CBM

Vilnius, Lithuania 2023

5TH CONGRESS OF BALTIC MICROBIOLOGISTS

Abstract book

Contents

GENETICS, BIOCHEMISTRY, AND PHYSIOLOGY OF MICROORGANISMS AND VIRUSES

Keynote speaker

ENVIRONMENTAL MICROBIOLOGY

Keynote speaker

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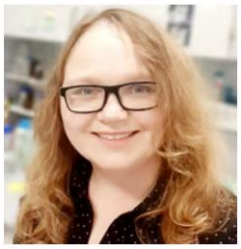

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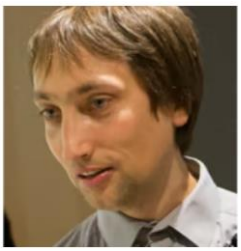

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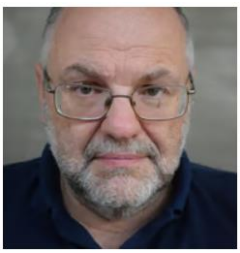

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Conference program

October 11, 2023		
12:00 – 13:00	**Registration**	
13:00 – 13:15	**Opening ceremony**	
13:15 – 14:00	Opening lecture: **Are you stressed? Towards a molecular understanding of the mysterious Di-adenosine tetraphosphate (Ap4A).**	**Prof. Dr. Gert Bange** Philipps-University Marburg, Max-Planck-Insitute for Terrestrial Microbiology, Germany
14:00 – 17:30	**Session: MICROBIOTA AND MEDICAL MICROBIOLOGY** **Moderators: Aurelijus Burokas, Arnas Kunevičius**	
14:00 – 14:30	Keynote speaker: **Gut microbiota, depression, and memory: Don't forget the viruses!**	**Dr. Jordi Mayner-Perxachs** Girona Biomedical Research Institute, Spain
14:30 – 14:50	Diabetes-related gut microbiota alterations associated with Alzheimer disease	**Alessandro Atzeni** Vilnius University, Lithuania
14:50 – 15:10	Vapor-phase of white thyme essential oil effect on *Candida albicans* and *Lactobacillus gasseri* colonization in a reconstituted human vaginal epithelium	**Liliana Fernandes** University of Minho, Portugal
15:10 – 15:30	Potential mechanism of antimicrobial action of silver nanoparticles obtained using *Geobacillus* sp. bacteria	**Kotryna Čekuolytė** Vilnius University, Lithuania
15:30 – 16:10	**Coffee break**	
16:10 – 16:30	Nova Natura (sponsor): Considerations for anaerobic culture in research and clinical labs	**Petra Miikkulainen** International Sales Manager, EMEA Baker
16:30 – 16:50	Insights into the microbial composition of the respiratory tract of bronchiectasis patients	**Julija Armalytė** Vilnius University, Lithuania
16:50 – 17:10	Versatile use of bacteriocin KvarM: intravenous treatment for *K. pneumoniae* sepsis or oral treatment for *K. pneumoniae* gut colonisation	**Aušra Ražanskienė** JSC Nomads, Lithuania
17:10 – 17:30	Synergism between aPDT and pulsed electric fields: an innovative strategy to overcome biofilm infections	**Wanessa Melo** State Scientific Research Institute Center of Physical and Technological Sciences, Lithuania
17:30 – 18:30	**Poster session**	

October 12, 2023		
8:55 – 9:00	**Information**	
9:00 – 12:30	**Session: GENETICS, BIOCHEMISTRY, AND PHYSIOLOGY OF MICROORGANISMS AND VIRUSES** **Moderators: Rimantas Daugelavičius, Jānis Liepiņš**	
9:00 – 9:30	Keynote speaker: **RHS Toxins – signed, sealed, delivered**	**Assoc. Prof. Dr. Dukas Jurėnas** Université Libre de Bruxelles, Belgium
9:30 – 9:50	Understanding alternative roles of respiration in bacteria: the electron transport chain of *Zymomonas mobilis*	**Uldis Kalnenieks,** University of Latvia, Latvia
9:50 – 10:10	An ancestral dual function of OMPM as outer membrane tether and nutrient uptake channel in diderm Firmicutes	**Augustinas Šilalė** Newcastle University, United Kingdom
10:10 – 10:30	Linea Libera (sponsor): Advanced technologies in clinical microbiota research	**Aistė Vitkūnaitė** Field Application Scientist
10:30 – 11:10	**Coffee break**	
11:10 – 11:30	Translational fidelity in the absence of Ψ32 and Ψ38-40 in the anticodon stem-loop of tRNAs	**Karl Jürgenstein** University of Tartu, Estonia
11:30 – 11:50	Stringent response in phage defense of *Pseudomonas putida*	**Hedvig Tamman** University of Tartu, Estonia
11:50 – 12:10	Enzymatic benzene ring reduction at a [4Fe-4S-O] cluster, a key reaction of aromatic degradation in anaerobes	**Jonathan Fuchs** University of Freiburg, Germany
12:10 – 12:30	Phenolic biofilm inhibitors - affecting similar biological processes of *E. coli* despite diversity in structure	**David Buchmann** University of Greifswald, Germany
12:30 – 13:30	**Lunch break**	
13:30 – 17:00	**Session: ENVIRONMENTAL MICROBIOLOGY** **Moderators: Eglė Lastauskienė, Daiva Burokienė**	
13:30 – 14:00	Keynote speaker: **From biodegradation to biocatalytic processes**	**Prof. Dr. Rolandas Meškys** Vilnius university, Lithuania
14:00 – 14:20	Arctic microbiomes – bioprospection and risk-assessment	**Lukasz Dziewit** University of Warsaw, Poland
14:20 – 14:40	Droplet emulsion pipeline reveals how environmental pollutants impact antimicrobial resistance in distinctive ways	**Simona Bartkova** Tallinn University of Technology, Estonia
14:40 – 15:00	Ardeola (sponsor): Precise monitoring of the SARS-CoV-2 in wastewater by ddPCR	**Petar Podlesniy** Field Application Specialist – Genomics, "Bio-Rad Laboratories, Inc."
15:00 – 15:40	**Coffee break**	
15:40 – 16:00	Bioremediation potential of microorganisms isolated from industrial environment	**Daria Kowalczyk-Chrząstowska** Wroclaw University, Poland
16:00 – 16:20	Organic acid production by gammaproteobacterial methanotrophs of lake and pond ecosystems	**Ramita Khanongnuch** Tampere University, Finland
16:20 – 16:40	Eco-friendly remediation of antibiotic resistance in wastewater: a constructed wetland approach	**Mikolaj Wolacewicz** University of Warsaw, Poland
16:40 – 17:00	Impact of microorganisms on the physical and mechanical properties of biocomposite boards impregnated with the linseed or tung tree oils	**Dovilė Vasiliauskienė** Vilnius Gediminas Technical University, Lithuania
17:00 – 18:00	**Poster session**	
20:00 – 23:00	**Gala dinner**	

October 13, 2023		
8:55 – 9:00	**Information**	
9:00 – 14:40	**Session: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY** **Moderators: Patrick Fickers, Triinu Visnapuu, Jaunius Urbonavičius**	
9:00 – 9:30	Keynote speaker: **Microbial bioreporters for environmental sensing**	**Prof. Dr. Angela Ivask** University of Tartu, Estonia
9:30 – 9:50	Molecular toolbox for gene expression from erythritol regulated promoters	**Patrick Fickers** University of Liège, Belgium
9:50 – 10:10	Structure and products of a maltase from yeast *Blastobotrys adeninivorans*	**Triinu Visnapuu** University of Tartu, Estonia
10:10 – 10:40	Labema (sponsor): Petrifilm. The solution built for efficiency. Don't waste time on media preparation	**Matt Bricknell** Professional Service Specialist EMEA, Food Safety, Neogen Corporation
10:40 – 11:20	**Coffee break**	
11:20 – 11:40	Long term viability studies of *Bacillus* species in biological self-healing concrete	**Augusta Ivaškė** Vilnius Gediminas Technical University, Lithuania
11:40 – 12:00	Research of *Geobacillus* lipases and esterases – new insights and possible applications	**Vilius Malūnavičius** Vilnius University, Lithuania
12:00 – 12:20	Revolutionizing the fight against *Pseudomonas aeruginosa*: unleashing the potencial of plant-expressed pyocins	**Šarūnas Paškevičius** JSC Nomads, Lithuania
12:20 – 12:40	Cold-active calcite precipitation: a study on *Sporosarcina* sp. ANT_H38 biotechnological potential	**Karol Ciuchcinski** University of Warsaw, Poland
12:40 – 13:40	**Lunch break**	
13:40 – 14:00	Antimicrobial activity of new synthesized heterocyclic hybrid compounds activated with blue laser light	**Agnieszka Kania** Pedagogical University of Krakow, Poland
14:00 – 14:20	Uncovering novel plasma membrane carboxylate transporters in the yeast *Cyberlindnera jadinii*	**Maria Sousa-Silva** University of Minho, Portugal
14:20 – 14:40	Discovery of novel type II bacterial toxin-antitoxin system from dark proteins	**Minhal Abdullah** University of Tartu, Estonia
14:40 – 15:20	**Coffee break**	
15:20 – 16:50	**Session: FOOD MICROBIOLOGY** **Moderator: Mindaugas Malakauskas**	
15:20 – 15:50	Keynote speaker: **Following the one health approach in food science: microbiome research as a connecting force**	**Assoc. Prof. Dr. Evelyne Selberherr** University of Veterinary Medicine Vienna, Austria
15:50 – 16:10	Lignocellulose to biochemicals, feed and food by oleaginous yeasts	**Volkmar Passoth** Swedish University of Agricultural Sciences, Sweden
16:10 – 16:30	Modification of 16S rRNA amplicon sequencing technology that quantitatively discriminates total and alive bacterial consortia	**Jekaterina Kazantseva** Center of Food and Fermentation Technologies, Estonia
16:30 – 16:50	High temperature lacto-fermentation improves the antidiabetic activities of red beetroot	**Eric Banan-Mwine Daliri** Vilnius University, Lithuania
16:50 – 17:00	**Closing ceremony**	

Oral presentations

ARE YOU STRESSED? TOWARDS A MOLECULAR UNDERSTANDING OF THE MYSTERIOUS DI-ADENOSINE TETRAPHOSPHATE (AP4A)

Prof. Dr. Gert Bange[1,2]

[1]Philipps-University Marburg, Center for Synthetic Microbiology (SYNMIKRO) & Department of Chemistry
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Diadenosine tetraphosphate, also known as Ap4A, is a nucleotide that is made up of two adenosine molecules linked by a phosphate chain. It is a naturally occurring molecule that is found in all living organisms, including all bacterial species. One of the key functions of Ap4A in bacteria is as a signaling molecule that helps to coordinate responses to stress. When bacteria are exposed to a variety of stressors, such as changes in temperature, pH, or nutrient availability, they can increase their levels of Ap4A. This, in turn, can trigger various stress response pathways, leading to changes in gene expression and metabolic activity that help the bacteria to adapt to the stress. Consequently, Ap4A has also been implicated in other bacterial processes, such as regulating virulence and biofilm formation. However, the exact roles and molecular mechanisms of Ap4A are still far from being understood. I will discuss our recent progress in understanding the molecular basis of Ap4A action in the bacteria and beyond.

Further reading:
Giammarinaro, P.I. et al. (2022) Nature Microbiology 7(9):1442-1452.
Leiva L.E. et al. (2023) Microbiol Mol Biol Rev. 87(1):e0004422.

GUT MICROBIOTA, DEPRESSION, AND MEMORY: DON'T FORGET THE VIRUSES!

Dr. Jordi Mayner-Perxachs

Girona Biomedical Research Institute, Spain

Growing evidence implicates the gut microbiome in cognition and depression. However, the field is dominated by animal studies focused on bacterial composition, not addressing microbiome functionality or the compositional nature of metagenomics datasets. To address these issues, we applied a multi-omics approach combining pre-clinical models with three human cohorts including patients with a wide range of depression symptomatology. Microbial functions and metabolites converging onto glutamate/GABA metabolism, particularly proline, were linked to depression. High proline consumption was the dietary factor with the strongest impact on depression. Whole-brain dynamics revealed rich club network disruptions associated with depression and circulating proline. Proline supplementation in mice exacerbated depression along with microbial translocation. Human microbiota transplantation induced an emotionally impaired phenotype in mice and alterations in GABA-, proline-, and extracellular matrix-related prefrontal cortex genes. RNAi-mediated knockdown of proline and GABA transporters in Drosophila and mono-association with L. plantarum, a high GABA producer, conferred protection against depression-like states.

In addition, viruses are a commonly overlooked component of the gut microbiota despite being the most abundant life entities on the planet. We found that subjects with increased Caudovirales and Siphoviridae levels in the gut microbiome had better performance in executive processes and verbal memory in a discovery (n = 114) and a validation cohort (n = 942). Conversely, increased Microviridae levels were linked to a greater impairment in executive abilities. Microbiota transplantation from human donors with increased specific Caudovirales levels led to increased memory in recipient mice in parallel to an up-regulation of memory-promoting immediate early genes in the prefrontal cortex. Supplementation of the Drosophila diet with the 936 group of lactococcal Siphoviridae bacteriophages resulted in increased memory scores and upregulation of memory-related genes in the brain.

DIABETES-RELATED GUT MICROBIOTA ALTERATIONS ASSOCIATED WITH ALZHEIMER DISEASE

Alessandro Atzeni[1], Jonas Mingaila[1], Silvia Arboleya[2], Akshay Kumar Vijaya[1], Rokas Buišas[3], Daiva Baltriukienė[1], Aurelijus Burokas[1]

[1]Department of Biological Models, Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania
[2]Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Spain
[3]Department of Neurobiology and Biophysics, Institute of Bioscience, Life Sciences Center, Vilnius University, Lithuania
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The rapid increase of Alzheimer's disease (AD) prevalence in the elderly population, and its relationship with diabetes has become an important topic that deserves further investigation. It seems that gut microbiota may play an important role in this association [1,2]. Accordingly, the exploration of the gut microbiota can be considered a valid strategy to understand the mechanism of AD progression, and to identify potential biomarkers that could help early diagnosis and treatments.

We hypothesized that alterations in gut microbiota as a consequence of the exposure to diet-induced diabetes [3] can lead to neuro-inflammatory processes that are crucially involved in the development of AD. Accordingly, we aimed to find bacterial strain/s of gut microbiota associated with cognitive tests indicative of AD that could potentially be used as biomarkers for early diagnosis of AD in pre- or diabetic.

Young, 8-week-old C57Bl/6J inbred male and female mice were assigned to each diet (high-fat (HF) or to the control group), with *ad libitum* food access. Additionally, these diet groups were divided according to prebiotic supplementation or non-supplementation. Cecum content was collected at 15 months, DNA extracted, and shotgun sequencing performed. Behavioral studies were conducted at 15 months to evaluate cognitive impairment by measuring novel object recognition (NOR) task, short memory, and social tests. A schematic representation of the study design is reported in Figure 1.

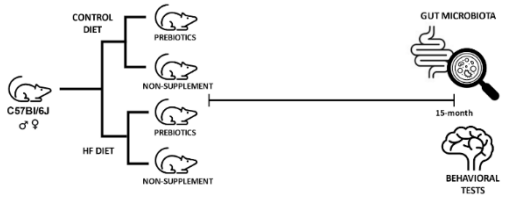

Figure 1. Design of the study conducted.

Statistical analyses based on linear models were conducted to test the association between the different diets and the behavioral tests, and the association between different diets and specific gut microbiota species. Finally, the association between diet-related species and behavioral test was assessed to identify potential gut microbiota features involved in the modulation of AD.

We observed that animals included in the control group non-supplemented shown higher NOR, and short memory compared to animals in the HF group (with or without prebiotics supplementation). Short memory was also higher in the control group + supplementation compared to both HF groups. Animals in the HF group + supplementation shown lower sociability compared to animals in both control groups. In addition, we observed higher abundance of 16 bacterial species in animals included in the control group supplemented compared to animals in HF group non-supplemented. The abundances of 3 bacterial species resulted to be lower in animals in the control group supplemented compared to HF group non-supplemented. Among the bacterial species related to the different diet groups we were able to identify one specific feature that shown higher abundance in HF group non-supplemented compared to the control group supplemented, that was negatively associated with sociability test.

In conclusion, the analyses conducted represent just a preliminary result but are enough indicative of a potential association between gut microbiota and AD. These results are quite promising and encouraging to keep investigating also taking into advantage a comprehensive dataset that could give the possibility of more complex investigations.

[1] Megur A, Baltriukienė D, Bukelskienė V, Burokas A. The Microbiota-Gut-Brain Axis and Alzheimer's Disease: Neuroinflammation Is to Blame? Nutrients. 2020 Dec 24;13(1):37. doi: 10.3390/nu13010037. PMID: 33374235; PMCID: PMC7824474.

[2] Burokas A, Moloney RD, Dinan TG, Cryan JF. Microbiota regulation of the Mammalian gut-brain axis. Adv Appl Microbiol. 2015;91:1-62. doi: 10.1016/bs.aambs.2015.02.001. Epub 2015 Mar 11. PMID: 25911232.

[3] Megur A, Baltriukienė D, Bukelskienė V, Burokas A. The Microbiota-Gut-Brain Axis and Alzheimer's Disease: Neuroinflammation Is to Blame? Nutrients. 2020 Dec 24;13(1):37. doi: 10.3390/nu13010037. PMID: 33374235; PMCID: PMC7824474.

VAPOR-PHASE OF WHITE THYME ESSENTIAL OIL EFFECT ON *CANDIDA ALBICANS* AND *LACTOBACILLUS GASSERI* COLONIZATION IN A RECONSTITUTED HUMAN VAGINAL EPITHELIUM

Liliana Fernandes[1], Raquel Costa[2], Sónia Silva[1], Nuno Pereira Mira[3], Sofia Costa-de-Oliveira[4], Mariana Henriques[1,5], Maria Elisa Rodrigues[1,5]

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[2]Costa Raquel, Aromas Aqua Spa – clínica saúde, Vila Verde, Braga, Portugal
[3]Instituto Superior Técnico, University of Lisbon, Lisboa, Portugal
[4]Faculty of Medicine, University of Porto, Portugal
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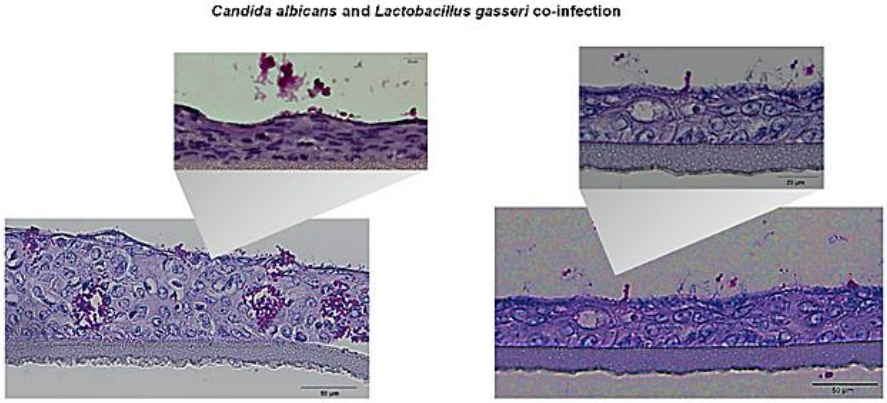
The balance of the vaginal ecosystem is the result of synergistic and complex interactions between microorganisms of the vaginal microbiota[1]. A healthy vaginal microbiota is predominantly constituted of *Lactobacillus* spp., which plays a key protective role in preventing gynecological diseases [1]. Also, *Candida* spp are present in the vaginal microbiota as a commensal microorganism, however, factors such as broad-spectrum antibiotics or immunosuppression can lead to its transition to a pathogenic microorganism, may lead to the development of Vulvovaginal Candidiasis (VVC)[2]. *Candida albicans* is the most frequent species that causes VVC[2]. VVC is often treated with antifungal drugs. However, there has been an increase in resistance to these drugs[3]. The high incidence of VVC combined with the increase in resistance to antifungal agents highlights the need to develop more efficient and safer therapies.

In previous studies, it was observed that the vapor phase of essential oils (VP-EO) can be a promising alternative to prevent biofilm-related VVC caused by antifungal-resistant strains[4],[5]. Nevertheless, it is extremely important that the development of new therapies consider the effect induced in other species that colonize the vaginal mucosa, such as *Lactobacillus* spp. So, as a main objective, a reconstituted human vaginal epithelium (RHVE) was used as an *in vitro* model of VVC to study the vapor phase of white thyme essential oils (VP-WTEO) effect on single and mixed *C. albicans* and *Lactobacillus gasseri* colonization, analyzed by DNA quantification, microscopy and determination of lactate dehydrogenase activity. Effectively, the results confirm the VP-WTEO effectiveness in significantly reducing *C. albicans*. Furthermore, there was no change in RHVE morphology after exposure to VP-WTEO, and more importantly there was no change in growth or morphology of *L. gasseri* (Fig.1). Overall, this study confirms and suggest that the volatile fraction of white thyme oil could be a promising solution to treat and prevent VVC, being a safe alternative for the remaining vaginal microbiota.



[1] B. Larsen and G. R. G. Monif, 'Understanding the bacterial flora of the female genital tract', Clin Infect Dis, vol. 32, no. 4, pp. e69-77, 2001
[2] B. Gonçalves, C. Ferreira, C. T. Alves, M. Henriques, J. Azeredo, and S. Silva, 'Vulvovaginal candidiasis: Epidemiology, microbiology and risk factors', Crit Rev Microbiol, vol. 42, no. 6, pp. 905–927, 2016
[3] C. Parolin et al., 'Isolation of Vaginal Lactobacilli and Characterization of Anti-Candida Activity', PLoS One, vol. 10, no. 6, p. e0131220,2015
[4] L. Fernandes et al., 'Vapor-Phase of Essential Oils as a Promising Solution to Prevent Candida Vaginal Biofilms Caused by Antifungal Resistant Strains', Healthcare 2022, Vol. 10, Page 1649, vol. 10, no. 9, p. 1649, 2022
[5] L. Fernandes, R. Costa, M. Henriques, and M. E. Rodrigues, 'Simulated Vaginal Fluid: Candida resistant strains' biofilm characterization and vapor phase of essential oil effect', Journal of Medical Mycology, p. 101329, 2022

POTENTIAL MECHANISM OF ANTIMICROBIAL ACTION OF SILVER NANOPARTICLES OBTAINED USING *GEOBACILLUS* SP. BACTERIA

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Due to the increasing resistance of yeast to antifungal drugs and the side effects they cause, scientists are trying to find new substances to fight yeast diseases. Since ancient times, silver has been known as an excellent antimicrobial material, and currently, silver nanoparticles (AgNP) are promissing potential antimicrobial alternative. AgNP can be obtained by both chemical and physical methods, but due to the environmental damage and higher cost of these methods, the biological synthesis of nanomaterials using plant extracts or microorganisms is receiving more and more observation.

AgNP have a complex antimicrobial effect. They simultaneously affect different parts of the cell, starting with the cell wall, membranes, and internal structures of the cell (Fig. 1). Also, AgNP can bind to macromolecules such as proteins and nucleic acids and cause oxidative stress, that is promoting the production of reactive oxygen species (ROS).

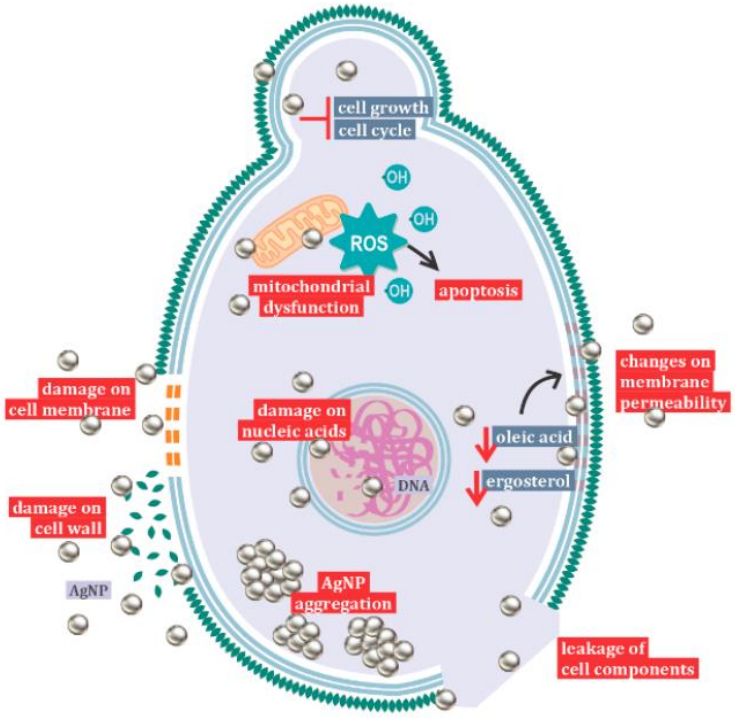

Fig. 1. Mechanism of action of AgNP against *Candida albicans* [1]

In this study, overall ROS formation was determined in *Candida guilliermodii* yeast cells after exposure to AgNP obtained using four different *Geobacillus* spp. bacterial strains (18, 25, 95, and 612), and it was also evaluated whether lipid peroxidation occurs when the yeast cells are exposed to the mentioned AgNP. In addition, the type of AgNP-induced yeast cell death was determined by observing the amount of activated caspases in *C. guilliermondii* and *Saccharomyces cerevisiae*.

[1] Fernandez, C.C.; Sokolonski, A.R.; Fonseca, M.S.; Stanisic, D.; Araújo, D.B.; Azevedo, V.; Portela, R.D.; Tasic, L. Applications of Silver Nanoparticles in Dentistry: Advances and Technological Innovation. *Int. J. Mol. Sci.* **2021**, *22*, 2485. https://doi.org/10.3390/ijms22052485

CONSIDERATIONS FOR ANAEROBIC CULTURE IN RESEARCH AND CLINICAL LABS

Petra Miikkulainen
International Sales Manager, EMEA Baker

Nova Natura

INSIGHTS INTO THE MICROBIAL COMPOSITION OF THE RESPIRATORY TRACT OF BRONCHIECTASIS PATIENTS

Julija Armalytė[1], Aleksandras Konovalovas[1], Laurita Klimkaitė[1], Tomas Liveikis[1], Brigita Jonaitytė[2], Edvardas Danila[2], Ruta Aldonytė[3]

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[2]Centre of Pulmonology and Allergology, Vilnius University Hospital Santaros Klinikos, Lithuania
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The human microbiomes, including the respiratory tract, are described and characterized in an increasing numbers of studies[1,2]. However, the composition and effects of the healthy or disturbed microbiome on pulmonary health and its interaction with the host are only beginning to be elucidated. In the field of chronic airway diseases, bronchiectasis stands out as a progressive condition characterized by microbial colonization and infection; however, the underlying causes of bronchiectasis (infections, inflammation, genetic predisposition) are still under investigation[3,4].

In our study we aimed to investigate the upper respiratory tract as a less invasive alternative for understanding lung health. By comparing the nose, nasopharyngeal and lower respiratory tract microbiota composition in bronchiectasis patients we sought to identify potential biomarkers linked to the disease. To accomplish this, we collected samples from 49 patients suffering from bronchiectasis disease and sequenced employing full-length 16S rRNA gene amplicon sequencing using Oxford Nanopore technologies.

The upper respiratory tract revealed higher microorganism composition complexity than the lower respiratory tract. The samples of each part of the respiratory tract were then grouped into clusters according to the similarity and relative abundance of microorganism composition. A single dominating bacterial species was common for the lower respiratory tract samples, *Pseudomonas aeruginosa* and *Haemophilus influenzae* being the most common colonizers. The patients with dominating *P. aeruginosa* also displayed more severe bronchiectasis according to Bronchiectasis Severity Index. While the composition of the nose or nasopharyngeal microbiome did not directly associate with the composition of lower respiratory tract microbiome, a subtle influence was observed, indicating several species of microorganisms which should be looked into as potential biomarkers of bronchiectasis severity.

[1] Budden, K.F., Shukla, S.D., Rehman, S.F., Bowerman, K.L., Keely, S., Hugenholtz, P., Armstrong-James, D.P.H., Adcock, I.M., Chotirmall, S.H., Chung, K.F., et al. (2019). Functional effects of the microbiota in chronic respiratory disease. Lancet Respir Med *7*, 907–920. 10.1016/S2213-2600(18)30510-1.

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VERSATILE USE OF BACTERIOCIN KVARM: INTRAVENOUS TREATMENT FOR *K. PNEUMONIAE* SEPSIS OR ORAL TREATMENT FOR *K. PNEUMONIAE* GUT COLONIZATION

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Klebsiella pneumoniae, a Gram-negative bacterium, poses a significant threat as a nosocomial pathogen due to its ability to rapidly develop resistance to various antibiotics. Gastrointestinal colonization by antibiotic-resistant strains among hospitalized patients not only increases the risk of nosocomial *Klebsiella* infections but also contributes to the dissemination of this pathogen within healthcare facilities. In this study, we investigate the efficacy of the bacteriocin KvarM in two different animal models to address *K. pneumoniae* sepsis and gut colonization.

In our previous research, we characterized klebicin KvarM, a wide spectrum bacteriocin with activity against *K. pneumoniae*, *K. quasipneumoniae*, *K. variicola*, *K. oxytoca*, and *K. aerogenes*. Notably, KvarM exhibited significant activity against 85% of antibiotic-resistant clinical *K. pneumoniae* isolates.

To assess the potential of KvarM in treating *K. pneumoniae* infections, we conducted experiments using murine models of sepsis and gastrointestinal colonization. In the sepsis model, mice were intraperitoneally infected with *K. pneumoniae* ATCC BAA 1705, and KvarM was administered intravenously either alone or in combination with meropenem. KvarM treatment resulted in a dose-dependent reduction in bacterial burden compared to the control group receiving the vehicle. Combination therapy with KvarM and meropenem exhibited superior efficacy compared to monotherapy with meropenem alone.

