International Conference of Life Sciences

The COINS 2025

Book of Abstracts

Table of Contents

Keyonote Speakers

Marrie C. Nordstrom

Food webs in our changing seas

Food webs are representations of feeding interactions among the species in an ecosystem. These trophic networks form the backbone of ecosystem functioning, and knowledge of how interactions are structured helps us understand how energy flows through the system and how stable the system is. Climate change as well as multiple other anthropogenic drivers are rapidly changing oceans and coastal seas across the globe. Coastal ecosystems are heavily impacted, and with a high proportion of the human population living by the coast, these ecosystems and their food webs will be experiencing even increasing pressures in the future. As we aim to safequard and maintain healthy and resilient oceans and seas, there is a pressing need to understand the functional consequences of changing biodiversity in marine systems. In this presentation, I will give examples of the multiple approaches we use to answer questions, such as:
1) How does marine food web structure and functioning vary over time and space?
2) How does species loss affect the stability of marine food webs?
3) How does warming influence biodiversity and interactions in coastal benthic communities?

Kelly Krause

Inside Nature: Visual communication in science publishing

In her presentation, Kelly will delve into the theme of "Visual Communication in Science Publishing." Founded in 1869, Nature is a multi-disciplinary journal that publishes a vast array of topics and content types, from original research to news and expert analysis. This makes Nature an exemplary case study for examining the broader landscape of science publishing—particularly with regard to visual content, including data figures, conceptual illustrations, photography, and moving images.

Kelly will take us behind the scenes to explore these visual elements in context, offering valuable tips and best practices for enhancing your own publication efforts. Attendees can look forward to gaining insights into how effective visual communication can elevate understanding and engagement across diverse audiences.

Eugine Koonin

The world of viruses and its evolution

Viruses and virus-like mobile genetic elements are ubiquitous parasites or symbionts of all cellular life forms and the most abundant biological entities on earth. The recent, unprecedented advances of comparative genomics, metagenomics and metatranscriptomics have led to the discovery of diverse novel groups of viruses and a rapid expansion of the chartered region of the virosphere. These discoveries provide for a vastly improved understanding of the evolutionary relationships within the virosphere. Arguably, we are approaching the point when the global architecture of the virus world can be outlined in its entirety, and the key evolutionary events in each of its domains can be reconstructed. I will present such an outline of the global organization of the virosphere and the corresponding megataxonomy, including 7 evolutionarily coherent virus realms, that has been recently approved by the International Committee on Taxonomy of Viruses, as well as additional candidate major taxa including new realms. The expansion of the prokaryotic virosphere that is being shown to include many groups of viruses, particularly, those with RNA genomes, previously thought to be eukaryote-specific, will be emphasized. I will further discuss the position of viruses within the wider space of replicators and the recent dramatic expansion of the "alternative virosphere" that includes viroids and diverse viroid-like viruses that seem to have evolved on multiple, independent occasions.

Kirsten Bos

History of syphilis and its close relatives from the perspective of ancient DNA

The origin and evolutionary history of the diseases associated with Treponemal pallidum, namely yaws, bejel, and syphilis, has been heavily debated. Most discussion centres on syphilis specifically, where evidence for the "Columbian" theory, which argues for a post-1493 introduction from the Americas, is weighed against "pre-Columbian" theories, which argue for its establishment in Europe in the Medieval period. While ancient DNA data have the potential to make meaningful contributions in addressing these controversies, molecular recovery of this pathogen is a known challenge. Today I will present five *T. pallidum* genomes from contexts in the pre- (n=4) and peri-contact (n=1) Americas. These genomes represent sister lineages for all currently known sublineages of *T. pallidum*, which supports their common origin in the Americas. Molecular dating suggests their divergence from a most recent common ancestor within the last 9000 years. Together these data present the most convincing evidence thus far for an American origin of *T. pallidum*, consistent with the "Columbian" theory.

Victor Ambros

Gene Regulatory Networks Controlling Developmental Timing in C. elegans

MicroRNAs, together with their Argonaute partner proteins, exert post-transcriptional regulation of gene expression via complementary base pairing between the microRNA and mRNAs. Genes that encode microRNAs function as negative regulators of hundreds of other genes, primarily by inhibiting the translation and/or stability of target mRNAs. MicroRNAs exhibit remarkably versatile regulatory roles within genetic regulatory networks (GRNs) – for example, to coordinate multigene functional modules, dampen gene expression noise, and/or conduct developmental switches. Genetic analysis of model organisms reveals that the function of microRNAs can be conditional – wherein a microRNA gene is required under certain environmental or physiological conditions, but relatively dispensable under other conditions. It is not uncommon for microRNA loss-of-function mutants to display emergent developmental or physiological defects when subjected to biological stresses and challenges that fall within the normal range of experience of the animal. These include temperature fluctuations within the animal's thermotolerant range, exposure to pathogens or toxins endemic to the animal's normal environment, or routine regeneration of tissues after injury. The observation that microRNA genes exhibit stress-dependent conditional phenotypes is interpreted to suggest prominent roles for microRNAs in enabling developmental and homeostatic processes to weather the contingencies of everyday life.

Stefan Kaufmann

Novel intervention measures against tuberculosis: Rational design of a novel vaccine and a prognostic signature

Tuberculosis (TB) remains a major health threat which kills more people than any other pathogen. Better control measures are therefore needed. These include better diagnostics, drugs and vaccines. More recent technical achievements have added biomarkers to this list.
Methods: I will describe design of host biosignatures based on transcriptomic and/or metabolomic mark ers and development of a recombinant vaccine candidate which has reached clinical phase III trial testing for protective efficacy.
Results: Host biomarkers have been defined which can diagnose and predict onset of active TB in household contacts of newly diagnosed index cases six to twelve months ahead of clinical diagnosis. Such biosignatures are composed of transcriptomic and/or metabolomic markers, selected and combined by computational models. In addition, a single serum-protein marker, activin A, has been identified which can be harnessed for TB diagnosis. The attenuated live TB vaccine VPM1002 is based on a genetically modified BCG in which the urease C gene had been replaced by the listeriolysin gene. VPM1002 has successfully completed phase I and II clinical trial testing and has entered phase III clinical trials for protective efficacy. First, VPM1002 is being tested in India whether it can prevent recurrence of TB in individuals who had been cured from active disease by drug treatment. This trial has been completed and data are currently being analyzed. Second, safety and prevention of infection by VPM1002 is being tested in HIV exposed and unexposed neonates at several sites in Sub-Saharan Africa. Recruitment has been completed and data are expected in 2025/2026. Third, prevention of TB in household contacts of newly diagnosed TB index cases is being assessed at several sites in India, coordinated by the Indian Council of Medical Research. This trial has been completed and data are currently being analyzed.Moreover, VPM1002 has also shown highly promising effects as replacement therapy for BCG against non-muscle invasive bladder cancer. This presentation will describe current progress in the development of biosignatures and vaccines as novel intervention measures against TB.

Dirk Schulze-Makuch

Microbial Motility as a Biosignature to Detect Both Extraterrestrial Life and Pathogens on Earth

Recent increases in computational power make the use of automated in-situ analyses with the help of AI feasible and provide an important tool for both planetary exploration and environmental health challenges. In the presentation I show how machine learning algorithms are used to identify various microbial species by their motility, the characteristic movement pattern of microbes. This approach has also the advantage that it is a biochemistry-independent method and thus could identify microorganism that have an unknown biochemistry. We showed that we can achieve an accuracy of 99% distinguishing microbial motility from Brownían motion. However, distinguishing different microbes from each other was much more difficult and only a maximum accuracy of 82% was achieved. In our efforts to use this technology for life detection at extraterrestrial locations, we tested the viability and motility of analog organisms under elevated salt stresses as they are expected to exist on Mars. In order to address the problem of getting microorganism to move, we used various attractants that motivated microbes to move toward them. Based on our experiments we found that the amino acid L-serine worked best as a chemo-attractant. We introduced a novel approach for utilizing µ-slides that diverges from the more traditional long-term chemotactic assay in favor of a shorter time frame assay that only requires a simple blob detection algorithm for microbial detection. A second goal of our technology is to detect microbial pathogens in surface water and waste water to ensure human health. Our initial work is focusing on cholera bacteria, which still kill more than 1 million people every year, mostly in underdeveloped countries. We are in the process of designing a detection system that is very accurate, but much faster than the traditional wet-chemistry and sequencing approaches.

