which reduces the yield of the respective product. Consequently, we are engineering the strain to reduce the activity of the alcohol dehydrogenases and to increase the stability of the strain. Together with the extensive toolbox developed for genetic modification of *Z. mobilis*, the platform strain is a step towards establishing this organism as a workhorse for production processes.

Acknowledgements

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Poster presentations

(1) Synergistic antimicrobial effect of essential oil blends against skin pathogens

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Keywords: antimicrobial, essential oils, skin pathogens

The rise of multidrug-resistant pathogens poses a growing threat to public health, highlighting the need for alternative antimicrobial strategies. Essential oils (EOs) are natural substances with known antibacterial, antifungal, and antiviral properties.

This study investigates the antimicrobial effect of five pure essential oils from *Melauleca alternifolia, Eucalyptus globulus, Pinus sylvestris, Citrum limonum, Thymus hyemalis* (M1–M5) and EO blends (P1–P5) against clinically relevant skin pathogens: *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Candida spp*.

EO microemulsions in water were prepared by ultrasound. MICs were estimated by broth microdilution method and MBCs by spot-inoculation on agar plates of the last five wells showing no growth.

Several EO blends showed synergistic activity, lowering MICs across multiple pathogens. MICs as low as 0,05 % were recorded for blends against *C. guilliermondii* and *C. lusitaniae*. Among the tested samples, several EO blends exhibited synergistic antibacterial activity, resulting in reduced MIC values against multiple pathogens. However, the MIC and MBC values of the blends against *S. aureus* did not show improvement compared to the pure oils. Notably, *E. coli* and *Candida* species showed high susceptibility to essential oils, with MICs ranged from 0,05 % to 0,2 % for both blends and pure EOs. *P. aeruginosa* was the most resistant across the tested organisms, with MICs of 4,7 % for blends and improved MBCs values of blends over pure oils (e.g. P2 vs M2 from 20,3 % to 10,9 %), indicating a synergistic effect. Mixtures containing *Melaleuca alternifolia* and *Pinus sylvestris* exhibited improved antimicrobial activity against several pathogens.

These results highlight the antimicrobial potential of EO mixtures and support formulation as a key factor in enhancing efficacy. Further research should explore the mechanisms of synergy and develop optimized blends against skin pathogens.

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(2) *Gardnerella* protein Cna as a candidate adherence factor

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Keywords: *Gardnerella*, surface protein, bacterial vaginosis, adhesin, structure modeling, fibrinogen

Gardnerella spp. are among the key bacteria in bacterial vaginosis (BV), a dysbiosis associated with adverse obstetric and reproductive outcomes. *Gardnerella*-dominant biofilm on vaginal epithelium is a diagnostic marker of BV. The colonization and biofilm formation by *Gardnerella* indicate adhesion potential, the determinants of which remain unexplored. To identify *Gardnerella* adhesins, we analyzed surface-exposed protein Cna, which was determined previously [1].

Cna structure modeling using AlphaFold3 revealed domains N2 and N3 with an immunoglobulin-like fold, which showed structural homology to the domains of staphylococcal MSCRAMM family adhesins SdrD and UafA. A single B domain shared structural similarity with the corresponding domain of Sdr proteins. The PKD-rich repeat region varied in length among isolates, while the C-terminus contained a non-canonical LVNTG cell wall sorting motif.

The *cna* gene was detected by PCR in 5 *G. vaginalis* isolates out of 34 *Gardnerella spp.* isolates. Additionally, Blast analysis of 144 genomes revealed the presence of *cna* in the genome species 2 and 7, with a total of 38 positive hits.

The *cna* gene fragment encoding amino acids 27–532 was amplified from *G. vaginalis* isolate 114.2 and expressed in *E. coli* Tuner (DE3). The recombinant protein rCna was purified and specific polyclonal and monoclonal antibodies were generated. Flow cytometry analysis using anti-Cna antibodies detected its presence on the surface of 29.9%–60.5% of *G. vaginalis* ATCC 14018 cells, suggesting differential expression of Cna within the bacterial population. rCna bound to the human fibrinogen in a dose-dependent manner, but showed no binding to fibronectin or collagen types I, III, or IV as determined by ELISA. Cna-positive *G. vaginalis* cells adhered to immobilized fibrinogen and fibronectin. However, the adhesion was not inhibited by the preincubation with rCna. Our findings suggest that Cna protein is unlikely to be the primary adhesion factor of *Gardnerella*.

Acknowledgements

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(3) Change in antibiotic resistance of *Staphylococcus* spp. in 2007-2025

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Keywords: *Staphylococcus* spp., resistance, antibiotics

A comparative analysis of antibiotic resistance of *Staphylococcus* spp. isolated in 2007, 2015, 2022 years and 2025 in the community – from the population and 1st-2nd year students was performed. 1736 strains of *Staphylococcus* spp. were tested: 260 *Staphylococcus aureus* (*S. aureus*) and 385 coagulase-negative staphylococci (CoNS) strains were isolated from the population and 440 S. aureus and 651 CoNS strains were isolated from the students.

Clinical specimens were collected by swabs, from the nostril. *Staphylococcus* spp. antibiotic susceptibility was tested according to CLSI (Clinical and Laboratory Standards Institute) [1] and European Committee on Antimicrobial Susceptibility Testing (EUCAST) [2] recommendations. Antibiotic resistance of Staphylococcus spp. strains was determined using Sensititre plates (TREK diagnostic system) [3].

It was found that during the study period, penicillin resistance of S. aureus isolated from the population and students decreased from 83.4-81.0% to 59.7-55.0% (P<0.05). The frequency of methicillin-resistant *S. aureus* (MRSA) strains ranged from 4.1 to 10,3%. *S. aureus* and CoNS resistance to gentamicin ranged from 39.9 and 34.5 to 11.6 and 9.9%. In the study groups, S.aureus resistance to ciprofloxacin ranged from 28.5 to 4.4%, to clindamycin - from 28.5 to 8.1%, to erythromycin - from 41.6 to 9.3%, to tetracycline - from 39.8 to 10.7% and S.aureus resistance to rifampin ranged from 29.5 to 3.5%. In the study groups, CoNS resistance to ciprofloxacin ranged from 16.5 to 4.3%, to clindamycin - from 25.0 to 7.2%, to erythromycin - from 59.6 to 19.8%, to tetracycline - from 43.1 to 20.5% and *S.aureus* resistance to rifampin ranged from 12.0 to 3.1%. All tested Staphylococcus spp. strains were sensitive to vancomycin and linezolid.

Monitoring of staphylococci (*Staphylococcus* spp.) provides important information about their prevalence and antibiotic resistance, identifies control priorities and the need to adjust antibiotic use.

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(5) Comparative analysis of microbial growth dynamics using laser speckle imaging and conventional liquid culture methods

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Keywords: laser speckle imaging, antimicrobial susceptibility, real-time monitoring, *Saccharomyces, Kluyveromyces marxianus, Yarrowia lipolytica, Vibrio natriegens, Escherichia coli*

Microbial growth assessment is critical for applications ranging from antimicrobial susceptibility testing to industrial bioprocess optimization. While conventional methods like optical density (OD600) measurements in 96-well plates are standard, they often lack the temporal resolution to capture rapid physiological changes. Laser speckle imaging (LSI), combined with subpixel correlation analysis, has demonstrated potential for early detection of antibacterial susceptibility by visualizing dynamic changes in microbial activity [1,2]. This study aims to compare the efficacy of LSI with conventional liquid culture methods for real-time monitoring of microbial growth under varying inhibitory conditions.

We are testing the ability of LSI system to detect the growth pattern of *Saccharomyces spp.*, *Kluveromyces marxianus, Yarrowia lipolytica, Vibrio natriegens*, and *Escherichia coli*. Comparison of LSI and conventional culture growth monitoring in 96-well plates by OD measurements are being done under standard conditions and in the presence of inhibitors, such as antimicrobial agents or environmental stressors. LSI is capturing spatiotemporal activity patterns of the colony growth, while OD600 measurements serve as a benchmark. The correlation between activity signals acquired by LSI and OD600 data is analysed to assess the sensitivity, speed, and accuracy of LSI in detecting growth inhibition.

Preliminary studies using *V. natriegens* and *E. coli* have shown that LSI can detect spatiotemporal changes in the colony structure, the effects which cannot be detected by a naked eye [2,3]. We aim to validate LSI as a high-throughput, non-invasive tool for real-time microbial growth assessment, with potential applications in antimicrobial susceptibility testing and industrial bioprocess monitoring. The results will provide insights into the advantages of LSI as tool for rapid and more reliable microbial culture monitoring, addressing the limitations of traditional approaches.

Acknowledgements

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(6) Inhibition of bacterial trimethylamine production by targeting anaerobic L-carnitine and γ-butyrobetaine metabolism

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Keywords: trimethylamine, trimethylamine oxide, L-carnitine, anaerobic L-carnitine metabolism, inhibitor design, microbiome

Trimethylamine (TMA) and its metabolite, trimethylamine-N oxide (TMAO), are chemical compounds of bacterial origin. They are produced in the human body by intestinal microbiota and are associated with several pathological conditions, including trimethylaminuria, atherosclerosis, renal failure, type 2 diabetes, and cancer. Preventing their production has been suggested to have a beneficial influence on health. Therefore, we propose to design and test novel compounds targeting a recently described TMA-producing bacterial pathway. Our approach focuses on inhibiting anaerobic carnitine metabolism into TMA by targeting BbuA (γ-butyrobetaine-CoA oxidoreductase) enzyme. We aim to demonstrate that suppressing TMA production from carnitine in anaerobic conditions can be achieved by inhibiting this enzyme, thus validating them as drug targets of TMA-associated diseases.

So far we have performed screening of more than 100 different compounds and identified several promising candidates able to suppress TMA production form carnitine by BbuA enzyme.

(7) Genomic characterization of bacteriophages isolated in Ukraine as an important step towards realizing their therapeutic potential

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Keywords: Bacteriophages, *Klebsiella*, *Pseudomonas*, Microorganism collection, Whole-Genome Sequencing, Genomic characterization, Comparative genomics, Phage therapy

The growth of antimicrobial resistance (AMR) across bacteria of healthcare and economic importance is a pressing issue of particular concern.

In Ukraine, the AMR challenges are further exacerbated by the ongoing military conflict, leading to a surge in nosocomial infections caused by multidrug-resistant (MDR) bacteria. Unregulated antibiotic therapy during medical evacuations, combined with overwhelmed healthcare facilities, has contributed to the rapid emergence and a widespread of MDR strains within the country. This urges the need to explore alternative strategies to overcome strains refractory to traditional antibiotics.

Phage therapy, a practice of using highly specific viruses of bacteria – (bacterio-)phages, to combat bacteria, is one such option proven to be among the most promising alternative or complementary strategies to traditional antibiotics. In addition to often showing high synergy with antibiotics, phages can “self-dose” via rounds of replicating on the target host population until its eradication.

However, a comprehensive characterization of any phage to potentially be applied for therapeutic purposes is required. This includes whole genome sequencing (WGS) to assess the safety of the chosen phage(s) that exhibit favourable microbiological properties. The availability of the complete and well-annotated genome of a bacteriophage assembled from WGS data is a main prerequisite for its informed and justified practical use.

In our current project, we conduct genomic characterization of a selected subset from the collection of previously isolated phages active against Ukrainian isolates of the three relevant bacterial pathogens of the ESKAPE group: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

This is the first phage genomic characterization effort of such a scale in Ukraine, allowing the selection of the most therapeutically promising candidate phages and paving the way for new treatments in the fight against the AMR crisis.