For the gastrointestinal colonization model, we orally administered Eudragit®-coated KvarM to C57BL/6J mice after establishing colonization with *K. pneumoniae* strain ATCC 43816. The Eudragit coating protected KvarM from gastroduodenal proteases and enabled controlled release based on pH. Quantification of *Klebsiella* in fecal samples using the *Klebsiella* haemolysin gene (*khe*) as a marker revealed significant reductions in khe levels following treatment with Eudragit-coated KvarM, indicating a substantial decrease in *K. pneumoniae* gut colonization.

In conclusion, our findings highlight the potential of bacteriocin KvarM as an intravenous treatment for *K. pneumoniae* sepsis. Moreover, orally administered KvarM, delivered using an Eudragit coating, shows promise as a preventive measure to target multidrug-resistant *K. pneumoniae* colonization in the gastrointestinal tract, potentially reducing the risk of infection and spread among hospitalized patients.

SYNERGISM BETWEEN aPDT AND PULSED ELECTRIC FIELDS: AN INNOVATIVE STRATEGY TO OVERCOME BIOFILM INFECTIONS

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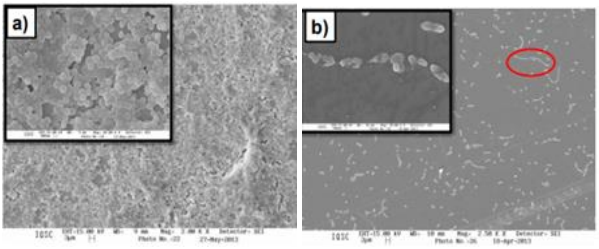
Currently, biofilms have been the cause of a wide variety of infections in the human body, reaching 80% of all microbial infections [1]. The bacteria *Staphylococcus aureus* is a leading cause of hospital-acquired infections. The biofilms present specific properties such as the extracellular polymeric substance (EPS), which increases the resistance to antimicrobial treatments [1]. Thus, the development of new approaches is urgent, and antimicrobial photodynamic therapy (aPDT) has been shown as a promising candidate. aPDT involves the synergistic combination of a photosensitizer (PS), molecular oxygen and visible light of an appropriate wavelength to produce highly reactive oxygen species (ROS), which leads to the oxidation of several cellular components [2]. Even though this therapy showed to be efficient to attack the EPS hampers the PS access to the deeper biofilm cells, promoting the re-grow of the microorganism community [2]. Therefore, to overcome this problem, it is necessary to combine the aPDT with a promising approach, such as electroporation (EP). The EP may enhance the permeability of the EPS-biofilm, allowing the PS to reach the deeper cells and consequently, the aPDT can completely disrupt the biofilm. This works aimed to evaluate the synergism between aPDT and EP against the *S. aureus* biofilm, detecting, mainly, the effect of this on the *S. aureus*-EPS components (proteins and carbohydrates).

The viability of S. aureus after only aPDT treatment or only EP was around 45.4% and 93.1% respectively, while the synergism between them promoted a significant decrease in the SI of the bacteria biofilm (~5.08%) (Table 1). This synergic effect can be visualized in Figure 1, showing S. aureus biofilm before (control) and after the treatment that significantly decreased the number of cells, caused morphologic damage to the bacteria and eliminated the presence of EPS. In addition, aPDT+EP reduced 91.71% and 95.05% of proteins and carbohydrates present in the EPS extracted from *S. aureus* biofilm. The effect of the red light or MB alone did not cause *S. aureus* biofilm reduction, as well as the EP alone.

Table 1: *S. aureus* biofilm survival index (SI). Carbohydrates and proteins content of EPS extracted from *S. aureus* biofilm.

Conditions	***Survival index(%)***	***Proteins* (μg/mL)**	***Carbohy-drates* (μg/mL)**
Control	100±0.50	123.1±2.58	78.9±3.8
Light only (630nm)	95.5±0.25	120.3±1.58	73.6±1.2
MB(1mg mL^{-1})	98.6±1.20	122.1±1.03	72.8±2.3
aPDT	45.4±1.02	30.8±5.03	15.5±4.2
EP	93.1±1.10	118.5±2.05	71.9±2.8
aPDT + EP	5.08±0.85	10.2±2.81	3.9±3.1

Figure 1. *S. aureus biofilm before (a) and after treatment of aPDT + EP (b).*



We may suggest that the EP possibly increased the EPS permeability allowing the PS to reach the biofilm bottom layer and consequently the deeper cells, intensifying aPDT effect.

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GENETICS, BIOCHEMISTRY, AND PHYSIOLOGY OF MICROORGANISMS AND VIRUSES

Keynote speaker

RHS TOXINS – SIGNED, SEALED, DELIVERED

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Bacteria live in crowded ecosystems where intense inter-species competition drives diversification of defense and offense mechanisms. By definition polymorphic toxins are multi-domain proteins with characteristic C-terminal toxic domain. Due to horizontal gene transfer and frequent genetic rearrangements the C-terminal toxic domains are quickly exchanged and provide competitive advantage. We have recently described structures with molecular signatures for novel secretion pathways of polymorphic Rhs (rearrangement hot spot) toxins. Combining protein interaction studies with cryo-electron microscopy we have revealed that the structure of Rhs protein resembles a molecular cocoon that seals the C-terminal toxic domain inside. Conserved internal auto-cleavages prepare the toxic domain for the release from cocoon into the prey [1]. The delivered toxic domains are typically enzymes that quickly damage key cellular processes. My primary interest are toxic domains with ADP-ribosyltransferases (ART) activity. ARTs constitute a large family of enzymes that use highly abundant cellular redox cofactor NAD+ for covalent transfer of ADP-ribose moiety on a target. We have revealed first examples of such Rhs ART toxins that target protein synthesis or cell division machinery. These toxins specifically ADP-ribosylate proteins or RNA target, alone or with help of conserved chaperones in the prey cell [2, 3]. Diverse structures and molecular mechanisms of growth inhibition by ADP-ribosylation suggest that these toxins have remarkably diversified to adopt novel mechanisms to eliminate the competitors.

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UNDERSTANDING ALTERNATIVE ROLES OF RESPIRATION IN BACTERIA: THE ELECTRON TRANSPORT CHAIN OF *ZYMOMONAS MOBILIS*

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The activity of microbial electron transport chains (ETCh) transcends several key cellular processes, including the catabolic ATP yield, intracellular redox balance, respiratory protection in free-living nitrogen-fixing bacteria, production of reactive oxygen species, and protection against oxidative (and indirectly, against other types) of stress. Although producing ATP via oxidative phosphorylation to attain higher biomass yields generally is the prime function of bacterial ETCh, there are telling exceptions where that is not the case, *Zymomonas mobilis* being one of those [1]. Apart from its powerful ethanologenic pathway, this bacterium possesses an active, yet apparently energetically inefficient, ETCh. Electron transport in *Z. mobilis* provokes special interest because of its unusual physiological manifestations and the low aerobic growth yield.

Under oxic conditions the *Z. mobilis* ETCh withdraws reducing equivalents from the alcohol dehydrogenase reaction, leading to accumulation of acetaldehyde, the growth-inhibiting metabolic precursor of ethanol [2]. Accordingly, a pronounced stimulation of aerobic growth is observed in the low-respiring *Z. mobilis* respiratory NADH dehydrogenase-negative mutant relative to the wild type. Although in nature its growth takes place in the presence of oxygen, in the course of evolution this bacterium for some reason has retained a high respiratory NADH dehydrogenase activity, thus not following the strategy of maximizing its aerobic growth rate.

Here we discuss the alternative physiological roles of the *Z. mobilis* ETCh, including the potential adaptive advantage of respiring bacteria under conditions of deep substrate limitation and periodical famine, as well as the pattern of adaptation of its ETCh to various stress conditions.

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AN ANCESTRAL DUAL FUNCTION OF OMPM AS OUTER MEMBRANE TETHER AND NUTRIENT UPTAKE CHANNEL IN DIDERM FIRMICUTES

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Tethering the outer membrane (OM) of diderm, or Gram-negative, bacteria to the peptidoglycan cell wall is important for cell envelope integrity. Well-studied mechanisms for OM attachment in *E. coli* include covalent linkage to peptidoglycan by Braun's lipoprotein, as well as non-covalent interactions between the OM proteins Pal and OmpA with peptidoglycan. However, recent phylogenetic research has shown that many bacterial phyla do not have these OM attachment systems and that a split in OM attachment mechanisms occurred early in the bacterial tree of life [1]. The Terrabacteria group, which includes phyla such as Firmicutes and Cyanobacteria, predominately has a single conserved OM attachment system involving the OM protein OmpM. Deletion of OmpM in the diderm firmicute *Veillonella parvula* has been shown to induce severe OM defects [1], but the molecular details of how OmpM mediates OM attachment remain unknown.

We determined the structure of OmpM from *V. parvula* by single particle cryogenic electron microscopy and solved the crystal structure of its peptidoglycan-binding domain. We further investigated the function of OmpM using binding studies, liposome swelling assays and bilayer electrophysiology recordings, and compared the results to the characterised *E. coli* general porin OmpF.

Our results shed light on a common but understudied mechanism of OM attachment [2]. They also provide insights into how loss of this mechanism could have led to the loss of the OM and emergence of monoderm bacterial lineages from ancestral diderm bacteria harbouring only the OmpM OM attachment system.

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ADVANCED TECHNOLOGIES IN CLINICAL MICROBIOTA RESEARCH

Aistė Vitkūnaitė
Field Application Scientist

Linea Libera

TRANSLATIONAL FIDELITY IN THE ABSENCE OF Ψ32 AND Ψ38-40 IN THE ANTICODON STEM-LOOP OF TRNAS

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More than 150 different modifications have been described in RNA molecules and no class of RNAs contains more – both in number and diversity – chemical alterations than tRNAs. Different functionally important regions in tRNAs are clustered by pseudouridines (Ψ), however, its specific functions remain elusive. Prior research in our research group has shown that two Ψ-synthases, TruA and RluA, affect mutation frequency in exponentially growing cells [1]. TruA and RluA isomerize uridine to pseudouridine in the tRNA anticodon stem-loop (ASL), in positions 38-40 (TruA) and 32 (RluA) [2,3]. Modifications in the ASL have been previously shown to affect the fidelity of translation [4]. Therefore, it is possible that the change in mutation frequency seen in the absence of these Ψ-synthases is a result of decreased accuracy of translation.

In this work we used a dual-luciferase reporter assay cloned into a broad host range plasmid to measure the translational fidelity in different bacterial species, namely *Pseudomonas putida* and *Pseudomonas aeruginosa*, in which the lack of Ψ in the ASL has been previously shown to affect mutation frequency, and *Escherichia coli*. We measured the level of translational frameshifting in different genetic contexts and stop-codon readthrough in wild-type and TruA/RluA deficient strains of the aforementioned bacteria using the same assay system in all species.

Findings reveal great diversity in terms of translational fidelity not only between different species of bacteria but also depending on the genetic context. We also show that the absence of TruA affects translational fidelity in some of the investigated genetic contexts, however the effect varies between different species, while the lack of RluA has no effect. Whether this points towards another mechanism linking Ψ-synthases to mutations or just shows that the link is not due to changes in global translational fidelity, but rather specific and discrete events, needs further research [5].

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STRINGENT RESPONSE IN PHAGE DEFENSE OF *PSEUDOMONAS PUTIDA*

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Stringent response is a universal stress response of bacteria that allows them to survive many different stressful conditions. It is governed by a molecule ppGpp that upon accumulation triggers a switch in bacterial metabolism from growth to stress survival. This pathway is most commonly activated upon nutrient limitation conditions. Surprisingly, some studies have connected stringent response also to the infection efficiency of bacterial viruses (bacteriophages, phages) [1-2].

Pseudomonas putida, an environmental bacterium, encounters many different stress conditions during its life and has been shown to have high stress tolerance. Indeed, if other bacterial species lacking the stringent response require special growth conditions and can easily acquire suppressor mutations [3], the *P. putida* ppGpp^0 strain has been shown to be prototrophic with no strong growth defects [4]. Taking advantage of the stable stringent response deficient strain, we set out to study the stringent response involvement in phage defense of *P. putida*. Until recently, no phages had been isolated that would infect *P. putida* laboratory strain PaW85 (isogenic to most commonly used KT2440). Thus, we are currently creating a collection of environmental phages that infect *P. putida* PaW85. This new library, consisting to date of around 24 species of novel phages from nine genera, was used to screen for stringent response involvement in phage defense.

We created a stringent response deficient *P. putida* derivate, characterized it thoroughly and screened our phage library for ppGpp-dependent effects on infection efficiency. Surprisingly, it appeared that while the stringent response generally provides bacteria with a basic low level of protection, it has a very strong protective effect against the infection of certain phages. Moreover, the lack of ppGpp metabolism enzymes decreased the infection efficiency of some phages, suggesting that the intact stringent response machinery might be important for the complete infection efficiency of certain phages in the collection.

Taken together, our results indicate that stringent response may affect phage defense through a variety of different ways and our further studies will be directed towards identifying these mechanisms that will lead to the understanding of the interplay between this bacterial stress response and phage defense of *P. putida*.

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ENZYMATIC BENZENE RING REDUCTION AT A [4FE-4S-O] CLUSTER, A KEY REACTION OF AROMATIC DEGRADATION IN ANAEROBES

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Anaerobic bacterial degradation of monoaromatic compounds proceeds via the central intermediate benzoyl-CoA (BCoA), catabolized by dearomatising benzoyl-CoA reductases (BCRs). Class I BCRs couple the reduction of the substrate to cyclohexa-1,5-diene-1-carbonyl-CoA to a stoichiometric ATP hydrolysis to ADP and P_i [1]. A “Birch-like” reaction mechanism via radical intermediates was proposed to achieve substrate reduction at $E^{0'}$= −622 mV, one of the most negative redox potentials of a redox couple in biology [2].

The active site-containing subunits of the ATP-dependent benzoyl-CoA reductase from the beta-proteobacterium *Azoarcus* sp. CIB [3] were heterologously produced in *Escherichia coli*. The crystal structure with the aromatic substrate bound was solved at 1.7 Å and revealed an active site [4Fe-4S] cluster coordinated by three cysteine residues and one hydroxy/water ligand. The structure suggests a hydrogen atom transfer-based mechanism via a neutral radical. Electron transfer to the active site is mediated by a double-cubane [8Fe-7S] cluster. The results reveal that nature uses metal-water complexes to achieve enzymatic Birch-like benzene ring reduction at the negative limit of the biological redox window.

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PHENOLIC BIOFILM INHIBITORS - AFFECTING SIMILAR BIOLOGICAL PROCESSES OF *E. COLI* DESPITE DIVERSITY IN STRUCTURE

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A few years ago, WHO declared the spread of multidrug-resistant (MDR), especially MDR-gram negative bacteria, as a major threat to human health [1]. Therefore, there is an urgent need to find new therapeutic options for the treatment of bacterial diseases. Medicinal plants have been used therapeutically for centuries, among others against infectious diseases. Since a quarter of all drugs approved by the FDA or EMA are of natural origin, natural compounds are a valuable source of new therapeutics. In addition, new treatment strategies are needed. For example targeting virulence properties such as biofilm formation, which accounts for about 65% of bacterial infections worldwide, can be attributed [2].

In our recent study we developed a machine learning-based prediction model to identify nature-like phenolic biofilm inhibitors of MDR *E. coli.* This concept reduced the time and cost of laboratory screening. More importantly, sevenfold more inhibitors could be identified compared to the conventional screening - the hit outcome was significantly increased and even unknown inhibitors were found [3].

Three of these compounds (octyl gallate, scutellarein, wedelolactone) and the widely described epigallocatechin gallate were subsequently investigated using a holistic RNA sequencing approach and additional RT-qPCR analyses to gain insight into the influenced biological processes. Several pathways related to biofilm formation have already been discussed in the literature [4]. In our study, structurally diverse compounds were shown to influence similar bacterial processes, mainly motility, transport systems, citrate cycle and arginine biosynthesis.

Thus, the data provide initial hints between phenotypic biofilm expression and biological pathways under the influence of these compounds. The extent to which the observed metabolic changes in particular are the cause of biofilm inhibition or a consequence to motile lifestyle needs to be further investigated.

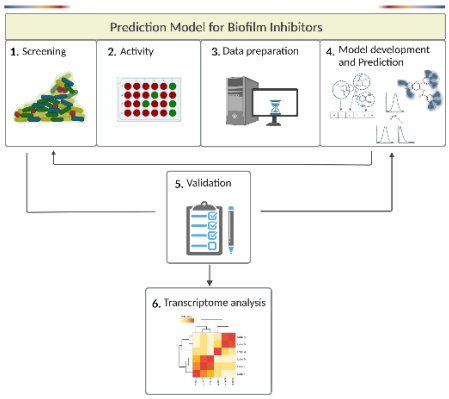

Figure 1. General workflow for the present study.

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FROM BIODEGRADATION TO BIOCATALYTIC PROCESSES

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Modern biotechnology is based on the application of enzymes derived predominantly from microorganisms. Both genetic and biochemical microbial diversity is an immense source of different proteins and biocatalysts. The analysis and exploration of said diversity especially an elucidation of the catabolic pathways is one of the main aims of our group. The studies are concentrated on several fields. The first one is related to the isolation of N-heterocyclic compound-utilizing microorganisms and the investigation of the catabolic pathways of these compounds in individual bacteria. Unique oxygenases active towards indole, pyridine and 4-hydroxypyridine and other pyridine compounds as a primary substrate have been characterized, genetically modified and applied for development of biocatalytic processes [1–4]. Screening for novel enzymes is also carried out by applying metagenomic techniques – effective selection systems combined with tailored substrates [5].

Aromatic N-oxides, organic sulfoxides and epoxides are desirable compounds with a potential for application in pharmacy and agriculture industries. As biocatalysis is making a great impact in on organic synthesis, there is still a lack of efficient and convenient enzyme-based techniques for the production of aforementioned compounds. A recombinant soluble di-iron monooxygenase PmlABCDEF overexpressed in *Escherichia coli* or *Pseudomonas putida* [6] was shown to be applicable for development of various biocatalytic processes without any side oxidation products. Being entirely biocatalytic, our approach provides an environmentally friendly and mild method for the production of S- and N-oxides as well as epoxides avoiding the use of strong oxidants, organometallic catalysts, undesirable solvents, or other environment unfriendly reagents.

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ARCTIC MICROBIOMES – BIOPROSPECTION AND RISK-ASSESSMENT

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The investigation of microbiomes from Arctic regions provides valuable insights into ecological interconnections, metabolic capacities and biotechnological opportunities present, as well as the mechanisms underpinning bacterial adaptation. It is also an excellent starting point for eco-epidemiological assessments (including exploration of bacterial resistomes) and bioprospecting of this pristine environment (including identification of bacterial strains suitable for bioremediation).

We performed a combined metagenomic and functional analyses and revealed that: (i) antibiotic (and metal) resistance genes are common not only in anthropogenically-shaped environments but also in pristine ones like Arctic; (ii) antibiotic resistance genes from Arctic bacteria are active in various bacterial hosts and (iii) their activity can be validated using *Escherichia coli*-hosted fosmid libraries.

The impact of our work extends to methodological advancements, since several novel tools for environmental microbiology have been developed through combining complex bioinformatic analyses and laboratory practice, including: (i) two databases of PCR primers for the detection of antibiotic and metal resistance genes (LCPDb-ARG and LCPDb-MET; http://lcpdb.ddlemb.com/) in various environmental samples [1, 2]; (ii) novel pipeline for genome-based bioprospection [3], which increases the chances of identification of bacterial strains with desired phenotypes, and (iii) a novel set of PCR primers for the detection of genes conferring resistances to antibiotics of last resort [4].

Acknowledgments

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DROPLET EMULSION PIPELINE REVEALS HOW ENVIRONMENTAL POLLUTANTS IMPACT ANTIMICROBIAL RESISTANCE IN DISTINCTIVE WAYS

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Environmental pollution is ubiquitous and a global health problem [1]. One key concern is that pollutants such as antibiotics, metals and microplastic can provide unique micro-ecosystems for enhancing antimicrobial resistance (AMR) of microbes [2][3]. Yet, the complicated interplay between pollutants and the severity of their AMR effect still requires a deeper understanding [1]. Here, we show how our user-friendly droplet emulsion pipeline can be used to investigate the impact of environmental pollution on AMR.

We use polydisperse water-in-oil droplets in our pipeline. They are like small miniature test tubes, which enable us to investigate (i) effect of metal on AMR by single cell-based (droplet) minimal inhibitory concentration (MIC) assays with GFP tagged *E. coli* pre-incubated in metal solutions (Fig.1a), and (ii) effect of plastic on AMR via comparison of single cell-based (droplet) MIC assays with and without adding 10 µm carboxylated polystyrene microspheres (PS) (Fig.1b). We then analyze all droplets via fluorescence imaging, combined with freely available software Ilastik [4], CellProfiler™ [5], and EasyFlow [6].

Results show that (i) effect on AMR depends on what metal is used for pre-incubation, and (ii) addition of PS seems to increase AMR. Our droplet emulsion pipeline enables simultaneous study of thousands of replicates with high resolution, thus highlighting its potential as a suitable tool for the intricate research of pollution and AMR.

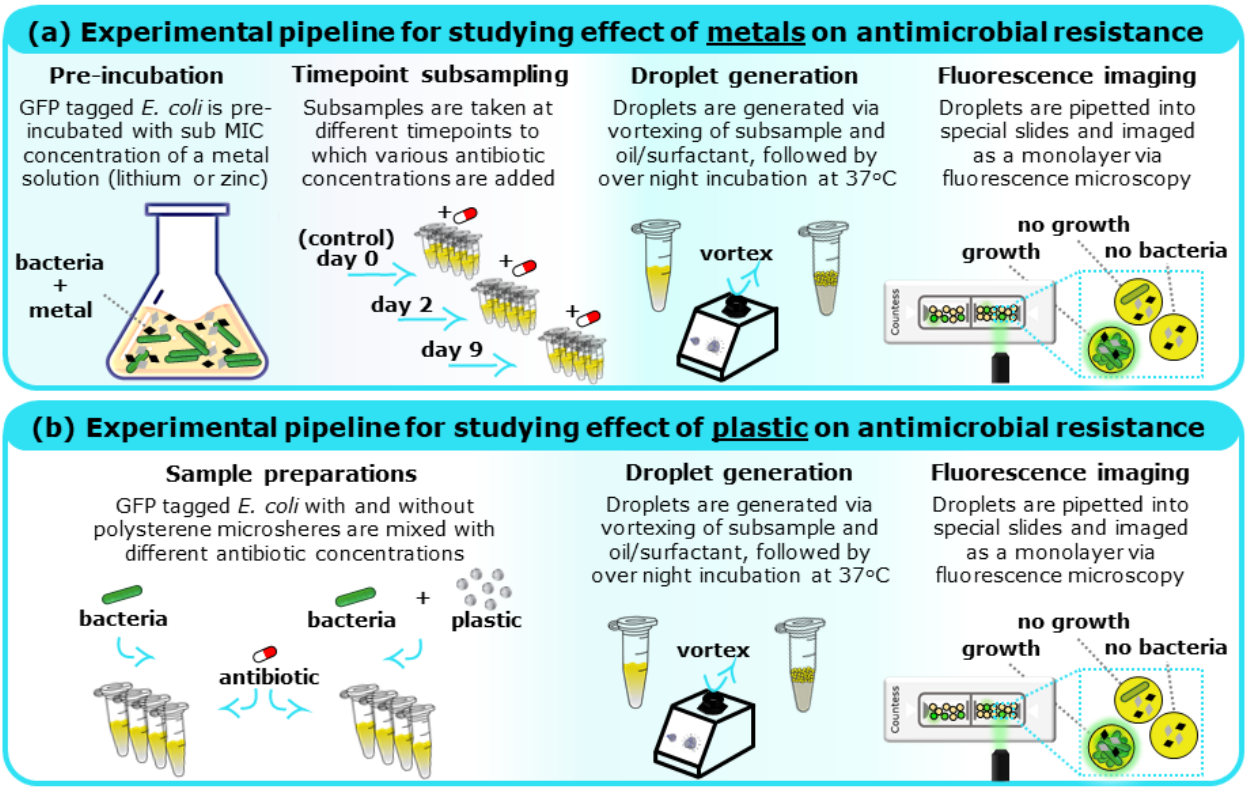

Figure 1. Depiction of experimental pipelines for investigating effects of pollutants on antimicrobial resistance.

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PRECISE MONITORING OF THE SARS-CoV-2 IN WASTEWATER BY ddPCR

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Ardeola

BIOREMEDIATION POTENTIAL OF MICROORGANISMS ISOLATED FROM INDUSTRIAL ENVIRONMENT

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The main purpose of industrial wastewater treatment is transformation of harmful substances into safe final products that do not have a negative impact on the environmental effect [1]. Despite the existence of many methods of industrial wastewater treatment, it is now realized that microbial metabolism provides a safer, more efficient, and less expensive alternative to physio-chemical methods for pollution abatement [2].

According to the general knowledge, microorganisms are adapting to the environments in which they are living and they are developing mechanisms that allow them to survive in difficult conditions. The goal of experiment was finding microorganisms inhabiting industrial environment as potentially effective in bioremediation processes of surfactants [3]. In the proposed work, microorganisms infecting surfactants were isolated. Using the Maldi-TOF mass spectroscopy, the following genus were identified: *Stenotrophomonas spp., Serratia spp., Achromobacter spp.* The isolated microorganisms were added to a flask A containing 600 ml of sterilized (by ozonation) industrial sewage and 8 g of a commercial bioremediation mixture (containing the genus of *Bacillus spp.)*. The wastewater was irradiated with UV light to get rid of residual ozone. The control flask B, contained 600 ml of sterile raw sewage and 8 g of above mentioned commercial mixture was prepared. The mixtures were incubated for 4 weeks in a laboratory bioreactor (Fig. 1) with oxygen supply and constant stirring. As an indicator of ongoing bioremediation, the reduction of the COD parameter (Chemical Oxygen Demand) was assumed. As a result of the research, the COD parameter decreased for flask A up to 77%, while for flask B it was 58%. The pH ranged from 6.85 to 7.84 for flask A and from 7.45 to 7.04 for flask B. The experiment was repeated three times.

The obtained data allow to conclude that the isolated microorganisms have a bioremediation potential for chemicals included in industrial wastewater.

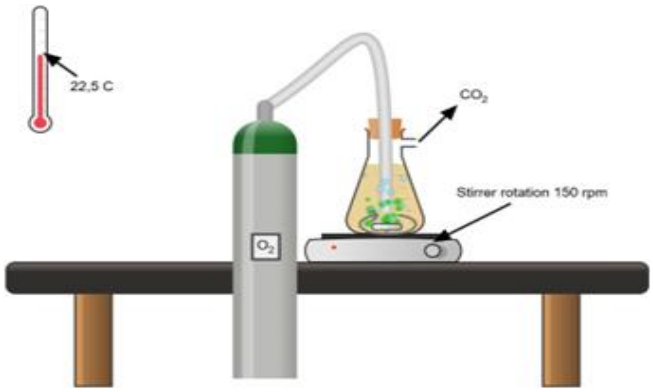

Fig.1 Scheme of laboratory bioremediation of waste water

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ORGANIC ACID PRODUCTION BY GAMMAPROTEOBACTERIAL METHANOTROPHS OF LAKE AND POND ECOSYSTEMS

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Aerobic gammaproteobacterial methanotrophs (gMOB) play a crucial role in regulating methane emission at the oxic-anoxic interfaces of freshwater ecosystems, including lakes, ponds, and wetlands. Under O_2-limiting conditions, gMOB might transition from their aerobic metabolism to fermentation, resulting in extracellular organic acids secretion. We recently studied a gMOB strain, *Methylobacter* sp. S3L5, isolated from boreal lake water columns. Our findings revealed its capability to convert methane into organic acids such as acetate, formate, malate, and propionate in O_2 limited environments [1]. Moreover, genomic analyses of the strain S3L5C and environmental metagenome-assembled genomes (MAGs) suggest that methane conversion to organic acids is prevalent among *Methylobacter* spp. in methane-rich freshwater ecosystems [1]. This raises a question: Is this trait unique to *Methylobacter* spp., or is it widespread among other gMOB species in freshwater ecosystems?

To fill the knowledge gap, we isolated representatives of two other gMOB genera, *Methylomonas paludis* S2AM and *Methylovulum psychrotolerans* S1L, from the boreal lake water columns. Both isolates could convert methane into various organic acids, including acetate, formate, succinate, and malate. In addition to the mentioned organic acids, lactate was detected from the strain S2AM cultivations. Genes linked to organic acid production were identified in these strains as well as in MAGs representing *Methylomonas* spp. and *Methylovulum* spp. from lake and pond ecosystems.

In conclusion, our findings demonstrate that the conversion of methane to organic acids is a common feature among gMOB in lakes and ponds, emphasizing their significance in channeling methane carbon into the microbial food chains of these freshwater ecosystems.