Kourelis Jiorgos

What does it take to bioengineer NLR immune receptor-nanobody fusions

Recurrent epidemics caused by plant pathogens pose a significant threat to global food security. While modifying natural components has been successful to retool the plant immune system, this approach can be rapidly rendered ineffective by the emergence of new pathogen strains. Made-to-order synthetic plant immune receptors, however, offer a promising solution for tailored resistance to pathogen genotypes present in the field. Here we define four technical and conceptual advances which enabled successful engineering of NLR immune receptor-nanobody fusions, termed Pikobodies, for this purpose. These advances include 1) evolutionary analysis to identify domain boundaries, 2) structural modelling to predict nanobody integration boundaries, 3) rapid screening using transient immune and disease assays, and 4) guided mutagenesis to abolish autoactivity triggered by some nanobody integrations. We show that plant immune receptors can serve as effective scaffolds for nanobodies that bind fluorescent proteins (FPs), and that the resulting immune receptor-nanobody fusions trigger immune responses and confer resistance against plant viruses expressing FPs. Since nanobodies can be raised against most molecules, immune receptor-nanobody fusions have the potential to generate resistance against most plant pathogens and pests delivering effectors inside host cells.

Kees Moeliker

The duck, his mate, and the pubic louse: Improbable Research and the Ig Nobel Prizes

Some scientific studies make people laugh, then think. The most surprising and funniest win an Ig Nobel Prize. Highlights in more than 30 years of this prestigious award include winning research into 'Why woodpeckers don't get headaches?', 'How a roller-coaster ride cures asthma', 'Pressure produced when penguins poo', 'Why scientists like to lick rocks', 'Using chromatography to separate drunk and sober worms', 'Sword swallowing and its side effects' and 'Self-Colonoscopy in the sitting position'.

Kees Moeliker tells what it needs and means to win this much-coveted prize. He will elaborate on his own Ig Nobel winning achievement – discovering and documenting the first case of homosexual necrophilia in the mallard duck – and how (t)his dead duck made him an expert on animal behaviour that is not taught in biology class.

Walid O. El Cheikh

How Pitching Can Be a Superpower! – Not a Source for Anxiety …

Pitching is an important skill for everyone, especially researchers, engineers, and/or scientists who want to realize their ideas into projects, maybe perhaps into successful businesses. In this Masterclass, you will get to know the topic like never before and you will get the first 1% belief to start applying it into your daily life, professionally and personally. The focus will be on the “How to pitch” vs. “What” is pitching. Almost all other coaches tell you what to do and what not to do, but in this session you will create your own pitch, practice with others and uncover your own strengths and weaknesses. You can expect fun but challenging exercises, and a dynamic but cozy environment to learn about one of the most crucial skills in life. Welcome to this life-changing session.

Donatas Zigmantas

Mapping energy transfer in photosynthetic bacteria *in vivo*

The remarkable quantum efficiency of solar energy collection in photosynthetic bacteria is assured by optimized arrangement of chromophores in light-harvesting complexes and by robust connectivity between complexes comprising photosynthetic units. However, information about this functional connectivity in intact cells is still very sparse. To map and characterize light-harvesting processes in intact photosynthetic bacterial cells we use multidimensional optical spectroscopy. We present physiological temperature measurements of intact cells of the thermophilic green non-sulfur bacterium ***Chloroflexus aurantiacus*** and purple bacterium ***Rhodobacter sphaeroides.*** Notably, the two studied photosynthetic bacteria feature different architecture of photosynthetic units, but very similar reaction centers. We were able to fully map the energy transfer processes between the light-harvesting complexes and down to reaction centers, with ensuing charge separation, leading to dark photosynthetic reactions. Then we apply global analysis to characterize dynamics of all the energy transfer channels in the intact photosynthetic machinery of both

Giovanni Poggiali

ROCKS & ORGANICS: THE ROLE OF MINERALS IN THE ORIGIN OF LIFE AND THE ENRICHMENT OF CHEMICAL COMPLEXITY IN THE SOLAR SYSTEM

From fine dust in the protoplanetary disk to complex rocky surfaces on planets, the interaction between minerals and molecules is a key factor in understanding chemical evolution and the enrichment of organic matter from the interstellar medium to the emergence of life. Investigating the physico-chemical interactions of life's building blocks under plausible prebiotic and space-like conditions is essential to unravel the processes that led to the life's origin on Earth and possibly on other planets. Indeed, minerals may have played a critical role in these processes, particularly on Earth and Mars. [1, 2, 3, 4, 5]. Surface-promoted reactions, including ion exchange, electron transfer, and photochemical processes [6], may have facilitated the increase of molecular complexity even before planetary formation, with interstellar dust grains acting as catalysts [7]. Observations of organic compounds on asteroids and comets further support the role of small rocky bodies in the evolution of organic material in the Solar System and in providing the building blocks of life on Earth and other terrestrial planets [8].

Understanding mineral-molecule interactions is also critical to assessing the fate of organics upon planetary delivery due to space weathering. While rocky surfaces are exposed to radiation and thermal gradients, UV photons penetrate only a few millimeters into regolith, leaving subsurface organics shielded [9]. However, UV-driven reactions at the surface can generate powerful oxidants capable of degrading organic matter. The study of photoprocessing and the survivability of prebiotic molecule is therefore essential for understanding the origins of life and assessing the preservation of potential biomarkers detectable by space missions such as NASA Mars2020 Perseverance rover.

In recent years, space-based observations of organics on rocky surfaces have multiplied, allowing direct comparisons between laboratory experiments and remote sensing data. This presentation will cover three main aspects: (i) the role of minerals as adsorbents, templates, shields, or catalysts in molecular complexity; (ii) the availability of minerals and evidence for mineral-organic interactions across the Solar System; and (iii) methods for assessing the role of minerals in prebiotic chemistry, with a focus on techniques applicable to space missions.

Maciej Lisicki

Twist and turn. Hydrodynamics of microscale locomotion.

A look into the microworld reveals plethora of swimming microorganisms, which display a rich variety of shapes and swimming gaits. An important subgroup are eukaryotic ciliates, which use thousands of active microscale filaments covering their bodies to achieve propulsion. Despite this diversity, the physics of microscale flows imposes universal limitations on their propulsion strategies. In my talk, I will review the basic properties of Stokes flows and their consequences on swimming. Next, I will show examples of ciliary motion in both artificial and biological systems, which can be modelled using the formalism of Stokesian hydrodynamics with imposed surface activity. I will comment on the role of this activity in shaping the flows they use for swimming, sensing, feeding, and streaming.

Short Talks

BIOSENSORS FOR SPACE: DEVELOPMENT OF L-AMINO ACID DETECTION SYSTEM FOR ISS

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Space poses significant health challenges for astronauts, particularly on long-duration missions where microgravity induces muscle atrophy, bone density loss, anemia, and vestibular disorders. Current diagnostic capabilities in space are limited; standard tests like blood panels are limited on spacecraft, and transporting blood samples to Earth is costly, with results delayed by weeks or months. One critical concern is muscle mass loss – reports from European Space Agency (ESA) indicate substantial muscle decline during long missions despite countermeasures, with decreases reaching 20-30% in some cases. Plasma free L-amino acids essential for muscle protein synthesis, change significantly before and after missions, likely reflecting muscle breakdown or metabolic shifts, though data during missions remain limited.

To address this gap, we present preliminary results from a project funded by the ESA, aimed at developing a portable, easy-to-use biosensor to measure total L-amino acids levels directly from human blood in space. This device could enable real-time monitoring of muscle status and metabolic health, providing critical insights into muscle degradation and nutritional needs without requiring Earth-based testing. *In situ* L-AA monitoring could enhance astronaut health management and offer new diagnostic capabilities for space medicine, supporting future missions to Mars and beyond. This project also has dual applications, potentially leading to technology capable to measure L-amino acids in clinical-related applications here on Earth. By advancing real-time diagnostic technology, we aim to contribute to space health and promote the development of portable biosensors for both space and terrestrial applications.

BEYOND FRAGMENTS: HOW LONG-READ SEQUENCING REVOLUTIONIZES DNA SEQUENCE VERIFICATION

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Long-read sequencing technologies are transforming not only genomics but also the sequence verification of various DNA constructs and small genomes. Whole-plasmid and unfragmented PCR amplicon sequencing offers significant advantages beyond time efficiency, revealing DNA properties previously inaccessible using canonical sequencing technologies. Widely used plasmids frequently form dimers and multimers with distinct properties compared to their monomeric counterparts. Additionally, whole-plasmid sequencing uncovers mutations within plasmid backbones that may significantly influence experimental outcomes. Similarly, full amplicon sequencing represents a valuable methodology for characterizing both specific and non-specific amplicons, functioning as a miniature next-generation platform for applications such as 16S rRNA analysis or sequence variant library assessment. This presentation will examine these technological advances and present several practical use cases and observations from real whole plasmid sequencing results in the Vilnius area, highlighting how modern sequencing approaches provide comprehensive tools for DNA sample characterization.

BEHIND THE MOONSHOT

Dominykas Milašius[1,2]

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Chasing moonshot ideas is thrilling—but let's be real, it's also hella hard. In this talk, we dive into the wild intersection of life science and space, where breakthrough ideas sound impossible until they're not. From drug discovery in zero gravity to extending human longevity on Earth and beyond, we'll explore how these industries can push each other forward—if we're bold enough to try.