Acknowledgements

The project “Complete genome sequencing and analysis of bacteriophages isolated in Ukraine as a crucial step towards realizing their therapeutic potential” is supported by the bilateral cooperation programme between Latvia and Ukraine in the field of science and technology, project number: LV_UA /2025/5.

(8) Evaluation and comparison of the effectiveness of conventional disinfectant and probiotic cleaning agents

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Keywords: probiotics, probiotic cleaners, infection prevention, conventional disinfectants, biocidal disinfectants

Elimination of pathogenic microorganisms from surfaces is essential for infection control. Currently, biocidal disinfectants are used for this purpose. Due to their limitations (short duration of action, selection of resistant organisms and potential health hazards), alternative solutions are being explored. One alternative is probiotic cleaners which contain spores of beneficial bacteria. The aim of this study was to compare the efficacy of conventional disinfectants and probiotic cleaning agents.

The study consisted of two stages. During the first stage, 15 desks in a lecture hall in the Faculty of Medicine, Vilnius University were cleaned using conventional disinfectant and probiotic cleaner containing *Bacillus coagulans* spores each for 7 days. In the second stage, over a period of 30 days, five desks were cleaned with a conventional disinfectant, probiotic cleaner and tap water (positive control) each. In both stages, cleaning was performed in the morning, and surface samples were collected after student activities. Samples were cultured on selective media, and bacterial species were identified using MALDI-TOF mass spectrometry. The ability of *B. coagulans* to colonize surfaces was evaluated, and the efficacy of different cleaning agents in reducing microbial load was compared.

The ability of *B. coagulans* to survive and colonise surfaces was confirmed: at the end of the first stage, *B. coagulans* constituted 11% of all surface bacteria, and 61% by the end of the second stage. During the first stage, both the conventional disinfectant and the probiotic cleaner showed similar efficacy (total surface bacteria was reduced by 89% with the disinfectant and 65% with the probiotic cleaner; *Staphylococcus* spp. were reduced by 90% in both cases), although the probiotic cleaner exhibited a slower onset of action. In the second stage, no statistically significant reduction in microbial load was observed in any group within the first two weeks.

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(9) Genome- and bioactivity-guided discovery of antimicrobial compounds from actinobacteria stored at the Microbial Strain Collection of Latvia

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Keywords: actinobacteria, natural products, antiSMASH, antimicrobial, natural products

The rise of antibiotic resistance has become a serious public health issue. To combat it, the discovery of novel, bioactive compounds is necessary. Actinobacteria is a Gram+ phylum of bacteria that has been historically proven to be a prolific source of antibiotics. Up to 75% of known antimicrobials today have been isolated from Actinobacteria. From Actinobacteria, *Streptomyces* genus is the one most research. Due to the increasing number of natural product rediscovery, nowadays natural products research has turned to lesser characterized bacteria or to bacteria from unexplored biotopes.

Microbial Strain Collection of Latvia holds more than 1700 different strains of microorganisms collected throughout the years at the University of Latvia. It also has more than 70 different strains of Actinobacteria, isolated from the Baltic region. Strains stored at the MSCL belong to the *Streptomyces* genus, as well as *Kitasatospora* sp., *Achromobacter* sp. and others. To determine the taxonomy, most strains were whole-genome sequenced. Additionally, to identify potential biosynthetic gene clusters encoding antimicrobial compounds, the BGCs were predicted using antiSMASH software. BGC analysis revealed diverse polyketide synthases (PKS), nonribosomal peptide synthetases (NRPS), and other secondary metabolite pathways.

To maximize the number of active biosynthetic gene clusters and produced natural products, strains were cultivated in either protein-rich or carbohydrate-rich media. After 7 days, the 2:1 ethyl acetate extracts were made from the supernatants. To functionally validate the antiSMASH findings, the prepared extracts were screened for antimicrobial activity against clinically relevant pathogens. A small panel of six microorganisms – two Gram+, two Gram- and two fungal species – was used. The bioactivity screening revealed multiple candidates for novel natural products discovery with antimicrobial properties. Further studies will focus on characterization of the rest of the Actinobacterial collection and metabolome analysis using LC-MS to characterize these bioactive metabolites.

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(10) Assessment of microbial contamination in eye makeup products and characterization of antibiotic resistance profiles and genotypes of microorganisms

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Keywords: cosmetics, microbial contamination, antibiotic resistance, eye makeup, 16S rRNA, genotyping, *Staphylococcus, Bacillus*

Cosmetic products, especially those containing natural extracts or oils, can provide favorable conditions for microbial growth and may therefore be contaminated with pathogens [1]. Eye makeup products, such as mascaras, eyeshadows, and eyeliners, can be sources of infections and allergic reactions [2]. The aim of this study was to assess the microbial contamination of eye makeup products, determine the antibiotic resistance profiles of microorganisms, and identify their genotypes.

A total of 71 products were examined: 30 mascaras, 30 eyeshadows, and 11 eyeliners/liquid liners. New, unused products were used as controls. Samples were collected using sterile swabs and inoculated onto five types of nutrient media. For bacterial identification, PCR with universal primers 27F and 1541R was applied, amplifying ~1500 bp fragments of the 16S rRNA gene [3].

The highest microbial contamination was detected in mascaras, with the presence of Staphylococcus and Bacillus species. Antibiotic susceptibility testing revealed varying levels of resistance, although gentamicin and norfloxacin were the most effective. Genotypic analysis showed the greatest diversity in mascaras and eyeliners/liquid liners, with the identification of *S. epidermidis*, *B. licheniformis*, and *B. subtilis*.

These findings highlight the need to maintain proper hygiene and preservation standards to reduce the risk of infections.

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(11) Short exposure to ionic and metallic copper or silver results in survival of antibiotic-tolerant *Escherichia* sub-populations

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Keywords: *Escherichia coli*, copper, silver, antibiotic tolerance, antimicrobial surfaces, phenotypic heterogeneity

The growing concern over antibiotic resistance highlights the need to study the underlying bacterial defense mechanisms. Survival in the presence of an antimicrobial agent (tolerance) is considered a stepping stone towards growing in the presence of an active agent (resistance). Exposure to antimicrobials, including those other than antibiotics, can confer survival advantages that subsequently also demonstrate increased antibiotic tolerance due to overlapping induced defense mechanisms.

Little is known about the effects of short exposure to antimicrobial metal ions and surfaces in different exposure conditions. This study investigated how a 10-minute semi-lethal exposure to semi-dry copper or silver surfaces or respective metal ions in solution affects bacterial tolerance to lethal concentrations of the antibiotics ciprofloxacin and ampicillin. Surviving bacteria were determined by plate counting. In addition, the lag phase distribution and antibiotic tolerance of cells surviving metal exposure was determined.

Short semi-lethal exposure to copper or silver prolonged the lag phase of the survivors independent of exposure conditions and increased subsequent bacterial tolerance to ciprofloxacin and ampicillin. Changes in antibiotic tolerance were transient and restored upon re-growth of the survivors. Due to short exposure time, induction of antibiotic tolerance mechanisms by gene expression changes was unlikely, suggesting that pre-existing phenotypic heterogeneity may underlie both the selective biocide survival and the subsequent increase in antibiotic tolerance. Further studies are needed to clarify the causal link between surviving short biocidal exposures and lag-time-associated antibiotic tolerance.

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(12) Hot water extract of *Lentinula edodes* enhances oxidative stress resistance in *Drosophila melanogaster*

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Keywords: medicinal mushrooms, *Lentinula edodes*, β-glucans, antioxidant activity, *Drosophila melanogaster*

L.edodes (shiitake) is a widely cultivated edible and medicinal mushroom, recognized for its rich content of bioactive compounds, including β-glucans and polyphenols, which have been reported to exhibit antioxidant properties in various experimental systems [1].

D.melanogaster serves as a model organism in diverse scientific disciplines. The mechanisms of oxidative stress—triggered by physical or chemical factors—and the corresponding cellular responses are evolutionarily conserved. In drosophila, as in mammals, redox homeostasis is maintained through multiple systems, including enzymatic antioxidants such as catalase and superoxide dismutase (SOD), non-enzymatic antioxidants like reduced glutathione (GSH) [2], and the molecular chaperone HSP70 [3].

A hot water extract (HWE) was prepared from the fruiting bodies of *L.edodes* (DSM 3565), then lyophilized, and stored at 4°C. FTIR of the preparation revealed 20–35% β-glucan content, while polyphenol concentration was relatively low (~0.5%). In parallel, β-glucan concentration was estimated by Glucan Enzymatic Method.

Wild-type drosophila embryos (10–12 per cm^2) were reared on commercial media supplemented with 0.1–5 mg/mL HWE at 25±1°C, 12:12 h light-dark cycle, and 60% relative humidity. Third instar larvae were subsequently exposed to oxidative stressors—H_2O_2, $CuSO_4$, or heat—to evaluate stress resistance.

HWE supplementation did not alter normal fly development, larval size, total protein content, pupation, and hatching times. However, pre-feeding with HWE enhanced oxidative stress resistance by activating redox homeostasis pathways, evidenced by increased CAT and SOD activities, elevated GSH levels, and upregulation of *hsp*70 gene expression. Larval locomotion, survival, and adult body size were also preserved under stress conditions.

These findings indicate that *L.edodes* HWE enhances oxidative stress resilience in *D.melanogaster*, supporting utility of the fruit fly model for studying the effects of bioactive compounds.

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(13) Antimicrobial silver sensitivity of silver or copper surface adapted *Escherichia coli* isolates

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Keywords: silver, copper, antimicrobial, Escherichia coli, growth inhibition

Amid the rise of antimicrobial resistance, assessing the potential for biocide resistance development is crucial for the safe use of antimicrobial surfaces. To examine how repeated surface exposure affects biocide tolerance, a selection of *E. coli* ATCC 8739 copper or silver surface adapted isolates [1] carrying mutations in *apt*, *cysN*, *ompC*, *zraS*, *ydjN* and *tnaA* genes were analysed. Growth and time-kill kinetics under ionic silver stress, silver surface exposure and analysis of tryptophanase function revealed diverse, context-dependent changes in silver tolerance.

Improved growth in silver-supplemented liquid medium did not always correlate with survival on semi-dry silver surfaces, highlighting the differences in adaptation and defence mechanisms against the same active agent in different exposure conditions. For instance, *ompC* porin mutants survived surface exposure better than wild type (WT) but displayed reduces growth in silver-supplemented medium, whereas *apt* and *cysN* mutants showed the opposite.

Focus was given to a missense mutation (M266I) in the tryptophanase gene (*tnaA*), which reduced the enzyme's activity, causing a temperature-dependent decrease in indole and hydrogen sulphide production. Reduced indole may affect biofilm formation [2] and favour emergence of persistence phenotypes [3] enhancing survival on silver surfaces.

$AgNO_3$ time-kill assay revealed a striking contrast between *tnaA* genotypes. Δ*tnaA* showed higher initial tolerance compared to WT and M266I but failed to resume growth, whereas WT and M266I, though less tolerant in early exposure, regrew within 24 h. This suggests that complete loss of function of tryptophanase delays killing by silver but may impair regrowth of the survivors, while the M266I mutation preserves recovery dynamics.

These genotype-specific benefits illustrate that silver tolerance is driven by varied context-dependent adaptive strategies.