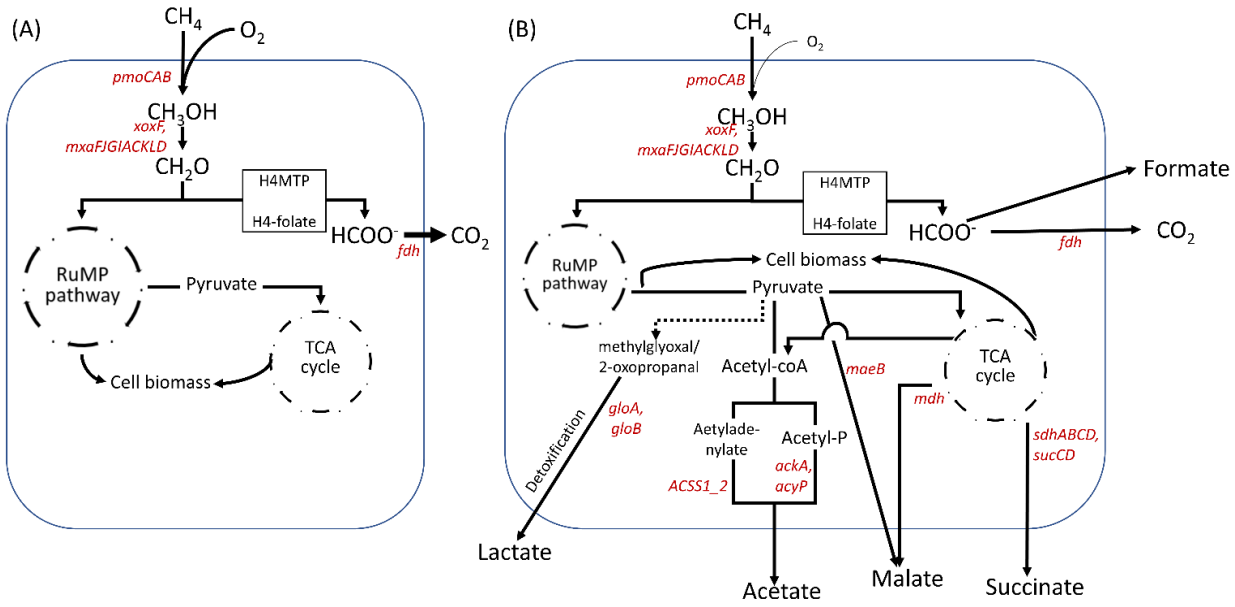

Figure 1. The proposed metabolic pathway for methane oxidation in *Methylomonas paludis* S2AM and *Methylovulum psychrotolerans* S1L (A) under O_2 saturation and (B) under O_2-limiting conditions, resulting in the generation of organic acids. The black line represents the genes found in S2AM and S1L genomes, while the dotted line represents the possible pathway (Modified from Kalyuzhnaya et al. [2])

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ECO-FRIENDLY REMEDIATION OF ANTIBIOTIC RESISTANCE IN WASTEWATER: A CONSTRUCTED WETLAND APPROACH

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Antibiotics in the environment pose a significant yet often overlooked threat. They select for the growth of antibiotic-resistant bacteria (ARB) and promote the spread of antibiotic resistance (AR) among microorganisms [1,2]. This environmental AR is a growing and global concern that aligns with the One Health concept.

Human activity is the primary source of antibiotic residues, ARB, and antibiotic resistance genes (ARGs). These compounds are released through human waste and transported through sewers to wastewater treatment plants (WWTPs). Despite WWTPs are usually efficiently in reducing the bacterial load, they struggle to remove pharmaceutical compounds, ARB, and ARGs. Moreover, their operation is expensive and unaffordable in many low- and middle-income countries. In contrast, constructed wetlands (CW) provide a cost-effective and eco-friendly approach to address this issue [3,4].

In this study, water and sediment samples were collected from a CW located in Empuriabrava (Girona, Spain). Samples were analyzed for the presence of antibiotic residues by LC-MS/MS [5] and subjected to DNA extraction and metagenomic sequencing to resolve their resistome.

A total of 37 antibiotics underwent screening as part of this study. Among these, 19 antibiotics from 9 antibiotic groups were detected. Significant reductions (from 88 to 99%) in the concentration of antibiotics were observed, particularly for macrolides and quinolones. Notably, azithromycin, ciprofloxacin and ofloxacin were completely removed.

Analysis of the sample metagenomes revealed about an 80% drop in waterborne ARGs during treatment, while sediment samples showed an increase in ARGs compared to the water samples.

Acknowledgements
This study was supported by the National Science Centre, grant nº 2021/03/Y/NZ9/00141 and the Joint Programme Initiative on Antimicrobial Resistance (JPIAMR) through the project PhageLand (Project ID: 956623).

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IMPACT OF MICROORGANISMS ON THE PHYSICAL AND MECHANICAL PROPERTIES OF BIOCOMPOSITE BOARDS IMPREGNATED WITH THE LINSEED OR TUNG TREE OILS

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Biocomposite boards made of hemp shives and corn starch are known as cellulose-based composites and are used as thermal insulating and structural building materials. We investigated the impact of microorganisms on the physical and mechanical properties of such boards with flame retardants. The boards were impregnated with drying oils such as linseed or tung tree as protective coatings. Properties of such biocomposites were evaluated before and after the action of either the bacterium *Pseudomonas putida* and the fungus *Rhizopus oryzae* or the mixture of them. The physical-mechanical properties such as compressive strength, thermal conductivity, water absorption, and swelling in thickness were measured. Also, the microstructure of the boards was investigated using electron microscopy. It was determined that the type of drying oil used for impregnation significantly affects the properties of biocomposite boards after 6 months of incubation with the *P. putida*, *Rhoryzae* or the mixture of them. Boards impregnated with linseed oil and after incubation with a mixture of microorganisms had a 10% decrease in compressive strength, 50% higher short-term water absorption, unchanged swelling in thickness, and slightly decreased thermal conductivity compared to the control without oil coatings. Cellulase activity of 25 U/mL, endo β-1-4-glucanase activity of 26 U/mL, and lipase activity of 101 U/mL was observed. Boards impregnated with the tung tree oil had a much more pronounced deterioration of the properties: 2x decreased compressive strength, 2x higher short-term water absorption, and 2.5x greater swelling in thickness. Also, cellulase activity of 28 U/mL, endo β-1-4-glucanase activity of 37 U/mL, and lipase activity of 91 U/mL was determined. We conclude that linseed oil provides better protection of the biocomposite boards from the impact of microorganisms compared to tung tree oil.

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MICROBIAL BIOREPORTERS FOR ENVIRONMENTAL SENSING

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Microbial bioreporters are usually bacterial or yeast cells that produce a detectable signal, which changes in response to certain environmental stimulus. Most often such cells are genetically modified to express a fluorescent or a chromoprotein, or a protein catalyzing bioluminescence reaction. In general, bioluminescence has been claimed advantageous over other reporter systems due to its high sensitivity and fast response time [1]. However, its high energetic burden to cells may undesirably modulate the sensitivity of bioreporters to environmental stress.

Based on working principle, microbial bioreporters can be divided into non-specific and specific whereas in the former the reporter system is expressed constitutively and in the latter, a specific genetic element is set to control the expression of the reporter. Non-specific bioreporters have been used to measure general changes in cell physiology, e.g., due to toxicity and most well-known of such kind is either recombinant or naturally bioluminescent bacteria introduced in rapid aquatic toxicity screening tests, such as Microtox® [2]. Specific bioreporters on the other hand enable a more specific detection of environmental stimuli and are usually constructed by fusing compound-responding genetic elements, usually a promoter and a regulatory gene, with bioreporter producing genes. Such bioreporters have been constructed to identify metals [3], organic solvents [4], antibiotics [5], or even explosives [6] and hormone-like compounds [7]. Also, a series of bioreporters specific to certain types of cellular stress pathways [8] have been constructed. Probably the broadest collection of bioreporters consists of 2000 *E. coli* promoter-GFP fusions [8].

In this presentation, advantages and limitations of microbial bioreporters in analysis of water and soil pollutants, in mode of action studies of chemicals and nanomaterials as well as for finding cellular stress pathways after antimicrobial treatments will be discussed.

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MOLECULAR TOOLBOX FOR GENE EXPRESSION FROM ERYTHRITOL REGULATED PROMOTERS

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Yarrowia lipolytica is an emerging cell factory to produce recombinant proteins (rProt) or specific bioproducts. Recently, we developed a set of regulated promoters for gene expression and synthetic biology that derives from the *EYK1* gene encoding erythrulose kinase. Promoter induction is repressed by glucose and glycerol and strongly induced by erythritol, a low cost 4-carbone sugar alcohol. Identification and characterization of the *EYK1* upstream activating sequences allowed the construction of tunable and hybrid promoters that significantly outperform the benchmark pTEF promoter in terms of gene expression level. The developed promoters can be also made bidirectional to allow gene coexpression. Several efficient recipient strains suitable for industrial applications have been developed together with easy to clone vectors based on chromogenic proteins. Depending on the genetic background of the recipient strain considered, the *EYK1*-derived promoters can be either constitutive, phase-dependent or regulated. For fast and efficient use, all those promoters have been made compatible with our existing *Y. lipolytica* Golden Gate assembly system. These molecular biobricks will be described in detail, and their utilization exemplified for the lipase CalB from *Candida antartica*. The developed promoters yield higher rProt productivity in *Y. lipolytica* than those obtained in the relevant industrial cell factory *P. pastoris.*

STRUCTURE AND PRODUCTS OF A MALTASE FROM YEAST *BLASTOBOTRYS ADENINIVORANS*

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An early-diverged yeast, *Blastobotrys* (*Arxula*) adeninivorans (*Ba*), has a biotechnological potential due to nutritional versatility, temperature tolerance and production of technologically applicable enzymes. The genome of the yeast harbors 88 glycoside hydrolases (GHs) including two GH13-family α-glucosidases (AGs). *Ba*AG2 has a high activity towards maltose, sucrose and *p*-nitrophenyl-α-D-glucopyranoside. The enzyme has a biotechnological potential due to its transglycosylating activity on maltose [1].

The aims of the study were to biochemically and structurally characterize *Ba*AG2. The diffraction data of the *Ba*AG2 crystals were collected at MaxIV (Sweden). Datasets with the resolution of 2.12 Å and 2.13Å were refined using molecular replacement (PDB: 7P01, 7P07). The transglycosylation products were separated/analyzed by chromatography methods. The chemical entities of oligosaccharides were determined by nuclear magnetic resonance.

The *Ba*AG2 effectively hydrolyses α-1,4 linkages in many maltose-like sugars (maltotriose and malto-oligosaccharides, maltulose, erlose, glycogen, amylopectin) and α-1,3 linkages in turanose and melezitose, but not α-1,6-linkages [1]. Interestingly, an α-1,1-linked sucrose analogue trehalulose was hydrolyzed while trehalose was not [2]. Several potentially prebiotic oligosaccharides with α-1,1, α-1,3, α-1,4, and α-1,6 linkages were disclosed among the products (Fig. 1) [2]. Trisaccharides isomelezitose, erlose and theanderose, and disaccharides maltulose and trehalulose were dominant transglycosylation products. *Ba*AG2 exhibited a catalytic domain with a $(\beta/\alpha)_8$-barrel fold and Asp216, Glu274, and Asp348 as the catalytic triad. Next to the substrate-binding pocket an enlarged space for potential binding of transglycosylation acceptors was identified.

In conclusion, we determined the first crystal structure of a yeast maltase and confirmed that *Ba*AG2 is able to efficiently synthesize several functional oligosaccharides rarely found in nature.

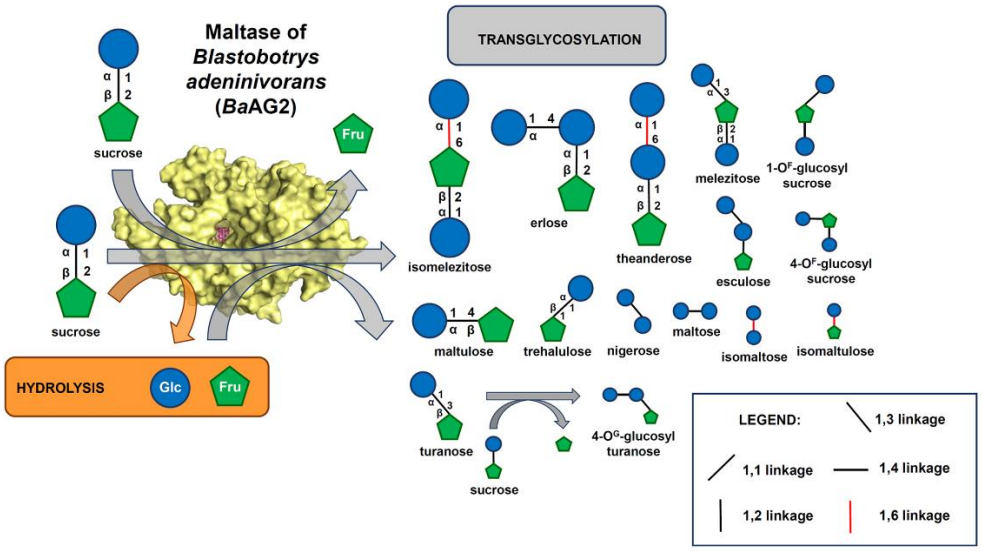

Figure 1. A schematic summary of *Ba*AG2 structure, catalytic activities on sucrose and product range [2].

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PETRIFILM. THE SOLUTION BUILT FOR EFFICIENCY. DON'T WASTE TIME ON MEDIA PREPARATION

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Labema

LONG-TERM VIABILITY STUDIES OF *BACILLUS* SPECIES IN BIOLOGICAL SELF-HEALING CONCRETE

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Biological self-healing concrete contains bacterial spores and mineral precusor that are encapsulated in a carrier. When a crack opens, the bacteria carry out a mineralisation process and precipitate $CaCO_3$, which fills the crack. However, the biggest challenge is to keep the bacteria viable in the concrete. High pH in concrete matrix and mechanical stress reduce the viability of the bacteria and the efficiency of self-healing. In this study, the viability of bacteria in a concrete matrix using different cement types, hydration temperatures and carrier coatings were investigated.

Expanded clay particles encapsulated by *Bacillus* spores, calcium lactate and yeast extract was used as carrier. This study reveals that bacterial spores viability is strongly affected by cement composition and concrete curing temperature. Out of the five cement species tested, the best viability of *Bacillus pseudofirmus* spores was obtained in a concrete mix using white CEM-I type cement which did not contain ZnO ions [1]. Therefore, this cement type was chosen to investigate the use of expanded clay coatings that could increase the long-term survival of bacteria in the concrete matrix. Out of the eight materials tested, styrene-acrylate and MgO-based coatings provided the best protection for *B. pseudofirmus* spores. They increased bacterial viability in the concrete matrix about 10 times [2]. Another factor affected the viability of bacterial spores is curing temperature of concrete. It was found that then concrete curing temperature reaches 80 °C bacterial viability drastically decreased [1]. In conclusion, the more viable bacteria present in the self-healing agent, the higher self-healing efficiency is. The chemical composition of the cement used and the inhibitory concentrations of the components should be analysed to achieve the highest self-healing efficiency. Actions should also be taken to prevent temperature increases in bio-concrete structures and keep bacteria viable.

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RESEARCH OF *GEOBACILLUS* LIPASES AND ESTERASES – NEW INSIGHTS AND POSSIBLE APPLICATIONS

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Recent global trends have highlighted the need for novel, safe and ecological innovations in environmental, medical, and industrial fields. Enzymes are one possible solution. These functional molecules can perform various reactions that can be utilized for more efficient and, more importantly, greener production or degradation of different compounds.

Lipolytic enzymes (lipases and esterases) are industrially important enzymes that can hydrolyze (or synthesize) ester bonds. *Geobacillus* sp. bacteria produce thermophilic lipases and carboxylesterases, that have many industrial attractive properties: they are active and stable at high temperatures, broad pH range, they function in organic solvents, detergents, etc. [1]. Furthering our understanding of these enzymes increases our knowledge of lipolytic enzyme structure-function relationship and provides new tools for industrial applications.

This work presents our newest studies related to lipolytic enzymes produced by several *Geobacillus* spp. bacterium. Protein engineering and immobilization experiments resulted in additional fundamental information about these enzymes as well as highlighted their application perspectives. In our studies we used directed-mutagenesis, random mutagenesis, and protein fusion methods to improve our enzymes and explore the fitness landscape of *Geobacillus* spp. lipolytic enzymes. Random mutagenesis yielded a more active lipase variant (GD95-RM) [2] and several improved esterases (GDEst-RM1, GDEst-RM2, GDEst-RM3). We have also created several fusion enzymes: LipGD95-GD66 (by fusing lipases GD-95 and GD-66) [3] and GDEst-Lip (by fusing esterase GDEst-95 with GD-95 lipase) [4]. These fused enzymes possess intermediate (or in the case of GDEst-Lip – improved) physicochemical and kinetic properties compared to the parental enzymes.

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REVOLUTIONIZING THE FIGHT AGAINST *PSEUDOMONAS AERUGINOSA*: UNLEASHING THE POTENTIAL OF PLANT-EXPRESSED PYOCINS

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Bacteria, like all living organisms, possess a fundamental drive to ensure their survival, regardless of whether they have beneficial or harmful effects. The rise of antibiotic resistance, a natural phenomenon that predates the widespread use of antibiotics, presents a significant global health risk. The World Health Organization (WHO) identifies carbapenem-resistant *Pseudomonas aeruginosa* as one of a top-priority pathogen, necessitating the development of new antimicrobial treatments.

Bacteriocins, which are proteins possessing distinctive mechanisms of action, are currently under investigation as potential antimicrobial compounds. *P. aeruginosa* produces a range of bacteriocins, such as nucleases, porins, inhibitors of peptidoglycan synthesis, lectin-like proteins, and bacteriophage tail-like complexes. Figure 1 presents the schematic representation of the experimental design employed in our investigation of bacteriocins. Notably, the pore-forming pyocin S5 and the peptidoglycan synthesis-inhibiting pyocin M4 exhibit potent antibacterial properties against both free-floating *P. aeruginosa* bacteria and those forming biofilms. These pyocins have also demonstrated their antimicrobial potential *in vivo* by effectively treating infections in *Galleria mellonella* larvae [1]. Additionally, we have enhanced the activity spectrum of PyoS5 by developing chimeric fusions that combine specific domains from related bacteriocins found in other *Pseudomonas* species [2].

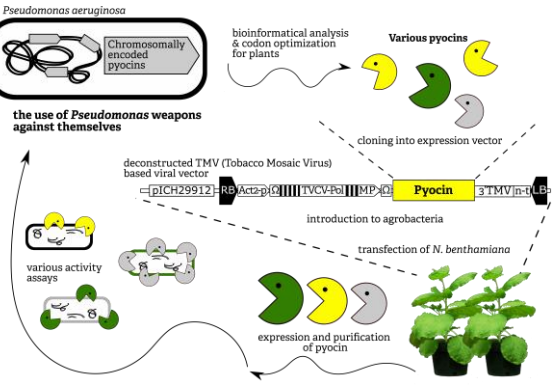

Figure 1. Experimental design scheme

Furthermore, both PaeM4 and the chimeric pyocin S5-PmnH show remarkable efficacy in treating *P. aeruginosa*-induced diseases in two distinct mouse models: keratitis, an eye infection, and lung infection. In conclusion, pyocins can be efficiently produced in large quantities using a transient expression system in plants. They retain their complete functionality and should be considered as a viable alternative to antibiotics for controlling pathogenic *Pseudomonas* infections.

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COLD-ACTIVE CALCITE PRECIPITATION: A STUDY ON *SPOROSARCINA* SP. ANT_H38 BIOTECHNOLOGICAL POTENTIAL

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Extreme Arctic and Antarctic conditions have prompted microorganisms to evolve unique enzymatic machinery, adapted to withstand the cold. This aligns with current biotechnological interests in enzymes with novel or efficient operation at low temperatures, offering cost-effective, enhanced reaction efficiency by minimising undesired side reactions [1]. Prominent among these microorganisms are *Sporosarcina* strains, known for calcite precipitation, an attribute sparking interest for potential use in bioremediation by precipitation of heavy metals [2, 3].

Building upon this understanding of *Sporosarcina*'s capabilities, we present a detailed analysis of *Sporosarcina* sp. ANT_H38, a novel strain isolated from Antarctic soil. In a first of its type analysis, we were able to assemble the complete genome sequence of this strain. Furthermore, we identified multiple mobile genetic elements, including plasmids, phages and transposons, granting an unprecedented insight into the mobilome of *Sporosarcina* strains. Notably, no plasmids were previously identified in any *Sporosarcina* genome. In the second part of the work, a detailed analysis of urease gene cluster and its role in calcite precipitation was performed using both *in vitro* and *in silico* methods.

As discovered during the course of our study, the strain exhibited promising safety characteristics, positioning it as a prospective candidate for future biotechnological applications, particularly those requiring low-temperature processes or self-healing surfaces. This study, therefore, not only enriches our knowledge of cold-active ureolytic bacteria but also opens new avenues for the utilisation of such microorganisms in sustainable biotechnology.

Acknowledgments

This study was supported by the National Science Centre, grants no. 2016/23/B/NZ9/02909 and Norway Financial Mechanism through the National Science Centre (Poland) GRIEG1 grant: UMO-2019/34/H/NZ2/00584 for L. Dziewit, as well as 2018/29/N/NZ8/00228 for P. Decewicz.

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ANTIMICROBIAL ACTIVITY OF NEW SYNTHESIZED HETEROCYCLIC HYBRID COMPOUNDS ACTIVATED WITH BLUE LASER LIGHT

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Based on literature, some thiohydantoin and quinolone derivatives reveal antimicrobial activity [1, 2]. Expecting the synergistic effect, the Knoevenagel synthesis was carried out, as a result of which new hybrid derivatives of 2-thiohydantoin and 2-quinolone were obtained. The chemical structures were confirmed by IR, NMR and ES MS. The compounds were characterized by UV-VIS and spectrofluorimetric methods. The measurements of $^1O_2$ generation quantum yields were conducted. Blue laser light was applied to enhance the potential antibacterial effect. Antibacterial activity was tested using the serial dilution method on standard bacterial strains: *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*, using microtiter plates. The experiments were conducted in two groups: irradiated one (445 nm) and non-irradiated control.

Under dark conditions weak to moderate bacteriostatic activity is observed, the highest for derivatives containing a methyl group in the 2-quinolone system and simultaneously without an acetate group in the 2-thiohydantoin ring. Irradiation with blue light enhances the bacteriostatic effect. Gram-positive bacteria are more sensitive than Gram-negative strains. The irradiation causes an increase in bacteriostatic or bactericidal activity because of the generation of reactive oxygen species [3].

The second tested group of heterocyclic compounds, dihydropyrimidinone derivatives, was synthesized by the Biginelli condensation reaction. This group is also reported to possess biological activity [4]. The chemical characteristic and microbiological tests in darkness were conducted for the obtained compounds, as described above. As a result, these derivatives reveal only bacteriostatic activity in the highest concentrations used in the experiments.

Further chemical modifications are desired to improve chemical properties and enhance the biological activity of the examined compounds.

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UNCOVERING NOVEL PLASMA MEMBRANE CARBOXYLATE TRANSPORTERS IN THE YEAST *CYBERLINDNERA JADINII*

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The yeast *Cyberlindnera jadinii* has great potential in the biotechnology industry due to its ability to produce a variety of compounds of interest, including carboxylic acids. In this work, we identified genes encoding carboxylate transporters from this yeast species (Fig.1). The functional characterization of sixteen plasma membrane carboxylate transporters belonging to the AceTr, SHS, TDT, MCT, SSS, and DASS families was performed by heterologous expression in *Saccharomyces cerevisiae* yeast.

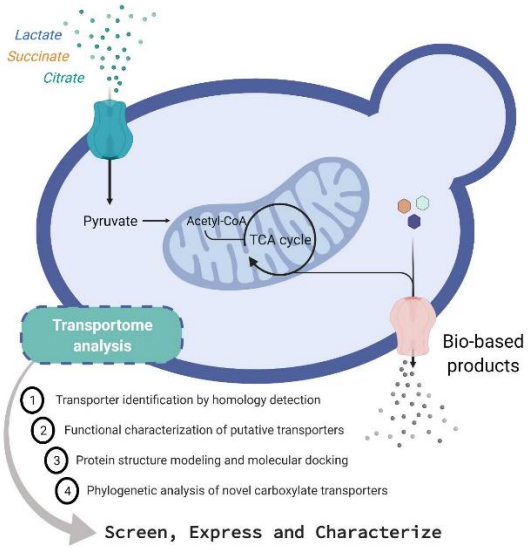

Figure 1. A simplistic scheme highlighting the steps followed for uncovering the relevant carboxylate transporters in *C.jadinii.*

The newly identified *C. jadinii* transporters present specificity for mono-, di-, and tricarboxylates. The transporters CjAto5, CjJen6, CjSlc5, and CjSlc13-1 display the broadest substrate specificity; CjAto2 accepts mono- and dicarboxylates; and CjAto1,3,4, CjJen1-5, CjSlc16, and CjSlc13-2 are specific for monocarboxylic acids. A detailed characterization of these transporters, including phylogenetic reconstruction, 3D structure prediction, and molecular docking analysis is presented here. The properties presented by these transporters make them interesting targets to be explored as organic acid exporters in microbial cell factories.

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DISCOVERY OF NOVEL TYPE II BACTERIAL TOXIN-ANTITOXIN SYSTEM FROM DARK PROTEINS

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Toxin antitoxin (TA) systems are two-gene elements widely found in bacterial genomes. Type II TA systems comprise protein toxin, neutralized by its cognate protein antitoxin. TA systems are implicated in playing a role in stress response[1], plasmid maintenance[2], and protection against bacteriophages via abortive infection[3]. The full diversity of TA systems is still yet to be discovered. This project has started with mining the AlphaFold2 database of protein structures for "functionally dark" (i.e., experimentally unexplored) proteins in [4]. The database and visualization of protein diversity are accessible at https://uniprot3d.org/atlas/AFDB90v4. By searching for novelty from sequence, structure, and semantic perspectives, we identified and experimentally validated a new superfamily of translation-targeting toxin-antitoxin systems, TumE-TumA; 'tume' means 'dark' in Estonian (**Figure 1**). As the next step, we are currently investigating the possible function of the TumE-TumA system in phage defence.

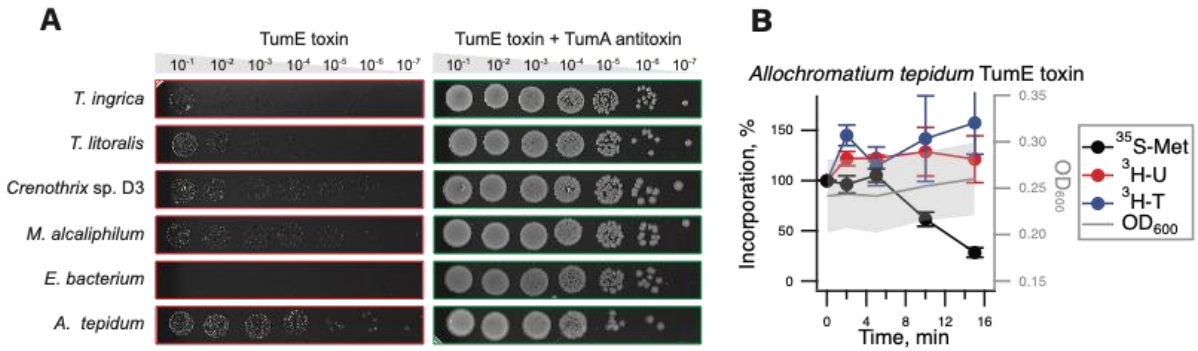

Figure 1. Discovery and characterisation of TumE-TumA TA system. (A) Validation of TumE-TumA TA pairs using toxicity neutralization assay. Toxin expression plasmids (pBAD33 derivates) were cotransformed into *E. coli* BW25113 cells with cognate antitoxin expression plasmids or the empty pMG25 vector. Bacteria were grown for five hours in liquid LB media supplemented with appropriate antibiotics and 0.2% glucose. The cultures were normalized to OD_{600} of 1.0, serially diluted, and spotted on LB agar plates containing appropriate antibiotics and 0.2% arabinose for toxin induction and 500 μM IPTG for antitoxin induction. The plates were scored after overnight incubation at 37 °C. **(B)** Metabolic labeling assays with wild-type *E. coli* BW25113 expressing *A. tepidum* TumE toxin.

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FOLLOWING THE ONE HEALTH APPROACH IN FOOD SCIENCE: MICROBIOME RESEARCH AS A CONNECTING FORCE

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The One Health concept is an integrated, unifying, and cross-sectorial approach for holistic solutions that affect the interface between humans, animals and ecosystems [1]. In food production, the microbial landscape is influenced by human intervention, and dependent on the product's origin, an individual food microbiome builds up during food processing, wherefore a One Health approach is necessary for tracing. Microbial dynamics occur during and after the production chain and these dynamics influence final product properties, contribute to shelf-life, can lead to food spoilage, and ultimately affect health and finally food security and food availability. Microbial contamination is often a hidden, stochastic process that is complex to trace and monitor and that can occur during each processing step [2].

It is estimated that ~1.3 billion tons of food are discarded worldwide each year, what corresponds to more than a third of all food produced for human consumption along the food supply chain [3]. The reduction of food waste is one of the major sustainable development goals of the United Nations [4]. A deep understanding how microbial dynamics contribute to final properties of a product, including spoilage as well as safety and quality issues, is of upmost importance for a sustainable food production. Basic concepts of microbial ecology as an integral part of food microbiology are now applicable, due to advances in high-throughput sequencing technologies and bioinformatics analysis tools.

In this talk, the importance of microbiome research in food science, e.g. for precise shelf life prediction, for the understanding of interactions of microbes with nutrition sources available in products and along production chains, and for modelling microbial flows is highlighted. Innovative product production based on microbiome research is discussed and case studies that have potential to solve major questions in food safety are presented.

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LIGNOCELLULOSE TO BIOCHEMICALS, FEED AND FOOD BY OLEAGINOUS YEASTS

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Yeast lipids produced from lignocellulose and crude glycerol can serve as sustainable alternatives to vegetable oils, which production is frequently accompanied with monocultures, land use changes, or rain forest clearings. We aim to understand the physiology of lipid production by oleaginous yeasts, optimise the production and establish novel applications of microbial lipid compounds [1].