Through personal stories, hard-learned startup lessons, and a few laughs along the way, we'll break down why the toughest challenges in biotech and space are exactly what make them worth chasing. Because in the end, the real beauty of life science startups isn't just in the success—it's in the audacity to try in the first place.

Get ready to think big, fail fast, and aim higher than ever. Because behind every moonshot is the struggle that makes it legendary.

HYPE, HOPE, AND HARD LESSONS IN AI-DRIVEN ENZYME DESIGN

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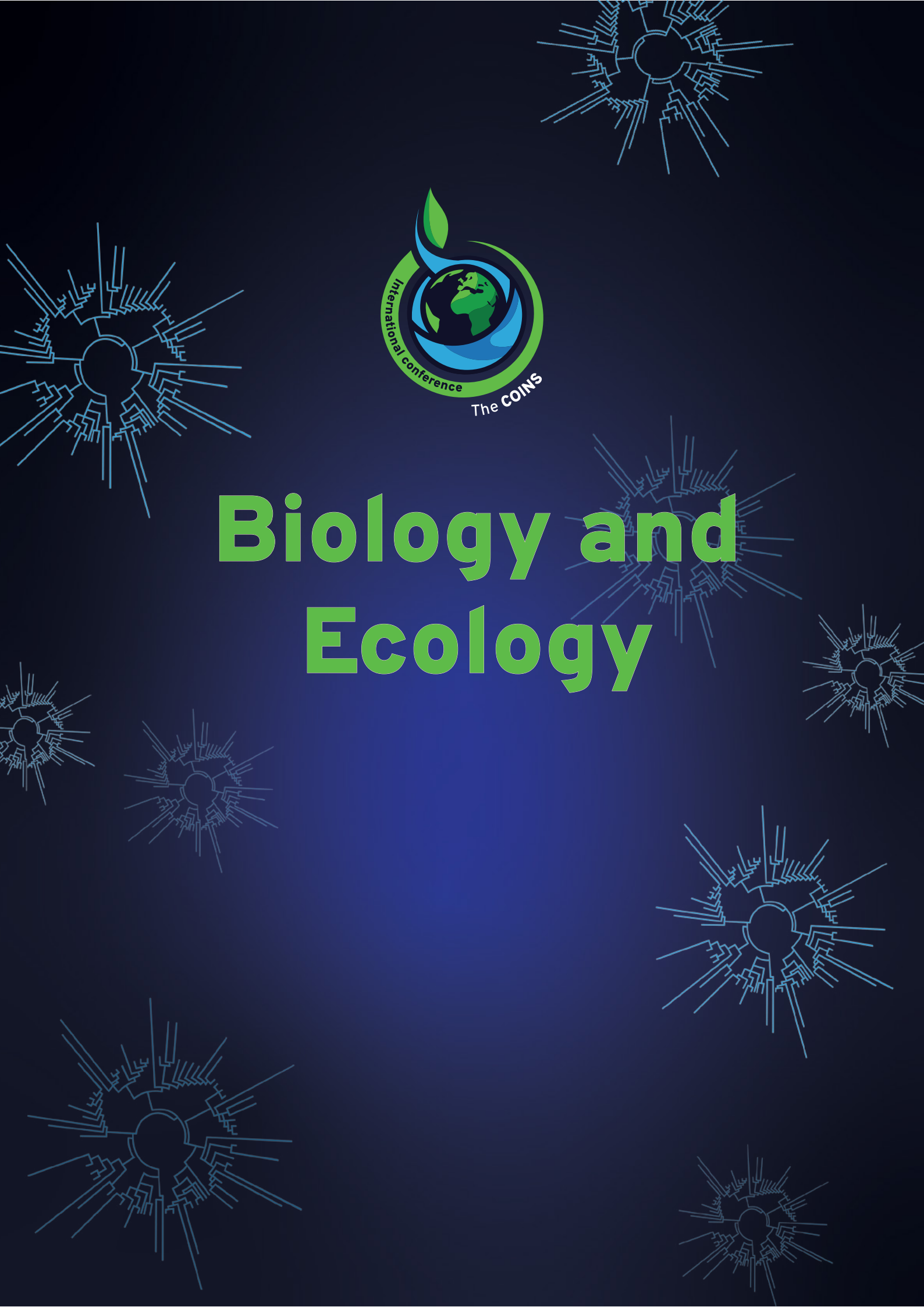
AI is transforming enzyme design, promising to accelerate the optimization and discovery of biocatalysts for diverse applications. From deep learning models predicting enzyme functions to generative AI designing novel proteins, the field is experiencing unprecedented innovation. Yet, challenges persist. Limited high-quality data, the complexity of enzyme behavior, and the gap between AI predictions and experimental validation continue to hinder progress. This presentation explores the practical impact of AI in enzyme engineering, distinguishing promise from reality. We'll discuss key breakthroughs, persistent challenges, and lessons learned in applying AI to enzyme design.



Biology and Ecology

A1

Preliminary Results on Detection of *Trichinella* and *Sarcocystis* Parasites in Mustelid Predators

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Trichinellosis is a widespread zoonotic disease caused by nematodes of the genus *Trichinella*. The life cycle of these parasites exclusively involves two generations of nematodes in the same host, which circulate in wild, synanthropic, and domestic animals. Sarcocystosis is a widespread pathogenic disease caused by protozoan parasites of the genus *Sarcocystis*. These parasites are characterized by two host prey-predator life cycles. Due to the increasing populations of the predators of the family Mustelidae, it is important to investigate how these mammals contribute to the spread of parasites. The objective of the present study was to evaluate the prevalence and intensity of infections in the muscles of mustelids and to identify parasite species composition in the animals examined.

In 2022-2024, 60 mammals of the Mustelidae family, 26 *Martes martes*, 19 *Neovison vison*, 13 *Mustela putorius*, one *Meles meles*, and one *Martes foina* were collected from different regions of Lithuania. The active method of artificial digestion has been used to investigate the prevalence and intensity of *Trichinella* spp. infection in mustelids. The infection intensity was estimated by counting *lpg* (number of larvae per gram of sample). The microscopic-compressor method was used to detect *Sarcocystis* spp. parasites. The intensity of infection was estimated by counting the number of sarcocysts per gram of sample. *Trichinella* and *Sarcocystis* species were identified by multiplex PCR and nested PCR, respectively.

Of the 60 animals tested, 10 (16.67 %) were positive for parasites investigated. Prevalence of *Trichinella* spp. and *Sarcocystis* spp. were 6.67 % and 10.00 %, respectively. Of the infected animals, the highest prevalence was found in *Mustela putorius*, 7.69 % for *Trichinella* spp., 15.38 % for *Sarcocystis* spp., and *Martes martes*, 7.69 % for *Trichinella* spp. and 11.54 % for *Sarcocystis* spp. No parasites were found in *Meles meles* and *Martes foina*. Molecular studies have identified two species of the genus *Trichinella* and one species of the genus *Sarcocystis*. Of the 40 *Trichinella* larvae 75.00 % belonged to *T. britovi* and 25.00 % to *T. spiralis*. The 18 *Sarcocystis* sarcocysts were identified as *S. lutrae* (100 %). In summary, *T. britovi* and *S. lutrae* are the most common species in Lithuania. However, the preliminary results show that mustelids also carry *T. spiralis*, the most dangerous *Trichinella* species for humans, which is usually transmitted through domestic animals.

A2

MOLECULAR CONFIRMATION OF THE TAWNY OWL (*STRIX ALUCO*) AS DEFINITIVE HOST OF NUMEROUS *SARCOCYSTIS* SPECIES

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The genus *Sarcocystis* consists of unicellular cyst-forming protozoan parasites characterised by two-host prey-predator life cycle. The intermediate host is infected through contaminated water or food and the parasites form sarcocysts in various host tissues (muscles, brain). The definitive host, predator or scavenger, gets infected through consumption of contaminated meat, and in intestines of animal oocysts and sporocysts are formed. *Sarcocystis* species detected in definitive host can be differentiated only by molecular tools. Worldwide, there is a lack of studies examining naturally infected raptors as definitive hosts of *Sarcocystis* spp. Therefore, the objective of the present study was to establish *Sarcocystis* species richness in the intestines of the tawny owl (*Strix aluco*) collected in Lithuania.

To prepare a sample the entire intestine was scaped then homogenized in distilled water using a hand mixer and centrifuged multiple times to separate and discard the supernatant. Then samples were treated with sodium hypochlorite (bleach) and incubated in a cold bath then repeatedly centrifuged and washed with distilled water until all traces of chlorine were eliminated. Concentrated oocysts and sporocysts were analysed using light microscope at ×400 magnification. *Sarcocystis* species were identified using nested PCR targeting *28S* rRNA and Sanger sequencing of purified PCR products. Phylogenetic placement of resulted sequences was analysed using neighbour-joining method with a help of MEGA11 software.