Acknowledgments

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(14) The influence of the environmental pH on the viability of *Candida* yeasts and the efficiency of antifungals

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Keywords: *Candida, C. albicans, C. glabrata,* multidrug resistance, acidic environment, nystatin

Candida yeasts are commensals in the human body. When the immune system is weakened, these microorganisms can become opportunistic, leading to superficial or disseminated infections that can be fatal. The mortality rate of these infections is especially high, as there are only four known classes of antifungals, and the cells can acquire multidrug resistance to all of them [1]. To successfully combat these infections, it is essential to gain a deeper understanding of the physiological characteristics of *Candida* yeasts.

This study aimed to investigate how different environments affect the viability of *C. albicans* and *C. glabrata* cells in real time in the presence of an antifungal drug. The binding of the lipophilic anion phenyldicarbaundecaborane (PCB^-) to yeast cells, the respiration rate, and the release of intracellular K^+ were monitored in thermostated cuvettes using dissolved O_2 sensing and ion-selective electrodes. It was determined that *Candida* cells become more susceptible to antifungal drug nystatin in an acidic environment: the highest binding of PCB^- was observed when the pH of the incubation medium was 2.5-3. In more acidic environments, the yeast cells bound the lipophilic anion without the help of antifungals. At pH 3, the respiration of *C. albicans* yeasts was much weaker than at pH 6, while *C. glabrata* cells at pH 3 barely used any oxygen at all. It was observed that at pH 3 *C. albicans* cells do not release intracellular K^+, while at pH 6, a slow, continuous leakage of this cation was observed. A leakage of K^+ was observed at both pH levels from *C. glabrata* cells. Additionally, it was observed that phenolic acids appear to have an antifungal effect on *Candida* yeasts' viability at acidic pH.

These findings expand our understanding of the viability of *Candida* species in various environments and suggest that an acidic environment enhances the efficacy of nystatin.

Acknowledgements

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(15) A retail prevalence study to investigate microbiological contamination levels in ready-to-eat (RTE) plant-based dairy and meat substitutes

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Keywords: *Listeria monocytogenes*, ready-to-eat, meat substitutes, dairy substitutes, plant-based

The demand for ready-to-eat (RTE) plant-based dairy and meat substitutes is increasing across the European Union (EU), yet scientific data regarding their microbiological safety remain limited. Unlike more traditional animal-based counterparts, many of these products are manufactured using diverse plant ingredients and processing technologies, some of which lack a final heat treatment step. This raises concerns about the potential presence of foodborne pathogens such as *Listeria monocytogenes*. Several incidents of contaminated plant-based cheeses linked to *L. monocytogenes* infections, including serious listeriosis cases in France in 2022 [1], highlight the importance of addressing these emerging risks.

This collaborative prevalence study involves representative laboratories from 15 EU Member States. 100 plant based dairy and meat substitutes were collected in each country at retail level and tested at the end of their labelled shelf-life to assess contamination under worst-case conditions. Analysis for *L. monocytogenes* and *Listeria* spp., with additional parameters such as pH, water activity, *Salmonella* spp., Shiga toxin-producing *Escherichia coli* (STEC), and spore-forming bacteria such as *Bacillus* spp. and *Clostridium* spp. were performed depending on laboratory capacity in each country. Preliminary results will include data on 22 dairy, 27 meat, 5 fish and 33 milk substitutes samples that were available in stores in Latvia at the moment of data collection in spring of 2025.

The obtained data will provide much-needed prevalence information to support future microbial risk assessments and help EU risk managers evaluate whether current measures, originally designed for animal-based products, are sufficient for this rapidly expanding product category. The study will also raise awareness of potential food safety risks associated with plant-based substitutes and support evidence-based consumer protection.

Acknowledgements

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(16) Characterization of a novel plant picorna-like virus isolated from sea buckthorn

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Keywords: sea buckthorn-associated picorna-like virus, protein expression, 3D structures, capsid proteins, nonstructural proteins

Sea buckthorn (*Hippophae rhamnoides* L.) is an agriculturally important crop, yet its viral diseases remain poorly studied. The first sea buckthorn virus was only reported in 2022, isolated from wild sea buckthorn leaves in Latvia that exhibited necrotic spots, and identified using next-generation sequencing (NGS). Based on its complete genome sequence and genomic organization, the virus was designated sea buckthorn marafivirus [1].

The same NGS dataset revealed a second virus genome, termed sea buckthorn-associated picorna-like virus (SBTaPLV). This is a novel, previously uncharacterized plant virus that shares genome organization and sequence similarity with picorna-like viruses. Its genome, 8,891 nucleotides in length, encodes two open reading frames (ORFs): ORF1 encodes the coat proteins (CPs), while ORF2 encodes a large polyprotein comprising three protein domains—RNA helicase, cysteine protease, and RNA-dependent RNA polymerase.

To further characterize SBTaPLV, structural and non-structural proteins were cloned and expressed in an *E. coli* system. The cysteine protease, helicase, and CPs were obtained in soluble form, with CPs likely assembling into virus-like particles, although optimization of buffer composition and expression conditions is still required. The RNA polymerase, however, was obtained in insoluble form.

Predicted 3D protein structures generated with AlphaFold 3.0 and visualized in PyMOL 3.0 revealed strong similarity to known proteins, with conserved amino acid motifs present within functional domains. The helicase contained conserved motifs “A,” “B,” and “C,” while the polymerase displayed the characteristic right-hand architecture with palm, thumb, and finger subdomains. The CP polyprotein may encode not only three distinct CPs but also a potential fourth protein located on the inner surface of the viral capsid.

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(17) Study of rhizosphere microorganisms with growth-promoting potential from winter wheat and canola

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Keywords: plant growth-promoting microorganisms, rhizosphere.

In the context of a growing global population and the depletion of natural resources, emphasis is being placed on sustainable and eco-efficient strategies to improve agricultural productivity in the face of climate change [1]. Conventional agricultural practices often rely on the extensive use of synthetic chemical fertilizers, posing significant risks to agroecosystems and human health. One promising alternative is the application of plant growth-promoting microorganisms (PGPMs), which can serve as eco-friendly substitutes and contribute to the restoration of agroecosystems [2].

This study aims to investigate and characterize PGPMs isolated from the rhizosphere of winter wheat (*Triticum aestivum*) and winter canola (*Brassica napus*), with particular focus on identifying strains capable of tolerating salinity and heavy metal stress conditions

Soil samples from the rhizosphere of wheat and canola were suspended in physiological saline and plated onto solid LB media supplemented with 0.5%, 5%, 10%, and 20% sodium chloride, along with varying concentrations of copper, nickel, and zinc. Morphologically distinct colonies were isolated from the plates containing the highest concentrations of NaCl, Cu, Ni, and Zn. Furthermore, their ability to produce siderophores, indole-3-acetic acid (IAA), and ammonia, to solubilize phosphate and potassium, as well as to exhibit catalase and ACC deaminase activities, was evaluated.

A total of 30 isolates from the canola rhizosphere and 43 isolates from wheat roots capable of tolerating 10% NaCl were selected. Among these, 29 isolates exhibited growth at 100 ppm Cu, 28 at 50 ppm Ni, and 16 at 100 ppm Zn. Furthermore, 60 isolates demonstrated plant growth-promoting properties. Based on their functional characteristics, 9 isolates were identified.

Our findings demonstrate that microorganisms isolated from the rhizosphere of winter wheat and canola exhibit plant growth-promoting activity.

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(18) From toxic cyanobacterial blooms to bioactive antifungal agents

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Keywords: plant diseases, phytopathogenic fungi, biocontrol, *cyanobacteria*, biomass

Anthropogenic eutrophication and global warming are simultaneously contributing to the formation of harmful algal blooms (HABs) worldwide, which have become the greatest threat to freshwater ecosystems. Certain bloom-forming cyanobacteria species can produce large amounts of biomass that, when decaying, reduce oxygen levels, alter the taste and odour of the water. HABs pose a serious environmental and health risk as bloom-forming cyanobacteria can synthesize a variety of cyanotoxins with neurotoxic, hepatotoxic, cytotoxic or dermatotoxic effects on humans and aquatic organisms. Interestingly, despite their toxic effects, cyanobacteria also exhibit antibacterial and antifungal properties, indicating their potential as biocontrol agents against plant pathogens.

The aim of this study is to investigate the application of HAB-derived biomass extracts in the development of sustainable plant disease control measures. The dominant biomass of *Microcystis*, a genus of cyanobacteria known for its high toxicity among other freshwater cyanobacteria species, was selected for testing. *Microcystis aeruginosa* (85%), *Microcystis* spp. (13%), *Aphanizomenon flos-aquae* and *Dolichospermum* spp. were the dominant cyanobacteria in the biomass collected from the Kaunas Reservoir in September 2024. The main objective was to evaluate the antagonistic activity of different extracts – aqueous, methanolic, methanolic-acetonic and ethanolic – at different concentrations against different plant pathogens.

The methanolic extract derived from the cyanobacterial biomass showed strong inhibitory activity against plant pathogenic fungi such as *Diaporthe* spp., *Fusarium* spp. and *Phytophthora* spp. These results emphasize the potential of cyanobacteria-derived methanolic extracts as environmentally friendly alternatives to chemical pesticides and encourage further research on their bioactive compounds and mechanisms of action.

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(19) Diversity and ligninolytic activity of saprotrophic fungi associated with *Ips typographus* and *Picea abies*

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Keywords: *Ips typographus*, *Picea abies*, fungi, saprotrophs, diversity, ligninolytic activity

Saprotrophic fungi decompose dead organic material consisting mainly of cell wall polysaccharides and biopolymers. Their main characteristic is the ability to degrade complex polymers such as lignin, the second most abundant biopolymer in nature, which has a very complex and heterogeneous structure. This process is facilitated by soft- and white- rot fungi through the action of ligninolytic enzymes. These microorganisms offer an environmentally friendly and cost-effective alternative to the traditional, energy-intensive chemical or mechanical methods used to process plant biomass in industries such as paper, pulp and biofuel production.

Insects play an important role as vectors of saprotrophic fungi that colonize dead plant material. The European spruce bark beetle (*Ips typographus*), which causes considerable damage to its host throughout Europe, spreads fungi with ligninolytic activity between trees. The aim of this study was to investigate the diversity of fungi colonizing *Ips typographus* and its host plant, *Picea abies* and to evaluate their potential for lignin degradation.

A total of 95 and 106 pure fungal cultures were isolated from adult *Ips typographus* beetles and the wood of *Picea abies*, respectively. Based on morphology, the beetle-associated isolates were grouped into 28 morphological groups, while the isolates from the spruce wood were divided into 25 groups. Bioinformatic analysis of the ITS region showed that both *Ips typographus* and *Picea abies* harbour fungi belonging to the class Sordariomycetes. The mycobiota of *Ips typographus* included species from the genera *Aspergillus*, *Cladosporium*, *Penicillium*, *Trichoderma*, while *Picea abies* was mainly colonized by fungi from the genera *Grosmannia*, *Pleurotus, Trichoderma*. The Bavendamm test with gallic acid showed that 112 of the 201 isolated fungi exhibited ligninolytic activity [1]. It was also found that 0.2% gallic acid inhibited the growth of 47 fungal isolates, stimulated 84 and had no effect on 25 [2].

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(20) How metal pollution affects antimicrobial resistance in droplets

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Keywords: collateral resistance, antimicrobial resistance, droplet microfluidics, metal pollution

Background and aims: Increase in antimicrobial resistance (AMR) is a global problem in need of immediate attention [1]. Studies indicate that low concentrations of certain pollutants, such as metals, in the environment can contribute to AMR, as this can induce collateral resistance when antibiotics and metals use the same resistant [3][4]. There is a need for further research into possible effect of metal pollution on AMR to understand the complex dissemination of AMR. Here we investigate whether zinc and lithium induce collateral resistance using droplet-based microfluidics. This allows precise control at microscale reactions where each droplet can act as a separate environment, enabling high throughput testing and minimizing use of reagents.