Using newly established methods for fermentation and intracellular lipid quantification [2,3], we found high variability in lipid formation even between very closely related oleaginous yeast strains on both, wheat straw hydrolysate and crude glycerol. In a fermentation with mixed carbon sources, the presence of hemicellulose hydrolysate stimulated glycerol assimilation in *Rhodotorula* strains [4, 5]. In initial experiments on carotenoid isolation, we identified β-carotene as the major carotenoid in *R. toruloides* [6]. However, using advanced extraction methods, supercritical CO_2-extraction, torularhodin and torulene were demonstrated to be the major carotenoids. Also in terms of carotenoid formation, we found a considerable strain diversity.

As a basis to understand the behaviour of the different strains and species, we sequenced a variety of genomes of *Rhodotorula* species. By a combination of long- and short read sequences, we could reconstruct most of the yeast chromosomes [7,8]. We identified 18-21 chromosomes and several circular structures. In general, a high diversity was found and some strains of the same species showed a phylogenetic distance to each other as to those of other species.

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MODIFICATION OF 16S rRNA AMPLICON SEQUENCING TECHNOLOGY THAT QUANTITATIVELY DISCRIMINATES TOTAL AND ALIVE BACTERIAL CONSORTIA

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Next-generation sequencing (NGS) is a highly effective and widely utilized technology in modern microbiology. Depending on the specific task, it can provide taxonomic distribution of microbial communities, genome characterization, or taxa-function connections. While 16S rRNA amplicon sequencing is the most accessible approach for providing the initial description of microbial communities, the outcome is the relative distribution of bacterial taxes based on the total DNA that includes dead and living species. However, this information is often insufficient to make conclusions about the microbiological load of living bacteria in food, shelf-life studies, or hygienic norms.

In our work, we modified 16S rRNA NGS technology combining it with spike-in control and viability qPCR to achieve quantification and taxonomic distribution of living bacteria in a sample (Figure1). For method development, we chose 20 isolated bacterial species and evaluated their viability using flow cytometry, microscopy, and qPCR with specific primers. These data were then compared to the developed quantitative NGS methodology.

The result of the work is a validated methodology that defines the taxonomic composition of living bacteria and estimates their numbers. We tested this approach in various food environments, tasks, and technological procedures and compared the results to classical microbiology plating. The data obtained from this approach shows reliable and unexpected results that merit further consideration and usage in everyday practice.

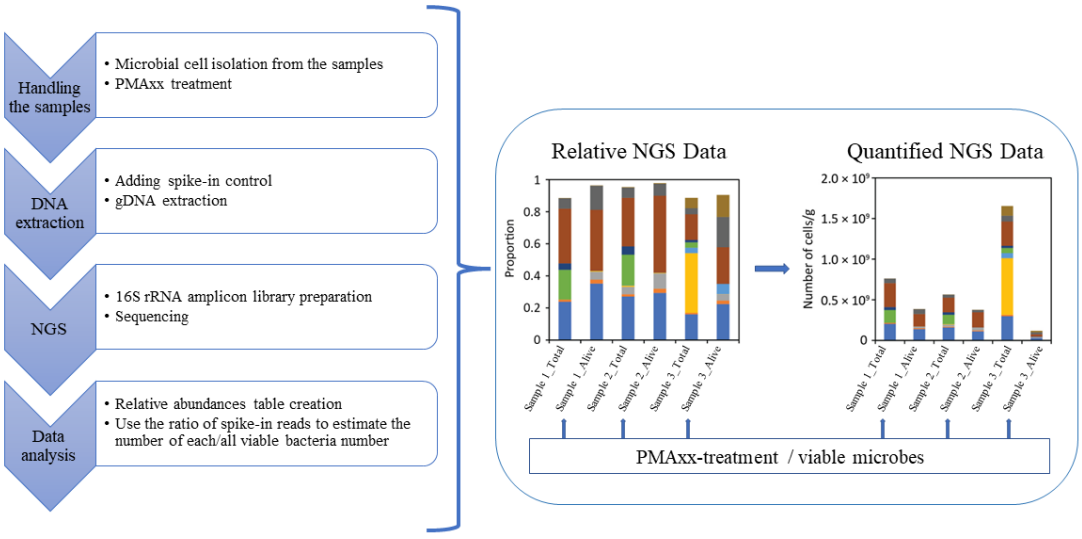

Figure 1. Schematic representation of estimated technology

HIGH TEMPERATURE LACTO-FERMENTATION IMPROVES THE ANTIDIABETIC ACTIVITIES OF RED BEETROOT

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Diabetes is becoming a global pandemic and therefore calls for diverse therapeutic strategies for mitigating the disease. In this study, lactic acid bacteria isolated from Lithuanian traditional fermented foods were screened for β-Glucosidase activities and were used to ferment beetroot to develop an antidiabetic product. After fermentation, LAB PN39 fermented samples demonstrated the strongest DPP-IV and α-Glucosidase inhibitory abilities of 50.0 ± 3.5% and 80.5 ± 5% respectively as well as the highest antioxidant capacity of 0.69 ± 0.04 mmol/L relative to all the other samples. Varying the fermentation conditions revealed that fermenting beetroot with LAB PN39 at 45 ºC for 72 h yielded the strongest DPP-IV and α-Glucosidase inhibition of 87.45 % and 80.5 ± 5.3% respectively and improved the antioxidant capacity to 1.46 ± 0.01 mmol/L. Whole genome sequencing analysis of LAB PN39 revealed the strain to be *Latilactobacillus curvatus* PN39. HPLC-MS analysis of PN39 fermented samples showed that the fermentation process generated high levels of dihydromyricetin (an antidiabetic flavonoid) which was absent in unfermented beetroot. Feeding the fermented product to T1D mice prevented a significant rise in their blood glucose levels after Streptozotocin injection and modulated the gut microbiota. Results from this study indicate that fermenting beetroot with *Latilactobacillus curvatus* PN39 at 45 ºC for 72 h would be an effective method for developing antidiabetic functional foods.

Poster presentations

1. BLACK SOLDIER FLY LARVAE (*HERMENTIA ILLUCENS) PROTEIN FRACTION MICROBIOLOGICAL STABILITY AND ANTIBACTERIAL ACTIVITY*

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The farming of Black Soldier Fly larvae can be one of the vital tools for building a circular economy and has led to their use in several areas such as biorefining, food waste valorisation, waste management, treatment of industrial by-products and bioconversion of agro-industrial residues. The initial operation after insect collection is devitalisation, and the subsequent treatment of the larvae depends on the final use of the insect product. According to literature [1], larvae can inhibit some micro-organisms and alter the composition of the substrate microbiota by releasing antimicrobial substances during cultivation. The treatment of the larval biomass also involves the use of high temperatures, which is likely to destroy part of the larval microbiota and ensure product safety for animal or human consumption.

The analysis of the microbiological parameters of the protein fraction showed that the main safety parameters are met. The number of coliforms in the product or in the environment is indicative of the sanitary conditions of the production. This investigation proved, that the protein fraction was free of coliforms at the beginning of the test and after 21 days of shelf-life study. *Salmonella* and *Listeria monocytogenes* were not detected in protein fraction samples. Mesophilic lactic acid bacteria and presumed bifidobacteria were not found in the protein fraction. The number of sulphite-reducing bacteria (*Clostridia*) is also indicative of the sanitary conditions of the production, since it is an indicator micro-organism and is not present in the protein fraction. Mould fungi were found in minimal numbers at the beginning of the survey, after a shelf-life study even decreased after 21-day period, as micro-organisms, including microscopic fungi, do not proliferate in dry material (moisture content 5,6 %). No yeasts were also detected, as the thermal process ensured elimination of initial contamination and secondary contamination was avoided. Thus, in the protein fraction of Black Soldier Fly larvae biomass was inspected with high number of total microorganisms reaching 10^5-10^6.

The antimicrobial properties of proteins and fats were assessed by agar diffusion. For the evaluation of the antimicrobial efficacy of the protein of Black Soldier Fly larvae protein fraction was diluted in 10% ethanolic solutions. Gram-positive cultures of *Bacillus subtilis*, *Staphylococcus aureus* and Gram-negative cultures of *Escerichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and a culture of a pathogenic yeast belonging to a microscopic fungus *Candida albicans* were used for the tests. The protein fraction solution demonstrated activity against bacterium, *Bacillus subtilis*, with an inhibition zone diameter of 12,5±1,5 mm. The data obtained suggest that Black Soldier Fly larvae protein fraction has slight potential to improve microbiological safety and stability of microbiological parameters in food systems.

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2. A MULTI-DOMAIN RAW STARCH-DEGRADING A-AMYLASE FROM MICROBACTERIUM SP. SINO2 – A BIOCATALYST FOR SUSTAINABLE STARCH DEGRADATION

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Starch is a renewable energy source and raw material used in various industries such as food, paper, pharmaceutical, etc. However, the industrial starch degradation process is not sustainable. Limited starch solubility limits the efficiency of starch-degrading enzymes. Therefore, high temperature and high-pressure conditions are required. Heat and pressure break down the semi-crystalline structure of starch and cause starch solubilisation and gelatinisation, making it accessible to various starch-degrading enzymes. The alternative sustainable solution offers the application of raw starch-degrading microorganisms or their enzymes that can degrade the semi-crystalline starch without the gelatinisation step as a prerequisite. Here, we present *Microbacterium* sp. SINO2 based on its ability to degrade soluble starch and different starch granules. We also investigate an enzyme AM1.3 – a multidomain α-amylase from *Microbacterium* sp. SINO2. Bioinformatic analysis revealed that AM1.3 has a multidomain structure and consists of one catalytic, four fibronectin type 3 domains, two adjacent CBM25 domains, and one CMB74 domain. According to the CAZy database, AM1.3 is a member of the GH13_32 subfamily, known for producing maltotriose, and domains CBM25 and CBM74 are identified as carbohydrate-binding domains known for their ability to bind granular starch. AM1.3 amylase activity screening on various substrates showed the ability to hydrolyse oligosaccharides of various lengths with α-1,4 glycosidic linkage. We assessed starch hydrolysis catalysed by AM1.3 using qualitative and quantitative methods such as TLC and HPLC-ELSD. Finally, we confirmed AM1.3 ability to degrade starch granules via pore formation using scanning electron microscopy and imaging of various starch granules after treatment with AM1.3. Our findings suggest that AM1.3 has the potential as a raw starch-degrading amylase for more sustainable biotechnological processes.

3. RESPONSE OF PLANKTONIC *PSEUDOMONAS PUTIDA* MSCL650 CELLS TO BENZALKONIUM CHLORIDE UNDER DIFFERENT CULTIVATION CONDITIONS

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A significantly increased tolerance of planktonic and immobilized bacterial cells to disinfectants and antibiotics represents a serious problem worldwide [1]. Biocide exposure may induce cross-resistance to clinically relevant antibiotics. Benzalkonium chloride (BAC) is one of the most commonly used quaternary ammonium compounds (QACs) in the pharmaceutical, cosmetic, and food industries. Typically, QACs kill bacteria by penetrating their plasmatic membrane and altering the phospholipid bilayer, which leads to membrane rupture and the eventual release of intracellular contents from the cell [2]. BAC is a broad-spectrum disinfectant lethal to Gram-positive and -negative bacteria and lipophilic viruses, in addition to being fungi- and algistatic [3].

The effect of environmental factors, such as temperature and nutrient composition under BAC stress could highlight the new aspects in cell resistance towards antimicrobials. The aim of this study was to compare the effect of BAC on the growth of planktonic cells of *Pseudomonas putida* MSCL650 under contrasting cultivation conditions, changing the temperature, broth composition, and BAC concentration. Three types of broth were tested, i.e., 5% and 100% Trypton Soya Broth (TSB), as well as the modified broth, which was statistically optimized. Physiological activity of bacterial cells was evaluated by fluorescein diacetate (FDA) hydrolysis, dehydrogenase and quinone reductase activity. All enzyme groups were inhibited by 50 mg/L BAC, except FDA hydrolysis activity, which was higher in the set with BAC comparing with control (100% TSB, at 23 °C). Additional type of broth, which was statistically optimized for biofilm formation, stimulated the biofilm formation, as compared to 5% and 100% TSB, being by 133% and 110% higher at 8 °C and by 378% and 386% at 23 °C, respectively. Interestingly, the culture of *P. putida* MSCL 650 at 37 °C showed a negative trend in biofilm formation in the optimized broth comparing with 5% and 100% TSB, and was by 34% and 38% less, respectively [4]. The DHA activity of planktonic cells in these cultures in the presence of BAC (50÷150 mg/L) was lower, than that in the sets with 100% TSB. Further research is needed to evaluate the risks related to the enhanced antimicrobial resistance of bacteria under adverse conditions.

Acknowledgments. The study was supported by the “State research project in the field of biomedicine, medical technologies and pharmacy” VPP-EM-BIOMEDICĪNA-2022/1-001 (Y3-VPP32f-ZR-N-090); „Optimization of biotechnological processes for effective utilization of renewable resources” Y5-AZ20-ZF-N-270; SAM 8.2.2. The third round project “Strengthening the capacity of the doctoral program of the University of Latvia in the framework of the new doctoral program model”.

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4. CHARACTERIZATION OF BACTERIAL CONSORTIA OF RYE, WHEAT AND OAT GRAINS DURING INDUSTRIAL GERMINATION PROCESS BY MODIFIED 16S RRNA AMPLICON SEQUENCING METHODOLOGY

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In the production of sprouted grains, microbes play an important role in the development of their taste, aroma, texture, and other quality parameters. In cooperation with a sprouted grain-producing company, we characterized the bacterial communities of rye, wheat and oat grains and mapped consortia changes at different stages of the industrial germination process. The 16S rRNA metagenomic sequencing showed that the bacterial communities isolated from the initial grains of rye and wheat are species-rich, diverse, and similar to each other, while the oat grains community is poorer and less diverse with the dominance of environmental microbiota for all grains. In the following production stages, the bacterial communities of different grains are more clearly distinguishable. The sprouts differ from the original grains' bacterial communities by the dominance of lactic acid bacteria and/or *Bacillus* species.

To describe the bacterial consortia, the modified 16S rRNA gene amplicon sequencing methodology on the Illumina platform was used. The novel developed by us methodology enabled to distinguish the living bacterial community from the whole consortia and to quantify the number of bacteria in the sample. Enumerated sequencing results compared to classical microbiology plating showed that for many samples the abundance of bacteria was at least an order of magnitude higher. It confirmed the common opinion that classical microbiology is too limited for describing the full bacteria communities. In addition, not all bacteria are culturable.

The microbiota of three types of grains during the industrial germination process was characterized, starting from the bacterial consortia of raw grain, going through the technological process up to the final sprout microbiota. Also, we applied developed by us modified 16S rRNA sequencing pipeline providing quantitative data distinguishing the living/total bacteria.

5. *GARDNERELLA VAGINALIS* ATCC 14018 TYPE II RESTRICTION-MODIFICATION SYSTEM

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The bacteria of the genus *Gardnerella* have been found in vaginal samples of healthy women and of those with bacterial vaginosis. *Gardnerella vaginalis* was previously considered a single species, but recently it has been differentiated into four species (*G. vaginalis, G. piotii, G. leopoldii, G. swidsinskii*) and nine genome species. Women with bacterial vaginosis are usually infected with multiple species. *Gardnerella spp.* have a very small core genome and horizontal gene transfer (HGT) is very common among the species. However species maintain their genetic distinctiveness [1]. The bacterial restriction-modification (R-M) systems are counted among the barriers to interspecies and intraspecies HGT. *Gardnerella* R-M systems were predicted by genome analysis, yet none were described experimentally.

We detected restriction endonuclease (REase) activity in *G. vaginalis* strain ATCC 14018 and attributed it to the predicted enzyme R.Gva14018I. Its target was defined as the GGCC sequence with probable blunt-ended cleavage. The methyltransferase M.Gva14018I which belongs to the R-M system was expressed in *E. coli*. It protected the host DNA from cleavage by R.Gva14018I. The wild-type R.Gva14018I gene was lethal to *E. coli* with background expression of M.Gva14018I. Spontaneously mutated R.Gva14018I-4 lacking the C-terminal alpha helix was successfully expressed in *E.coli*. The mutation might have resulted in less efficient DNA cleavage. R.Gva14018I-4 displayed wild-type REase specificity. The enzyme cofactor requirements and bioinformatics analysis indicated that R.Gva14018I belongs to the PD-(D/E)XK REase family.

REase-like activity was discovered in 5 of 31 *Gardnerella* strains. The specificities did not match the pattern of R.Gva14018I. The isolates were negative for the genes encoding M.Gva14018I and R.Gva14018I in PCR assays. In conclusion, we identified and characterised the specific R-M system of *G. vaginalis* ATCC 14018.

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6. INVESTIGATION OF POTENTIAL CRISPR-ASSOCIATED NUCLEASE FROM *BACILLUS* PHAGE VB_BAUM_KLEB27-3

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Bacteriophages are widespread and found everywhere, including unique environments such as gypsum karst lakes. Recently, a novel *Bacillus* phage vB_BauM_KLEB27-3 (KLEB27-3) has been isolated from a gypsum karst lake in Lithuania. Notably, KLEB27-3 possesses a genome exceeding 200 kb, classifying it as a jumbo phage. The expansive genome of such phages suggests the potential presence of diverse genome editing elements, including Cas proteins. These elements may engage with the CRISPR/Cas system, potentially leading to interactions that could influence the system's functioning. Such interactions might involve the redirection of the CRISPR/Cas system's activity, thus targeting said system against bacteria or smaller bacteriophages and enabling previously infected phages to survive and spread effectively.

This study involved analysing the genome of a newly isolated bacteriophage KLEB27-3. One specific gene *g92*, was identified and selected for further investigation. Bioinformatic and phylogenetic analyses indicated that this gene (*g92*) is similar to a CRISPR-associated exonuclease found in another *Bacillus* phage vB_BceM_WH1 (with 56% amino acid identity). The gene product, termed gp92, aligns with the Cas4 family of proteins known to partake in CRISPR adaptation while exhibiting exonuclease activity. Despite an absence of closely related homologues of gp92 in publicly available databases, it is believed to have unique properties worth exploring in more detail. In order to find out the potential activity of gp92, the gene was cloned into two different inducible expression vectors (pET21a and pCDFDuet-1), resulting in recombinant proteins with a His-Tag attached to either the N- or C-terminus. Through a subsequent purification, the soluble form of C-His tagged gp92 was demonstrated to be ATP-independent. Notably, both gp92 C-His and gp92 N-His variants exhibited potential endonuclease activities, dependent upon the presence of magnesium and manganese ions.

Results of this study not only expand our understanding of bacteriophage proteins against bacterial defence systems, but also offer novel insights into the potential applications of the mentioned enzyme across various domains, including medicine, biotechnology, and ecology.

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7. SEARCH, IDENTIFICATION AND ANALYSIS OF PYRETHROIDS-DEGRADING MICROORGANISMS

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Pyrethroids are widely used for pest control in agriculture. Different pyrethroids have high insecticidal potential, however, they do not degrade immediately after application and their continuous use can have detrimental effect on non-target organisms [1, 2]. As one of the solutions to the problem, microbial lipolytic enzymes (carboxylesterases EC 3.1.1.1, lipases EC 3.1.1.3), among some other enzymes, due to their broad substrate specificity, can be a new and effective means of removing pyrethroids from the environment [3].

In this study samples of plant growing substrate from two Lithuanian farmlands as well as some microorganisms from the collection of the Department of Microbiology and Biotechnology were used for the conventional search of the target bacteria. For this purpose, enrichment culture using pyrethroids as a sole carbon source was employed for the isolation of the bacteria degrading permethrin and bifenthrin. Isolated cultures were selected and identified by 16S rDNA sequencing and phylogenetic analysis. 5 cultures belonging to *Pseudomonas*, *Staphylococcus*, *Micrococcus* sp. were selected as permethrin- and 6 cultures belonging to *Micrococcus*, *Bacillus* sp. and *Staphylococcus saprophyticus* AG1 as bifenthrin-degrading. To determine that particularly lipolytic enzymes synthesized by the selected bacteria are involved in the degradation of pyrethroids, zymographic analysis using tributyrin and permethrin as substrates was used.

Three cultures belonging to *Pseudomonas* sp. (according to the phylogenetic analysis most likely belonging to *Pseudomonas knackmussii*, *Pseudomonas fluorescens*) were determined to have enzymes of ~50-70 and 55 kDa in the zymograms active towards both tributyrin and permethrin. *Staphylococcus warneri* and *Micrococcus aloeverae* were shown to have enzymes (70-100 kDa) active towards tributyrin only. Some of the determined species in this work have not yet been established to degrade pyrethroids using lipolytic enzymes of the determined size.

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8. MICROBIAL CONTAMINATION OF A TRADITIONAL FLOUR *"RHALI"* MADE FROM CASSAVA, IN MOZAMBIQUE

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Cassava is an important staple food that contributes to food security for 97% of small-scale Mozambican farmers [1]. Processing cassava roots into food-base products is a common practice within farming communities, both to increase shelf-life and reduce cyanogenic compounds [2]. In southern Mozambique, farmers usually process cassava into cassava roasted flour, locally known as *"rhali"*. Handling and processing practices of the cassava root are likely to introduce microbial contamination. We assessed the microbial contamination of *rhali* made in local farmer associations and consumed either locally or sold in rural markets. Microbial sampling was carried out both during winter and summer seasons, and Aerobic Bacteria, *Staphylococcus aureus*, Yeast, Moulds, *Escherichia coli, Bacillus* spp., *Bacillus cereus, Enterobacteriaceae,* and Lactic Acid Bacteria (LAB) were enumerated (Fig 1).

The results revealed seasonal variation in terms of microbial diversity in all stages of cassava root processing. In samples collected in summer, Moulds, LAB, Aerobic Bacteria and *Bacillus* spp. were isolated, whereas in samples collected in winter, other groups of microorganisms such as yeasts and *S. aureus* were present.

Wickerhamomyces anomalus, Rhodotorula mucilaginosa, Pichia exigua, Meyerozyma caribbica and *Torulaspora deslbrueckii* were the most frequent yeast species found in the cassava processing stages. Contamination by *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp., and *Alternaria* sp. was more frequent than other groups of microorganisms in all sampled cassava processing associations. The collected market cassava samples had the lowest diversity of microorganisms of the study.

Aflatoxin-producing moulds were observed infrequently in this study, and only at low counts, thus, the risk for aflatoxin contamination in cassava samples appears to be low. From a food quality and safety point of view, this staple food can therefore be considered clean and safe for human intake.

Figure 1. Stages of processing cassava roots into cassava roasted flour *"rhali"* in local farmer associations.

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9. PURIFICATION AND APPLICATION OF FUNGAL HYDROPHOBIN RODA FOR IMPROVEMENT OF GLUCOSE BIOSENSOR

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Hydrophobins are proteins composed of 70–130 amino acids and containing 8 cysteines, linked by 4 disulfide bonds, which are characteristic of the entire hydrophobin family [1]. The main advantage of hydrophobins is their ability to form amphiphilic layers on various surfaces and thus to change their properties from hydrophilic to hydrophobic and vice versa due to the bifunctional molecular structure of these proteins [2]. Due to their properties, hydrophobins are used in the pharmaceutical, cosmetic and food industries as they are able to stabilize emulsions in product formulation [3]. Hydrophobins are also used in biosensor surface modifications [4] and in tissue engineering for the development of tissue scaffold with higher hydrophilicity [5].

In this work, the hydrophobin RodA of *Aspergillus fumigatus* was investigated as matrix for glucose biosensor. Gene responsible for the synthesis of the RodA was identified, expressed in *Escherichia coli*, and corresponding purified recombinant protein was used as a matrix of gold electrode of the engineered glucose biosensor. The engineered biosensor with a RodA matrix (Au/RodA/GOx) was compared with biosensor without a RodA matrix (Au/GOx), both biosensors had immobilized glucose oxidase (GOx) enzyme. Cyclic voltammetry analysis confirmed the successful immobilization of GOx enzyme for both biosensors and chronoamperometry was used to calculate the K_M values and the maximum generated currents (I_{max}). For Au/GOx, the K_M value was 6.99 mM and the I_{max} was 34.8 $\mu A \cdot cm^{-2}$, K_M value for the Au/RodA/GOx biosensor was 2.37 mM and the I_{max} was 0.432 $\mu A \cdot cm^{-2}$.

The obtained Au/RodA/GOx K_M value showed that GOx immobilized in Au/RodA/GOx biosensor had a higher affinity for the substrate, indicating that hydrophobins are a suitable choice for gold electrode surface modification. The experiments to further improve glucose biosensor are under way.

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10. ADAPTATION OF PMA QPCR FOR APPLICATION IN THE STANDARDIZATION OF PLANT-DERIVED MICROBIOTA INOCULUMS

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Environmental microbiology is often limited to DNA-based characterization of microbial communities because of the complicated cultivation of environmental microorganisms. However, this approach has a major caveat: DNA-based characterization methods usually do not discriminate between live and dead microorganisms [1]. Propidium mono azide qPCR (PMA qPCR) allows for the successful discrimination of live and dead cells based on the selective permeability of dead cells and consecutive inhibition of DNA amplification [2]. The method is successfully applied in the field of human microbiota transplantation allowing standardized fecal microbiota transplants [3, 4]. However, the application of PMA qPCR for standardization and viability testing of plant-derived microbiota inoculums has not yet been studied. The aim of the study was to adapt PMA qPCR for application in the standardization of plant-derived microbiota inoculums.

Experimental testing of PMA qPCR for European elder microbiota inoculums revealed that elderberry root extract does not affect PMA qPCR efficiency. The optimal inoculum concentration is 3 mg*ml^{-1} since it allows for efficient PMA photoactivation as well as successful DNA isolation, and the optimal PMA concentration for efficient live-dead cell discrimination is 100 µM. Further optimization of the method will involve characterization of sensitivity and limit of detection of PMA qPCR in plant-derived microbiota inoculums.

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11. COMPLEX GENETIC MAKEUP OF MEROPENEM- AND IMIPENEM-RESISTANT PLASMIDS ORIGINATING FROM WASTEWATER TREATMENT PLANT

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The increasing prevalence of antibiotic-resistant bacteria in the environment is a significant public health issue[1]. Wastewater treatment plants (WWTPs) are considered to be sources of antibiotic resistance genes (ARGs)[2], and promoters of their accumulation and spread. This study aimed to explore the diversity and genetic composition of meropenem- and imipenem-resistant plasmids isolated from a WWTP in Oświęcim, Poland.

Plasmids were first isolated using antibiotic-enriched culture method followed by transformation into *E. coli.* This revealed one imipenem- and four meropenem-resistant plasmids. Complete, circular sequences of each plasmid were determined using Oxford Nanopore sequencing. Subsequently, metagenomic data was subjected to bioinformatic analyses, including Comprehensive Genome Analysis (BV-BRC) followed by manual annotation, detection of ARGs, identification of genes responsible for conjugal transfer mobilization, and determination of replication incompatibility groups.

The analysis revealed an abundance of mobile genetic elements (MGEs), including those carrying ARGs. Notably, multiple different ARGs were co-localized within particular plasmids, including these conferring resistances to β-lactams, carbapenems, aminoglycosides, sulfonamides, chloramphenicol, quinolones, and trimethoprim. These findings highlight the complex genetic makeup of ARG-carrying plasmids from WWTPs. They also emphasize the importance of continuous surveillance and management of antibiotic resistance in environmental reservoirs.

Performed bioinformatic analyses will serve as a baseline for further laboratory experiments. This includes confirming phenotypic antibiotic resistance, evaluating the mobility of MGEs using an entrapment vector, assessing the host range of these plasmids, and determining the plasmids' mobility in various microcosm experiments.

Acknowledgments
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12. EXPLORING THE GENOMIC VARIABILITY OF *NEISSERIA MENINGITIDIS* STRAINS IN LITHUANIA

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The epidemiology of meningococcal infections varies widely based on geographic location, timeframe, and the age of susceptible populations. This is also influenced by widespread meningococcal vaccine usage and the isolation measures implemented during the COVID-19 pandemic.

Over the past decade, many European countries have employed whole genome sequencing (WGS) for *N. meningitidis* surveillance. WGS availability enhances our understanding of its biology and diversity. This study aims to assess the genomic relationships between *N. meningitidis* strains from Lithuania and strains identified across the Europe region.

321 *N. meningitidis* isolates collected in Lithuania between 2009 and 2021 were subjected to multilocus restriction typing (MLRT) following the method of Benett et al.[1]. Based on MLRT genotyping, 10 infrequently observed strains were selected for WGS analysis. The sequenced genomes were integrated into the PubMLST database [2] to assess genomic diversity and relationships among Lithuanian and European circulating *N. meningitidis* strains, and the draft genomes have accession numbers: 119504, 120132 - 120140. The genomic diversity of Lithuanian *N. meningitidis* strains was assessed by analyzing a collection of 43 publicly available genomes. The analysis revealed the presence of 21 different sequence types (STs) circulating in Lithuania. Among them, ST34 (76.19%) was the most prevalent. Notably, isolates 120132, 120136, and 120140 had unique combinations of 7 housekeeping genes, identified as novel STs in the PubMLST database: ST16969, ST16901, and ST16959, respectively.

Using the GrapeTree [3] tool on PubMLST, a minimal spanning tree (MST) was generated for a publicly available collection of 9325 *N. meningitidis* isolates, based on a cgMLST v2 scheme. MST revealed no significant geographic relationships among them. Overall, a conspicuous global dissemination of *N. meningitidis* was observed, with the root of the MST originating from Sweden. The analyzed isolates belonged to 42 clonal complexes (CC), with the ST-11 and ST-41/44 CCs being predominantly prevalent. In contrast, the ST-32 CC, highly common (69.4%) in Lithuania, exhibited a lower prevalence (7.9%) across the broader European region. These Lithuanian isolates formed a distinct clade, along with samples from various European countries. All 10 Lithuanian isolates studied here belonged to distinct related clades.