Based on microscopical examination, oocysts/sporocysts of *Sarcocystis* spp. were found in 3 of 22 birds analysed. Based on molecular analysis at least one species of *Sarcocystis* were detected in 13 (59.1%) of studied tawny owls and in two of the birds (9.1%) two distinct species were discovered. The species we discovered were previously detected *S. funereus, S. glareoli, S. halieti, S. kutkienae, S.* sp. ex *Corvus corax* and two new genetically distinct *Sarcocystis* species *Sarcocystis* sp. LT24Sa1 and *Sarcocystis* sp. LT24Sa11. Phylogenetically, *Sarcocystis* sp. LT24Sa1 and *Sarcocystis* sp. LT24Sa11 were most closely related to *Sarcocystis* spp. using rodents as their intermediate hosts and birds of order Accipitriformes and Strigiformes as their definitive hosts. The most frequently species found (36.4%) was *S. glareoli* forming sarcocysts in brain of mainly bank vole (*Clethrionomys glareolus*) and probably other rodent species. In general, *Sarcocystis* species identified in raptors tested use rodents and birds as their intermediate hosts, which corresponds to the diet of the tawny owl. Notably, of the *Sarcocystis* species detected, *S. glareoli* and *S. halieti* are pathogenic for their intermediate hosts.

Thus, the tawny owl plays an important role in transmission of *Sarcocystis* species, including the pathogenic ones. Furthermore, molecularly based investigations of intestines of raptors are relevant in the disclosure of the diversity of *Sarcocystis* spp.

A3

GLACIAL RELICT COPEPOD CRUSTACEANS IN THE LAKES OF LITHUANIA

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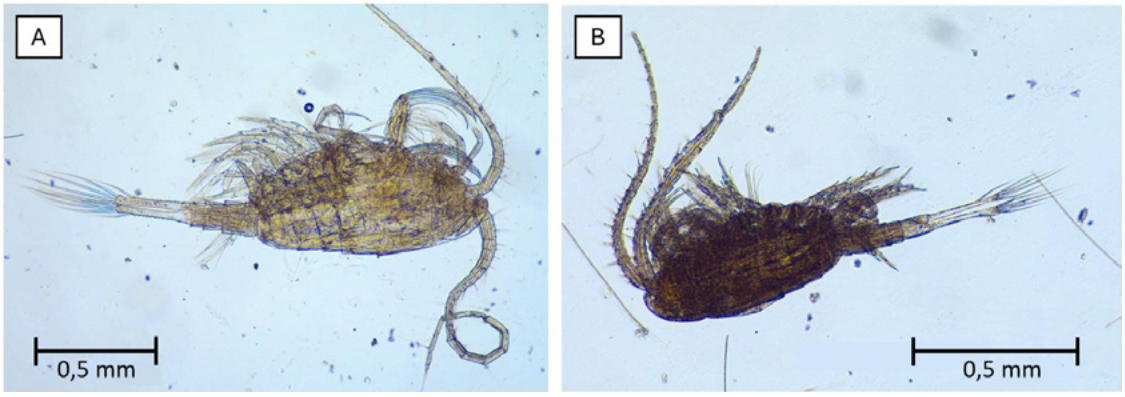
Two species of glacial relict copepod crustacean, *Limnocalanus macrurus* (Sars G.O., 1863) and *Eurytemosa lacustris* (Poppe, 1887) (Fig. 1) are still found in Lithuanian lakes of glacial origin [1]. These species are interesting from a scientific point of view. The status of these crustacean populations may be informative on the lake ecological status. In addition, relict crustaceans also constitute an important part of trophic web as food for fish and greatly impact fish production [2]. Unfortunately, the survival of these crustaceans is threatened by eutrophication and global warming [3].

The aim of this work was to summarize information and perform research on the distribution of *L. macrurus* and *E. lacustris* and their abundance in Lithuania lakes.

Investigations performed in 2022 revealed that relicts inhabit all 22 studied lakes, *Limnocalanus macrurus* was found in 14 lakes, and *E. lacustris* was present in 8 lakes. Relict copepoda crustaceans have been found in all their historical habitats. New habitats for these relics have also been identified: *L. macrurus* was found in Juodieji Lakajai and Žeimenys, and *E. lacustris* in Alaušas, Avilys and Galstas.

Figure 1. A - *Limnocalanus macrurus*; B - *Eurytemora lacustris* (photo by V. Arcerytė).



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[2] Audzijonytė, A. (1999) 'Aukštesniųjų ledynmečio reliktinių vėžiagyvių gausumo ir populiacijos struktūro tyrimai Lūšių, Šakarvų, Akmenos ir Verniejaus ežeruose', Ekologija, 2, 36-41

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A4

VEGETATIVE COMPATIBILITY BETWEEN PATHOGENIC FUNGI OF THE SPECIES OF *DIAPORTHE ONCOSTOMA*

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Phytopathogenic fungi cause serious plant diseases that can have a negative the entire plant. These include *Diaporthe oncostoma*, a globally occurring pathogenic fungus species that causes tree diseases. Some of these fungi have been identified as opportunistic pathogens in humans. For these reasons, pathogenic fungi are a major ecological and industrial problem. Therefore, knowledge of the ability of fungi of the genus *Diaporthe* to form vegetatively compatible pairs is particularly important, as this allows the pathogens to exchange genetic information through heterokaryosis and form hybrids. During vegetative compatibility itself, hyphae of isolates with the same loci can fuse and form viable heterokaryons. This leads to increased virulence or resistance to fungicides. In Lithuania, however, there is a lack of detailed information on pathogenic fungi of the genus *Diaporthe*, especially on their ability to form vegetatively compatible pairs. The aim of this work is therefore to determine the vegetative compatibility of *Diaporthe oncostoma* fungi among the selected isolates.

For the study, 30 *Diaporthe oncostoma* isolates were selected from the fungal collection of the of the Laboratory of Plant Pathology at the Nature Research Centre. The fungi were collected from 8 different locations in Lithuania and 4 different hosts: *Robinia pseudoacacia*, *Robinia ambigua*, *Caragana arborescens*, *Caragana frutex*. The aim of the vegetative compatibility study was to evaluate the mutual compatibility of 30 different isolates and to perform molecular studies to identify MAT genes. In the first part of the study, vegetative compatibility tests were carried out in which the fungal isolates were inoculated in pairs against each other on culture media. After incubation, the fusion zones were analyzed to determine whether there was vegetative compatibility between the isolates or whether barriers formed, indicating incompatibility. This allowed a preliminary categorisation of the isolates into compatibility groups. In the second part of the study, molecular methods were used to analyse the MAT genes associated with the type of fungal propagation. DNA isolation, PCR and electrophoresis were used to determine which MAT genes were present in the isolates analysed. The data from the first and second part are compared, and general conclusions are drawn. The results of these genetic studies made it possible to refine the grouping of vegetative compatibility and provided additional information on the genetic diversity of these isolates and their ability to form hybrids.

A5

A STUDY OF THE DIVERSITY AND PROPERTIES OF FUNGI ISOLATED FROM THE SOIL

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INTRODUCTION: Fungi that inhabit the soil primarily decompose wood and organic waste, playing a crucial role in the carbon, mineral and nutr0069ent cycles of ecosystems. Compared to fungi occupying other ecological niches, fungal decomposers often possess a more diverse enzyme repertoire, enabling them to break down complex organic materials efficiently. One particularly important function of these enzymes is the degradation of lignin, a structural component of plant cell walls. In fungi, phenoloxidases facilitate the degradation of lignin and toxic phenolic compounds, enhancing soil nutrition and health. Beyond their ecological role industrial applications include food processing, wastewater disposal and the management of wood industry by-products. In the latter case, lignin is often a by-product that is burnt for heating and/or power generation. However, this practice not only underutilizes the potential of lignin but also contributes to carbon emissions. Instead of being treated as waste, lignin-rich by-products could be further processed through fungal-derived enzyme applications. As mentioned above, phenoloxidase enzymes can degrade lignin, and applying these enzymes to wood industry by-products could lead to higher-value feedstocks, such as bio-based chemicals and biodegradable materials. This approach not only increases economic benefits but also reduces waste incineration and environmental impact. By integrating enzymatic lignin degradation into industrial processes, more sustainable strategies can be developed that transform waste into valuable resources.

AIM: The aim of the study is to analyze the diversity of soil fungi and to form morphogroups based on macro- and microscopic characteristics. In future steps it will be analyzed whether these isolates have phenoloxidase activity and this will be evaluated using an index.