Methods: We use GFP-labelled *Escherichia coli*, $ZnCl_2$ and LiCl as metal solutions, and cefotaxime and kanamycin antibiotics. We perform a single cell-based (droplet) minimal inhibitory concentration (MIC) assay for each antibiotic, with *E. coli* pre-incubated for 9 days in sub-inhibitory concentrations of each metal. Droplets are imaged via confocal microscope and data is analysed via software CellProfiler™ [5].

Results: Droplet MIC assays following pre-incubation with sub-inhibitory metal concentrations suggest that metals influence *E. coli* resistance. Pre-incubation in lithium decreases susceptibility to cefotaxime and kanamycin on day 9 while pre-incubation in zinc increases the resistance to cefotaxime and kanamycin on day 9.

Conclusion: This study highlights the need to address environmental sources of metal contamination to avoid the spread of AMR. In our experiment, low concentrations of metals affected resistance to antibiotics in different ways depending on the metal assessed. Using droplet-based microfluidics moreover proved to be a high throughput and cost-effective method for generating reproducible data and thus a useful method for future studies exploring environmental factors influencing spread of AMR.

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(21) Antifungal activity of *Streptomyces* species against plant pathogens

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Keywords: *Streptomyces*, antifungal activity, pathogens

Ash dieback is a devastating disease threatening ash populations across Europe. The fungus *Hymenoscyphus fraxineus* is the primary causal agent, leading to widespread ecological and economic losses. Traditional control strategies have been ineffective.

Using underexplored *Streptomyces* spp. strains, we performed a series of *in-vitro* experiments to explore the antimicrobial efficiency and antagonistic mechanisms against *H. fraxineus* and other plant fungal pathogens – *Botrytis cinerea, Mycosphaerella* sp., *Fusarium oxysporum* and *Fusarium graminearum*. The most effective bacterial strains belonged exclusively to the genus *Streptomyces*, with *Streptomyces* sp. MSCL 350, MSCL 1739 and MSCL 1720 ranking among the top performers based on mean inhibition across all fungi. These findings support the hypothesis that selected *Streptomyces* spp. strains possess broad-spectrum antifungal activity and could serve as promising candidates for the development of biological control agents against *H. fraxineus* and other plant pathogens.

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(22) Structure-function analysis of *Streptomyces scabiei* 87.22 cutinase by site-directed mutagenesis

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Keywords: cutinase, mutagenesis, enzymes, polymers, site-directed mutagenesis

Cutinases (EC 3.1.1.74) – members of the α/β hydrolase family, characterized by the ability to hydrolyse cutin. In recent years some cutinases have been shown to degrade synthetic polymers, such as poly(ethylene terephtalate), poly(butylene succinate), poly(ε-caprolactone) and others [1]. Due to an increasing need for enzymatic polymer degradation, cutinase discovery, characterization and improvement of enzymatic qualities is becoming more important. Understanding how enzymatic function depends on structure could help design more efficient enzymes for implementation in bioremediation and other branches of industry [2].

In this study, we introduced four mutations (Gln88Ala, Asp95Ala, Ser123Ala, Leu80Ala) into the sequence of *S. scabiei* 87.22 cutinase [3]. Mutations were selected by targeting potentially important regions that could affect enzymatic activity or substrate specificity of this enzyme. Mutant enzyme variants were designed by site-directed mutagenesis, cloned into expression vector (pET-21c(+)), protein biosynthesis performed in *E. coli* BL21(DE3) strain and proteins purified by immobilized metal ion exchange chromatography. The enzymatic activity was determined spectrophotometrically; optimal temperature, thermostability, pH range, substrate specificity for synthetic *p*-NP esters, tolerance to organic solvents and potential for polymer degradation were investigated.

The results provide valuable insights in the structure-function relationship of *Streptomyces scabiei* 87.22 cutinase. Gln88Ala substitution in the conserved motif of catalytic pentapeptide leads to a complete loss of enzymatic activity. Ser123Ala mutation shifted substrate preference towards longer-chain synthetic *p*-NP esters. Asp95Ala substitution increased thermostability. Leu80Ala mutation had detrimental effects for thermal activity and thermostability These findings demonstrate that Gln88, Asp95, and Ser123 amino acids play crucial role for enzymatic activity of *S. scabiei* 87.22 cutinase.

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(23) FTIR spectroscopy – a multifunctional tool in microbiological research

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Keywords: FTIR spectroscopy, microbiological analysis, metabolic profiling, high-throughput screening (HTS-XT), infrared microscopy, Diamond anvil cell (DAC)

Microbiological research frequently requires reliable methods for strain differentiation, monitoring of metabolic states, and characterization of structural adaptations such as biofilm formation or stress-induced biochemical remodelling. Fourier-transform infrared (FTIR) spectroscopy provides a powerful, multifunctional, and label-free analytical approach by probing the vibrational signatures of proteins, lipids, nucleic acids, and polysaccharides within intact microbial samples.

A wide range of FTIR modules is available, each of which can be used independently or in combination. Potassium bromide (KBr) pallets remain a classical method for transmission measurements enabling detailed analysis of dried microbial biomass or extracted biomolecules, though preparation is labour-intensive and moisture sensitive. High-throughput screening extensions (HTS-XT) extend FTIR to larger scale studies, allowing rapid and reproducible acquisition of biomass spectra under standardized conditions with minimal sample preparations. Infrared microscopy. equipped with either simple IR or attenuated total reflection (ATR) objectives, enables spatially resolved biochemical mapping at the micrometre scale, supporting investigations of sample heterogeneity. For scarce or valuable specimens, diamond anvil cell (DAC) technology permits high-quality spectral acquisition from minimal biomass quantities, thereby extending applicability to rare isolates and microcultures.

When combined with advanced chemometric methods and curated spectral libraries, this FTIR toolkit supports both supervised and unsupervised multivariate analysis, enabling discrimination at the genus, species, and even strain level, while providing quantitative insights into metabolic dynamics. The deployment of such a comprehensive framework enhances the resolution of microbiological investigations, effectively bridging classical phenotypic assays with modern vibrational spectroscopic profiling.

(24) Misconceptions about nucleic acids signatures in FTIR spectroscopy

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Keywords: FTIR spectroscopy, DNA, RNA. phosphate

Fourier Transform Infrared (FTIR) spectroscopy is widely used to characterize the biochemical composition of cells and tissues. A persistent misconception referred in literature is the assignment of the 1080 cm^{-1} band as a direct and unique marker of DNA and RNA content in biomass. Though in reality, this region reflects the symmetric phosphate stretching vibration (PO_2^-) not only of nucleic acids but also of membrane phospholipids, while additionally overlapping with C–O stretching vibrations from carbohydrates such as glycogen. Moreover, it should be noted that nucleic acids contribute only a small fraction to the total cellular mass, and therefore their spectral signal is often minor compared to those of proteins, lipids, and carbohydrates, which dominate the FTIR fingerprint region. Misinterpretation of the 1080 cm^{-1} band can therefore lead to erroneous conclusions about the concentration, integrity, or mutation of nucleic acids.

Evaluation of individual macromolecular compound reference spectra and cell and tissue spectra shows that accurate assessment of nucleic acids requires consideration of the broader phosphate region (1250-1080 cm^{-1}) and nucleic acid base vibrations (1600-1500 cm^{-1}) in the context of overlapping macromolecular contributions. Therefore, the characteristic band of nucleic acids is in region of 1250-1230 cm^{-1}. This clarification emphasizes that FTIR provides a holistic biochemical fingerprint rather than a DNA/RNA-specific probe, and that careful band assignment is essential for meaningful biological interpretation.

(25) Culture-dependent and 16S rRNA gene NGS based characterization of bacterial communities from peatlands in Latvia

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Keywords: peatlands, soil microbiome, NGS, bacterial isolation, iChip

Peatlands are unique ecosystems in Latvia that play a crucial role in global carbon and water cycles. They harbour unique microbial communities adapted to acidic, low-nutrient and high moisture conditions. Despite their ecological importance and biotechnological potential, the microbiome of Latvian peatlands remains poorly characterized. This study aimed to investigate the microbial composition and isolate key bacterial taxa from selected peatlands in Latvia.

Soil samples were collected from multiple *Sphagnum*-dominated peatlands. The total soil DNA was extracted for 16S rRNA v3-v4 amplicon sequencing to characterize taxonomic composition of the bacterial communities. Additionally, bacterial strains were isolated from peatland soil samples using two isolation techniques: dilution-to-extinction method on various media and iChips.

16S rRNA gene amplicon sequencing of total DNA from peatland soil revealed a dominance of *Acidobacteria* and *Proteobacteria* phylum. Slight community structure variations were observed among the sampling sites. Applied culture dependent techniques allowed for isolation of bacterial strains assigned to more than 70 morphotypes in total. Applying extinction-to-dilution method, the highest isolation success was achieved using R2A media. iChip method enabled the isolation of a greater diversity of microorganisms, many of which were not isolated using traditional methods. The isolated morphotypes were 16S sequenced and belonged to the genera with known roles in peatland ecological processes.

Our results contribute to the overall understanding of microbial ecology in peatlands and lay groundwork for future exploration of the biotechnological potential of the isolated bacterial strains.

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(26) Biofunctionalization of polymeric filtration membranes

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Keywords: biofunctionalization, enzyme immobilization, fouling mitigation, membrane fouling

Membrane filtration is a key technology in water treatment and bioprocessing, offering efficient separation of suspended and dissolved components. However, membrane fouling, caused by the accumulation of organic, inorganic, and biological matter, is a common challenge in filtration processes. Various strategies have been explored to mitigate membrane fouling, ranging from surface hydrophilization and incorporation of antimicrobial nanoparticles to chemical grafting of functional groups that minimize interactions with foulants.

Meanwhile, enzymes are already widely applied in membrane cleaning-in-place (CIP) protocols, where protease, lipase etc. are used to catalyse specific foulants. In contrast, enzymatic membrane functionalization aims to shift the focus from cleaning to prevention: enzymes are immobilized on membrane surface, enabling them to degrade organic foulants before a stable fouling layer develops. This mitigation approach may be particularly relevant in industries with high organic loads, such as food processing, biotechnology, or wastewater treatment.

In this study, polymeric membranes were fabricated via the non-solvent induced phase separation method. Biofunctionalization was achieved using lipase from *Candida rugosa*, immobilized onto the membrane surface to catalyse lipid-based foulants. The catalytic activity of the treated membranes was assessed using p-nitrophenyl palmitate as a model substrate, where enzymatic hydrolysis releases p-nitrophenol, and quantified via spectrophotometric detection.

The biofunctionalized membranes exhibited measurable enzymatic activity, demonstrating that the enzyme retained functionality after immobilization. These results confirm the feasibility of enzyme-based membrane modification. Future work will focus on assessing the stability of the immobilized lipase under operational conditions and evaluating the performance of the membranes in filtration experiments.

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(27) Functional capacity of plastic-degrading bacteria derived from landfill and municipal wastewaters

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Keywords: biofilm, enzymatic activity, microplastic, respiration intensity

The mechanisms of plastic biodegradation by microorganisms remain poorly understood because of high variability in environmental conditions. To this date, no microplastic-specific removal strategy is employed. Nevertheless, microplastic removal efficiency is stated to be approx. 88%, with most of it accumulating in the activated sludge [1].

This study aimed to assess a physiological response of pure cultures and natural microbial communities to the presence of different types of microplastics. Microorganisms were derived from Getliņi EKO landfill (Riga, Latvia) and some municipal wastewater treatment plants.