This study underscores the importance of genomic characterization in understanding circulating *N. meningitidis* lineages, leading to new targets for lineage monitoring, diagnosis, or vaccine development. Ultimately, this research enriches the PubMLST database with WGS data of Lithuanian isolates.

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13. THE ROUTE OF GUT MICROBIOTA TRANSFER IMPACTS THE EFFECT ON ADULT MICE BEHAVIOR

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Autism spectrum disorders (ASD) are a collection of neurodevelopmental disorders with complex etiology. Current research indicates that children with ASD have an increased likelihood of gastrointestinal disturbances and exhibit lower diversity of gut microbiota. Notably, changes in the ratio of *Firmicutes*/Bacteroides and composition of primary bacterial phyla are a sign of dysbiosis and are widely observed in ASD patients. Previous studies indicate the importance of host-microbiota interactions in neuropsychiatric disorders and the potential of microbiota to modulate the symptoms of various neuropsychiatric disturbances. Furthermore, lower diversity of gut microbiota can contribute to the cognitive pathology and symptoms observed in the ASD. However, the lack of models suitable to investigate the role of gut microbiome changes in ASD remains the main hurdle in establishing biomarkers or therapeutic targets. Hence, we transferred fecal samples from ASD patients to mice after microbiota cleansing with a mixture of antibiotics and analyzed their behavior, gene expression in CNS and gut microbiome changes. We found that transfer of gut microbiota from ASD patients can induce changes in the behavior of adult mice, impact gene expression in different brain areas based on the strategy of the fecal microbiota engraftment.

14. EVALUATION OF LACTOSE-CONTAINING MEDIA FOR MIXOTROPHIC PRODUCTION OF LIPID-ENRICHED *CHROMOCHLORIS ZOFINGIENSIS* CCAP 211/14 BIOMASS FOR USE IN AQUACULTURE FEEDS

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Recent development of salmonids aquaculture requires increasing amounts of lipid-enriched feed. It is established that lipid-enriched microalgae biomass can be used in aquaculture feeds in order to increase nutritive value and lipid content. Previous studies show improvement in growth, immunity and meat quality of salmonids, when microalgae are added to the feed [1]. Currently microalgal biomass commercial production is associated with several limitations, e.g. high production costs and low biomass productivity, when microalgae are cultivated photoautotrophically [2]. Mixotrophic microalgae cultivation on alternative substrates, such as dairy industry by-products, has a potential for significant intensification in biomass production with increased lipid content. This study focuses on mixotrophic cultivation of *Chromochloris zofingiensis* CCAP 211/14 on modified Bold's Basal medium (3N-BBM-V) supplemented with lactose (10 g/L) and resulting lipid, protein and carbohydrate content in biomass.

Mixotrophic cultivation of *C. zofingiensis* CCAP 211/14 in lactose supplemented medium was compared to photoautotrophic growth (control). Results show significant increase of biomass production in mixotrophic cultivation group (3.74 ± 0.13 g/L dry biomass) compared to control (1.60 ± 0.17 g/L). Additionally, during photoautotrophic growth lipid concentration in the biomass was significantly lower (9.75 ± 1.10 %), compared to mixotrophic group (33.36 ± 1.30 %). However, no significant changes ($p > 0.05$) in content of carbohydrates (~30%) and proteins (~20%) in the biomass were detected. Moreover, lactose uptake (~50%) from the medium was evaluated during mixotrophic cultivation as well as extracellular (61.46±1.70 U/L) and intracellular (36.52±1.42 U/L) activity of β-galactosidase.

It can be concluded that 3N-BBM-V supplementation with lactose results in significant increase of *C. zofingiensis* CCAP 211/14 biomass production and lipid content. Moreover, lactose consumption and β-galactosidase production was confirmed. Further studies are in progress to evaluate *C. zofingiensis* ability to grow on different lactose containing dairy by-products.

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15. IN VITRO SCREENING AND CHARACTERIZATION OF LACTIC ACID BACTERIA FROM LITHUANIAN FERMENTED FOOD WITH POTENTIAL PROBIOTIC PROPERTIES

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Probiotics are essential live microorganisms which colonize gut and produce metabolites that has beneficial effects on the host. Lactic acid bacteria (LAB) are among the abundant, Gram-positive bacteria which are widely distributed in nature and naturally thrive in traditional fermented foods. LAB offers special functions that are considered beneficial to the host, hence falling into the category of probiotics. LAB are facultative anaerobes with exceptional characteristics that are essential for the functioning of mammalian health. Due to its therapeutic effects, their application is still studied in treating and curing numerous diseases [1][2]. The present work aimed to identify probiotic candidates from Lithuanian fermented food samples and its in-vitro screening, functional characterization properties and safety analysis.

LAB strains were isolated from the fermented foods procured from the local market, Hales Turgus, Vilnius, Lithuania. These selected sample went through the screening steps of tolerating the harsh conditions of the gastrointestinal tract, which includes survival in low pH of 2, pepsin, bile salts and pancreatin. The isolates showing >50% survival in the gastrointestinal conditions proceeded with the determination of functional characterization steps in in vitro condition of antimicrobial activity, antioxidant potential, gut colonization ability, proliferation in presence of prebiotic, tryptophan metabolite production for exhibiting psychobiotic ability. The selective strains were then identified by 16s RNA and whole genome sequencing techniques to confirm its strains and at last tested for safety analysis.

23 morphologically different LAB colonies were isolated from various fermented food samples. Among these 23 isolates, only 12 showed resistance to the probiotic screening tests of survival in low pH of 2, tolerance to pepsin, bile salts and pancreatin. In the characterization methods, these 12 strains exhibited antimicrobial activity against five pathogens- *Staphylococcus aureus* ATCC 29213, *Salmonella typhimurium* ATCC 14028, *Streptococcus pyogens* ATCC 12384, *Streptococcus pyogens* ATCC 19615 and *Klebsiella pneumonia* ATCC 13883 with average zone inhibition of 10.75 to 19.5 mm, respectively. Following with the determination of trolox equivalent antioxidant concentration with the scavenging effect of the cell free supernatants on a 1,1-Diphenyl2-picryl-hydrazyl radical was executed. All the 12 strains showed free radical scavenging activity and antioxidant capability. For the gut colonization potency, auto-aggregation property was measured at three different timelines- 4, 12 and 24 hours, and the adhesion on HCT116 colon cells was calculated by percent adhesion. Only 5 isolates showed promising adhesion capability. These 5 isolates proceeded with growth and tryptophan production in presence of prebiotic galactooligosaccharides at 2% and 4% concentration. All 5 isolates showed better growth in the presence of 2% and 4% concentration of GOS and only 11w isolate produced tryptophan metabolite. The selective strains were identified by 16s RNA and whole genome sequencing techniques confirming to be new strains. At last, in the safety analysis, only three isolates passed the antibiotic susceptibility, mucin degradation, gelatin hydrolysis, and hemolytic activity.

Taken together, results from this study show that *Lacticaseibacillus paracasei* 11w had the best probiotic potential when compared to the rest of the LAB isolates. Further in vivo studies are however required for their health promoting effects in mammals. Accordingly, due to these unique probiotic properties, selected three strains should be proceeded with in vivo testing, and future clinical trials.

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16. ANTIFUNGAL ACTIVITY OF LANTHANUM AGAINST *CANDIDA ALBICANS* CELLS IN A NON-SESSILE AND BIOFILM MODEL

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Aim: Opportunistic infections, affecting mucosal to dermal surfaces, pose a deliberate threat to a worldwide healthcare system [1]. Biofilm formation on organic and inorganic surfaces has substantially improved virulence and is a crucial pathogenesis factor for the opportunistic fungus *Candida albicans* [2]. In this study, we aim to evaluate lanthanum nitrate (LN) and lanthanum nitrate hexahydrate (LNH) potential against non-sessile *C. albicans* cells and a pre-formed 24-h biofilm. Lanthanum exhibits anti-fungal activity by generating excessive reactive oxygen species, formerly described as capable of damaging cell components and disrupting cellular mechanisms [3, 4].

Methods: Antifungal susceptibility against non-sessile *C. albicans* cells was assessed using the broth dilution method. The minimal inhibitory concentration (MIC) was evaluated after 24 hours of incubation using a viability assay. For sessile model susceptibility evaluation, *C. albicans* biofilms were developed in a flat-bottomed 96-well plate for 24 hours. Pre-formed biofilms were treated with 1 x MIC, 1,5 x MIC, 2 x MIC, and 2,5 x MIC concentrations of LN and LNH, respectively. Treated biofilms were collected after 24 hours of incubation. Metabolic activity and viability within the biofilm were assessed using the XTT reaction and viability assay.

Results: For non-sessile *C. albicans* cells, the MIC was 12 mg/mL for LN and 14 mg/mL for LNH. The biofilm model showed increased resistance to both substances. Concentrations needed for disturbing pre-formed biofilm were dose-dependent, with LN being more compelling. The metabolic activity of LN-treated biofilms was reduced to 14% and 23% for LNH. The viability assay displayed equivalent data, ensuring legitimate results.

Conclusion: Lanthanum nitrate and lanthanum nitrate hexahydrate demonstrate antifungal activity against non-sessile *C. albicans* cells and a pre-formed biofilm. Implying that it has the potential to be an alternative to current antifungal remedies.

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17. CHARACTERIZATION OF VOLATILE ORGANIC COMPOUNDS (VOCs) EMITTED BY FUNGUS *OPHIOSTOMA QUERCUS*

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Ophiostoma quercus (Ascomycota, Ophiostomatales) is a worldwide distributed fungus that can colonise a wide range of tree species, mostly hardwood hosts. The fungus is considered to be non-pathogenic, but it causes sapwood-staining, which also leads to the significant economic losses in the timber industry [1]. To date, there are no effective measures to control this microorganism and effective preventive measures are being sought to limit its spread.

Ophiostomatoid fungi are characterised by a high morphological and genetic diversity, which makes *O. quercus* particularly difficult to identify among other fungi. In order to apply more effective methods for faster and more accurate detection and identification of this fungal species, the aim of this study was to compare the effectiveness of molecular and volatile organic compound (VOC) applications.

Two strains of *Ophiostoma quercus* fungi (NRCIB Oqu1 and NRCIB Oqu2) isolated from *Quercus robur* trees from Lithuania were used in this study. The fungal strains were identified molecular methods: the internal transcribed spacer (ITS) of ribosomal DNA using primers ITS1 and ITS4 [2], and the 28S large subunit partial nuclear ribosomal DNA (LSU) using primers LROR and LR5 [3]. In addition, regions of four genes were amplified: β-tubulin (TUB2) using primers Bt-2a and Bt-2b [4], α-actin (ACT) using primers ACT-512F and ACT-783R, calmodulin (CAL), using primers CAL-228F and CAL-737R, and the translation elongation factor 1-α (TEF1-α) gene using primers EF1-728F and EF1-986R [5]. Pure cultures of *O. quercus* were used to determine the composition of fungi emmited volatile organic compounds (FVOCs) employing technique of gas chromatogtapy-mass spectrometry (GC-MS) [6]. *In vitro* studies revealed 12 VOCs specific to this species.

For the first time in Lithuania, different methods have been used to characterize *O. quercus*, providing a broader picture of how to identify this fungal species more effectively.

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18. DOES THE ORIGIN OF THE PINE TREE INFLUENCE THE GENOTYPIC DIVERSITY OF ENDOPHYTIC FUNGI?

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Climate change and global trade are important factors in the spread of alien plant pathogens worldwide. Sentinel plants provide an opportunity to study the interactions of introduced and native plants with plant pathogens and other endophytic microorganisms. As it would help to identify invasive plant pathogens more quickly and to assess the impact on nature, as there is a lack of in-depth knowledge on the comparative genetic diversity of the endophytic microorganisms found in these plants, which is the key to the success of the survival and spread of a potential invasive plant pathogens and the plants itself.

The plant material for the study was collected from 7 *Pinus* species (native *P. sylvestris* L. and introduced *P. mugo* Turra, *P. strobus* L., *P. nigra* J. F Arnold, *P. banksiana* Dougl. x *contorta* Lamb., *P. ponderosa* Douglas ex C. Lawson, *P. parviflora* Siebold & Zucc.) in the Kairėnai Botanical Garden of Vilnius University. A total of 115 fungal isolates were examined in this study and classified into 13 morphological groups based on microscopic and macroscopic phenotypic traits such as color, shape and size of colonies, hyphae, and spores. Sequencing of fungal ITS1 and ITS2 region using the ITS1/ITS4 primers [1] resulted in the assignment of all these morphological groups to 11 fungal genera. The genotypic diversity of endophytic and phytopathogenic fungi isolated from *Pinus* spp. plants was investigated using 10 ISSR-PCR [2] primers, showing a certain level of intra-group differentiation.

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19. THE ROLE OF PEROXISOMES IN YEAST RESISTANCE TO VARIOUS STRESSES

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Peroxisomes are highly dynamic, DNA free, single membrane surrounded organelles. They may be involved in various metabolic pathways that predominantly depend upon cellular and environmental conditions. Peroxisomes are small organelles between 0,1 – 0,2 μm in size. Cell can contain from 1 – 2 peroxisomes to several dozen peroxisomes. Yeasts are convenient model to study peroxisomes because cell transfer from a glucose containing medium to a medium containing a peroxisome proliferator (oleate, methanol) induces synthesis of peroxisomal enzymes and the growth and division of peroxisomes. For example, peroxisomes may occupy between 30 and 80 % of the cell volume in cells growing in the presence of peroxisome proliferator under certain conditions. After multiple rounds of organellar growth and division, individual cells may contain over 20 peroxisomes [1; 2; 3].

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. Yet nothing is known about other organelles, like peroxisomes, during stress response [4]. The aim of this study was to obtain first information concerning the role of peroxisomes in various stresses - hyperosmotic, oxidative, thermal and dehydration/rehydration.

In this study we used *Saccharomyces cerevisiae* and *Ogataea polymorpha* strains with affected peroxisome division (*pex11Δ*), inheritance (*inp1Δ)*, biogenesis (*pex3Δ)* and peroxisomal matrix protein import (*pex6Δ*).

We concluded that yeasts with intact and functional peroxisomes after their induction are able to withstand better dehydration and subsequent rehydration stress. Overall, our results showed for the first time that if yeast cells were incubated in a medium with a peroxisome inducer before transfer into the state of anhydrobiosis, it gave advantage of better withstanding dehydration-rehydration stress. The different ability of mutant strains to withstand dehydration/rehydration treatment if they are incubated in medium with peroxisome inducer prior to dehydration does not depend on resistance to oxidative, hyperosmotic stress or heat shock. There must be another mechanism by which peroxisomes favor better transition to the anhydrobiosis state and subsequent reactivation from this state.

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20. DEGRADATION STUDY OF LINSEED OIL-BASED CROSS-LINKED POLYMER COMPOSITES FILLED WITH INDUSTRIAL WASTE MATERIALS IN THREE DIFFERENT SOILS

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Today‘s world is facing the huge accumulation of non-degradable polymers, the depletion of crude oil reserves, and the continuous rise in consumer demand for plastics. As a result, environmentally degradable bio-based polymers and their composites are being developed [1]. However, it is equally important to assess the degradability of newly developed polymers and their composites as well as compare the degradation rate of such materials across different environments. The aim of the study was to evaluate the degradability of polymer composites in three different soils.

The degradability of polymer composites was evaluated by a soil burial test according to EN ISO 846:1997 [2]. The polymer composites were prepared by direct mixing of epoxidized linseed oil and an aqueous solution of 1-hydroxyethane-1,1-diphosphonic acid, followed by the addition of 5 wt% of industrial waste materials (pine needles, pine bark, grain mill waste, and rapeseed cake). The experiment was performed in coniferous and deciduous forest soil as well as grassland soil for 180 days. Changes in chemical composition and surface morphology of polymer composites were evaluated by attenuated total reflectance infrared spectroscopy (ATR-IR) and scanning electron microscopy (SEM), respectively.

The weight loss (%) of polymer composites after 180 days of a soil burial test was significantly higher in coniferous forest soil, which was characterized as neutral (pH 7), having lower organic C (1,54%) and N (0,117%) values and more stable cellulolytic activity compared to deciduous forest and grassland soil. The polymer matrix showed the greatest weight loss (%) in all of the tested soils, which was also supported by the results of ATR-IR and SEM. The reduction or disappearance of the signals assigned to C=O, C-O-C, P=O, and P-O-C bonds indicated the cleavage of the polymer matrix. The obtained results suggest that the matrix of polymer composites has degraded faster than the fillers.

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21. STATINS AS POSSIBLE ANTIFUNGALS AGAINST *CANDIDA* SPP. YEASTS

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The mortality rate of invasive fungal infections caused by pathogenic *Candida* yeasts is 30-90% even when antifungal therapy is applied [1]. One of the biggest problems encountered in treating these infections is the ability of cells to acquire resistance to known antifungal drugs. The most common mechanism of resistance is multidrug resistance pumps. Usually, in resistant strains activity of such pumps is upregulated, leading to increased antifungal drug efflux out of cells. Because of that, the concentrations of drugs within the cells are too low to inhibit the growth of pathogenic *Candida* yeasts. One possible way to increase the concentrations of antifungals inside the cells is an inhibition of the activity of efflux pumps using alternative substrates, i.e. drugs used for the treatment of chronic diseases.

The aim of this study was to evaluate the ability of statins - drugs to lower the level of cholesterol in the blood – to inhibit the efflux of antifungals. The effects of statins alone or in combinations with antifungals were tested against different strains of *C. glabrata* and *C. albicans*, including mutants in ABC family efflux pumps – CDR1 and CDR2.

The results of our experiments revealed that all three of the chosen statins (atorvastatin, fluvastatin, and rosuvastatin) had fungicidal activity against *C. albicans* wild-type cells, and the deletion of CDR1 efflux pump made the strains more sensitive to the drugs. Statins had fungicidal activity against *C. glabrata* strains at the highest tested concentrations (256 µg/ml) only and the deletion of efflux pumps had no impact on the sensitivity in this species. The combinations of statins with the known antifungal fluconazole reduced the growth of *C. albicans* wild-type cells but did not have this effect on the growth of *C. glabrata* yeasts. These findings show that statins can strengthen the effects of antifungal drugs and might act as efflux pump inhibitors, but further research is needed.

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22. PREPARATION AND CHARACTERIZATION OF ANTIBACTERIAL WOUND DRESSING MEMBRANES FOR TATTOOS

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A tattoo is a permanent pigmentation of the skin resulting from the entry of exogenous substances into the skin to form indelible marks or designs. During the procedure, the skin is under a lot of stress, because it is constantly punctured with needles and dye is injected into the skin, so after the procedure, not only the desired drawing appears on the skin, but also an open wound, which must be properly protected and treated [1, 2].

The aim of the project was to develope and characterize polymeric wound dressing membranes for tatoo care and healing. Solutions of various concentrations were developed to form wound dressings, which had viscosity in the range of 38.5±0.2–49.1±0.4 mPa·s and a pH of 5.11±0.17–5.85±0.21. The physical characteristics of the membranes, such as thickness, transparency, yellowness, elasticity and hardness were found to be affected by the composition of liquorice extract and polyvinylpyrrolidone. The composition most suitable for the formation of membranes containing active compounds was identified based on the results obtained, and two new formulations were prepared: with 2% lidocaine hydrochloride and with 0.025% octenidine dihydrochloride. The wound dressing membranes containing lidocaine hydrochloride were found to be thicker and less transparent than the membranes with octenidine dihydrochloride. In vitro release kinetics revealed that after 24 hours there was a release of 63±1% of lidocaine hydrochloride and 89±2% of octenidine dihydrochloride, respectively. All membranes were found to moisture the skin and has antibacterial activity against Staphylococcus aureus. Furthermore, the dressing containing octenidine dihydrochloride was also found to be effective against Enterococcus faecalis and Pseudomonas aeruginosa.

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23. EFFECTS OF *LENTINULA EDODES* EXTRACT ON IMMUNE RESPONSE IN *DROSOPHILA MELANOGASTER* LARVAE

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Shiitake mushroom *Lentinula edodes* (Berk.) Pegler is a widely cultivated edible basidiomycete and is considered a valuable source of biologically active compounds. The immunomodulatory activity of shiitake polysaccharides, especially beta-glucans, has been studied in various animal models [1].

Drosophila is a non-mammalian model organism used in biological, medical, and pharmacological research. Drosophila has innate immunity; the response involves haemocytes, antimicrobial peptides (AMP), and melanisation reaction [2].

L. edodes DSM 3565 hot water extract (HWE) was obtained from fruiting bodies. Depending on the planned test HWE was standardized at 0.1 to 5 mg/ml by dry weight, pasteurized, lyophilized and stored at 4°C. The concentration of beta-glucans was ca. 20% of the dry weight of HWE as estimated by FTIR spectroscopy and enzyme-based colorimetric analysis.

Fertilized eggs of wild type drosophila were placed on HWE-containing corn meal media, the obtained larvae (≤ 10 per cm^2) were reared at 25±1°C, 12:12 h light:dark cycle and 60% relative humidity.

Immune response of 3rd instar larvae was assessed. HWE-fed larvae had more pronounced melanisation reaction - intense wound site blackening, alongside with increased number of sessile crystal cells and phenol oxidase activity. HWE-induced increase of haemolymph antibacterial activity was revealed indicating elevated AMP synthesis. No adverse effects on the development, viability, and body size of HWE fed larvae were observed.

Research is being continued in the drosophila larvae model to optimize tests for screening immunomodulator activity by natural infection approach and to characterize the changes of immune response gene expression after HWE feeding.

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24. RECOMBINANT HEMEPROTEIN PRODUCTION FOR PLANT-BASED MEAT: FERMENTATION TECHNOLOGY & ANALYTICS

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Modern livestock production systems are associated with crucial environmental problems and contributes between 15 to 24 % of total greenhouse gas emissions [1]. Additionally, meat production by processing animals and birds into food, is associated with ethical concerns and is increasingly criticized for being inhumane [2].

An alternative to livestock production can be cell-based (also referred as *in vitro* meat) and plant-based meat. Unfortunately, *in vitro* meat production systems suffer from various technological problems [3]. The drawbacks of *in vitro* meat production have encouraged the research of other alternatives like plant-based hemeproteins (hemoglobins) to mimic the colour and flavour close to that of meat.

Plant hemoglobins, also known as leghemoglobins, are present in the root nodules of leguminous plants. Upon cooking, the leghemoglobin protein (LegH) unfolds by releasing its heme cofactor to catalyse reactions that can transform the same plant-based sources biomolecules into the compounds that comprise the flavour and aroma of traditional meat [4]. As of now, the most perspective process of manufacturing LegH is through fermentation using recombinant microorganism strains.

During the research, a recombinant (LegH producing) strain of *Komagataella phaffii* was cultivated by using a defined medium composition. The said medium, in contrast to industry accepted formulas, utilizes ammonium salts (rather than ammonium hydroxide), which makes it possible to control the pH and ensure optimal nitrogen levels independently.

Multiple fed-batch fermentations with model predictive control (MPC) [5] were performed in a bench top 5 L bioreactor. The maximal biomass concentrations achieved in said cultivations were 79 gDCW/L at the end of the glucose feeding stage. The maximal obtained LegH concentration reached 0.08 g/L.

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25. TARGETED EDITING OF vB_EcoM_VpaE1 LYTIC COLIPHAGE

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Bacteriophages (phages) are viruses that infect bacteria and control their abundance and diversity. Lytic phages, which induce cell lysis after replication, are promising control agents for pathogenic bacteria and simple viral models for basic and applied research. Of particular interest are numerous discovered genes that are unique to specific phages or shared among closely related ones. These genes hide potentially useful functions, but they can also be dangerous when using phages for therapeutic purposes. Targeted editing of phage genomes can help uncover these functions and improve phages [1].

Genome editing of lytic phages was complicated due to their unique biological properties. Recently, the marker-based and marker-less strategies have been developed. However, the methods must be adapted to the specific phage group and require knowledge of phage biology. Here, we present the ways for engineering vB_Ecom_VpaE1 (VpaE1) phage belonging to the unexplored *Felixounavirus* genus [2, 3]. The phages from this genus infect *Escherichia coli* or *Salmonella* strains including clinically important ones [4, 5]. The VpaE1 phage is an attractive model for investigation of this genus as it infects laboratory strains of *E. coli*.

The BRIP method proved most suitable for introduction of *in vitro* designed mutations into the phage genome by homologous recombination. This involved electroporating a PCR fragment containing homology arms and either a marker gene (*trxA* or *lacZ* alpha fragment) or the gene of interest with an *amber* mutation into VpaE1-infected *E. coli* host strains. After recombination, the mutant phages have been selected according to their plaque phenotype on the *E. coli trxA*$^{-}$ or *lacZ*ΔM15 strains. Inactivation of the essential gene was based on the insertion of *amber* (stop) codon, followed by selecting mutants on suppressing strains. Using these methods, the knock-out mutants of essential and non-essential genes were constructed enabling further studies of their functions.

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26. EXPLORING BACTERIOPHAGE-BASED BIOCONTROL: A ZEBRAFISH MODEL FOR BACTERIAL INFECTIONS AND PHAGE THERAPY DEVELOPMENT

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Bacterial infections can cause harm to human health and affect the economy. In order, to understand the disease process, possible treatments and to determine effective control strategies, it is necessary to develop effective models for the study of bacterial infections. Aquaculture is the fastest growing sector in animal food production. Today, approximately 50% of the world's fish consumption originates from controlled aquatic settings. Among the concerns faced, bacteria such as *Aeromonas* spp, *Pararheinheimera* spp., and others have the potential to infect freshwater fish, leading to substantial losses within the aquaculture sector and posing risks to food security.

This study attempted to create a model of infection using bacteria isolated from a gypsum karst lake Kirkilai from North Lithuania and zebrafish embryos. Zebrafish embryos at 2 days post-fertilization (dpf) with tail injuries were immersed in the bath. At this developmental stage the mouth has not yet opened, so only physical injury could have facilitated the entry of bacteria into the embryo. Nine different strains were tested at a concentration of 10^6 CFU/ml with two embryos per well within 96-well plates. Embryo viability was monitored for 72 h. In contrast to healthy embryos, the *Pararheinheimera soli* strain induced lethality in embryos with tail lesions. *P. soli* resulted in mortality rates of up to 77% in embryos. To reduce the mortality of zebrafish embryos, phage treatment was administered. The initial phage treatment displayed moderate efficacy, although further comprehensive research is still needed to enhance its potential.

The development of bacterial infection models is crucial for understanding the disease mechanisms, evaluating potential treatments, and developing effective phage-based biocontrol strategies. These models play a central role in advancing our knowledge of bacterial infections and improving our ability to combat them.

27. ACQUISITION OF POLYUNSATURATED FATTY ACIDS FOR AQUAQULTURE SALMONID FEED ENHANCEMENT

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A method for acquiring a polyunsaturated fatty acid (PUFA) fraction and separating it from monounsaturated (MUFA) and saturated (SFA) fatty acid fractions was explored and tested on microalgae (Fig 1)[5]. Omega-3 are valuable PUFA and an essential component for salmonid fish feed [6]. Microalgae are known producers of these fatty acids.

Subsequent saponification, transmethylation, winterization and urea complexation stages were performed.

A 8.2% and 4.4% free fatty acid (FFA) content was determined in *Chlorella sp.* and *Spirulina sp.* microalgae biomass samples respectively.The FFAs were methylated to fatty acid methylesters (FAME). Urea was added to FAME to achieve urea complexing (UCF) and non-urea complexing (NUCF) fractions. UCF consists primarily of SFA and MUFA, however, NUCF is enriched with PUFAs. FAME of *Chlorella sp.* were separated into NUCF and UCF with recovery of 91%. Recovery of NUCF and UCF from FAME of *Spirulina sp.* was 45%. NUCF content in *Chlorella sp.* and *Spirulina sp.* biomass was determined to be 0.75% for the former and 0.44% for the latter.

Urea complexation can be used as a viable method to acquire PUFA enriched fatty acid fractions from microalgae.

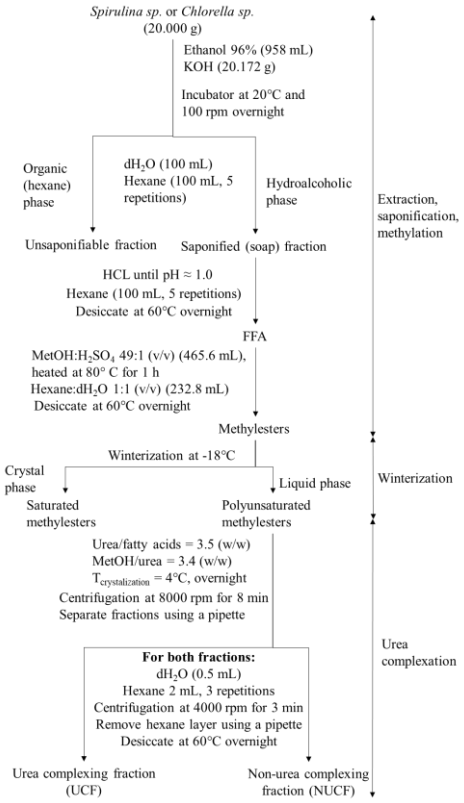

Fig 1. Scheme for acquisition of UCF and NUCF from *Chlorella sp.* and *Spirulina sp.*[1]

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28. LIGNINOLYTIC PROPERTIES OF LOCAL RHODOCOCCUS ISOLATES

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Lignin, is a complex aromatic polymer that together with cellulose and hemicellulose forms the main constituent of wood. At that, lignin is considered as the most abundant renewable low-cost aromatic raw material on Earth. Biological degradation of lignin polymer is relatively slow process due to its irregular and recalcitrant structure. However, several microbial species can efficiently depolymerize lignin and/or utilize lignin-derived toxic aromatic compounds. The bacterial species capable to utilize lignin originate primarily from soil, wastewater, compost, and mining sediments. Microbial species from extreme environments or polluted areas often possess high metabolic versatility - this enables to synthesize various storage compounds which contribute to cell viability under severe stress conditions [1].