MATERIALS AND METHODS: The fungi studied were obtained from mixed forest soil near the Nature Research Centre and a total of 74 fungal cultures were isolated. Each isolate is grown at 24 °C for 2–3 days (or longer, depending on the growth rate of the isolate) on 3 plates with Chapek agar as control and 3 plates with Chapek agar containing 0.2% gallic acid, which is hydrolyzed in the presence of phenoloxidase enzymes and turns brown or slightly blue in color. The diameters of the colony and the hydrolysis zone are measured and an index is calculated for each isolate: I = d(hydrolysis zone [cm]) / d(colony size [cm]). The fungi of interest will be identified using DNR sequencing. It is planned to use other indicators to determine which specific enzymes these fungi produce, as gallic acid indicates the presence of peroxidase, laccase, tyrosinase and other enzymes.

RESULTS AND EXPECTATIONS: Currently, a total of 28 morphogroups have been generated, half of which (14 groups) contain only 1 isolate; the other groups contain 2 – 9 isolates. Based on the existing literature, the fungi collected are expected to have medium phenoloxidase activity, with an index value around 1. While soil fungi have several enzymes of interest for this study, which could potentially increase the index, most of the fungi collected were found to grow very rapidly. This rapid growth could negate the positive effects that would increase the index value.

CONCLUSION: This study emphasisis the potential of soil fungi to contribute to effective lignin degradation through their phenoloxidase activity. The categorization of 74 fungi into 28 morphogroups forms the basis for the identification of fungi with interesting enzymatic capabilities that could be exploited. By assessing phenoloxidase activity using an index, isolates that can efficiently degrade lignin will be identified. These results could inform future strategies to reduce waste, lower carbon emissions and promote more sustainable practices in industries such as wood processing.

A6

ENHANCING ROOT GROWTH IN SPRING BARLEY THROUGH ENDOPHYTIC FUNGAL INOCULATION AND VOLATILE ORGANIC COMPOUND (VOC) TREATMENT

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The use of natural plant growth promoters, such as endophytic fungi, is gaining significant attention in agricultural research due to their potential to enhance crop growth while reducing reliance on chemical fertilizers and pesticides. Endophytic fungi can positively influence plant growth and stress resistance, which is particularly relevant in the context of sustainable agriculture. In this study, we aimed to evaluate the growth-promoting effects of three endophytic fungi—*Cadophora fastigiata*, *Paraphoma fimeti* and *Plectosphaerella cucumerina*—on the growth of spring barley (*Hordeum vulgare*). In the first experiment, surface-sterilized barley seeds were inoculated with fungal spore suspensions, and the plants were grown in a greenhouse for 30 days in the multi-cavity trays. All three newly tested fungi positively influenced barley growth, with the most significant impact observed in root development. Inoculations with *P. cucumerina* and *C. fastigiata* outperformed other inoculations, resulting in a 50% increase in root dry weight, compared to 20% in the other treatments. The second experiment was conducted in plate-in-plate assays, where barley seedlings were exposed to *C. fastigiata* and *P. cucumerina* VOSs. The analysis of primary and lateral roots revealed that both fungal VOCs stimulated root development. After 7 days, barley treated with *P. cucumerina* VOCs exhibited a 110% increase in primary roots compared to the control, while *C. fastigiata* treatment resulted in a 70% increase in lateral roots. Evaluation of root hair number revealed a two-fold increase following exposure to *C. fastigiata* and *P. cucumerina* VOCs. This suggests that VOCs play a key role in mediating the root growth-promoting effects of endophytic fungi. Expanding this research to other crops could lead to the further implementation of fungi-based biostimulants in modern agricultural practices.

A7

GROWTH-PROMOTING EFFECTS OF THREE ENDOPHYTIC FUNGI ON ITALIAN RYEGRASS (*LOLIUM MULTIFLORUM*)

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Many studies confirm that almost all plants are hosts of endophytic fungi, and their importance to plant communities became evident when their stimulatory effects on plant growth were demonstrated. However, the stimulating effect of many fungal species on plant growth is insufficiently studied or not studied at all. We studied the impact of *Cadophora fastigiata*, *Paraphoma fimeti*, and *Plectosphaerella cucumerina*, previously isolated from the roots of *Festuca*/*Lolium* grasses, on the growth of Italian ryegrass (*Lolium multiflorum*). Sterilized seeds of *L. multiflorum* were inoculated with a spore suspension of three endophytic fungi and their mixture. Before harvesting, the inoculated plants were grown in a greenhouse in the original cylindrical element system. After sixty-three days of growth following inoculation, the stimulatory effects of the three endophytic fungi on *L. multiflorum* plants (N=30) were evaluated: aboveground and root biomass were weighed; the number of nodding and total stems were counted; root length and shoot height were measured. All three fungi were found to positively affect the growth of Italian ryegrass plants, with the most pronounced impact observed in their root size. All fungal inoculations significantly promoted root growth of *L. multiflorum*, resulting in a 20–30% increase in dry weight compared to non-inoculated plants. Treatments with *C. fastigiata*, *P. fimeti*, and the spore mixture resulted in a 20% to 26% increase in the biomass of both fresh and dry shoots. In contrast, the *P. cucumerina* treatment showed a relatively smaller biomass increase of 11%. Thus, our study demonstrated the potential of the grass root-derived endophytes *Cadophora fastigiata*, *Paraphoma fimeti*, and *Plectosphaerella cucumerina* as growth promoters of Italian ryegrass. These studies can be extended to other major crops and grasses by evaluating different fungal isolates.

A8

DETECTION OF *SARCOCYSTIS* SPP. IN THE INTERNAL ORGANS OF COMMON RAVENS (*CORVUS CORAX*)

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Members of the genus *Sarcocystis* are cyst-forming intracellular coccidian parasites, which infect various animals and humans [1]. *Sarcocystis* spp. are characterized by a two-host prey-predator life cycle. The intermediate host (IH) acquires the infection through the ingestion of food or water contaminated with sporocysts, while the definitive host, which is a predator or scavenger, becomes infected by consuming the infected tissues of the IH [2]. Currently, the genus *Sarcocystis* includes more than 200 species, though the precise number of species is not well-defined [3]. Some *Sarcocystis* species are pathogenic for their IHs. Currently, detection of *Sarcocystis* parasites in the IH relies on microscopical and molecular methods analysing muscle tissue. During the previous studies carried out in Lithuania, five *Sarcocystis* species (*S. corvusi*, *S. cornixi*, *S. halieti*, *S. kutkienae,* and *Sarcocystis* sp. ex *Corvus corax*) have been identified in the muscles of birds of the family Corvidae [4]. Of the identified *Sarcocystis* spp., *S. halieti* is known to cause encephalitis in the little owl (*Athene noctua*), while the possible pathogenicity of other parasite species has not yet been examined, as previously only muscle samples of corvids have been investigated. Thus, this study aimed to detect and identify *Sarcocystis* parasites in various organs of corvids by means of molecular analysis.

Eight common ravens (*Corvus corax*) were collected during 2023-2024, and the following organs were used in the study: brain, heart, kidney, liver, and lungs. The obtained organs were homogenized using the artificial pepsin digestion protocol in two ways: separately as individual organs and as a pooled sample from a single common raven. The resulting homogenate was used for gDNA extraction. Species-specific nested PCR was utilized to amplify partial sequences of the *ITS-1* region to identify *Sarcocystis* spp. Parasite species were confirmed by Sanger sequencing.

Sarcocystis kutkienae was detected in seven of eight (87.5%) common ravens examined when organs were digested separately. In contrast, when organs of the same bird were pooled, the detection rate of *S. kutkienae* was lower, as only five birds (62.5%) were found to be infected with the mentioned *Sarcocystis* species. Notably, *S. kutkienae* was discovered in all organs analysed. Most commonly this parasite species was molecularly confirmed in heart muscles (7/8, 87.5%), followed by liver and lung (4/8, 50% each). Also, the parasite was identified in two brain and single kidney samples, respectively. Thus, this study presents the first molecularly based identification of *Sarcocystis* parasites in corvids. Further histopathological examinations are planned to examine the possible hazard effects of the detected *Sarcocystis* species on common ravens.

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A9

MOLECULAR IDENTIFICATION OF PARASITES IN VILNIUS CITY SOIL SAMPLES

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There are more than 600 000 cats in Lithuania, but a large number of them live outside. There are currently no studies on what parasites cats can spread, or whether they might be parasites dangerous to humans. In Vilnius, the municipality initiated the establishment of winter houses at stray cat feeding sites. In addition to feeding and resting, the cats also defecate near these houses, potentially contaminating the environment with various parasites.

The study analysed 90 soil samples collected at the winter cat houses in Vilnius. The samples were tested for the presence of *Toxoplasma gondii*, *Sarcocystis bovifelis*, *S. hirsuta*, *S. myodes*, *S. truncata*, *S. entzerothi* protozoa and *Taenia taeniaeformis, Dipylidium caninum, Mesocestoides* spp. cestodes.