Among the bacteria selected from landfill leachate, *Bacillus* was the predominant genus identified, whereas *Pseudomonas* dominated the metagenome. Comparative testing revealed the highest non-specific esterase activity in cultures of *B. licheniformis* and *B. altitudinis*. A newly developed consortium derived from Getliņi EKO landfill demonstrated relatively high degrading activity toward low-density polyethylene (LDPE) resulting in a weight loss of untreated microplastic granules of up to 19.44 % after 42 days of incubation. In turn, weight loss of polyethylene terephthalate (PET) and high-density polyethylene (HDPE) was found to be 5.99 % and 2.58 %, respectively. Thermal, acidic, and basic pre-treatments of plastic granules led to distinct differences in bacterial physiological responses, showing contrasting trends in enzymatic activity of planktonic cells [2].

Another aspect of our study was related to the impact of PET microplastics on the biochemical activity of the microbial community in real activated sludge while using synthetic wastewater with a chemical oxygen demand (COD) of 500 mg/L. Fluorescein Diacetate (FDA) hydrolysis was assessed as an indicator of microbial activity and an indirect measure of microbial biomass. Urease activity is a rough indicator for microplastic degradation [3]. FDA hydrolysis declines for all tested PET concentrations when measured after 7 days. The same pattern was true for urease activity, meaning, that microbes being part of the microbial nitrogen cycling system were also affected by this lowered activity. These results demonstrated the toxic effect of PET powder on the microbiota of activated sludge.

Further research could focus on assessing other parameters of microbial activity in relation to microplastics.

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(28) First look at antibiotic consumption and resistance genes in urban wastewater in Latvia

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Keywords: antibiotic resistance, antibiotic consumption, monitoring urban wastewater, digital PCR

Antimicrobial resistance has become a significant burden on public health. Therefore, it is crucial to monitor the spread of resistance and investigate the factors driving it[1]. Wastewater monitoring has emerged as an effective approach to detect pathogens and AMR at the population level[2]. This study aimed to determine the effect of antibiotic consumption on ARG abundance in urban wastewater.

The concentration of selected ARGs—*sul*1, *tet*W, and *erm*B—was determined in urban wastewater samples using digital PCR. Influent samples were collected from wastewater treatment plants in Latvia from autumn 2022 to winter 2024. In this study, associated nation-level antibiotic consumption data and linear regression models were used to determine the effect of antibiotic consumption on ARG concentration.

It was determined that season had a statistically significant effect on doxycycline (F-value = 6.66; p = 0.0245) and azithromycin consumption (F-value = 6.22; p = 0.0285). Additionally, doxycycline consumption statistically significantly affected *tet*W concentration in wastewater with a three-month lag period (F-value = 6.63; R^2 adj = 0.41; p = 0.0367). No statistically significant effect was found between *sul*1 and sulfamethoxazole and trimethoprim, as well as between *erm*B and clarithromycin, azithromycin and clindamycin consumption ($p > 0.05$). Additionally, no statistically significant effect ($p > 0.05$) was found between antibiotic consumption and the year, implying no significant difference in antibiotic consumption between years 2022-2024.

Observed results show complicated relationships between antibiotic resistance, antibiotic consumption, and the urban environment. These findings show the difference in interactions between antibiotic consumption and their respective ARGs, implying that an individual approach is necessary for each ARG being monitored.

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(29) Isolation of bacterial strains from phenol-contaminated environments

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Keywords: *Pseudomonas*, phenolic compounds, lignin

Lignin is an important component of plant cell walls and represents the most abundant renewable aromatic biopolymer on Earth. It is also a primary by-product in biomass processing industries.

Industrial wastewater, particularly from pulp and paper, textile, and chemical industries, often contains phenolic compounds. These substances are toxic, recalcitrant, and environmentally persistent, posing significant challenges for treatment processes and microbial communities.

The isolation of bacterial stains from phenol-contaminated environments offers promising avenues for both bioremediation and lignin valorization. These strains often possess specialized metabolic pathways capable of degrading or transforming phenolic compounds. Understanding these pathways is critical for evolving sustainable lignin bioconversion and improving the efficiency of wastewater treatment.

In this study, several *Pseudomonas* sp. strains were isolated from industrial phenolic wastewater. Initial identification was carried out using the MALDI Biotyper system. Preliminary results with the first strain indicated its ability to catabolize various types of lignin, including hydrolysis lignin, kraft lignin, and lignosulfonate. These data were obtained using classical analytical techniques, including size-exclusion chromatography and gas chromatography-mass spectrometry.

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(30) Catabolism of lignin-derived aromatics by *Rhodococcus*

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Keywords: *Rhodococcus* sp., lignin-derived aromatics, catabolism

Lignin is considered as the most abundant renewable and low-cost source of aromatic carbon on Earth. However, due to its irregular and recalcitrant structure, the biological degradation of lignin is a relatively slow process. Nevertheless, several microbial species are capable of efficiently depolymerize lignin and/or metabolize toxic lignin-derived aromatic compounds. These aromatics-degrading bacteria are typically found in soil, wastewater, compost, and mining sediments. Such microbial species often exhibit high metabolic versatility, allowing them to synthesize various storage compounds that support cell viability under stressful conditions [1].

Current study focuses on evaluating the ligninolytic potential of *Rhodococcus* sp. strains isolated from various local unpolluted and polluted environments. The ability of these strains to catabolize lignin-derived aromatics was assessed using three types of technical lignins: hydrolysis lignin, kraft lignin, and lignosulfonate. Lignin-degrading activity was evaluated using analytical chemistry techniques including GC-MS, HPLC, and SEC. Comparison of aromatic compound profiles before and after bacterial treatment enables the identification of *Rhodococcus* sp. strains with different aromatics-utilization potential, while also outlining potential catabolic bottlenecks.

A systematic and comparative understanding of the resulting catabolic products is essential for selecting complementary strains for microbial consortia capable of more effective lignin utilization. As a next step, we aim to perform comparative transcriptomic analyses of selected *Rhodococcus* sp. strains to uncover not only novel components, but also new interactions within the complex metabolic network involved in the utilization of aromatic compounds. The ultimate goal is to develop and validate robust high-throughput methods for screening bacterial strains for lignin-converting capabilities.

Acknowledgements

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(31) Comparative analysis of *Agrobacterium tumefaciens* strains for CRISPR/Cas9 – mediated transformation in *Lolium perenne*

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Keywords: *Agrobacterium tumefaciens*, *Lolium perenne*, CRISPR/Cas9, transformation efficiency, gene editing, callus regeneration

As climate change intensifies abiotic stressors in agriculture, precision genome editing may assist in developing stress-resilient non-transgenic crops [1]. However, low efficiency of *Agrobacterium* – mediated transformation (AMT) and subsequent plant regeneration remain obstacles for genome-editing, in particular, in monocots [2]. Plant genotype and *Agrobacterium* strain are the two most relevant variables affecting the AMT efficiency [3]. We examined the AMT efficiency and genome-editing capability of four *A. tumefaciens* strains AGL1, EHA105, ITC340, LBA4404 in perennial ryegrass, focusing on targeted inactivation of the *CBF6* (C-repeat/DRE/LTRE-binding protein) gene using a CRISPR/Cas9 system. AMT success was assessed via *ZsGreen* reporter gene fluorescence and PCR, and the overall AMT efficiency was based on the final transformation count from the fluorescence and different types of regeneration capabilities. 189 transformed calli were generated, with strain-specific distributions: 82 (AGL1), 70 (EHA105), 26 (ITC340), and 11 (LBA4404). AMT efficiency varied among strains, with ITC340 achieving the highest proportion of regenerable, edited plants (23.08%), followed by AGL1 (6.1%) and EHA105 (1.43%). LBA4404 yielded no regenerants. This highlights substantial differences in virulence and host compatibility across *Agrobacterium* strains and demonstrate the importance of bacterial strain selection in achieving successful gene editing in monocots. We provide a potential microbiological framework for optimizing CRISPR delivery via *A. tumefaciens* supporting ITC340 as a promising candidate for future transformation efforts in perennial grasses.

Acknowledgements

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(32) Isolation and endemic diversity of arbuscular mycorrhizal fungi in latvian soils

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Keywords: Arbuscular mycorrhizal fungi diversity, fungal isolation, soil microbiota

Due to growing global demand for food and the EU incentives to reduce reliance on synthetic fertilizers and pesticides there is increasing need for novel innovative agricultural solutions. It is well known that arbuscular mycorrhizal fungi (AMF) form mutualistic associations with most land plants and play an important role in plant growth, nutrition and stress response. This potentially offers sustainable solution for organic farming strategies contributing to yield improvement and plant protection with reduced agrochemical inputs. However, there is a limited number of AMF species that can be cultivated for such commercial preparations. Moreover, the efficacy of the available AMF preparations appears to be crop specific and urges to explore the endemic AMF diversity. Therefore, the isolation and characterization of novel AMF from agricultural soils holds the potential for developing sustainable AMF-based microbial preparations for crop-specific applications crop growth and protection.

To this end, we isolated AMF spores from agricultural and natural soils from multiple field sites across Latvia, covering a diverse range of monocot and dicot cover crops as well as natural meadows. Spores were isolated using the wet sieving and sucrose gradient centrifugation method, followed by spore surface sterilization. Spore community composition and morphotypes were determined via morphological analysis under light microscope. Observed variations in spore density and diversity in individual sites suggest that soil properties and land-use may influence AMF distribution and species composition. We further aim to examine and characterize the metagenomic diversity of the endemic AMF communities as well as isolate and propagate them in vitro to test their effects in reducing fertilizer inputs on various crop plants in greenhouse tests towards novel AMF-based microbial preparation prototypes for sustainable agriculture.

(33) Photothermal inactivation of *E. coli* using Ti-based MXene-coated PCL membranes

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Keywords: MXene, photothermal effect, *E. coli*, antibacterial surface, PCL membrane, calcein AM, propidium iodide, laser

MXenes, a family of two-dimensional transition metal carbides and nitrides, are increasingly studied for their unique physical properties, including high photothermal conversion efficiency, surface reactivity, and potential antibacterial applications[1]. Among various configurations, titanium-based Mxenes have demonstrated strong light-to-heat conversion under near-infrared irradiation, making them promising candidates for photo-responsive antimicrobial surfaces. In this study, we investigated the photothermal bactericidal effect of electrospun polycaprolactone (PCL) membranes coated with Ti-based MXenes against *Escherichia coli*.

PCL membranes were incubated with *E. coli* for 4 hours in Mueller-Hinton broth to allow bacterial adhesion. The membranes were then subjected to laser irradiation under two conditions: (1) 5 minutes at 10 Hz, 2 W and (2) 10 minutes at 50 Hz, 4 W. Temperature monitoring revealed that MXene-coated membranes reached up to 80 °C and 70 °C under the first and second laser regimes, respectively. In contrast, control PCL membranes without MXene showed no significant temperature increase under the same conditions, confirming the material's critical role in thermal generation.

After irradiation, the membranes were gently washed, and the bacterial suspension was plated onto solid agar media. In parallel, molten agar was poured directly over laser-treated membranes to test for surface-bound bacterial viability. No bacterial growth was detected in either condition involving MXene-coated membranes, while control membranes (non-MXene-coated) showed dense colony formation, indicating preserved viability. To further assess cell integrity, membranes were stained with Calcein AM (viable cells) and propidium iodide (non-viable cells) and analyzed via fluorescence microscopy. No Calcein-positive (live) bacteria were observed on the irradiated MXene-coated membranes, while PI staining indicated widespread membrane damage and cell death.