Our current project is focused on assessing ligninolytic properties of Gram-positive bacterial isolates originating from various local (mainly Baltic) unpolluted and polluted areas. For initial screen, different technical lignins (organosolv, hydrolysis, Kraft) as sole carbon sources have been used. The ability of these new bacterial strains to utilize lignin was assessed using different analytical chemistry methods. Our preliminary screen revealed that seven *Rhodococcus* strains isolated from environments such as compost or contaminated soil/water have significant abilities to use lignin as a carbon source and utilize lignin-derived aromatic compounds.

Several representatives of *Rhodococcus* species have biotechnological potential due to their ability to degrade wide range of organic compounds, and biosynthesize different storage compounds with industrially relevant potential (triacylglycerols, carotenoids, polyhydroxyalkanoates, metal-based nanoparticles or antimicrobials) [2, 3]. The network and mechanisms of cooperating enzymes during bacterial lignin utilization and valorization have not been fully elucidated yet. To improve ligninolytic properties or increase biosynthetic capacity of any microbial strain, its metabolism must be well understood (for developing efficient genetic tools). In order to do so, our ultimate goal is to perform comparative proteomic analysis of selected lignin-grown *Rhodococcus* strains for finding new pieces for this large and complex lignin-utilizing metabolic puzzle.

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29. GROWTH AND MORPHOLOGICAL CHANGES OF *ESCHERICIA COLI* BL21 (DE3) IN THE PRESENCE OF MICROPLASTIC PARTICLES

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In recent years, microplastics (MP) have received considerable attention worldwide as one of the top four global threats. [1] The diversity of MP and the number of different microorganisms determine the complexity of the interaction between plastics and microorganisms. [2] While there is an increasing number of scientific studies related to the interaction between microplastics and microorganisms, the potential toxic effects of microplastics on microorganisms in the environment are still poorly understood.

In this study, the main focus is to determine whether different microplastic particles affect the growth, morphology of *E. coli* BL21 (DE3) bacteria, and what processes might contribute to these alterations. *E. coli* BL21 cells are cultured in M9 medium, and four types of microplastic particles (PS, PE, PVC, PP) with a diameter ≤ 500 µm are used. Samples are taken at each hour of bacterial growth for analysis of morphology, growth, pH changes and extracellular polymeric substances formation.

It can be concluded that microplastic particles did not alter the growth of *E.coli* BL21 cells so it might be said that no toxic substances for this bacteria were released from the microplastics that could disrupt cellular metabolic processes, and no other factors influenced the growth or inhibited it. During this study, no pH changes induced by microplastics were observed. Visualizing the morphology of *E.coli* BL21 revealed that microplastic particles promote greater bacterial accumulation, possibly due to the involvement of EPS in biofilm formation.

Quantitative analysis of protein and DNA content in the samples aimed to elucidate the potential influence of microplastic particles on extracellular polymeric substance (EPS) formation. Despite the initial hypothesis suggesting a correlation between microplastic presence and elevated EPS production due to greater bacterial accumulation, the obtained results revealed no significant alterations in protein or DNA quantities.

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30. *IN SILICO* ANALYSIS OF NOVEL MICROBIAL LIPOLYTIC AND LIPOLYTIC-LIKE ENZYMES

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Lipolytic and lipolytic-like enzymes, such as esterases, lipases, polyethylene terephtalate hydrolases (PET hydrolases), cutinases, and lipases are a biocatalyst group of undeniable importance to various uses in industry or science. Applications of these enzymes range from production of household chemicals to precise synthesis of different pharmaceutical compounds. Bacterial and archaeal lipolytic and lipolytic-like enzymes are thought to be a promising group of these biocatalysts for industry because of their high tolerance of various inhibitors, organic solvents, and ability to work at high temperatures and wide pH range [1]. All known lipolytic enzymes are characterized as having similar catalytic mechanism, similar tertiary structures and ability to hydrolyze similar lipid substrates [2].

During this study several putative protein sequences from free-access databases were selected. Firstly, the sequence had to be either annotated as or be highly similar to putative lipolytic or lipolytic-like enzymes; secondly, source of the sequence had to be derived from either bacterium, archaea or metagenomic sample; lastly, a requirement of novelty was set – sequence source had to be either uncharacterized or low characterized organism or metagenome. According to these requirements, five sequences were selected: *Umezawaea tangerina* cutinase, *Saliningranum rubrum* cutinase, *Candidatus Muproteobacteria* cutinase/PET hydrolase, esterase/lipase family protein from uncultured marine group II/III euryarchaeote KM3_69_A05, and hypothetical PET hydrolase from *Steroidobacter gossypii.* After selection, sequences were subjected to structure-predicting algorithms and aligned to sequences of well-characterized enzymes. After that, conservative regions and possible catalytic amino acid residues were determined. All collected data was then analyzed to gain primary confirmation of enzyme belonging to specified lipolytic enzyme group and function prediction.

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31. BIOCIDAL YEASTS INHABITING ROSEHIPS AND ROWANBERRIES

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Rosehip (*Rosa canina* L.) and rowanberry (*Sorbus* L.) are forest berries rich in antioxidants, phenolic compounds, organic acids, minerals, vitamins, fatty acids, and dietary fibers [1]. The consumption of forest berries addresses global food safety issues by controlling the spread of harmful microorganisms and viruses [2]. Various microorganisms, especially yeasts, act as biological control agents by producing protein toxins and other biological substances, influencing their survival, and thus determining the quality of plant-based food and human health [3].

Recently, there has been significant interest in yeasts with biocontrol properties, which are widespread among various yeast genera such as *Saccharomyces*, *Candida*, *Hanseniaspora*, *Pichia*, *Metschnikowia*, *Torulaspora*, and others [3]. The biocidal phenotype of yeasts can be encoded in the chromosome, viral double-stranded RNA (dsRNA), or plasmid DNA [4]. Those yeasts can compete with various microorganisms (bacteria, fungi, or protozoa) and control their growth [5]. Biocompatibility properties are applied in various fields, including environmental protection, medicine, industrial biotechnology, and agriculture [6,7].

The aim of this work was to examine which yeasts multiplied selectively from the surface of rosehips and rowanberries exhibit biocidal properties, evaluate their antimicrobial activity against control and other natural yeast strains, and determine the origin of biocidal phenotype. Different yeast strains belonging to *Metschnikowia pulcherrima, Torulaspora delbrueckii, Saccharomyces cerevisiae, S. paradoxus, Hanseniaspora uvarum, Pichia anomala,* and *P. kluyveri* species were isolated, and identified by applying molecular methods. A broad antagonistic activity of tested strains was investigated at different pH values. dsRNA was extracted from biocidal yeasts and profiling of viral killer systems was performed.

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32. CHARACTERIZATION OF CHITOSAN BIOPOLYMER ISOLATED FROM CELL WALLS OF SELECTED BASIDIOMYCETES

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The cell walls of filamentous fungi are mainly composed of different polysaccharides according to the taxonomic group. Basidiomycota contains fibrillar polymers chitin and β (1,3) – β (1,6) glucans and matrix polymers α (1,3) - glucans and xylomannoproteins [1]. Chitin makes up about 1–15% of the fungal cell mass with a higher concentration in the cell walls, and it supports the strength, shape, and integrity of cell structure [2]. Chitosan is produced by the deacetylation of chitin; in this process, some N-acetylglucosamine moieties are converted into glucosamine units. Commercial chitosan is mainly produced from the chemical deacetylation of chitin from crustacean sources [3].

This study aimed to obtain (1) the mycelial biomass from *Phanerochaete chrysosporium* in submerged cultivation, and (2) commercially cultivated fruiting bodies of *Agaricus bisporus* for chitosan extraction from the cell walls via chemical method in a two-step procedure. Subsequently, the extracted biopolymer was subjected to quality assessment using various analytical methods including elemental analyses (N content), Fourier transform infrared spectrometry (FTIR), X-Ray diffraction analysis (XRD) for crystallinity measurement, acid-base titration for determination of deacetylation degree (DD) and viscometry for determination of molecular weight.

Results showed that the dry mycelial biomass concentration of *P. chrysosporium* reached 1.03 g 100 mL^{-1} after 14 days of cultivation. The chitosan yield in the mycelium of *P. chrysosporium* was 0.38%, while in the fruiting bodies of *A. bisporus*, it was higher, reaching 1.7%. The nitrogen in isolated chitosan was over 6% both in the mycelium of *P. chrysosporium* and the fruiting bodies of *A. bisporus*. The FTIR spectra of chitosan extracted from both mycelium and fruiting bodies coincided with the spectrum of commercially produced chitosan (Sigma Aldrich). All characteristic peaks identifying chitosan were visible in the FTIR spectra. The crystallinity of chitosan was 63% in *A. bisporus* fruiting bodies and 34% in *P. chrysosporium* mycelium. The chitosan from *A. bisporus* exhibited higher crystallinity and a lower amorphous part, making it more durable but less flexible compared to the chitosan from *P. chrysosporium*. The DD in chitosan samples from *P. chrysosporium* mycelium and *A. bisporus* fruiting bodies was 71.8% and 63.4%, respectively, which closely approached the minimum values required for chitosan to be soluble in dilute acid. The molecular weight of chitosan samples ranged from 9050 g/mol (*A. bisporus*) to 10700 g/mol (*P. chrysosporium*).

In conclusion, the chitosan yield from the fruiting bodies of *A. bisporus* was 4.5 times higher than that obtained from the cultivated mycelium of *P. chrysosporium*. The analytical methods employed in this study effectively assessed the quality of the isolated chitosan, which demonstrated consistency with fungal chitosan. The leftover stalks from *A. bisporus* food production hold promise as a potential raw material for chitosan extraction. The extracted chitosan can serve as a functional biopolymer additive, offering an alternative to synthetic wet and dry strength agents in packaging materials.

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33. ANTIBACTERIAL ACTIVITY OF ENDOPHYTIC *BACILLUS* SPP. ON FOODBORNE PATHOGENS BACTERIA

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Endophytes are a group of microorganisms that colonize in different areas of plants, reside in healthy plants and do not cause a pathogenic effect, which is why such organisms are of increasing interest to scientists in different research areas [1]. Numerous studies have confirmed that endophytes play an important role in the synthesis of secondary metabolites for various commercial purposes [1-5]. Metabolic potential of new endophytes strainsis not thoroughly examined.

The aim of this work was to investigate the antibacterial activity of endophytic bacteria against the foodborne pathogenic bacteria strains *in vitro*.

The research objects were endophytic bacteria strains isolated from bilberry (*Vaccinium myrtillus* L.), cranberry (*Vaccinium oxycoccos*), lingonberry (*Vaccinium vitis-idaea*) leaves from locations in the Baltic-Nordic countries: *Bacillus halotolerans* Bil-LT1-1, *B. halotolerans* Bil-LT1-2, *B. velezensis* Cran-LT1-8, *B. amyloliquefaciens* Ling-NOR4-15 (accession numbers in NCBI database SAMN36292479 - SAMN36292482); reference bacteria strains *Listeria monocytogenes* ATCC 13932*, Bacillus cereus* ATCC 11778*, Salmonella. enterica* subsp. *enterica* serovar *Typhimurium* ATCC 14028*, Staphylococcus aureus* subsp. *aureus* ATCC 25923*, and Escherichia coli* ATCC 25922*.*

The antibacterial activity of endophytic bacteria was determined by agar diffusion method. *B. velezensis* Cran-LT1-8 showed the highest antimicrobial activity against the all tested pathogenic bacteria. The most sensitive to the antimicrobial activity of endophytic bacteria were *Staphylococcus aureus* subsp. *aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 13932. Our study showed the potent antibiotic activity of endophytic bacteria strains in food industry.

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34. USE OF MACROALGAE IN A THREE-STAGE BIOREACTOR FOR THE BIOGAS PRODUCTION

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An excess of macroalgae growing in water bodies is an environmental problem, as it is difficult to remove the excessive amounts of them in natural ways. It was observed previously that mixing macroalgae with the other organic substrates can produce high yields of biogas (mostly methane). *Cladophora glomerata*, macroalgae growing in the Lithuanian freshwaters, was used for this purpose. They were mixed with cattle manure and an activated sewage sludge as source of microorganisms for the biogas production in a three-stage bioreactor. Bradford, Folch or Dinitro Salicylic Acid (DNS) methods were used for the determination of concentrations of total proteins, lipids, and glucose (reduced sugars), respectively. Concentrations of those were determined at different bioreactor organic loads: 2.87, 4.06 and 8.13 kg VS/m^3 d. The highest total lipid concentration (6.69%) was reached when the organic load was 8.13 kg VS/m^3 d. The average biogas yield at this load was 198.7±2.2 L/Kg VS_{added}, and the methane yield was 154.1±5.4 L CH_4/Kg VS_{added}. Concentration of reduced sugards decreased from 9.68 to 6.67% during this proccess indicating that some of it has been consumed by microorganisms. The lowest total protein concentration (1.12%) was found when the organic load of the bioreactor was 4.06 kg VS/m^3 d. The decreased concentration of total proteins showed the activity of microorganisms during which the yield of biogas and methane increased to 386.4±3.8 L/Kg VS_{added} and 281.5±8.2 L CH_4/Kg VS_{added}, respectively. Our studies have shown that the yield of biogas and methane in a three-stage bioreactor depends on the organic load of the bioreactor. Such dependence was determined by the concentration of glucose, fat and protein in the substrate.
This project has received funding from the Research Council of Lithuania (LMTLT, grant number S-LU-22-2) and Ministry of Education and Science of Ukraine.

35. ALPHA-L-FUCOSIDASES: AN ALTERNATIVE BIOCATALYST FOR THE SYNTHESIS OF FUCOSYLATED OLIGOSACCHARIDES

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α-L-Fucosidases are enzymes that naturally catalyse the hydrolysis reaction of the glycosidic linkage between various oligosaccharides and fucosyl residues. They can also catalyse glycosidic bond synthesis reactions after altering reaction conditions, such as varying substrates, organic compounds, and salt concentrations. This unique capability of α-L-fucosidases has significant potential in synthesising fucosylated oligosaccharides, a major component of oligosaccharides found in human milk and highly beneficial in infant milk formula and functional foods. Studies have shown that fucosylated oligosaccharides possess antiviral, antimicrobial, and immune response-modulating functions. Despite their importance, sustainable methods to synthesise these stereospecific compounds in high yields are lacking. Enzymatic methods for synthesising these compounds could offer a more sustainable and stereospecific advantage. This study introduces five bacterial α-L-fucosidases selected via metagenomic library screening. We analyzed the sequences and structures of these enzymes using bioinformatic methods. Finally, we evaluated the capability of the α-L-fucosidases to perform the trans-fucosylation reaction, resulting in oligosaccharide compounds like those found in human milk. We also investigated the products of trans-fucosylation reactions using TLC and HPLC-MS techniques. Our findings offer valuable insights into utilising α-L-fucosidases to synthesise fucosylated oligosaccharides.

36. ALTERATIONS OF THE SALIVA MICROBIOTA IN PATIENTS WITH PERIODONTAL DISEASES

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Periodontitis is one of the most prevalent inflammatory diseases among humans [1]. While certain microorganisms have been linked to the pathogenesis of periodontal diseases, it is now clear that these illnesses are caused by dysbiosis [2][3]. The present study aimed to describe the oral saliva microbiome diversity in periodontal disease patients.

Unstimulated whole saliva sialometry and clinical examination were done for 34 patients with salivary flow disorders. The human oral metagenomic DNA from saliva samples was isolated and the metagenome sequencing of the V3-V4 regions of 16 rRNA was conducted by Novogene Company (UK).

Patients were assigned into 3 groups according to their periodontal status using the Community Periodontal Index (CPI code: 1 = bleeding on probing; 3 = periodontal pocket (PD) 4-5 mm; 4 = PD 6+ mm). Of the 34 participants 62% had periodontitis (CPI code 3 + 4) and 38% had gingivitis (CPI code 1). Metagenome sequencing data disclosed that community diversity in the saliva of the CPI 3 group was lower compared to the CPI 1 and CPI 4 groups. At the phylum level, *Firmicutes* were predominant across all groups, with CPI 3 group showing particularly high dominance. Patients with gingivitis had a higher abundance of *Proteobacteria* and *Fusobacteriota* in the phylum level, whereas *Bacteroidota* were more abundant in the CPI 4 group. The genera *Aggregatibacter*, *Haemophilus*, *Actinobacillus* were more abundant in the CPI 3 group, while *Pseudomonas* and *Neisseria* were associated with gingivitis. *Streptococcus*, *Prevotella_7* and *Rothia* were the most frequent genera in all groups. *Leptotrichia* was enriched in CPI 1 group. In the CPI 3 group, the genera *Streptococcus*, *Haemophilus* and *Porphyromonas* were most predominant. Conversely, the CPI 4 group showed higher of *Prevotella_7, Veillonella, Fusobacterium* and *Prevotella* compared to other groups. Nonetheless, both periodontitis groups were related to higher proportions of genera *Porphyromonas* and *Treponema*.

In summary, this study revealed moderate alterations in the salivary taxonomic composition across various periodontal disease states. CPI 3 group exhibited lower microbial diversity and were positively correlated with periodontal pathogens, including *Porphyromonas* and *Treponema*.

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37. DEGRADATION CHARACTERISTCIS OF TECHNICAL LIGNINS BY *THERMOBIFIDA SPECIES*

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Lignocellulosic biomass (LCB) is an abundant raw material available on Earth composed of an aromatic biopolymer called lignin linked by covalent and non-covalent bonds with cellulose and hemicellulose [1]. The recalcitrant nature of LCB is mainly imposed by the lignin which is the main challenge for its efficient degradation and utilization [2]. Hence, there is a need to develop new technologies that utilize LCB/lignin as a raw material. Despite the fact that fungi are most efficient lignin degraders, the ligninolytic bacteria are more adaptable than fungi, as they are highly tolerant to diverse environments and susceptible to genetic manipulation [3]. Gram-positive bacteria belonging to *Thermobifida* genera are important degraders of LCB and produce a repertoire of lignin degrading enzymes [4].

We perform flask experiments to test the ability of two *Thermobifida* species to utilise technical lignins (organosolv, kraft and hydrolysis) from different biomasses (hardwood and grass). Our goal is to characterize the lignin depolymerization and utilization products as well as to decipher the cocktail of enzymes responsible for the utilisation of lignin derived aromatics. Size exclusion chromatography (SEC) and gas chromatography-mass spectrometry (GC-MS) are performed to evaluate the changes in lignin mass distribution and composition of aromatic compounds upon microbial treatment. The secreted cocktail of ligninolytic enzymes will be identified using comparative proteomics.

Both bacterial species tested were able to grow on lignin containing defined minimal medium supplemented with growth inducer. SEC results revealed differences in lignin molecular weight distribution in microbial treated samples. Bacterial treatment of lignin produced low molecular weight aromatic compounds such as 4-hydroxybenzoic acid, vanillic acid, and 4-coumaric acid compared to abiotic samples detected by GC-MS. Enzymes from extremophiles, such as *Thermobifida* species, hold a potential for bioremediation or degradation of lignin-derived compounds.

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38. PURINE DEPLETION PROMOTED OXIDATIVE STRESS TOLERANCE DOES NOT DEPEND ON MITOCHONDRIAL FUNCTIONS

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Understanding the physiological and metabolic responses of auxotrophic microorganisms is object of increasing interest in microbial research. Among auxotrophic phenotypes, adenine auxotrophy exhibits unique characteristics - cells demonstrate an organized response to purine starvation, probably similar to nitrogen or carbon starvation. Moreover, our surprising findings indicate that the ability to withstand various stresses following purine starvation persists even in petite cells.

In this study, we employed purine auxotrophs with mutations in the adenine biosynthesis pathway, with and without the additional petite phenotype. We investigated the effects of short-term purine and nitrogen starvation on yeast fitness, as well as acute and chronic stress responses. Our results highlight the importance of the location of knock-out mutations within the adenine synthesis pathway, shedding light on the critical factors influencing cellular response to purine scarcity. Furthermore, we explored the involvement of mitochondrial metabolism in the intracellular response to purine starvation. Both petite and non-petite cells exhibited enhanced stress resistance and varying degrees of adaptability to challenging environments. While the precise mechanisms by which cells signal purine starvation remain elusive, our data provides significant clues regarding the underlying processes.

By employing a combination of different experiments and approaches, we uncover the intricate interplay between purine metabolism, mitochondrial activity, and stress resistance in adenine auxotrophic yeast. These findings not only expand our understanding of cellular responses to nutrient scarcity but also have implications for the evolutionarily conserved nature of purine starvation response mechanisms.

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39. ANALYSIS OF THE RECOMBINANT ALLERGEN COMPONENT BET V 4 PRODUCED IN DIFFERENT PROTEIN EXPRESSION SYSTEMS

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Allergy is an exaggerated response of the immune system to otherwise harmless substances present in the environment [1]. Results of the previous statistical researches show that the prevalence of allergies varies between different countries from 20% to 40% [2]. According to the World Health Organization, by the year 2050 one out of two people worldwide will suffer from allergies [3]. Accurate diagnostic tests are needed for precise diagnosis and efficient treatment. Allergens used for diagnostic tests and immunotherapy are produced from natural sources and used as whole-allergen extracts. Standardization of these extracts is difficult and their usage can lead to false positive/negative results and ineffective treatment [4]. Single recombinant allergen components can be used in allergy diagnostics and help to overcome these problems.

Escherichia coli is often used for the production of the recombinant allergen components. It is easy and inexpensive to cultivate and usually produces high yields of recombinant proteins in short time. However, some allergen components could not be produced in *E. coli* because of the lack of post-translational modifications and production of misfolded proteins. Yeast expression system has the advantages of prokaryotes and eukaryotes – easy and inexpensive cultivation, high yields of recombinant proteins and most of the eukaryotic post-translational protein modifications. Plants also provide an alternative option for producing recombinant allergen components, particularly those that originate from plants.

In the present study, allergen component Bet v 4 from the European white birch (*Betula verrucosa/Betula pendula*) was analyzed. Single allergen component Bet v 4 and Bet v 4 fused with maltose binding protein (MBP) were produced. Recombinant proteins were synthesized in *E. coli*, *Nicotiana benthamiana* and different yeast expression systems. Proteins were purified using Ni^{2+} chelate affinity chromatography and their antigenicity was tested.

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40. DETERMINATION OF FUNGISTATIC AND ANTIBACTERIAL PROPERTIES OF BIO-TEXTILES WITH AMBER PARTICLES

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The substances in the composition of bio-textile provide a good source of nutrients for the various microorganisms, which can lead to external environmental conditions harmful to human health, therefore, there is a wide demand for innovative bio-textiles with antimicrobial properties in various industries. Amber found in the Baltic region is attributed with health-promoting properties, the bioactive substances in the composition, such as succinic acid, are known as a natural antibiotic and immunity booster [1,2]. The presence of amber particles has proven that they do not cause allergic symptoms to the user and have a beneficial effect on skin cell regeneration [3]. Amber is considered a potential protective agent. Micro- and nanoparticles of amber and silica dioxide are being studied as potential row material for integration into bio-textile fibbers.

The aim of the study was to determine the fungistatic and antibacterial properties of new innovative bio-textiles. Fabric stripes of raw materials produced by A/B Linas (LT) & JLU Technologies Ltd. (LV) was tested.

Fungistatic properties were tested according to modified ISO 14119:2003 method (A1 and A2) [4] and fungal isolates *Aspergillus niger* from the Collection of Microorganism Cultures of Latvia (LMCC 324), *Chaetomium cochliodes* (LMCC 1536), *Chaetomium globosum* (ATCC® 6205TM) were used.

Control fabric and amber bio-textile with added silicon dioxide (SiO_2) nanoparticles (1, 2, 3%) were used in experiments to determine the antibacterial activity according to ISO 20743: 2013 [5]. Bacterial cultures: *Enterococcus faecalis* (ATCC®29212TM), *Escherichia coli* (ATCC® 25922TM), *Staphylococcus aureus* subsp. *aureus* (ATCC®25923TM), *Klebsiella pneumoniae* (ATCC® 700603TM) were used in tests.

Amber-containing bio-textiles do not show significant resistance to microscopic fungi. Amber bio-textile shows statistically significant antibacterial properties ($P < 0.05$). The addition of silicon dioxide nanoparticles (20 nm) shows significant antibacterial activity on gram-negative *Klebsiella pneumoniae*, and also on gram-positive *Staphylococcus aureus* ($P < 0.05$). Negative corelation among size of nanoparticles and amount of colony forming units were found.

The study was financially supported by the EUREKA project E!11170 — Innovative multifunctional bio-textile, integrated with silica dioxide and succinate development, and its impact on biosystemsll (IFSITEX!).

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41. EFFECT OF DIFFERENT LASER WAVELENGTHS ON SELECTED BACTERIAL STANDARD STRAINS

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The aim of the presented studies was to check the potential bacteriostatic or bactericidal effect of irradiation with visible laser light on the standard bacterial strains: *Bacillus subtilis* ATCC 6633*, Escherichia coli* ATCC 25922*, Enterococcus faecalis* ATCC 29212*, Klebsiella pneumoniae* ATCC 13883*, Staphylococcus aureus* ATCC 25923*, Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas fluorescens* 13525*.*

Sterile 96-well microtiter plates fulfilled with labeled suspension of bacteria were used to determine the antimicrobial activity of laser light at the wavelengths 420 nm, 535 nm, 635 nm, and 670 nm. After 9 and 18 hours of incubation at 37°C, the turbidance values were determined spectrophotometrically. Irradiation with blue light (420 nm) leads to a bacteriostatic effect observed in case of *E. coli, K. pneumonia* and *S. aureus*, whereas a bactericidal effect is noticed for *B. subtilis, P. aeruginosa* and *P. fluorescens*. Green light (535 nm) is bactericidal only on *B. subtilis*. The exposition to red light 635 nm and 670 nm has no bacteriostatic potential.

The bacterial strains sensitive to blue light possess endogenous pigments such as zeaxanthin, astaxanthin, β-carotene, staphyloxantin, pyoverdin, pyorubin and melanin. Bacterial strains sensitive to green light have phenazine, violacein, anthocyanins, and/or also melanin [1]. Additionally, the light perception is achieved by photoreceptor proteins. Some species of bacteria have evolved the capability to respond to a proper light wavelength [2]. The observed effects result from the presence of the bacterial pigments and the bacterial sensitivity to the proper light wavelength

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42. A STUDY ON ANTIBIOTIC RESISTANCE GENE ABUNDANCE IN LANDFILL LEACHATES

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As a consequence of the prevalent utilization of antibiotics, bacteria are progressively developing an enhanced resistance towards these pharmaceuticals, posing a significant hazard to public health. In this regard, an important area of research focuses on the routes by which anthropogenic sources produce antibiotic resistant genes (ARG). Because of the diversity of the sources and the paucity of information at the genetic, biochemical, and community levels, assessing the risks posed by antimicrobial resistance (AMR) in the community is currently difficult [1]. In the context of AMR, it is important to assess the relative contribution of the environment versus the impact of various stress-inducing elements (such as bacteriophages, biocidal agents, heavy metals, detergents, pesticides, temperature, pH level, and others) [2].

Leachate from landfills is thought to be a substantial point source of pollutants, especially for surface and ground waterways that could be hazardous to the environment. The main goal of our work was to characterise the leachates from the Getliņi municipal solid waste landfill, which is managed by Getlini EKO Ltd. (Riga, Latvia). Leachates were tested in terms of micropollution, the quantity of ARGs, the composition of the microbial community, and the potential for AMR induction.

Among the pharmaceutical compounds detected in leachate, ibuprofen and diclofenac were the most abundant, i.e., 13.13 and 6.08 µg/L, respectively. The taxonomic profile of leachate has revealed a predominance of Proteobacteria (53.32%), followed by Firmicutes (10.59%) and Euryarchaeota (9.37%). In the context of AMR, the species/genus/families from the list of antibiotic-resistant “priority pathogens” [3] have been selected from the sequencing results. The most abundant family from this list was *Enterobacteriaceae* (1.32%). In the metagenomic analysis, 31 distinct families of ARG were identified. The minimum inhibitory concentrations (MIC) towards antibiotics of culturable bacteria was evaluated by MIKROLATEST MIC® (ERBA LACHEMA) and Sensititre™ (Thermo Fisher Scientific). The resistance of culturable bacteria in the leachate towards amikacin, aztreonam, cefepime, colistin, polymixin B, cefotaxime was detected. Incubation of bacterial cultures in the sterilized leachate resulted in decreasing MIC towards several antibiotics compared with the control.

Further study is needed to assess any potential risks related to the ARGs dissemination from landfills via hydrological processes, e.g., runoff and infiltration.

Acknowledgments. The study was supported by the “State research project in the field of biomedicine, medical technologies and pharmacy” VPP-EM-BIOMEDICĪNA-2022/1-001 (Y3-VPP32f-ZR-N-090); „Optimization of biotechnological processes for effective utilization of renewable resources” Y5-AZ20-ZF-N-270; SAM 8.2.2. The third round project “Strengthening the capacity of the doctoral program of the University of Latvia in the framework of the new doctoral program model”.

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43. ANTIFUNGAL ACTIVITY OF *BACILLUS SUBTILIS* AND *BACILLUS AMYLOLIQUEFACIENS* AGAINST PHYTOPATHOGENIC FUNGI

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The aim of the study was to evaluate the antifungal activity of *Bacillus subtilis* and *Bacillus amyloliquefaciens* against phytopathogenic fungi under *in vitro* conditions. The loss of agricultural products due to phytopathogenic micro-organisms causing plant diseases necessitates the evaluation of the inclusion of bacteria in the formulation of biofertilisers and the feasibility of the substitution of chemical fungicides [1].