After analysis of soil samples, we found *Sarcocystis bovifelis* in six samples, *S. morae* in four samples, *Sarcocystis* sp. in two samples and *S. myodes* in one sample. According to the bioinformatic analysis of DNA sequences, *Sarcocystis* sp. was genetically closest to *Sarcocystis* species whose intermediate hosts are members of the Cervidae family. Based on the species identified, it is assumed that stray cats were infected with *Sarcocystis* parasites by feeding on the muscle tissue of rodents (*S. myodes*), cattle (*S. bovifelis*) and cervids (*Sarcocystis* sp. and *S. morae*). Pathogenic *Toxoplasma gondii* and the cestode species tested were not detected.

A10

CHARACTERIZING ECOSYSTEM-SPECIFIC PATTERNS OF ANTIBIOTIC RESISTANCE

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Antibiotic-resistant bacteria (ARB) are a growing concern in agricultural ecosystems, which shape bacterial communities and serve as hotspots for antimicrobial resistance (AMR), even in environments where antibiotics are not used. ARB can spread through soil, water, and animal feed, increasing the risk of resistance gene transfer to pathogenic bacteria, which poses a significant threat to human and animal health. Understanding ARB dynamics in these systems is essential for assessing natural background resistance levels and evaluating risks. This study investigates ARB distribution and resistance patterns under ecological farming conditions in Lithuanian farmland ecosystems, focusing on soil, feed, and freshwater samples.

Forty-seven bacterial strains resistant to ampicillin, streptomycin, tetracycline, and chloramphenicol were isolated from three pastoral dairy farmlands and identified using 16S rRNA sequencing with comparative analysis via the NCBI database. Statistical assessments included relative frequency distribution, Chi-square test, correspondence analysis, and correlation analysis. Minimum inhibitory concentration (MIC) test was conducted to determine resistance levels for each antibiotic across all strains, with breakpoints defined by Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

The strains belonged to 13 different genera, with *Bacillus*, *Enterobacter*, and *Acinetobacter* being the most prevalent, while *Enterococcus*, *Escherichia*, *Klebsiella*, and *Morganella* were least detected. The highest abundance of ARB was observed in soil, followed by feed and freshwater. A significant association between ARB genera and environmental sources was observed (Chi-square = 42.28). Correspondence analysis showed that *Solibacillus, Morganella,* and *Providencia* were most associated with soil, *Escherichia* with feed, *Klebsiella* and *Enterococcus* with freshwater. Correlation analysis showed strong relationship between *Enterococcus*, *Solibacillus*, and *Providencia* genera ($r \geq 0.95$), suggesting potential co-occurrence and co-resistance mechanisms. The MIC tests of each strain revealed that the highest resistance rate was detected against ampicillin, followed by chloramphenicol, streptomycin, and tetracycline. Our results suggested that about 90 % of bacteria exhibit resistance to more than one antibiotic, with 18 strains resistant to all four antibiotics, 11 strains were resistant to three, 11 resistant to two, and only 5 strains resistant to a single antibiotic.

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A11

Vegetation–environment interactions: vegetation groups in *Carex rostrata* rich fen communities

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Rich fens are remarkable habitats with heterogenous, high species-richness vegetation and unique species composition. Many rich fens habitats (Alkaline fens (7230), Transition mires and quaking bogs (1740), Calcareous fens with *Cladium mariscus* and species of *Caricion davallianae* (7210)) based by Annex 1 of the EU Habitats Directive [1] are under protection in Europe. Moreover, many rare and endangered species are also found in these habitats. In Lithuania, natural fen habitats occupy less than one percent of the country's territory and have been poorly studied [2]. A considerable part of the rich fens in Lithuania is dominated by brown mosses and *Carex rostrata* in the herb layer. According to Peterka et al. [3], these communities can be attributed to the *Sphagno warnstorfii–Tomentypnion nitentis* alliance. Local ecological conditions within communities lead to the formation of specific vegetation patches. The aim of this study was to investigate the vegetation groups (vegetation patches) of rich fen communities with *Carex rostrata* and to ascertain their ecological conditions and differences in bryophyte cover. This research was conducted at seven study sites. Species cover and abundance were recorded in 39 study plots (4 × 4 m) using the Braun-Blanquet (1964) scale. For each study plot, topographical characteristics, as well as physical (pH, conductivity) and chemical parameters of water—including concentrations of Ca^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, NH_4^{+}, NO_3^{-}, and PO_4^{3-}— were measured. TWINSPAN analysis was performed to identify plant species groups. The ecological differences between the groups were clearly confirmed by PCA ordination.

According to the composition of the herb layer TWINSPAN identified vegetation groups characterized by *Agrostis stolonifera–Calla palustris*, *Carex lepidocarpa*, *Carex limosa*, *Eleocharis quinqueflora*, *Phragmites australis*, and *Typha* species. Additionally, the group combining vegetation patches rich in various herb and shrub species was distinguished. The PCA ordination diagram revealed that the composition and distribution of vegetation groups were primarily determined by variations in hummock cover and iron concentration, while other parameters showed only slight differences between groups. The patches of particular vegetation groups were characterized by a different composition of bryophytes. *Hamatocaulis vernicosus*, EU Habitat Directive species [1] was recorded in most of vegetation groups (except group with characteristic *Carex lepidocarpa*). The other most frequent species were *Calliergonella cuspidata*, *Marchantia polymorpha*, *Plagiomnium ellipticum*, *Tomentypnum nitens*, nevertheless their abundance varied. Bryophyte layer of the groups with characteristic hummocky topography (those with *Phragmites australis*, *Typha* sp. and rich in shrub-herb species) was formed by *Helodium blandowii*, *Palludella squarrosa* and *Sphagnum* species.

In conclusion, such studies help identify the key indicator components that reflect anthropogenic impacts and the influence of climate change on fen habitat vegetation, allowing for the anticipation of future threats and changes.

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A12

HABITAT-DEPENDENT AND TEMPORALLY DECREASED DISPERSAL PATTERNS IN INCREASING WHITE-TAILED EAGLE *HALIAEETUS ALBICILLA*

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Dispersal is a fundamental ecological process that affects animal population structure, genetic diversity, and species distribution by preventing inbreeding and promoting gene flow [1]. It plays a crucial role in wildlife conservation, as understanding dispersal patterns helps identify suitable habitats, mitigate human–wildlife conflicts, and support ecosystem stability. For apex predators such as the White-tailed Eagle, dispersal can influence prey species dynamics and habitat occupation, making it essential to understand for effective conservation planning.

Our study investigated the dispersal patterns of White-tailed Eagles in Lithuania, examining the effects of age, natal habitat, and possible changes over time on dispersal distances. Using ringing and ring recovery data collected from 1988 to 2023, we analysed dispersal distances, temporal changes and habitat dependence in 315 White-tailed Eagles. Additionally, we tested for age-related variations in dispersal distance within pre-mature age groups (1-4cy), and between adult (5+cy) and pre-mature birds.

Dispersal was age-independent, with no significant differences in distance between pre-mature and adult birds. However, eagles that hatched in coastal areas dispersed significantly shorter distances than those that hatched inland. Moreover, over the last decade, dispersal distances have decreased significantly, with this decrease being more pronounced for birds originating from inland habitats (see Figure 1).

Our results suggest that dispersal patterns in White-tailed Eagles in Lithuania are influenced by the natal habitat, with higher quality habitats, rich in prey resources and nesting opportunities, likely leading to decreased dispersal distances [2]. Temporal patterns suggest that the growing population in the region—the population increased from solitary pairs in the late-1980s to approximately 200 pairs in recent years [3]—and therefore increasing densities in breeding areas, may be contributing to more localised movements [2] by serving as an indicator of habitat quality, acting as a social cue relating to resources and breeding partners to other eagles [4]. In addition, other factors may have further decreased dispersal distance, such as milder weather conditions during autumn-winter and an increase in prey abundance. This highlights the role of natal habitat quality and population density in shaping movement behaviour, with potential future implications for population dynamics, habitat occupation and interspecies interactions in the region.