These results confirm the potent photothermal bactericidal activity of Ti-based MXene coatings upon laser activation. The heat generated was sufficient to induce rapid and complete bacterial inactivation, both on the membrane surface and in the surrounding medium. The experiment underscores the potential of MXene-functionalized biomaterials as light-activated antimicrobial systems for surface sterilization and wound protection. Importantly, the selectivity of the response—absent in uncoated PCL membranes—highlights the necessity of MXene inclusion for photothermal efficacy. Further development of such systems may offer promising routes for non-antibiotic infection control in biomedical devices and smart wound dressings.

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(34) The role of unfolded protein response in the stress resistance of *Saccharomyces cerevisiae* associated with the transition into the state of anhydrobiosis

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Keywords: *Saccharomyces cerevisiae*, unfolded protein response, UPR, Ire1, anhydrobiosis

The unfolded protein response (UPR) in yeast *Saccharomyces cerevisiae* is a cellular stress response triggered by the accumulation of unfolded proteins in the endoplasmic reticulum (ER). Ire1 is an ER located transmembrane protein that senses ER stress and triggers the UPR. This response aims to restore ER function and protein homeostasis by increasing the capacity of the ER and promoting the degradation of misfolded proteins [1].

Extensive knowledge has been gained about the role of the plasma membrane and its proteins in the transition of yeast cell into the state of anhydrobiosis [2]. Yet nothing is known about UPR in the stress resistance in yeast cells associated with the transition into the state of anhydrobiosis. The aim of this study was to obtain first information concerning the role of UPR in various stresses - hyperosmotic, oxidative, thermal and dehydration/rehydration cycles.

The *Saccharomyces cerevisiae* strains used in this study were BY4741, BY4742 and its respective derived ire1Δ strain (obtained from EUROSCARF). Strains were subjected to various stresses, for example, hyperosmotic, oxidative, thermal and dehydration/rehydration with or without of addition of dithiothreitol (DTT), trehalose, proline, xylitol in various combinations.

Our results showed that the ire1Δ mutants were sensitive to dehydration. Addition of DTT to yeasts cultures before drying decreased twofold the ability to withstand dehydration and subsequent rehydration. It is noteworthy that trehalose (disaccharide), proline (amino acid) and xylitol (sugar alcohol) facilitated the ability of cells to cope with excessive unfolded protein load in cells during transition into the state of anhydrobiosis. The ability of mutants and parent strains to withstand drying and subsequent rehydration treatment does not depend on resistance to oxidative, hyperosmotic stress or heat shock prior to dehydration. Indicating that the UPR plays an important role during dehydration and transition into state of anhydrobiosis, excessive unfolded protein load in the cell can be dangerous.

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(35) PCR-based screening for NRPS and PKS gene fragments in the urban microbiome of Vilnius city

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Keywords: microbiome, urban microbiome, urbanisation, secondary metabolites, polyketide synthases, non-ribosomal peptide synthetases.

The studies of the urban microbiome and the microbiome of the built environment have gained specific interest in the past few years. These studies focus on various objects: air, transit systems, soil, sewage, honeybees, etc., and as scientific data accumulates, it can be argued that urban microbiomes are becoming a separate ecological niche that interacts with humans, soil, and other microbiomes. Urbanisation is one of the main human-driven processes that reshape the natural distribution of soil bacterial communities. However, it is still unclear what impact the urbanisation process has on microorganisms and their ability to produce such compounds as secondary metabolites.

Secondary metabolism of bacteria and fungi is a rich source of new biologically active compounds that can be used in pharmaceuticals, biotechnology, and agriculture, providing not only clinically important drugs but also natural products with ecological functions. Polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS) are two main enzyme systems for the synthesis of secondary metabolites, and they can influence additional ecosystem benefits and interactions between microorganisms.

In this study, primary (autumn) and repeat (spring) soil samples were collected from four microdistricts of Vilnius city to identify PKS and NRPS genes.

The PKS gene search was performed by PCR using 23 primer pairs. PCR products were obtained using seven primer pairs. Three primer pairs targeted type I PKS, two targeted type II PKS, and two targeted type III PKS. PCR products were obtained in all samples using the ARO-PKS and KAF1/KAR3 primer pairs (targeting type II and type I PKS, respectively).

The NRPS gene search was performed by PCR using 13 primer pairs, and PCR products were obtained with six primer pairs. PCR products were obtained in all samples using only one primer pair A3F/A7R.

(36) Potential prebiotic effect of non-psychoactive cannabinoids and hemp by-products

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Keywords: cannabinoids, hemp, recovery of by-products, probiotics, prebiotics

Rich in fibers and bioactive terpenophenolics, hemp (*Cannabis sativa*) was primarily grown as a fiber crop for the production of textile and ropes and is now increasingly used in different fields, including food, feed, cosmetics, and pharmaceuticals, resulting in the accumulation of by-products. In addition to traditional hemp uses, there has been recent interest in the influence of its products on microbiota modulation, suggesting new roles for the plant. Focusing on industrial hemp (THC < 0.3%) and non-psychoactive cannabinoids (CBD, CBG, CBN), this study aims to assess the antimicrobial activity of cannabinoids against six lactic acid bacteria (LAB) cultures and one yeast culture, as well as the prebiotic index of hemp by-products.

To this end, using the agar diffusion method, no distinct inhibition zones were observed for LAB, except an inhibition zone of 7.5±0.5 mm induced by 1% CBD for *L. acidophilus*. In terms of *S. cerevisiae* yeast, the inhibition zones of 8.0-10.0 mm in both control and cannabinoid samples indicated that yeast cells were more susceptible to ethanol than to cannabinoids. Subsequently, the liquid medium supplementation with 1% cannabinoids showed that CBG inhibited the proliferation of *L. gasseri* and *L. plantarum*, also decreased the viability of *L. fermentum, L. reuteri,* and *L. acidophilus* by 5-6 lg CFU ml^{-1}. Furthermore, the growth of *L. reuteri* and *L. acidophilus* was additionally hindered by CBD and a mixture of three cannabinoids. Conversely, no significant changes in yeast growth rate were observed.

Lastly, the prebiotic indices of hemp husks, seed pomace, and pellets with *L. brevis* revealed that enzyme-treated hemp husks exhibit the highest prebiotic activity (0.382±0.001), comparable to commercial inulin (0.379±0.023), highlighting the strong prebiotic effect of the husks.

In conclusion, certain cannabinoids and hemp by-products possess great potential as novel prebiotic products. Future research will explore their effects on gut modulation.

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(37) Interplant signal transfer and defence response activation in mycorrhizal *Betula pendula* and *Populus tremula x tremuloides* systems

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Keywords: *Betula pendula*, *Populus tremula* x *tremuloides*, mycorrhiza, plant defence

Along with climate change and a growing demand for timber, Northern Europe is experiencing an expanding necessity for fast-growing and stress resistant commercially grown tree species. Additionally, global warming is predicted to drive northward expansion of deciduous tree species such as aspen and silver birch as well as expand the role of endomycorrhizal fungi in traditionally ectomycorrhizal temperate forest ecosystems in the Baltic region.

These factors necessitate development of innovative biotechnological approaches to enhance forest resistance and resilience. However, progress in understanding the genetic basis of stress responses in temperate deciduous trees remains limited, largely due to the absence of reproducible laboratory model systems and well-characterized molecular stress markers in trees.

In this study we aimed to validate previously described plant defence gene markers and characterise novel ones to study their expression profiles in silver birch and hybrid aspen trees linked via a common mycelial network. We used sequence mining and phylogenetic analyses to identify homologues of biotic stress-response genes in the genomes of both tree species and utilized *in vitro* propagated silver birch and hybrid aspen clones to test the effect of the presence and integrity of a common mycelial network on defence response regulation in naïve receiver trees after applying stress stimuli - wounding and bacterial flagellin - to the leaves of a connected sender tree. Additionally, we assessed the functional roles of inter-tree signals by performing fungal pathogen growth bioassays on neighbour primed receiver trees.

These findings represent a methodological advancement for studies of common mycelial network mediated interplant signalling and molecular defence responses in ecologically and economically important tree species and offer a pipeline for gene homologue discovery and expression profile assessment for future research on tree-microbe interactions.

(38) Comparing liquid-surface and solid-state cultivation methods for filamentous fungus *Phlebiopsis gigantea* spore production: oidia case study

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Keywords: filamentous fungi, spore production, liquid-surface cultivation, solid-state cultivation, biological control, *Phlebiopsis gigantea*

In fungal preparations, such as plant protection agents, the active ingredient is mainly fungal spores [1]. In this study, the authors evaluated spore (oidia) production of the Latvian isolate of *Phlebiopsis gigantea* PG 182 using liquid-surface and solid-state cultivation processes.

For both processes, the inoculum was prepared in the same way – by making a fungal suspension (oidia and fine mycelium fragments) from a 14-day static culture in liquid medium. The effects of medium depth (5 or 7 mm), malt extract syrup (Ilgezeem Ltd.; carbohydrates 71 wt% including 36 wt% sugars, proteins 3.3 wt%, with no fats, saturated fatty acids or salts) concentration (25, 50, and 75 g/L) and cultivation duration (1, 2, 3 and 4 weeks) on oidia concentration (oidia per ml of medium) were evaluated in the liquid-surface cultivation experiments. Cultivations were carried out in 450 ml jars, loosely covered with lids containing filter paper, under static conditions in an incubator (ES-20, Biosan) at 28 °C. The highest spore yield $(1.60 \pm 0.04)\times10^7$ was obtained on day 28 at a malt extract concentration of 50 g/L and medium depth of 0.5 cm. Under the same conditions, the yields on days 14 and 21 were $(0.22 \pm 0.09)\times10^7$ and $(0.83 \pm 0.06)\times10^7$ respectively, and were 35–70 % higher than under other combinations of malt extract concentrations and medium depths.

Solid-state cultivation experiments were carried out using pine wood shavings with sieved fractions of 7-2 mm (48 %), 13-7 mm (24 %) and >13 mm (3 %) in a thermostated water-jacketed incubator at 28 °C. Similar spore yields were obtained at substrate depth levels of 4 cm and 8 cm. Assessing initial substrate moisture contents of 50, 60, 70, and 80 %, the most suitable was 60 %. The effects of pine sawdust enrichment with wheat bran (0, 5, 10, 15, and 25 %) and cultivation time (14 and 21 days) were evaluated under conditions of 8 cm substrate depth and 60 % moisture. Without bran addition (0 %), the oidia yield (oidia per g of substrate) on days 14 and 21 was $(1.67 \pm 0.56)\times10^7$ and $(2.11 \pm 0.21)\times10^7$ respectively. Addition of 5 % bran approximately doubled the yield to $(7.41 \pm 0.18)\times10^7$ and $(5.04 \pm 0.81)\times10^7$ at the respective time points. Increasing the bran content to 10–25 % gave similar results on days 14 and 21 – reaching its maximum $(14.9 \pm 1.75)\times10^7$ on day 14 with no further increase observed on day 21.

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(39) Valorisation of low-value plant biomass residues for the development of mycelium-based biocomposites

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Keywords: mycelium, plant biomass, biocomposites, birch sanding dust, wheat straw, valorisation

This study evaluated the physicochemical properties of mycelium-based biocomposites (MBB) produced from *Trametes versicolor* mycelium grown on low-value biomass feedstocks: birch sanding dust (BSD) or wheat straw (WS), with co-substrates of hemp shives or birch sawdust. Four distinct MBB formulations were prepared for each substrate (BSD1–BSD4 and WS1–WS4).