The study was carried out using two methods. The disc method, where agar discs derived from bacterial and fungal pure cultures were plated on Malt Extract Agar media, and the agar diffusion method, where the culture liquid of individual bacterial species and their consortia and a suspension of bacterial cultures inoculated in fungal pure cultures were used. The bacteria used were from the Microbial Strain Collection of Latvia; *B. subtilis* MSCL 897 and *B. amyloliquefaciens* MSCL 1635, and the phytopathogenic fungi; *Pyrenophora tritici-repentis* MSCL 1625, *Fusarium graminearum* MSCL 435, *Cladosporium herbarum* MSCL 276, *Alternaria tenuis MSCL* 280 and *Microdochium nivale* MSCL 437.

The highest antifungal activity of *B. subtilis*, *B. amyloliquefaciens* and *B. subtilis* + *B. amyloliquefaciens* culture liquids and bacterial culture suspensions was found against *P. tritici-repentis*, *F. graminearum* and *A. tenuis*, and *B. subtilis*, *B. amyloliquefaciens* and *B. subtilis* + *B. amyloliquefaciens* cultures against *A. tenuis* (Fig 1). The most effective control of fungal phytopathogens was achieved by agar diffusion.

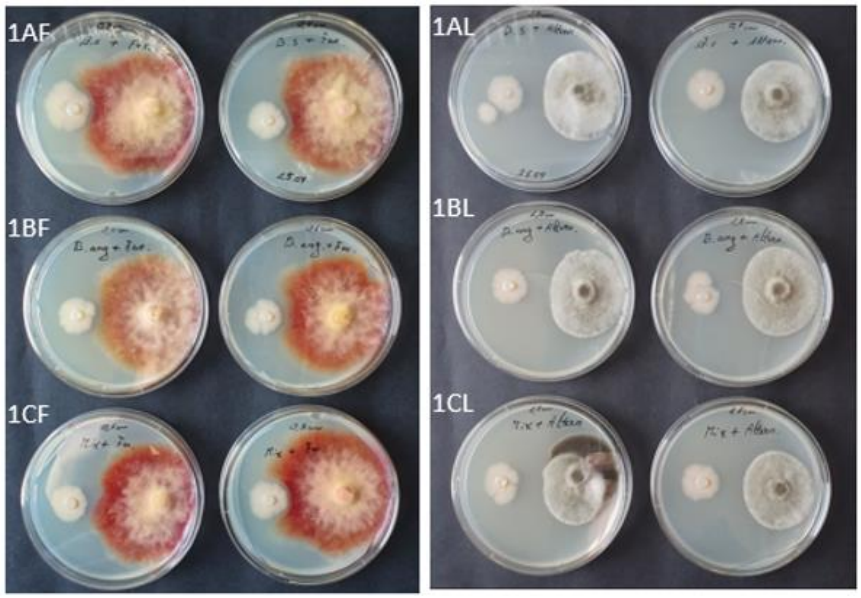

Figure 1. Inhibition zones of *B. subtilis*, *B. amyloliquefaciens* and *B. subtilis* + *B. amyloliquefaciens* against phytopathogenic fungi. 1A - *B. subtilis*, 1B - *B. amyloliquefaciens*, 1C - *B. subtilis* + *B. amyloliquefaciens*; F – *F. graminearum,* L – *A. tenuis* after 10 days; obtained in duplicate.

Acknowledgments
Supported financially by the project “Strengthening the doctoral capacity of the University of Latvia within the framework of the new doctoral model”, project identification No. 8.2.2.0/20/I/006, UL registration No. ESS2021/434, co-financed by the European Social Fund.

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44. EXPERIMENTAL EVOLUTION OF *ESCHERICHIA COLI* ATCC 8739 ON SOLID SILVER, COPPER, STAINLESS STEEL, AND GLASS SURFACES

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Despite perceived efficacy of copper and silver surfaces their actual bactericidal activity is highly dependent of environmental conditions [1]. The mechanism of action of metallic antimicrobials is expected to reduce the risk of developing resistance but effects of stress on the survivors are less studied and the resulting genetic changes could have undesirable consequences.

In this study a standard strain of *E. coli* was repeatedly exposed to silver, copper, stainless steel, and glass surfaces in two conditions simulating real life contamination scenarios. Retrieved survivors were propagated clonally on solid medium to avoid competition and selection for growth speed. After 30 exposure cycles changes in genetic material, motility, biofilm formation, metal tolerance and antibiotic resistance of the evolved populations were studied.

No differences in accumulation of single nucleotide variations (SNV) or larger genomic rearrangements on different surface materials were observed indicating lack of surface-specific emergence of stable hypermutator phenotypes in the evolved populations. A higher number of SNVs were detected in low-organic dry compared to high-organic humid conditions in general. Despite no surface-specific differences, a higher number of genomic rearrangements was detected in high-organic humid compared to low-organic dry conditions in general.

Due to the relatively wide biocidal bottleneck in exposure cycles most of the accumulated mutations were not fixed complicating interpretation of quantitative bioassays. No significant changes in swimming motility, biofilm formation nor consistent larger than two-fold differences in metal tolerance or antibiotic resistance profiles were observed in evolved populations compared to the ancestral strain.

To quantitatively evaluate effects of individual non-fixed mutations, selected mutations (e.g., in cellular barrier functions and efflux) were re-introduced into the ancestral strain and will be further characterized.

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45. CHARACTERISTIC OF VIRULENCE FACTORS AND ANALYSIS OF BIOFILM PRODUCTION OF SALMONELLA SPP. STRAINS ISOLATED FROM WILD SNAKES IN POLAND

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Zoonoses are healthcare problem worldwide. *Salmonella* spp. are one of the zoonotic agent isolated from animals and the most frequent causative agent of intestinal infections in Poland [1, 2]. Reptiles are a reservoir of *Salmonella* serovars which can cause RAS (*Reptile-associated salmonellosis*). There is still a need to expand the data on epidemiology of RAS and on the microbiota of free-living reptiles in Poland and Europe.

Ability to form biofilms is one of the virulence factors of *Salmonella* spp. The main components of ECM (extracellular matrix) of *Salmonella* spp. biofilm are curli fimbriae and cellulose [3]. We indicate temperature and nutrient availability as determinants of phenotype switching between biofilm and planktonic lifestyle and we indicate biofilm as a factor that enables asymptomatic colonization by strains remain virulent in planktonic phase.

The total of 57 *Salmonella* spp. strains were collected from wild *Natrix natrix* (NN), *N. tessellata* (NT), *Coronella austriaca* (CA) and *Zamenis longissimus* (ZL) and identify by MALDI TOF MS. We used PCR, bactericidal action of 50% normal human serum (NHS) tests and analysis of biofilm with Congo Red Agar, static cultivation and Bioflux 1000 system (microfluidic conditions). Increased production of bacterial biofilm at both room temperature and 28°C and in nutrient-poor media has been demonstrated. Rugose morphotype in 28°C, saw morphotype in 37°C and production of biofilm ECM components in microfluidic conditions were observed (Fig. 1). The presence of virulence genes *invA, sipB, prgH, span, orgA, tolC, iroN, sitC, sifA, sopB, spiA, cdtB, msgA* and the resistance to NHS action were also detected.

The study confirmed that snakes in Poland are a source of potentially pathogenic *Salmonella* spp. and may be considered as a part of the epidemiological chain of RAS infections in Europe. The study also demonstrated influence of environmental conditions on the ECM production of exotic *Salmonella* serovars.

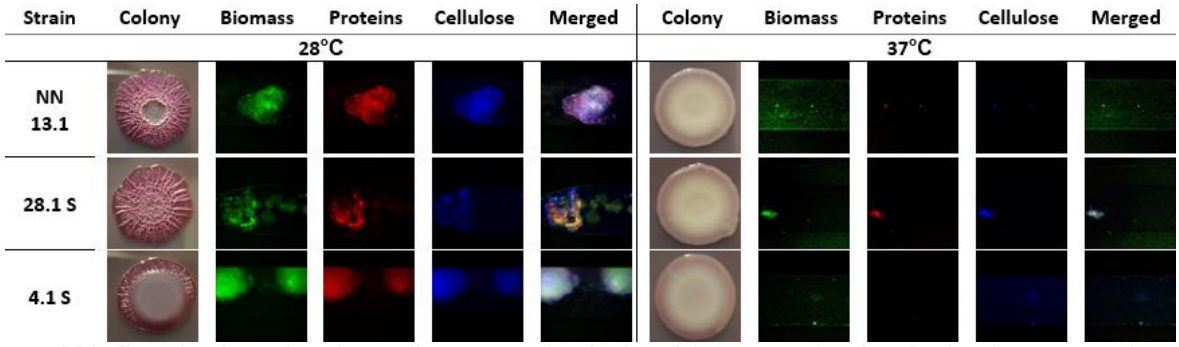

Figure 1. Colony morphotype and biofilm of selected *Salmonella* spp. strains isolated from NN after incubation in 28°C and 37°C in Luria-Bertani Broth with microfluidic conditions, stained by SYTO9 (green), CALCOFLUOR WHITE (blue) and SYPRO RUBY (red) to indicate the bacterial biomass, cellulose, and proteins, respectively.

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46. INVESTIGATION OF TAILSPIKE PROTEIN GP45 FROM *AEROMONAS* INFECTING PHAGE VB_AVES_KLEA5

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Due to the rising prevalence of bacterial resistance to antibiotics, it is crucial to explore alternative approaches for combating them. One potential option is to use bacteriophages and their components as alternative method, although, not enough research has been done in this area. Therefore, we aimed to study a specific *Aeromonas* infecting phage vB_AveS_KLEA5 (KLEA5) by investigating the probable depolymerase-like activity of its tailspike protein gp45 *in vitro*.

Utilizing bioinformatics analysis, it has been determined that the tailspike protein, designated as gp45, lacks matches within publicly available databases. Notably, the highest similarity (29.55%) is found in *Dompsiwa* phage TSP7_1's tailspike protein. Furthermore, analysis of the primary structure of gp45 homologues revealed the plausible presence of a conserved Tail_spike_N domain. This domain, frequently identified within receptor-binding proteins, might potentially harbor depolymerase functionalities, thereby playing a crucial role in the recognition of bacterial cell surface elements. These findings highlight the unique properties of gp45 associated with depolymerase-related characteristics, which calls for a thorough follow-up investigation. To study the potential activity of gp45 *in vitro*, the *g45* of KLEA5 phage has been successfully cloned into two inducible vectors, pET21a and pCDF (with His-Tag attached to the C- or N- terminus, respectively), for expression in *E. coli* BL21 (DE-3). Following purification, the resultant proteins were subjected to analysis via SDS-PAGE, a methodology that confirmed the presence of soluble proteins, characterized by an approximate molecular weight of 70 kDa. To explore the functional capabilities of the recombinant gp45, a spot test on double-layer agar employing the *Aeromonas veronii* strain KR2-5 as a substrate, was executed. Post a 20-hour incubation period maintained at a temperature of 22 C˚, visible turbid zones were observed as a hallmark indicative of depolymerase activity. Encouragingly, both gp45N-his and gp45C-his variants elicited the formation of such turbid zones, thus corroborating their inherent depolymerase attributes. An investigation extends into the interplay between gp45 and cellular components, specifically investigating the potential interactions with cell surface receptors. Building upon these promising findings, the trajectory of this research encompasses refinement of experimental protocols and conditions, thereby leading up to a better understanding of gp45's functional role.

The outcomes derived from this study not only extend our knowledge about *Aeromonas*-infecting bacteriophages, but also offer innovative perspectives to their plausible therapeutic and biotechnological applications for combating bacterial infections.

47. ANTIMICROBIAL PROPERTIES OF Fe_2O_3 PARTICLES DEPOSITED ON A TEXTILE

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Recent pandemics, reduced development of new antibiotics and increasing number of deaths caused by resistant bacteria mean that scientists have to rethink alternative measures to fight antimicrobial resistance [1]. Agents of nosocomial infections are of particular importance and therefore, measures to reduce their survival rate on different surfaces should be encouraged [2]. The aim of this study was to investigate the antimicrobial effect of textile coated with Iron Oxide (Fe_2O_3) nanoparticles (NP) using non-thermal plasma deposition approach. Surface morphology measurement was performed with SEM (Hitachi S-3400 N) using a secondary electron detector. Energy dispersive X-ray spectroscopy (Bruker Quad 5040) was used for the analysis of elemental composition and elemental mapping of NP coated samples.

Iron Oxide NP's were deposited on both sides of the surface of cotton textile (dimensions: 12 cm × 15 cm) using a physical vapour deposition system. A pulsed-DC power source (P = 200 W) was used for plasma generation. During the deposition process, 99.999% purity oxygen was supplied into the vacuum chamber to maintain a constant pressure of 40 Pa. The distance between the Fe electrode (dimensions 12 cm × 15 cm; 99.99% purity) and the sample was 5 cm and NPs were deposited varying deposition time up to 120 min.

Pathogenic bacteria and yeasts were used to evaluate antimicrobial activity of the coated textile. The coated cotton was aseptically cutted into 10x10 mm squares on which 50 µL of 0.5 McFarland density (~1.5 × 10^8 CFU/mL), microbial suspensions were placed with exposure of 60 minutes in enclosed Petri dishes at room temperature. The textile squares then were placed into tubes with isotonic solution and mixed for 20 seconds with tube shaker. Thereafter, serial dilutions were performed for counting of alive bacteria by overnight incubation on Mueller Hinton Agar II (Thermo Fisher, UK). Uncoated textile was used as a control.

The results of antimicrobial activity of the textile coated by Fe_2O_3 NPs are presented in Figure 1.

Control	*100*
E. cloaceae	*15,2*
S. enterica	*58,1*
C. freundii	*0,01*
A. hydrophila	*0*
A. baumanii	*0*
P. aeruginosa	*27,3*
K. pneumoniae	*0*
S. aureus	*0*
S. haemolyticus	*0*
E.faecalis	*0*
E. faecium	*4*
C. albicans	*4,6*

Figure 1. Antimicrobial activity spectrum of the textile coated by Fe_2O_3 NPs. A total numbers of alive microorganisms on textile (%) in comparison with uncoated textile (control, growth rate 100%).

The obtained results demonstrated that textile coated by Fe_2O_3 NPs were able to inactivate all tested microorganisms. In total, 100 percent of inactivation was towards staphylococci (including MRSA), *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Acinetobacter baumanii* and *Aeromonas hydrophila*. Less effect it has against *Salmonella enterica*, *Pseudomonas aeruginosa* and *Enterobacter cloaceae* (41.9%, 72.7% and 84.8 % of inactivated bacteria respectively). Coated textile was also effective against yeasts (95.4% of inactivation).

According to the data obtained it may be concluded that textile coated by Fe_2O_3 NPs is active against wide spectrum of bacteria, but further research is needed by using larger numbers of different strains of the pathogens tested.

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48. ABUNDANCE OF CULTURABLE FUNGI IN THE MINERAL SOIL OF FOURTEEN DIFFERENT TREE SPECIES PLANTATIONS

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Exploring the link between above- and belowground biodiversity has been a major theme of recent ecological research, due in large part to the increasingly well-recognized role that soil microorganisms play in driving plant community processes [1].

The aim of this study was to investigate the abundance of culturable fungi in the upper layers (0-5 cm) of mineral soil of homogeneous plantations in 7 native and 6 alien trees monocultures. The study was carried out in central Lithuania (54°53′ N, 23°49′ E) in the hemiboreal zone of European transitional deciduous mixed forests [2], in the first week of January, March, May, July, September and November. The age of the studied plantations was 60 years and the area of the stands varied from 0.14 to 0.31 ha. The soil serial dilution method was used.

The highest fungal abundance was found in the soil of the studied plantation in early spring (March) and late autumn (November). The lowest abundance of fungi was found at the end of summer, influenced by the dry weather in August. The most abundant fungi were in soil of plantations generating the highest mass of annual tree litter, like *Thuja occidentalis*, *Aesculus hippocastanus* or *Larix eurolepis*, while the least were found in soil of fast-decaying litter tree plantations*: Tillia cordata*, *Alnus glutinosa* and *Sorbus intermedia*.

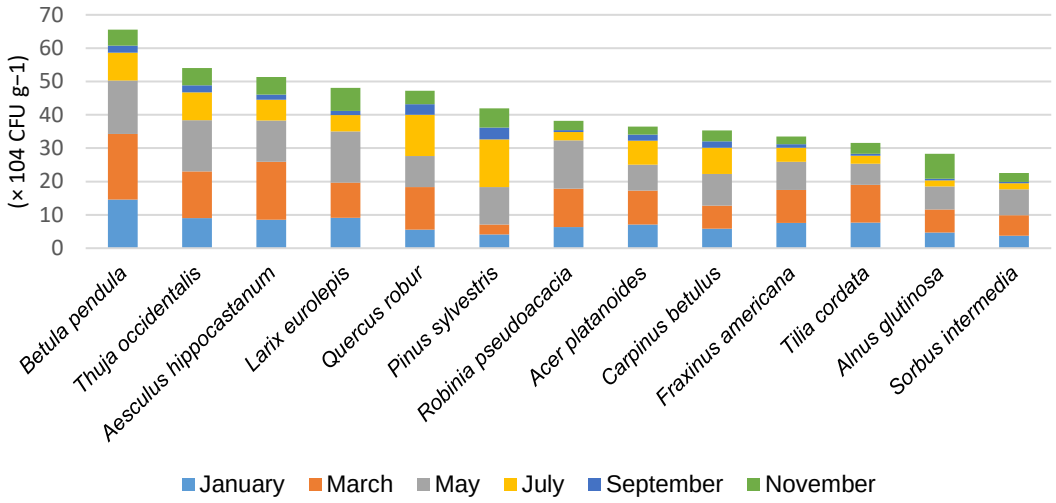

Figure 1. Total number of fungal colony-forming units (× 10^4 CFU g^{-1}) in plantation mineral soil layers under different tree species.

Betula pendula unexpectedly had the highest fungal number among the plantations studied, although compared to other trees it has a low mass of annual litter and relatively high litter degradation rate. The high fungal community found in the *B. pendula* plantation soil may be due to specific relationships with other soil micro-organisms and the biochemical composition of the litter [3].

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49. PILOT-SCALE BIOPROCESS INVESTIGATION FOR *BACILLUS SUBTILIS* MSCL 897 SPORE PRODUCTION

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Bacillus subtilis (Bs) is an endospore-forming bacterium that benefits plants and is used in the production of plant biostimulants and biofungicides [1]. Endospore production in Bs cultivations is a crucial step in ensuring the desired quality traits and economic feasibility of the microbial preparation. In a series of shake flask [2] and 100 L bioreactor (Fig. 1) experiments, we investigated the economically feasible broth composition for spore production of *B. subtilis* MSCL 897, a Latvian soil isolate. High spore yield is also a prerequisite for secondary antifungal metabolite production, being an important aspect in biocontrol product (biofungicide) formulation. We studied the effects of legume-based flours (broad bean, grey pea, and soy) as the main source of nitrogen; sugar-beet molasses, sucrose, or glucose as the carbon source; yeast extract, peptone, and corn steep liquor as additives providing growth factors; and $(NH_4)_2HPO_4$ or urea as extra nitrogen sources on spore yields. Our results show that a culture medium composed of broad bean flour (10 g/L), molasses (10 g/L), and minor yeast extract or corn steep liquor (0.5 g/L) additive led to high spore productivity of about 2.0±0.2 x10^9 CFU/mL at 48 h (Fig. 2-3) and a sporulation efficiency >80-90 %. Antifungal properties against *Cladosporium herbarum* (Fig. 4), *Fusarium graminearum*, *F. culmorum*, *Aspergillus niger* and *Alternaria alternata* plant pathogens were also detected. The authors conclude that investigated low-cost medium made mainly from locally (temperate climate zone) available and renewable food- and feed-grade ingredients can be further evaluated for use in *B. subtilis* MSCL 897 biostimulant and biofungicide production.

Figure 1. 100 L bioreactor setup.

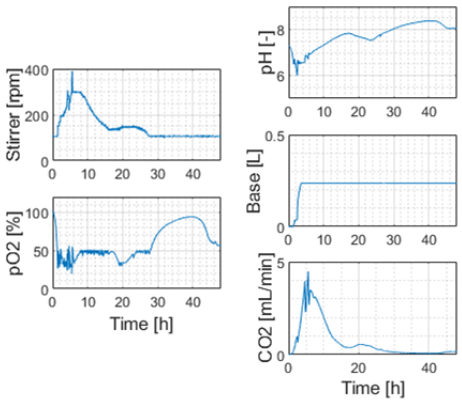

Figure 2. Successful sporulation reflected by active respiration (stirrer rate and off-gas CO_2 parameters) and metabolism (base-alkali consumption parameter).

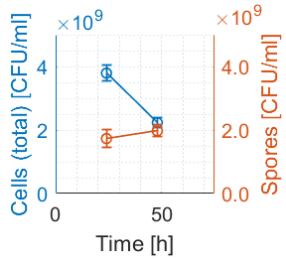

Figure 3. Total cell number and spore counts.

Figure 4. Anti-fungal activity of cell-free Bs cultivation broth exhibited against *C. herbarum* plant pathogen.

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50. APPROBATION OF MICROALGAE CULTIVATION IN CLOSED AQUACULTURE SYSTEMS AND EVALUATION OF THEIR EFFICIENCY IN FISH FEED

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Currently aquaculture market is valued at approximately 289.6 billion USD (2022) and is projected to reach estimated value of 421.2 billion USD by 2030 at a compound annual growth rate of 5.5% over the eight-year period. However, several problems remain prevalent in aquacultures, e.g. overfishing due to production of fish feeds, environmental pollution, fish heath and malnutrition, product quality, that may significantly affect the development of this area. Among several innovative approaches, that can mitigate existing problems, fish feed supplementation with microalgae biomass attracts high interest [1]. In recent years it was established that fish feed supplementation with microalgal biomass at moderate concentrations, usually 5 – 15 %, can provide required nutrients, e.g. proteins, lipids, including polyunsaturated fatty acids (PUFA), pigments, vitamins, antioxidants, as well as stimulate growth and immune system, and reduce water pollution in the aquacultures [2]. Within the scope of the study *Spirulina platensis* and *Chlorella vulgaris* biomass was successfully produced in 140 L column photobioreactor, as well as different media and cultivation methods were tested to induce lipid synthesis. Consequently, obtained biomass was tested as a supplement in rainbow trout diet at 5 and 10 % concentrations, compared with standard commercially available formulations Positive effects of evaluated microalgal supplements were observed in trout's growth dynamics . The highest enhancement of growth parameters was observed in groups supplemented with *S. platensis* and *C. vulgaris* at 5% concentration. Further studies will focus on the development of cultivation methods of other microalgal species, capable in producing high amounts of polyunsaturated fatty acids and other bio-active compounds, as well as development of fish feed formulations for different stages of rainbow trout growth.

This study was performed within the EMFF funded project no. 20-00-F02201-000001 supported by the Ministry of Agriculture and Rural Support Service of the Republic of Latvia.

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51. THE POTENTIAL OF *INULA BRITANNICA* L. EXTRACTS AS NATURAL INHIBITORS FOR MODULATING BACTERIAL VIRULENCE FACTORS

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Nowadays, the world is facing the challenge of treating bacterial infection due to antibiotic resistance. As a result, it is necessary to discover new ways to inhibit microbial pathogenicity without promoting microbial resistance. One promising approach is to disrupt the cascade of the Quorum sensing (QS) system, a bacterial communication mechanism through which bacteria alter their gene expression in response to changing environmental conditions. This enables bacteria to adapt easily and control their virulence, including pathogenicity, antibiotic resistance, biofilm formation, synthesis of enzymes, etc.

Each step in the QS mechanism can be negatively influenced, resulting in reduced bacterial virulence, particularly in terms of pathogenicity. It has been known for centuries that plants possess various activities such as antibacterial and anti-virulent. Using plant metabolites to disrupt QS system is a novel strategy for combating bacterial virulence. Therefore, the aim of our study is to investigate the potential of different extracts from *Inula britannica* L., a well-known medicinal plant, as natural virulence inhibitors. We tested their effectiveness on QS-controlled virulence factors such as violacein production, biofilm formation, swarming motility and catalase activity. Biofilm reduction was estimated using a Crystal violet assay on three pathogenic bacterial strains - *E. coli* 25922, *C. violaceum* 30191, *P. aeruginosa* PAO1. The results have shown that the IBr1 and IBr1-SL extracts had the highest effectivity on biofilm formation across all tested strains. These findings were confirmed by fluorescence staining analysis, indicating a decrease in cell viability and a possible reduction of the biofilm matrix in the treated samples. Additionally, we employed scanning electron microscopy, and the obtained images revealed that treated bacteria exhibited elongation in cell size, disruption in cell division, an inability to form bacterial septa and morphological deformation on the cell surface structure. Furthermore, we assessed their anti-virulence potential by evaluating their effect on the violacein synthesis and swarming motility in *C. violaceum*.

In summary, our study highlights the potential of *Inula britannica* L. extracts as natural inhibitors of bacterial virulence. These findings uncover a promising way for combating bacterial virulence by disrupting the QS system, thus reducing the impact of pathogenicity.

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52. EFFICIENT VALIDATION, CURATTION AND REUSE OF (META)GENOMIC DATA

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With the advancement of DNA sequencing, the technology has firmly established itself in laboratories around the world, bringing significant benefits. The evolution of this field of biotechnology, characterized by increased throughput leading to lower service costs, has brought years of exponential growth in publicly available (meta)genomic datasets. As the number of funded projects increases, so does the demand for data generation and subsequent analysis.

One major challenge is decoding the composition of entire microbial communities. This includes not only delving into specific microorganisms and their metabolic traits, but also their mobile genetic components, such as plasmids and bacteriophages. These elements exert significant influence on relationships and evolutionary trajectories. To unravel these complexities, it is necessary to accurately distinguish and annotate their genomes.

While automated annotation systems and tools are available to identify diverse mobile genetic elements, their effectiveness depends on the reference databases on which they are based. While machine learning-based tools have made progress in surpassing the limitations of purely reference-based methodologies, their performance remains tied to the quality of the reference datasets on which they are trained. These datasets require meticulous validation, often performed manually by researchers.

The process of accurate annotation requires researchers to use a number of different programs and databases, making manual annotation a laborious and time-consuming undertaking. In our initiative, we set out to overcome this challenge by combining the advantages of both automated and manual annotation systems. Our solution, MAISEN, is a web-based annotation management system [1]. Its goal is to combine results from multiple programs into a unified platform, thereby improving the efficiency of manual *de novo* (meta)genome annotation. MAISEN was created to allow users to verify annotations, such as automatic predictions of mobile genetic elements, and generate reusable and reliable novel reference sequence collections. As an example, we used SigMa to identify prophages in bacterial genomes, allowing the identified prophages to be used immediately to improve subsequent annotation analyses.

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53. BERRIES AS A RESERVOIR FOR VALUABLE YEASTS

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Studying yeast populations on berries is crucial for fermentation, food safety, and flavor development. Moreover, exploring yeast communities associated with berries sheds light on microbial interactions and offers opportunities for agricultural applications, making it a valuable area of research for multiple industries. By manipulating carbon sources, it is possible to modulate the production of specific biocidal compounds, making them more potent or targeting different types of microorganisms. Analyzing pigments in yeast provides valuable insights into their physiological processes, metabolic adaptations, and sensory attributes, enabling the development of visually appealing and functionally diverse fermented products.

Various microbiological and molecular biology methods were used to isolate and identify berries-inhabiting yeasts. In 2019-2021, a total of 13 yeast genera of fermenting and 8 non-fermenting yeast were identified. *Metschnikowia sp.* was the most detected (35.8%) on the berries, followed by *Hanseniaspora sp.* (17.9%) and *Pichia sp.* (11.3%). After 1 month of fermentation, *Aureobasidium* (38.2%) and *Metschnikowia* (25,8%) became dominant in the samples. Out of 105 purified yeast strains, 21 showed a biocidal phenotype that differed depending on pH and target yeast species. Five strains of *Pichia* and *Saccharomyces* genera exhibited biocidal activity which varied in response to carbon sources in cultivation media. Among *Saccharomyces* strains glucose, sucrose, and maltose were found to have the most impact on biocidal activity, whereas for *Pichia* - only glucose. Occurring on berries in small quantities*, R. diobovata, R. mucilaginosa,* and *R. glutinis* yeasts were examined for the effect of temperature on pigment synthesis. It was determined that carotenoids accumulated the best in *R. diobovata* and *R. glutinis* at 25ºC, while in *R. mucilaginosa* - at 30ºC. Our findings highlight the diverse nature of yeast population on berries and demonstrated the high potential of particular counterparts.

54. BACTERIA RECYCLE SULFUR-MODIFIED NUCLEOTIDES

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In all domains of life, transfer RNAs (tRNAs) contain posttranscriptionally sulfur-modified nucleosides such as 2- and 4-thiouridine. It was previously shown that TudS (formerly DUF523) domain containing proteins are bacterial desulfidases which utilize 4-thiouracil and 2-thiouracil as sources of uracil when overexpressed in uracil auxotrophic *E. coli* cells. Yet, the precise in vivo role of TudS enzymes has remained elusive. We examined two TudS enzymes from *Aeromonas* sp. and *Pseudomonas* sp. in vitro. The kinetic studies revealed that 4-thio-UMP rather than sulfurated tRNA, thiouracils or thiouridines is the preferred substrate of TudS. Furthermore, we have shown that *Pseudomonas putida* KT2440 and *Bacillus subtilis* can employ endogenous TudS desulfidase in vivo for utilization of exogenous 4-thiouracil and 4-thiouridine. We propose that TudS proteins are widespread desulfidases involved in recycling and detoxifying tRNA-derived 4-thiouridine monophosphate nucleosides for RNA synthesis.