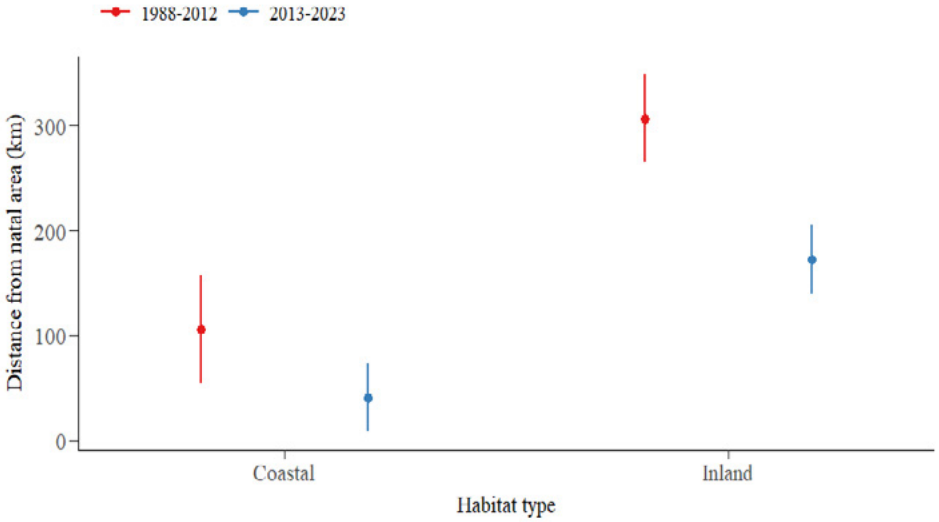

Figure 1. Dispersal distances (kilometers) of birds that hatched in coastal and inland habitats during the early stages of population establishment—1988–2012, and after the population had become firmly established—2013–2023.

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A13

HYMENOPTERA (INSECTA) IN LARGE DIAMETER DEAD TREE TRUNKS OF DECIDUOUS TREE SPECIES IN LITHUANIA

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An important component of the forest ecosystem and an indicator of sustainable forestry is dead wood, which helps maintain ecosystem diversity and increase their suitability [1]. Insects of the order Hymenoptera are characterized by an extremely high diversity in dead wood. This group is extremely rich in parasites, decomposers, and predators. They play an important role in the wood decay process and in controlling forest pest populations [2]. In the past, Hymenoptera insects in dead wood were rarely studied in Lithuania. Therefore, to conserve Hymenoptera, it is important to study their habitat needs. The aim of the study was to assess the diversity of Hymenoptera in the dead wood of four species of deciduous trees of the second decay class in protected areas in Lithuania.

The study was conducted in protected areas of Lithuania: Būda Botanical-Zoological Reserve; Dubrava Reserve Area; Punios Šilas Strict Nature Reserve. These areas are part of the „Natura 2000“ ecological network of European importance. Insects were caught in 2021 from May to September. 18 trunk-emergence-type traps were installed on 4 tree species (*Alnus glutinosa*, *Betula sp.*, *Populus tremula*, *Quercus robur*) of large-diameter deadwood trunks of the second decay class, covering a 1-meter section of deadwood (Fig. 1, B). The collected insects were described in the laboratory based on morphological characteristics to the family or subfamily.

During the study, 1157 individuals of the order Hymenoptera from 12 superfamilies and 24 families were identified. The Hymenoptera Aculeata group consisted of 231 individuals, most of which belonged to the family Formicidae (208), the Parasitica group consisted of 926 individuals with the most abundant family Ichneumonidae (195) (Fig. 1, A). On average, the largest number of individuals were caught from *Q. robur*. The least number of different taxa (families) was averaged from *A. glutinosa*, while the average number of different taxa was similar in other trees. Individuals from twelve families were caught from all four tree species, and seven from the wood of only one tree species. The family Ichneumonidae consisted of 14 different subfamilies. The highest average number of Ichneumonidae individuals caught was from *Q. robur*, and the lowest was from *P. tremula*.

The study found that dead wood of deciduous trees is characterized by a high diversity of Hymenoptera, especially parasitic wasps. Further studies are needed to assess the diversity of Hymenoptera insects associated with deadwood in more detail, so that these indicators can be used for conservation management of habitats.

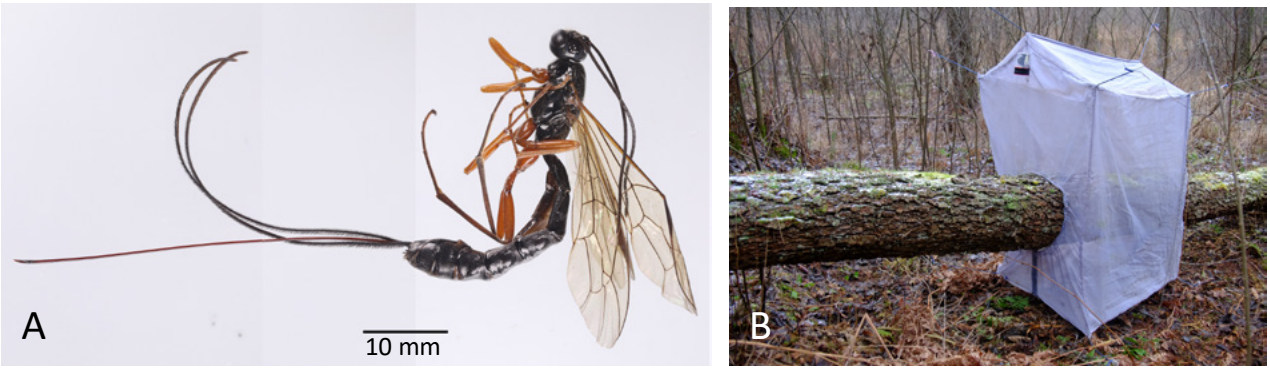

Figure 1. (A) Pimplinae (Ichneumonidae), Canon EOS 80D camera, Canon Mp E 65 mm Macro Lens; (B) trunk-emergence-type trap on *A. glutinosa* trunk.

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A14

WILD BEES AND WASPS (HYMENOPTERA: ACULEATA) IN RESTORED INLAND SAND DUNES IN SOUTHEASTERN LITHUANIA

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The biggest threat to biodiversity is habitat loss. The most endangered are xerothermic habitats, which provide refuge for many hymenoptera insects [1]. Hymenoptera Aculeata are important in arthropod population management and plant pollination. [2]. Habitat management has the potential to provide suitable conditions for Hymenoptera to establish. Interaction of Hymenoptera and other biodiversity elements is an important indicator of ecosystem health, therefore studies of this community may be important in assessing habitat quality and necessary conservation actions. [3]. The aim of the study was to assess the diversity of Hymenoptera in newly opened inland dunes in southeastern Lithuania.

The research was conducted in Dzūkija National Park, in the Alkūnės Kampas, Marcinkonys, and Dravių Kampas geomorphological reserves. In these areas, the LIFE integrated project "Optimizing the Management of the Natura 2000 Network in Lithuania" was implemented, and in 2023 - 2024 three continental dune plots were opened for the restoration of habitats of European Community importance. Insects were caught in 2023 and 2024 from June to August. Ten yellow and white pan (Moericke) traps were used in open dune plots and surrounding forests (control) (Fig. 1). The collected insects were identified in the laboratory based on morphological characteristics to the species level.

During the study, 667 individuals of the order Hymenoptera from 103 species, 17 families, and 5 superfamilies were identified. The largest number of families was from the Apoidea superfamily (13), with the family Sphecidae having the largest number of individuals (220). The most species-rich family was Pompilidae (29). Of the identified species, 51 species were captured only in open inland dunes and 24 species were captured only in forest habitats. In the open dunes, the Lithuanian Red Data Book species *Scolia hirta* (Schrank, 1781) was caught in traps. 28 species were captured in both forest and open continental dune habitats, with the majority of species being relatively more abundant in the dunes. The average number of individuals per trap per day was higher in open continental dune habitats.

The study found that insect species associated with open sandy areas (bees and wasps), which provide important ecosystem services, increased in the open dunes. Continental dune habitats are not fully established, so further research is important to assess the importance of such projects.

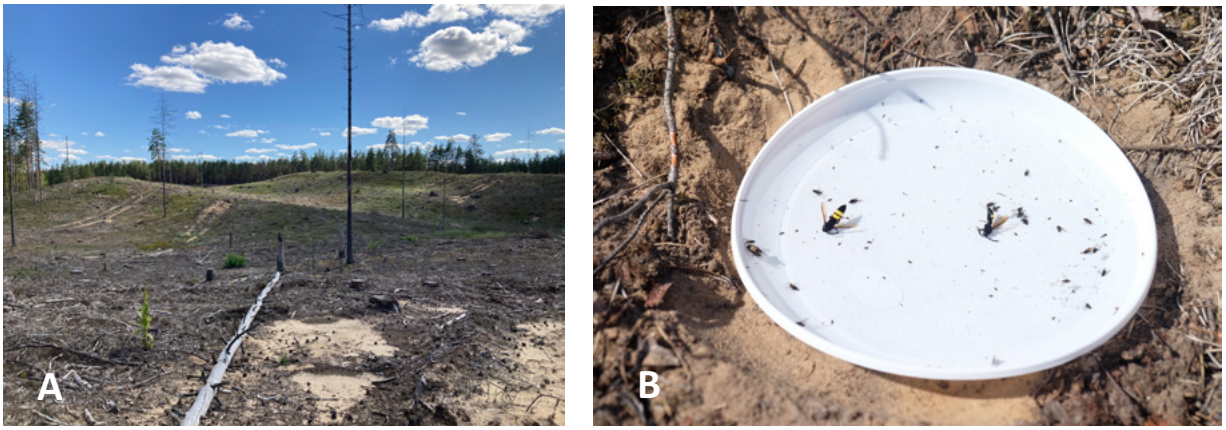

Figure 1. (A) View of open inland dunes; (B) Pan (Moericke) trap in inland dune habitat.