Mechanical tests showed that BSD composites exhibited higher compressive (0.10–0.24 MPa) and flexural (0.05–0.10 MPa) strengths compared to WS composites (0.02–0.08 MPa compressive; 0.03–0.09 MPa flexural), with the strongest material being BSD1 (0.24 MPa).

Water absorption after 24 h immersion reached up to 600% for BSD2 and 650% for WS2, both with hemp co-substrate; volumetric swelling remained below 10% in all samples. High water uptake suggests limited use of MBBs in construction without further hydrophilization, but it may be beneficial for applications such as heavy metal biosorption.

Mold susceptibility tests revealed colonization within 2 days of exposure, starting with *Rhizopus* followed by *Trichoderma* species.

Hygroscopic analysis showed moisture content between 13–17% at 90% relative humidity (RH), rising slightly above 20% at 95% RH, with WS-based composites, particularly WS2, demonstrating greater hygroscopicity to humidity than BSD-based materials.

Overall, BSD substrates provided superior mechanical performance and improved moisture resistance, indicating their greater suitability for biomass residue valorisation in sustainable biomaterial applications.

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(40) Effect of substrate composition and chitosan coating on the properties of *T. versicolor* mycelium composites

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Keywords: *mycelium composites, substrate, chitosan coating*

Mycelium-based composites are emerging as sustainable alternatives to synthetic materials, yet their performance depends strongly on substrate and treatment. Composites were produced using the basidiomycete *Trametes versicolor* on two substrates: pure wheat straw (WS) and a 1:1 mixture of straw with pine chips (WSP), both supplemented with wheat bran. Some samples were coated with chitosan to assess its effect on material properties.

Fungal abundance was evaluated microscopically with Leica S9i imaging after lactophenol blue and safranin staining. Image analysis (ImageJ) showed lactophenol blue was most effective, and WS supported higher mycelial colonization than WSP, likely due to pine's antimicrobial compounds. Mechanical testing (Zwick/Roell Z010) measured bending and compressive strength. Differences between substrates and chitosan-coated groups were not statistically significant, though WS tended to show higher bending strength ($p = 0.148$), while WSP performed better in compression, indicating substrate properties outweighed mycelial density.

Water absorption tests showed no strong substrate effect, though WSP absorbed slightly more ($p = 0.053$). Chitosan coating reduced liquid water absorption ($p < 0.05$). In contrast, hygroscopic analysis revealed thicker coatings increased uptake from air, reaching 36.7% for WS and 25.0% for WSP ($p < 0.05$), worsening humidity sensitivity.

Overall, WS favored colonization and bending performance, whereas WSP provided higher compressive resistance. Chitosan reduced liquid water absorption but enhanced hygroscopicity. These results highlight the complex interactions of substrate, fungal growth, and coatings, and the need for optimized combinations when designing functional mycelium composites.

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(41) Mycelium biocomposites derived from agricultural and wood processing byproducts with magnetic properties as potential biosorbent

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Keywords: mycelium biocomposites, magnetic biosorbents, synthesis, magnetic properties

Adsorption of heavy metals on biosorbents is attractive approach to remediate the wastewater. Birch sanding dust (BSD) and wheat straw (WS) are studied as biosorbent and they are suitable for mycelium bio-composites (MB) production. Magnetic sorbent has the advantage of separating substances from the solution by a magnetic field without additional centrifugation or filtration. The present study aimed to develop the MB from BSD and WS and modify them with Fe_3O_4 micronanoparticles.

Wheat straw was obtained from the *Latgale Agricultural Science Center*, Ltd., Vilani, Latvia, and birch sanding dust from the plywood producer *Latvijas Finieris*, JSC. MB was obtained using *Trametes versicolor* by method [1]. Magnetic particles were synthesized by co-precipitation method. MB was suspended in water, added $FeCl_2$ and $FeCl_3$ and NH_3 water solution under Ar atmosphere. Obtained Fe_3O_4 MB magnetic particles were separated with a magnet, washed and dried. The magnetic properties of the obtained magnetic MB were analyzed using VSM [2].

Microscopy showed that were obtained Fe_3O_4 particles with size <30 μm. The obtained Fe_3O_4 micronanoparticles particles showed magnetization curves with no hysteresis typical for superparamagnetics. Saturation mass magnetization of obtained magnetite Fe_3O_4 was as high as 65.2 emu/g. Saturation magnetization of the magnetic BSD MB was 7.8 emu/g. Saturation magnetization of WS MB was much lower 0.16 emu/g. Calculated Fe_3O_4 concentration in WS MB was only 0.2%. In comparing synthesis of Fe_3O_4 with raw wheat straw showed Ms = 7.9 and Fe_3O_4 concentration 12% that is close to the magnetic BSD MB.

Obtained results showed that co-precipitation of magnetite with birch sanding dust mycelium bio-composites is a perspective method for magnetic bio-sorbent synthesis on base of mycelium composite. Synthesis of magnetite with wheat straw mycelium bio-composites was not so successful. Probably wheat straw mycelium bio-composite inhibit synthesis of magnetite.

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(42) Effect of enzymatic lactose hydrolysis on microalgal growth in concentrated cheese whey permeate

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Keywords: *Graesiella emersonii, Galdieria sulphuraria*, β-galactosidase, heterotrophic and mixotrophic cultivation, dairy by-products

High production costs of valuable microalgal biomass can be decreased by utilizing cheap agricultural wastes, such as dairy industry by-products [1]. Although certain microalgae demonstrate the ability to hydrolyze lactose, the hydrolysis rates remain comparably low. Enzymatic hydrolysis of lactose into glucose and galactose offers a potential strategy to enhance carbon utilization and support biomass production [2].

In this study, the growth performance of two lactose-utilizing microalgae, *Graesiella emersonii* MSCL 1718 and *Galdieria sulphuraria* SAG 107.79, was evaluated in enzymatically hydrolyzed concentrated cheese whey permeate. Results demonstrated that the lactose hydrolysis has significantly increased *G. sulphuraria* biomass productivity under heterotrophic growth conditions from 0.13 ± 0.01 to 0.21 ± 0.01 g L^{-1} d^{-1}. In contrast, lactose hydrolysis negatively impacted the biomass productivity of *G. emersonii* in the experimental medium (0.28 ± 0.01 g L^{-1} d^{-1}) compared to mixotrophic cultivation on untreated lactose-containing permeate (0.35 ± 0.01 g L^{-1} d^{-1}) under both mixotrophic and heterotrophic cultivation conditions.

These findings highlight the importance of optimizing medium preparation strategies to specific microalgal strains. Future research will focus on microalgae that lack lactose-metabolizing capabilities, making enzymatic hydrolysis a potentially more impactful strategy.

Acknowledgements

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(43) Metabolic response of *Clostridium autoethanogenum* to different syngas mixtures in controlled bioreactor cultures

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Keywords: acetogens, gas fermentation, syngas, *Clostridium autoethanogenum,* bioreactors

Considering the current environmental challenges, waste can serve as a valuable resource within the framework of a circular economy. For instance, gasification of waste streams enables the conversion of organic and inorganic waste into syngas, a gas primarily composed of carbon monoxide (CO), carbon dioxide (CO_2), and hydrogen (H_2). Syngas production from waste is particularly attractive due to its biotechnological potential for downstream applications as it can be converted into valuable products via gas fermentation using acetogen bacteria. Among these, *Clostridium autoethanogenum* has emerged as one of the most prominent gas-fermenting biocatalysts, capable of utilizing the Wood–Ljungdahl pathway to convert CO and CO_2 into industrially relevant chemicals such as ethanol and acetate. In addition, the concentrations of gases in syngas mixtures can vary depending on the feedstock, pre-treatment, and the gasification process. The aim of this work was to simulate syngas compositions derived from relevant waste feedstocks in Estonia, excluding real syngas impurities, and to analyse the metabolic response of *C. autoethanogenum* to the gas mixtures in bioreactor cultures. Batch fermentations were carried out with five different synthetic syngas mixes to evaluate how different concentrations of $CO/CO_2/H_2$ affect carbon balance, final product titers, rates, and yields. Our results demonstrated that the carbon flux was redirected mainly toward the production of ethanol, acetate, and CO_2, and the molar ratio of ethanol to acetate was higher in four out of five gas mixtures. Ethanol production accounted for an average of ~20% to ~44% of the total carbon balance, showing an increasing trend proportional to the H_2 uptake rate. Additionally, fermentation kinetics and optimization strategies will be discussed, providing insights into how syngas composition influences the efficiency of gas fermentation by acetogens.

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(44) Life cycle assessment of microbial protein production using hybrid living materials

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Keywords: alternative proteins, life cycle analysis, greenhouse gases, microbial consortia

The rising global demand for sustainable protein highlights the need for innovative microbial technologies that also address climate change challenges. Hybrid Living Materials (HLMs) are designed as interfaces between artificial supports and living microorganisms, enabling the simultaneous capture of greenhouse gases and protein production. In this system, porous membranes host a synergic microbial consortium of methanotrophic and photoautotrophic organisms, which together transform CH_4 and CO_2 into protein-rich biomass suitable for feed applications.

A cradle-to-gate life cycle assessment (LCA) was performed to evaluate the environmental performance of HLM-based protein production. The study identifies environmental hotspots in the laboratory-scale production process, proposes mitigation scenarios, and establishes a baseline for benchmarking HLM-derived protein against conventional sources, such as fishmeal and soymeal. The LCA encompassed microbial pre-cultivation, biofilm formation, biomass harvesting and drying, as well as the preparation and modification of the supporting membranes. Two functional units (FUs) were applied: FU1 – production of 1 kg microbial protein via HLMs, used to identify process-level hotspots, and FU2 – production of 1 kg protein equivalent to that in fishmeal, used for comparison with conventional proteins.

Results indicate that in microbial protein production, electricity demand during cultivation and drying accounts for more than 70% of the environmental burden. In addition to energy, several impact categories were identified as critical during the biofilm formation process, including freshwater eutrophication, marine and freshwater ecotoxicity, and human carcinogenic toxicity. While environmental challenges remain in several categories, advantages in land and water use were observed when compared to soymeal and fishmeal. These findings illustrate how LCA can act not only as an evaluation tool but also as a guide for directing microbial innovation towards circular and climate-resilient protein production.

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(45) Digitalisation of biology: the dawning of the Taltech Biofoundry

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Keywords: enzyme design, protein engineering, lab automation, synthetic biology, machine learning, non-conventional yeasts, biorefinery, big data

Several industries and sectors have adopted biotechnology to manoeuvre their processes towards a more sustainable future. As it expands, biotech increasingly generates valuable biological information. However, this valuable data can easily lose its impact if not properly managed and used. The DigiBio project, an Estonian (University of Tartu, Taltech) – Danish (DTU) Teaming collaboration, aims to tackle this challenge by creating a research and technology platform to digitalise and automate synthetic biology Design-Build-Test-Learn (DBTL) cycles. Establishing a protein engineering focused Biofoundry at Taltech is one of its pillars, which typically requires high-throughput equipment. But how to start such an endeavour when, in the beginning, the BioEngineering lab had nothing but manual pipettes and a classical Synthetic Biology background? This poster presents how we transitioned to a semi-automated lab in a year, through networking, learning the principles of automation and re-evaluating established paradigms. Synergistically, we have developed a functional bioinformatics platform for enzyme design. This platform allows for the investigation of protein properties and models improvements in catalytic activity and thermostability. The Taltech Biofoundry and other DigiBio facilities are intended for both commercial and academic collaboration. By leveraging these facilities, we expect to contribute to the quality and advancement of biotechnology research and industry in the Baltic region.