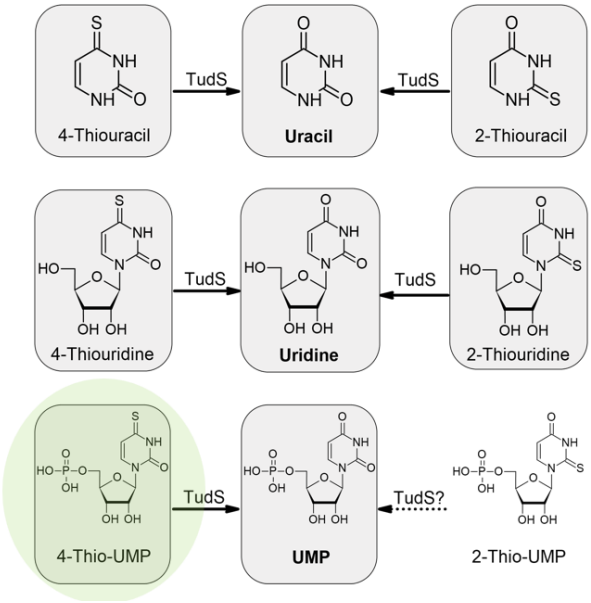

Figure 1. Reactions catalyzed by TudS in vitro. 4-Thio-UMP is the preffered susbstrate (green).

55. *GEOBACILLUS* LIPOLYTIC ENZYMES – ATTRACTIVE BIOCATALYSTS FOR THE DECOMPOSITION OF POLYCAPROLACTONES

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Plastics are ubiquitous polymers used in all fields of science, industry, and commerce. The production of plastics is steadily increasing and has reached 390.7Mt in 2022 [1]. Therefore, the environmental pollution caused by plastic waste is becoming an increasingly urgent problem. Different carboxyl hydrolases can catalyse the hydrolysis of ester linkages which are crucial for the integrity of polyester plastics like poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA) or polyethylene terephthalate (PET). The aim of this work was to investigate the ability of GD-95RM lipase [2] and GDEst-lip fused enzyme [3] to hydrolyse PCL films (Mn 45.000 (PCL450000) and Mn 80.000 (PCL80000)).

In this study, both enzymes were purified using immobilized Ni^{2+} ion affinity chromatography (IMAC). The PCL films were prepared using the solvent casting method [4] according to the procedures described by Khan et al. [5] and Abdel-Motaal et al. [6]. The dried and sterilized PCL_{45000} and PCL_{80000} films were transferred into 15 mL flask tubes and filled with enzyme solution (3 mL of Tris-HCl buffer (50 mM, pH 8) containing 0.05, 0.1, 0.15, or 0.5 mg of target enzyme). The prepared samples were incubated at 30 °C or 50 °C temperatures with agitation (130 rpm) and the hydrolysis efficiency was measured after 24 h. After treatment, PCL films were removed, washed with deionized water, and dried until a constant weight was observed. The mass loss of PCL films was calculated following equation (1) [7].

Eq. 1. Weight loss of PCL films (%)= $100 \times ((W_i - W_{pd}))/W_i$

W_i – the weight of pre-degraded PCL films, W_{pd} – the weight of post-degraded PCL films

Obtained results indicated that after 24 h at 50 °C, both enzymes hydrolysed more than 40% of PCL films when 0.05 mg of the target enzyme was used. At 30 °C, both GD-95RM and GDEst-lip enzymes (0.05 mg) were able to hydrolyse 20–40% of PCL films within 24 h. The obtained data also indicated that 0.1 mg is a suitable amount of enzyme to degrade PCL films efficiently, as the weight of PCL films decreased by more than 70%.

In conclusion, GD-95RM and GDEst-lip lipolytic biocatalysts can be defined as an attractive new polyesterases for PCL hydrolysis.

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56. CHARACTERISATION OF RNA-BINDING AMIDOHYDROLASE FROM *ARCHAEOGLOBUS FULGIDUS*

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Human activating signal cointegrator homology (ASCH) domain is widely dispersed in all domains of life and some prokaryotic viruses. Based on the known properties and functions of homologous domain-containing proteins in RNA metabolism, the ASCH domain is thought to be able to interact with RNA molecules as a transcription co-regulator [1]. Recent studies have identified bacterial ASCH domain-containing protein from *Zymomonas mobilis* as a monomeric ribonuclease ZmASCH [2] while its analogue YqfB from *Escherichia coli* has been described as the smallest known amidohydrolase with N^4-acetylcytidine (ac4C) as its primary substrate [3]. However, no experimental data on archaeal proteins belonging to the ASCH superfamily are available to date. In this study we aimed to characterize an ASCH domain-containing protein from a thermophilic archaeon *Archaeoglobus fulgidus*. To achieve this, recombinant protein purification and activity assays with *N*-acylated cytosine and cytidine derivatives were performed. To determine whether the target protein can bind RNA, we applied the electrophoretic mobility shift assay (EMSA) using radioactive isotope-labelled *E. coli* tRNA. Our findings show that *A. fulgidus* protein has a narrow substrate range compared to YqfB, although both share the same catalytic triad. Interestingly, the archaeal YqfB analogue exhibits RNA-binding properties, however, no ribonuclease activity was detected in the conditions tested. These results provide novel insights into a functional variety of the widespread ASCH superfamily, yet further research is required to identify structural motifs responsible for RNA binding activity and the possible cellular role of archaeal ASCH domain-containing proteins *in vivo*.

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57. DIFFICULTIES WITH B-GLUCAN ISOLATION AND ANALYSIS

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β-glucans are biologic response modifiers demonstrating a wide range of activities including immune response activation through specific receptors. However, β-glucan source and extraction methods have major impact on overall structure and physicochemical characteristics of the acquired molecules, including backbone and side chains length, number of side chains and purity. As the result, obtained β-glucans have different molecular weight, water solubility and biological properties. β-glucans extraction process is the first problem that researchers are facing. After isolation it is important to analyze the β-glucan structural integrity which is another challenge. Conventional methods for β-glucan chemical characterization are FT-IR and NMR spectroscopies. Both are suitable for analysis of soluble and insoluble β-glucans in solution and solid-state. Analysis in solid-state don't require prior preparation of the sample, however some parts of the spectrum can be difficult to interpret. Impurities represent another difficulty in β-glucan analysis due to some of the band overlapping of different molecule types in FT-IR spectroscopy as well as resonance similarities in NMR. Yet powerful, still these methods do not give detailed answer on length of side chains in branched β-glucans. Traditionally spectrophotometric and enzymatic methods are used for the quantification of acid hydrolyzed β-glucan. However, these methods have several downsides. One is lacking the specificity for β-glucans in the presence of other glucose containing molecules. Another, incomplete hydrolysis of some high molecular weight β-glucans.

β-glucan isolation requires accurate multistep processing of the source as even a small error has an impact on the final structure and properties of isolated molecules. And the method of choice for structural analysis of β-glucans depends on the goal of the research.

58. CHARACTERISATION OF FOUR α-L-FUCOSIDASES FROM ALPACA FEACAL MICROBIOME METAGENOMIC LIBRARY

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α-L-Fucose (Fuc) is a unique carbohydrate having a L-configuration and lacking a hydroxyl group on the C-6 carbon. It is found in the structure of oligosaccharides, and Fuc is also the most common terminal sugar of glycoconjugates [1]. Fucosylated glycoconjugates, present in both prokaryotic and eukaryotic (including humans) organisms, are essential for many biological processes, such as host-microorganism interactions in bacterial pathogenesis, cell-to-cell communication in plants, neurological and immunological processes in humans [1, 2, 3].

α-L-Fucosidases are exo-acting glycoside hydrolases that catalyse the removal of α-L-fucose from oligosaccharides and glycoconjugates. These enzymes can be found in a wide variety of organisms and tissue types. The diversity of α-L-fucosidases produced by gut microorganisms may provide these species an advantage in colonising and adapting to the gut environment [1]. In humans, α-L-fucosidase deficiency may lead to two pathological events: fucosidosis and cancer. These enzymes are applied in medicine, research, and biotechnology, as they show potential for the enzymatic synthesis of valuable oligosaccharides through transfucosylation [2].

Four new fucosidases Fuc25A, Fuc25C, Fuc25D and Fuc25E were obtained screening metagenomic library from alpaca feacal microbiome. Most of their homologues are proteins from Clostridia class bacteria. Phylogenetic analysis showed that Fuc25A, Fuc25D and Fuc25E are quite closely related, while Fuc25C is more distant from the others. Recombinant proteins were synthesised in *Escherichia coli* strains and purified. The kinetic parameters of α-L-fucosidases were determined using *p*-nitrophenyl-fucopyranoside as substrate. Fuc25D presented the highest catalytic efficiency (0,364 $\mu M^{-1}s^{-1}$), whereas Fuc25C the lowest (0,001 $\mu M^{-1}s^{-1}$). The enzyme activity assays revealed that all fucosidases were mesophilic and showed the highest activity at neutral pH. They were the most stable in a slightly alkaline environment (pH 8), and at 0 °C temperature, with a decrease in activity at higher temperatures. The fucosidases in question also possess transfucosylation activity.

59. EFFECTS OF SUPPLEMENTATION OF AQUACULTURE RAINBOW TROUT DIET (*ONCORHYNCHUS MYKISS*) WITH MICROALGAE BIOMASS ON FISH GROWTH DYNAMICS

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Use of microalgae in production of aquaculture fish feeds for can mitigate certain problems associated with fish i.e. salmonids malnutrition, environmental pollution, fish meat quality and appearance, as well as reduce overfishing of aquatic species used for commercial fish feed production. However, literature shows that a selection of suitable microalgae strains and supplementation in appropriate concentrations are required in order to obtain higher aquaculture productivity [1,2]. During this study *Spirulina platensis* and *Chlorella vulgaris* microalgal biomass were added to commercially available rainbow trout's feed and successfully evaluated in the aquaculture. During the production of fish feed 25 and 50% of fish meal present in commercial feed were replaced with respective microalgae biomass, at a final concentration of 5 and 10 % w/w, respectively. Rainbow trout groups with initial weights of 45 and 65 g were fed with experimental microalgae containing feed, alongside with control group being on the standard diet, once per day "*ad libidum*" for 60 days or longer A significant increase in trout's mass and length was observed in all groups supplemented with microalgae. The highest increase in fish mass was observed in groups supplemented with *S. platensis* and *C. vulgaris* at 5% for 65 g fish resulting in 293.9 ± 14.4 and 285.0 ± 15.2 g per fish, respectively. Additionally, the upper mentioned feeds resulted in highest trout's length among groups, resulting in 26.8 ± 1.0 and 26.5 ± 1.2 cm for 65 g fish as well as 24,2 ± 0.6 and 24,3 ± 0.7 45 g fish, respectively. It can be concluded, that supplementation of microalgae at 5% can be viewed as optimal concentration to enhance growth dynamics of rainbow trout. Further studies will focus on improvement of fish feed formulations and selection of other suitable microalgal strains for production of such bio-active compounds as lipids, polyunsaturated fatty acids, astaxanthin and vitamins.

This study was performed within the EMFF funded project no. 20-00-F02201-000001 supported by the Ministry of Agriculture and Rural Support Service of the Republic of Latvia.

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60. NOVEL COLLECTION OF BACTERIOPHAGES INFECTING *PSEUDOMONAS PUTIDA*

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Pseudomonas putida is an environmental bacterium with a wide range of different metabolic pathways. Being metabolically highly versatile, these bacteria have great promise both as cell factories in biotechnological production and for bioremediation approaches to degrade a variety of aromatic pollutants. Different metabolic pathways of *P. putida* have been extensively studied, but surprisingly, until recently almost no phages had been isolated for the common laboratory strain *P. putida* KT2440 and no phage defence mechanisms have been characterized yet.

Here, we introduce a novel collection of environmental phages that infect *P. putida* PaW85 (isogenic to KT2440), consisting currently of over 21 species of dsDNA phages, that can be grouped into 9 phage families. The collection was isolated from soil and water samples using a predictably weakened derivative of the bacterium: a prophage-negative strain that additionally lacks 13 toxin-antitoxin systems (TAS) from the genome. According to literature, both these chromosomal entities have previously been associated with phage defence of different bacteria. We show that three of 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. Additionally, we looked into the protective effect of stringent response on phage defence and determined that although it generally protects bacteria on a low level, it has a very strong protective effect against the infection of some phages.

Our further goals are to expand the phage library and to describe in detail *P. putida* phage defence mechanisms.

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61. UNVEILING THE ROLE OF GUT MICROBIOTA IN SHAPING FOOD ADDICTION-LIKE BEHAVIOR IN MICE

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A growing body of evidence indicates that there are similarities in the intestinal microbial composition among healthy individuals, suggesting that a particular microbiota is required for proper host health, while dysbiosis is frequently linked with various immune, endocrine and nervous system disroders [1-4]. Previous studies conducted in our laboratory have found differential microbiota signatures of food addiction in vulnerable and resilient mice despite identical experimental and living conditions. We found a significantly higher amount of *Blautia* spp. in non-addicted mice, suggesting a possible protective role in the development of food addiction [unpublished data]. Therefore, we aimed to increase *Blautia* spp. abundance in the gut of mice by prebiotic supplemenation or bacteria itself to evaluate this possible protective role.

Prebiotics A and B, and *Blautia* sp. were administered to mice, to increase *Blautia* spp. population in the gut. Mice underwent an operant protocol of food addiction for 98 days. A qPCR was performed to confirm the effectiveness of targeted prebiotics and probiotic in increasing *Blautia* spp. abundance in the gut.

None of the mice receiving prebiotics or probiotics achieved the addiction criteria, compared to control. qPCR results confirmed the increased *Blautia* genus concentration in the gut. Results indicates that *Blautia* spp. is a beneficial bacteria with a protective role in the development of food addiction.

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62. FUNCTIONAL BIOPROSPECTING OF COLD-ACTIVE ENZYMES USING A FOSMID-BASED METAGENOME LIBRARY OF COLD-ACTIVE MICROORGANISMS

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Psychrophilic and psychrotolerant microorganisms found in Arctic marine and coastal environments are inexhaustible source of novel, cold-active enzymes. Bioprospecting of such enzymes is promising for various purposes, opening new paths in environmental biotechnology, biomedicine and agriculture. For example, cellulases or pectinases from psychrophiles are good candidates for use in recently developing regenerative agriculture. These enzymes applied to the post-harvest fields may enhance the degradation of the post-cultivation organic matter and raise its accessibility for soil microorganisms, ultimately improving the soil quality for the next production cycle.

Here, we report a bioprospecting approach utilizing the fosmid libraries produced for Arctic marine metagenomes. The soil samples were collected in the vicinity of the Polish Polar Station Hornsund (Spitsbergen, Svalbard, Norway) located on the coast of the Greenland Sea [1]. By transforming *Escherichia coli* cells with the fosmid library and plating them on the minimal medium containing either carboxymethyl cellulose or pectin as a sole carbon source, we identified colonies expressing cellulase or pectinase originating from psychrophilic soil microbiota.

This bioprospecting approach has an enormous advantage: we found native psychrophilic genes which are functional in *E. coli* cells. In the next step, a sequencing of fosmids isolated from cellulase- and pectinase-positive clones delivered native DNA sequences used for designing expression systems for the overproduction of cold-active cellulase and pectinase using *E. coli* as a host. Using this approach, we mitigate the risks of *de novo* synthesis of genes identified in metagenomes, which may fail due to the DNA sequencing and read assembly errors.

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63. GENETIC CHARACTERIZATION AND SPREAD OF MULTIDRUG-RESISTANT BACTERIAL PATHOGENS IN LITHUANIA: INSIGHTS FROM *ACINETOBACTER BAUMANNII* AND *ESCHERICHIA COLI* ISOLATES

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The emergence and dissemination of multidrug-resistant (MDR) bacterial pathogens have become a global healthcare challenge. This study aimed to investigate the genetic characteristics, antimicrobial resistance profiles, and spread of MDR isolates in Lithuania, focusing on two prevalent pathogens: *Acinetobacter baumannii* (MDR-*Ab*) and *Escherichia coli* (MDR-*E. coli*).

For the MDR-*Ab* study, 194 non-duplicate MDR-*Ab* isolates were collected from various clinical specimens in Lithuania during 2014, 2016, and 2018. Antimicrobial susceptibility testing revealed a high prevalence of resistance against multiple classes of antimicrobial agents. Molecular typing using BOX-PCR method identified 6 distinct genetic clusters, indicating a diverse genetic landscape among the isolates. Multiple-locus variable-number tandem repeat analysis (MLVA) further characterized the isolates into 8 different genetic clusters, with Cluster 2 being the most predominant. The study also identified the spread of specific MDR-*Ab* clones across different healthcare facilities and regions, suggesting nosocomial transmission (Kirtikliene et al., 2021; Smith et al., 2019).

For the MDR-*E. coli* study, 256 non-repetitive MDR-*E. coli* isolates were collected from bloodstream infections in Lithuania. Antimicrobial susceptibility testing demonstrated resistance against β-lactams, fluoroquinolones, and aminoglycosides. Polymerase chain reaction (PCR) analysis detected resistance genes, including $bla_{CTX\text{-}M}$, bla_{TEM}, bla_{SHV}, and bla_{OXA}, indicating the presence of extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases. Molecular typing using BOX-PCR method revealed 14 distinct genetic clusters, and MLVA identified 6 different genetic clusters. Clonal dissemination of MDR-*E. coli* isolates was observed, indicating both local and cross-regional spread (Kirtikliene et al., 2022; Johnson et al., 2018; Peirano et al., 2021; Grundmann et al., 2017).

Overall, these studies, along with other global epidemiological reports (European Centre for Disease Prevention and Control, 2021), highlight the alarming prevalence, genetic diversity, and clonal dissemination of MDR-*Ab* and MDR-*E. coli* isolates in Lithuania. The findings underscore the urgent need for enhanced infection control measures, targeted surveillance, and the development of effective treatment strategies. Continuous monitoring of the genetic characteristics and spread of these MDR pathogens is essential for mitigating their impact on patient care and public health.

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64. MOBILE GENETIC ELEMENTS WITHIN RARE PLASMIDS IN A CLINICAL *ACINETOBACTER BAUMANNII* ISOLATE

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Acinetobacter baumannii is a gram-negative, opportunistic pathogen that was added to World Health Organization critical watch list due to its multiple antibiotic resistance. *A. baumannii* is able to gain various resistance factors, employing different mobile genetic elements (MGEs), by recombining and transferring DNA within and among the bacteria. Analysis of these MGEs could help identify different ways to mobilize DNA that could be found in unique plasmids. This, in turn, could hint at new emerging patterns of resistance acquisition.

The isolate AB36 plasmids were profiled according to Bertini *et al.* [1], using PCR-based replicon-typing scheme and pulsed-field gel electrophoresis (PFGE) method. They were then sequenced using Illumina Novaseq and Nanopore MinION technologies. Hybrid assemblies were annotated using NCBI PGAP (v. 2023-05-17.build 6771). Sequence analysis was performed using ISFinder [2], Phaster [3], sequences were examined using Snapgene (v. 7.0.2).

Primary results of clinical isolate AB36, showed that it contains plasmids belonging to GR1, GR12/GR18, GR13 and GR24 replicon types, while PFGE revealed only 3 plasmids, sized ~11 kb, ~50 kb and ~115 kb. Hybrid sequencing confirmed replicon types, but plasmid sizes differed. AB36 contained 169 kb, 117 kb, 113 kb, 16 kb, 18 kb and multiple 2 kb sized plasmids. The 169 kb, 18 kb and 16 kb plasmids were cointegrates, containing 2 or 3 replicons within. Furthermore, only the 3 largest plasmids contained 1-3 prophages, as well as any insertion sequences. 18 kb and 16 kb sized plasmids both had 4-6 p*dif* sites, capable of forming *dif* modules.

These findings show that plasmids are capable of hosting numerous MGEs. While no antimicrobial resistance genes could be found within plasmids, other genes were found in mobile modules. For example, BrnTA toxin-antitoxin system was found in two possible *dif* modules, proving that other genes could be mobilized and transferred to other bacteria in a similar manner.

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65. PLANT EXTRACTS WITH INHIBITORY EFFECTS ON STRUCTURE AND CELL VIABILITY OF *S. AUREUS* BIOFILMS

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The aim of this study is to investigate the inhibitory effects of extracts from the medicinal plant *Inula britannica* L. on the structure and cell viability of *Staphylococcus aureus* biofilms. Plant extracts were prepared by the subsequent extraction with solvents of different polarities (chloroform and methanol) at room temperature. *S. aureus* is a microorganism associated with persistent and recurrent infections, owing to its ability to produce biofilms. Bacterial biofilms are embedded in extracellular polymeric substances (EPS), which protect the microorganisms from environmental hazards. The structural and functional characteristics of biofilms determine the high drug tolerance of biofilm infections. This requires the search for novel ways capable to overcome the barrier created by the EPS. The biofilm of *S. aureus* was treated with 250 µg/ml of plant extracts. The Crystal violet assay test showed the reduction of the biofilm biomass in a concentration-dependent manner. The viability study showed a different proportion between viable and non-viable bacterial cells in the plant-treated biofilms. Scanning electron microscopy investigations confirmed the loosening of the extracellular substance in the treated samples. Some plant extracts from *Inula britannica* achieved inhibition of hemolytic activity. The results underlined that newly discovered natural anti-biofilm agents could help in devising novel strategies for treating biofilm-associated infections.

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66. EVALUATION OF ANTIBACTERIAL ACTIVITY OF BACTERIOCINS IN COMBINATION WITH ANTIBIOTICS AGAINST *PSEUDOMONAS AERUGINOSA*: *IN VITRO* ANALYSIS FOR POTENTIAL *IN VIVO* APPLICATION

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Bacterial keratitis, commonly caused by *Pseudomonas aeruginosa*, poses a significant threat to ocular health. Broad-spectrum antibiotic eye drops are typically prescribed as the first-line treatment. We investigated the potential of *Pseudomonas* bacteriocins, specifically PaeM4 and S5-PmnH [1] [2], to enhance the antibacterial efficacy of three commonly used antibiotic eye drops: gentamicin, tobramycin, and ciprofloxacin.

In vitro analysis was conducted using a time-kill test to evaluate the antibacterial activity against *P. aeruginosa* PAO1 strain. Minimal inhibitory concentrations of the antibiotics and bacteriocins were determined and used to select appropriate concentrations for combination testing. Bacterial colony forming units per milliliter were quantified to compare the effectiveness of samples treated with antibiotics or bacteriocins alone versus their combination.

The results demonstrate that combining tobramycin, gentamicin, or ciprofloxacin with PaeM4 and S5-PmnH bacteriocins exhibited slightly higher antibacterial activity compared to the antibiotics or bacteriocins used alone. Notably, no antagonistic effects were observed, suggesting compatibility between the antibiotics and bacteriocins.

These findings provide valuable preliminary data for potential *in vivo* models and further investigation. The enhanced antibacterial activity observed *in vitro* suggests that combining antibiotic eye drops with PaeM4 and S5-PmnH bacteriocins may offer a promising approach for the treatment of *Pseudomonas aeruginosa*-induced bacterial keratitis. However, additional studies are required to evaluate the efficacy and safety of these combinations *in vivo*.

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67. BACTERIAL AMIDOHYDROLASES AND MODIFIED 5-FLUOROCYTIDINE COMPOUNDS: NOVEL ENZYME-PRODRUG PAIRS

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Gene-directed enzyme prodrug therapy is an emerging strategy based on the transfer of genes encoding an enzyme that can convert a drug into a potent cytotoxin only in targeted cancer cells. Although the development of enzyme-activated prodrugs is promising, there are several limitations, such as the lack of suitable enzyme variants and the limited choice of chemical bonds that could be activated. Therefore, the aim of this study was to determine whether a number of new bacterial amidohydrolase-based enzyme-prodrug combinations have the potential to reduce the viability of eukaryotic cancer cells. First, several N^4-acylated cytidine derivatives were selected as potential prodrugs. Then, HCT116 and MCF7 human cancer cell lines, which stably express the genes encoding target bacterial amidohydrolases YqfB and D8_RL, have been established. Finally, the transduced cells expressing the bacterial amidohydrolases were exposed to several concentrations of the new prodrugs and their viability was assessed using the MTT assay. The results show significant decrease in the viability of HCT116 cells expressing either the YqfB or the D8_RL amidohydrolase, compared to the control cell line transduced with a vector without a gene insert. However, the viability of MCF7 cell lines with inserted genes did not differ from control cells for all prodrugs tested. In conclusion, our results suggest that bacterial YqfB and D8_RL amidohydrolases, together with the modified cytidine-based prodrugs, may serve as promising enzyme-prodrug systems for gene-directed enzyme prodrug therapy, however, the success of the therapy may depend on the type and/or origin of the cancer cells.

68. PROFILE OF LIPIDS AND CAROTENOIDS FROM DIFFERENT STRAINS OF *RHODOTORULA TORULOIDES* EXTRACTED WITH SUPERCRITICAL CARBON-DIOXIDE (SC-CO_2)

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In the recent times, supercritical carbon dioxide (SC-CO_2) extraction has gained significant attention as an alternative method for effective extraction of thermolabile compounds like lipids and carotenoids [1] [2]. In this study, lipids and carotenoids were extracted using SC-CO_2 from three different *R. toruloides* strains: CBS 14, CBS 349, and CBS 6016^T, which is a hybrid strain of CBS 14 and CBS 349. The extraction process involved specific conditions such as pressure, temperature, CO_2 flow rate, and the addition of ethanol as a co-solvent. The lipids from the yeast cells were extracted at 30 MPa pressure, 45 °C temperature and at a CO_2 flow rate of 2 ml/min.

Following the extraction of lipids, the carotenoid extraction process commenced at 30 MPa pressure, 50 °C temperature, CO_2 flow rate of 2 ml/min and 99.5 % ethanol as co-solvent at a flow of 0.2 ml/min. Both the extraction steps were carried out for a total duration of 180 minutes each.

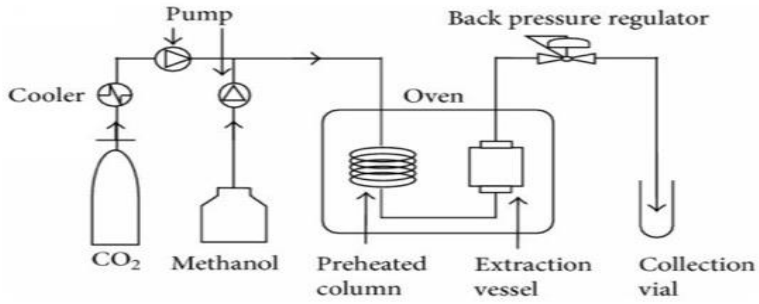

Figure 1. Schematic representation of an idealized supercritical fluid extraction instrument

The extracted lipids were analyzed using gas chromatography (GC), while the carotenoids were identified and quantified using ultra-high liquid chromatography (UHPLC). Four main carotenoids, namely β-carotene, γ-carotene, torularhodin, and torulene, were identified in all three strains, with torularhodin being the major carotenoid produced. This differs from our previous study where torularhodin and torulene were degraded during extraction, and thus, β-carotene was identified as the major carotenoid [3]. The total carotenoid concentration in the three strains varied, with CBS 14, CBS 6016^T, and CBS 349 exhibiting concentrations of 58.04, 43.57, and 4.85 µg/g, respectively. Similarly, the total lipid concentration at the end of cultivation varied, with CBS 14, CBS 6016^T, and CBS 349 strains showing concentrations of 6.43, 7.33, and 2.05 g/L, respectively. Oleic acid was found to be the major fatty acid in all three strains, followed by palmitic acid and linoleic acid.

The study revealed that the hybrid strain CBS 6016 displayed a lipid and carotenoid profile that closely resembled its parental strain, CBS 14 which suggest that CBS 6016^T predominantly inherited and exhibited the genetic traits linked to CBS 14, rather than CBS 349 and then further will possibly transfer it to a next generation.

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69. PURINE DEPLETION ELICITS RESILIENCE PHENOTYPE SIMILAR TO OTHER ESSENTIAL NUTRIENT STARVATIONS.

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Yeast, including the laboratory workhorse *Saccharomyces cerevisiae*, is an organism that has evolved to thrive in both times of abundance and scarcity. It possesses the remarkable ability to detect the availability and quality of nutrients, adjusting its growth rate and stress resistance accordingly. In yeast research, purine auxotrophy, resulting from mutations in the purine biosynthesis pathway, serves as a commonly used genetic marker.

Among these mutations, those in the *ade1* and *ade2* genes are the most widely employed because they lead to the formation of distinctive red colonies. In this study, we explore the behavior of a range of adeX mutants (*ade1, ade2, ade4, ade5-7, ade6, ade8 and ade16ade17*) when exposed to environments deficient in purine. Surprisingly, these mutants can sense purine scarcity, causing them to arrest their cell cycle and develop a robust stress resistance phenotype, where resistance to wide variety of stresses is high.

We show that transcriptional response of adenine synthesis mutants is similar to nitrogen starvation with respect of downregulated genes, where most of the transcripts are associated with protein synthesis, while upregulated genes vary among the starvation agents and mutants.

The robustness of this stress resistance phenotype is exemplified by petite cells, which, despite not relying on oxygen in their energetic metabolism, exhibit remarkable resistance to oxidative stress when subjected to purine starvation.

This phenomenon may have ecological significance, as many eukaryotic organisms, including intracellular parasites that exploit host cell purines, cannot synthesize their own purine and must scavenge it from their surroundings. Some of these parasites can endure extended periods of purine scarcity, suggesting that the loss of purine synthesis capacity and the development of stress-resilient traits may confer selective advantages in specific ecological niches.

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Abstract book

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