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(46) Metabolic modelling of a two-organism consortia for the production of chemicals using sunlight and carbon dioxide

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Keywords: genome-scale metabolic modelling, circular bioeconomy, metabolic engineering, microorganism consortium

The global chemical systems are primarily linear, fossil-dependent and emissions-intensive. Finding sustainable solutions to produce chemicals using a circular bioeconomy is necessary for a sustainable future.

The objective of this project is the capture and utilization of natural resources and waste for developing energy-efficient and scalable living materials that catalyze chemical production. This includes developing a synthetic microbial consortium and bioreactor prototypes for continuous monomer production. This concept supports the EU's GHG emission reduction targets, the European Green Deal and the Circular Economy Action Plan.

The metabolic engineering of two consortia-building microorganism interactions uses constraint-based stoichiometric modelling. Genome-scale metabolic models of *Synechocystis* sp. PCC6803 and *Pseudomonas taiwanensis* VLB120 were combined to develop a two-cell metabolic model. Maximization of the growth rate for both cells was used as the objective function. Reactions for adipic acid production and transport reactions for the exchange of fumarate, acetate, and oxygen between the two cells were added to the model. The model was used to simulate growth and adipic acid production under anaerobic mixotrophic conditions. The results show that the growth of *P. taiwanensis* depends on the transport of oxygen, which is produced during oxygenic photosynthesis by *Synechocystis*. *Synechocystis* consumes most of the glucose in the medium, and acetate and fumarate produced by it are transported to *P. taiwanensis*. It is evident that the ratio of *P. taiwanensis* per *Synechocystis* is higher under lower rates of glucose and photons. Adipic acid production is the highest when the photon uptake rate is high and the total glucose uptake rate is low. This two-cell metabolic model can be used for evaluation of the production of different bioproducts under various growth conditions.

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(47) Responsible research and innovation in practice: development of sustainable productive catalytic living materials

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Keywords: Responsible Research and Innovation (RRI), catalytic living materials, societal readiness, sustainable materials, synthetic microbial consortia, ethics

Transitioning to bio-based chemical production demands not only technological innovation but also systemic inclusion of responsibility towards society and the environment. Catalytic living materials, developed in the M-ERA.NET LivMat project, are a promising platform cultivating AI-guided microbial consortia within 3D porous structures for CO_2 sequestration and sustainable synthesis of bio-monomers like ε-caprolactone or adipic acid. While this technology has the power to create notable change, we must consider both positive and negative potential consequences and establish integrative practices to address society's concerns, needs, and long-term sustainability goals within research.

We present an approach to guided implementation of the Responsible Research and Innovation (RRI) principles adapted for a biotechnology research project. Building on the Societal Readiness Thinking Tool [1], the approach combines internal reflection through a sequence of thematic questionnaires with external dialogue facilitated by a dedicated stakeholder RRI board, including industry, consumer, and policy representatives. This dual process is developed to facilitate systematic reflection and proactive action across four key RRI dimensions - ethics, gender, open science, and public engagement.

This approach is expected to provide the project team valuable insights directly influencing their research practices. These insights may include the adoption of new protocols for transparent data management, ethics, and the early recognition of socio-economic impacts of gender-related gaps, environmental significance, and public perception.

By integrating RRI systematically rather than ad hoc, this approach offers a transferable model for responsible innovation in multidisciplinary biotechnology projects. It provides researchers with a practical toolset to anticipate societal impacts, align technology pathways with sustainability goals, and increase public trust in emerging biotechnologies.

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(48) Extending substrate range of the *Magnetospirillum gryphiswaldense* strain MSR-1

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Keywords: magnetotactic bacteria, *Magnetospirillum gryphiswaldense* MSR-1, genetic modification, dihydroxyacetone kinase dhaKLM, dihydroxyacetone, micro cultivation

Magnetotactic bacteria (MTB) are a multitaxonomic group of prokaryotes, which are capable of producing unique organelles called magnetosomes. Each magnetosome contains a single magnetic nanocrystal with defined structure and magnetic properties [1]. Therapeutic molecules can be attached to magnetosomes and guided to tumor sites using magnetic fields. This targeted drug delivery approach minimizes the unwanted side effects of anticancer therapy, such as the cardiotoxicity of anthracyclines [2].

Despite their promising applications in cancer treatment, the slow growth rate, narrow substrate range, and low yield of magnetotactic bacteria hinder their practical implementation [3]. In particular, *Magnetospirillum gryphiswaldense* MSR-1, one of the most studied MTB species, uses a limited number of carbon sources: acetate, pyruvate, succinate, fumarate and lactate [4]. Enabling consumption of alternative C or N sources is an alternative approach to improve the production of biomass and / or to reduce the cost of cultivation.

In this study we aimed to expand the substrate range of MSR-1 strain. We first tested the ability of certain carbon compounds (glucose, fructose, lactose, galactose, and xylose, glycerol, and dihydroxyacetone) to sustain the growth of *M. gryphiswaldense* MSR-1. We found that none of these substances can serve as a carbon source for the bacteria. Accordingly, we next turned to a metabolic engineering approach to expand the MTB substrate range. We successfully expressed dihydroxyacetone kinase from *E. coli dha*KLM gene in MSR-1. The genetically modified strain we obtained is capable of growing with dihydroxyacetone at concentrations of 5–10 mmol/L to OD565 of 0,26.

This versatile engineering toolbox used in the study will facilitate the cultivation and use of MTB bacteria in biotechnological and environmental applications.

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(49) Application of reverse engineering approach in *Clostridium autoethanogenum* to design superior cell factories

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Keywords: *Clostridium autoethanogenum*, reverse genetic engineering, gas fermentation, chemostat, metabolomics, proteomics, biofuels, biochemicals

Clostridium autoethanogenum, a model gas-fermenting acetogen, is emerging as a promising microbial chassis for the industrial bioproduction of fuels and chemicals from gaseous C1 waste feedstocks. However, due to our limited understanding of its complex metabolism and the scarcity of advanced genetic engineering tools, its full potential as a cell factory has yet to be fully explored. We previously utilised adaptive laboratory evolution (ALE) to obtain C. autoethanogenum strains with faster growth and superior characteristics for industrial applications and identified mutations potentially associated with the improved phenotypes [1]. In this study, we leveraged this genetic information for reverse strain engineering by creating similar genetic perturbations in wild-type C. autoethanogenum using CRISPR/Cas9n. We selected target mutations based on the putative roles of the respective genes in metabolic pathways and signalling networks. Strikingly, growth and metabolic profiling of the two reverse-engineered strains in autotrophic batch and continuous cultures revealed superior characteristics similar to their ALE isolate counterparts. Furthermore, proteomic analysis of these reverse-engineered strains provided valuable insights into the interplay of central metabolism and regulatory networks. Our study highlights the benefits of combining experimental evolution with reverse genetic engineering to dissect the complex metabolism of C. autoethanogenum towards its biotechnological application.

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(50) Engineering an orthogonal hypoxia-inducible synthetic promoter in *Saccharomyces cerevisiae*

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Keywords: *Saccharomyces cerevisiae*, synthetic promoter, hypoxia, HIF-1, HRE, orthogonal gene expression

A broad library of genetic parts compatible with a given host organism is essential for the flexible and rational design of synthetic biology circuits. Among these tools, inducible promoters are of particular importance, as they enable precise control over gene expression, improving the productivity and reliability of engineered systems. Equally critical is ensuring orthogonality, so that synthetic circuits do not interfere with the host's native molecular processes. *Saccharomyces cerevisiae* is a widely used chassis for genetic engineering, yet existing inducible expression systems predominantly rely on nutrient-regulated promoters. New systems that respond to cultivation parameters are therefore of great interest. Particularly promising for industrial applications are promoters that react to low oxygen levels, since yeast cultures naturally deplete oxygen during growth.

To address this need, we developed a series of synthetic promoters by combining the minimal CYC1 promoter with varying numbers (3, 6 or 9) of hypoxia response element (HRE) copies in a modified *S. cerevisiae* strain expressing the animal-derived transcription factor hypoxia-inducible factor 1 (HIF-1). In this system, HREs and HIF-1 together form a regulatory module responsive to low oxygen levels. To evaluate their performance, the reporter gene yEGFP was placed downstream of the engineered promoters. The reporter's expression intensity was measured under aerobic and anaerobic conditions using RT-qPCR and fluorescence assays.

Although the constructs were successfully assembled, reporter gene induction was weak and leaky, it did not strongly correlate with oxygen availability or HRE copy number. These findings suggest the need for further optimization of promoter architecture and transcription factor expression to achieve a robust, orthogonal hypoxia-inducible system in yeast.

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(51) Analysis of glycyl radical choline-TMA lyase CutC, B12-independent 1,2-propanediol dehydratase and B12-dependent diol dehydratase with chymotrypsin digestion indicates common substrate-induced structural shifts

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Keywords: radical enzymes, structural shifts, chymotrypsin, bacterial microcompartments

Radical enzymes, including glycyl radical enzymes (GREs) and B12-dependent enzymes, catalyze a wide range of biochemical transformations through radical-based mechanisms. An unusual property—conditional resistance to chymotrypsin digestion—has previously been reported for two GREs. However, whether this feature is broadly conserved among related radical enzymes and what factors trigger it has remained unclear. In this study, we investigated five radical enzymes: four GREs and one B12-dependent diol dehydratase. Proteolytic assays demonstrated that substrate binding significantly enhances resistance to chymotrypsin degradation, suggesting a conserved conformational shift from an open, protease-sensitive state to a closed, protease-resistant form. X-ray crystallographic analysis of a GRE-type 1,2-propanediol dehydratase from *Raoultella planticola* confirmed that active site occupancy correlates with increased protease resistance. Importantly, non-substrate analogs such as 1,3-propanediol and β-methylcholine failed to induce protection, underscoring the specificity of ligand-induced stabilization. These findings reveal a broadly conserved mechanism of substrate-induced conformational stabilization in GREs and B12-dependent radical enzymes and offer a scalable strategy for ligand identification with potential applications in enzyme engineering.

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(52) Investigation of metagenomic GH 151 family fucosidases

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Keywords: α-L-fucosidase, metagenomic, transfucosylation

α-L-Fucosidases are glycoside hydrolases that catalyze the removal of L-fucose from oligosaccharides and glycoconjugates. These enzymes are classified into five families based on sequence. Although little is known about fucosidases belonging to the GH 151 family, it is assumed that they can catalyze transfucosylation reactions, during which an L-fucose group is transferred from one substrate to another. This ability is important for utilizing enzymes in synthesizing human milk oligosaccharides and modifying the carbohydrate chains of glycoconjugates.

This study investigated three metagenomic α-L-fucosidases: FucKUR, FucLINKA, and FucMSL2. Recombinant proteins were successfully synthesized in *E. coli* BL21(DE3) bacteria and purified by affinity chromatography. FucKUR and FucLINKA were found to be homotetramers, while FucMSL2 formed both homotetrameric and homohexameric structures. AlphaFold3 was used to generate protein monomer models that revealed differences in the C-terminal domain loop. The fucosidases were found to be mesophilic, with the highest activity at weakly acidic or neutral pH. An enzyme kinetics study demonstrated that FucKUR has the highest catalytic efficiency. A substrate specificity study revealed that the enzymes cannot hydrolyze human milk oligosaccharides or alkylfucosides. Finally, the α-L-fucosidases studied were found to be capable of catalyzing transfucosylation reactions with 2'-deoxyuridine as the fucose group acceptor but not of fucosylating carbohydrates.

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