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A15

MOLECULAR IDENTIFICATION OF *CRENOSOMA VULPIS* IN THE RESPITORY TRACT OF THE RED FOX (*VULPES VULPES*)

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Crenosoma vulpis are widely distributed lungworms infecting carnivores, mainly red foxes (*Vulpes vulpes*) [1]. Nematode larvae infect the tissues of terrestrial gastropods, which are intermediate hosts [2]. After being eaten, the larvae grow up in the respiratory tract of the final host [2]. In the present study, trachea and lung samples of seven red foxes hunted in various regions of Lithuania between 2023 and 2024 were examined for nematodes. The lungworms were characterized morphologically by stereomicroscopy, light microscope, and molecular analysis (12S ribosomal DNA (rDNA)). Out of seven red foxes, three (42.8%) were infected with parasitic worms belonging to the genus *Crenosoma*. Overall, 31 nematodes were detected in the trachea and bronchi of the red fox and were morphologically identified as 16 male and 15 female *Crenosoma vulpis*. On the basis of 12S rDNA sequences, *Crenosoma vulpis* was identified in the trachea and bronchi of the red fox in Lithuania for the first time

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A16

Heavy metal pollution in the muscles of Twaite shad, *Alosa fallax*, migrating for spawning in Lithuanian coastal waters

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Twaite shad, *Alosa fallax* (Lacépède, 1803), is a diadromous pelagic fish, with a wide distribution throughout Europe, from Iceland to Morocco, including the Baltic Sea [1]. In the first half of 20th century Twaite shad was the most abundant and one of the most important fish of the family Clupeidae in the North and Baltic Seas as well as in the Curonian Lagoon, Lithuania [2]. Around the 1950s the abundance of Twaite shad started noticeably decline due to, presumably, increased pollution from nutrients, heavy metals, and other pollutants in the lagoons of the southern Baltic, blockage of migration routes by hydroelectric dams, and destruction of spawning habitats [2]. For a long time Twaite shad was considered almost extinct. Species was included to Red Data Book of Lithuania since 1992 [3].

There are various inputs of environmental contaminants, including heavy metals such as arsenic, cadmium, lead, copper, zinc, chromium which can have a negative impact on wildlife, including marine organisms, due to their ability to bioaccumulate [4]. Fish may accumulate substantial amounts of metals, especially in their muscles and adipose tissues, fish can therefore be used as semi-quantitative bioindicators, reflecting the occurrence and bioavailability of contaminants [5], [6].

Although this species has been affected by damage from marked destruction of spawning habitats and pollution, little is still known about the contaminants in muscle tissues. Moreover, given its growing economic importance, especially in the south-eastern Baltic Sea, there is a lack of information on its toxicity as a food for human consumption.

The aim of this study was to determine the contamination of six heavy metals - arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), copper (Cu) and zinc (Zn) - in the muscle tissues of this species, to determine whether Alosa fallax could be used as a semi-quantitative bioindicator in the coastal waters of Lithuania and the level of quality for human consumption.

The samples were collected from the Curonian Lagoon around Cape Ventė. In this area, Twaite shad spawn or pass on migration to spawning grounds towards the open lagoon areas opposite the Nemunas delta. Samples collected during the spawning migration in June 2022. Heavy metal concentration analysis was performed by Perkin Elmer Optima 1000 DV ICP-OES inductively coupled argon plasma spectrometer (ICPMS).

The results showed a tendency of low levels of heavy metal concentrations accumulated in the muscle tissue of Twaite shad. No significant correlation was found between metal concentrations and body size. Finally, the concentrations of none of the metals in muscle tissues exceeded the EU and FAO/WHO limits. This demonstrates that Twaite shad is a SAFE for human consumption. However, further detailed studies of other important contaminants in different tissues, such as liver and gills, could be useful for assessing bioaccumulation of pollutants in Twaite shad.

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A17

EVALUATION OF THE EFFECTIVENESS OF MOLLUSCICIDES WITH IRON PHOSPHATE

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The native range of *Arion vulgaris* Moquin-Tandon, 1855 is considered from southwest France, but intensive spread and damage both to the biodiversity and agriculture in foreign countries [1], raises the need to include it in the list of invasive species. Although various measures are used to eradicate slugs, it continues to spread unstoppably – for example, in recent years it has been found in Armenia [2]. The aim of this study was to evaluate the efficacy of three molluscicides with iron phosphate approved in Lithuania under field conditions as laboratory studies have revealed very poor effectiveness [3].

The experiment took place in Vilnius, in the area of Bajorai Gardens (Coordinates: 54.756537, 25.246075), from 10 July to 10 August 2024 with slugs of different body mass (Fig. 1). Each slug was provided with a portion of food that comprised 5 g of lettuce ("Iceberg") and five pellets of one molluscicide type which were supplemented every three days. It was tested three types of molluscicides with different concentration of iron phosphate approved in Lithuania, here named as M1, M2, M3 (Fig. 1). Each treatment contained 20 slugs of the same size group in tin fences driven into the ground and tightly covered with water-permeable agro textile, in a shaded area of the garden. Each treatment took 14 days and was carried out 2 times. Data were analyzed using SPSS Statistics 23 software [4].

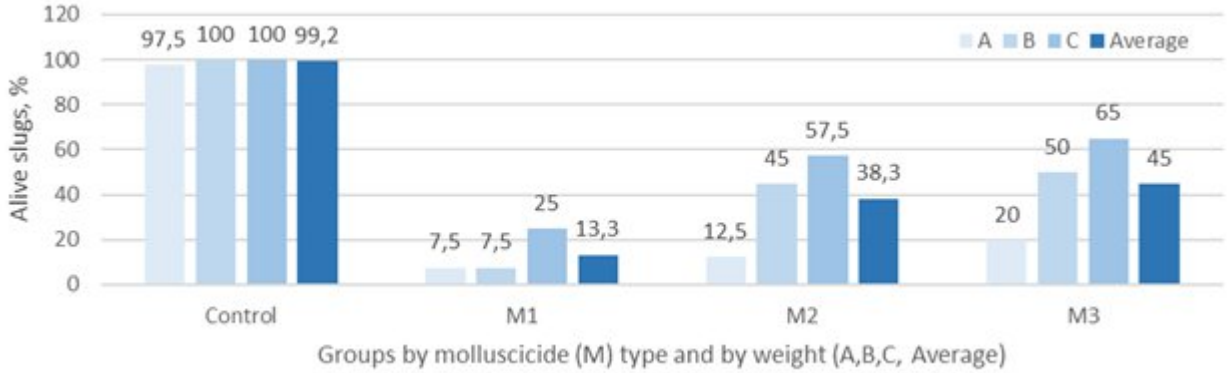

Figure 1. Percent of alive slugs after treatment of molluscicides with different concentrations of iron phosphate: M1 – 24,2 g/kg; M2 – 29,7 g/kg; M3 – 9,9 g/kg. Groups by weight: A – 1,07 ± 0,39 g; B – 3,1 ± 0,41 g; C – 4,77 ± 0,58 g.

In general, the effectiveness of all molluscicides in field was higher than in laboratory. The results (Fig. 1) showed that M1 was the most effective (86,7% average mortality) molluscicide, although it contains not the highest concentration of iron phosphate (24,2 g/kg). The molluscicide with the lowest concentration of iron phosphate (M3: 9,9 g/kg), the only one from tested types available to ordinary consumers (the others are intended for professional use), had the worst effectiveness (55% average mortality). The smallest slugs were the most susceptible to all molluscicides, but effectiveness varied 2-3 times.

In summary, studies have revealed: 1) when choosing the most effective molluscicide, its effectiveness should be tested not only in laboratory but also in field conditions; 2) it is obvious that not only the active ingredient determines the effectiveness, but also the overall composition of the ingredients; 3) to achieve the best results, molluscicide should be used most intensively when there are small slugs – all tested molluscicides are the most effective in the smallest age group of slugs (~ 1 g), and least effective in the largest (~ 5 g); 4) in order to improve biodiversity conservation strategies and destroy an invasive slugs, it is necessary to hire specialists who have a permit to use pesticides.

Acknowledgments: Purchase of molluscicides was supported by grant from the BASF SE (Germany) and the Vilnius University (Lithuania) R&D Service Agreement No (1.57) 15600-INS-43.

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A18

EVALUATION AND COMPARISON OF THE GENOTOXIC EFFECTS OF PHTHALATES ON BONE MARROW CELLS OF 2nd GENERATION RATS

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Phthalates are chemical compounds widely used as plasticizers in various PVC (polyvinyl chloride) products. They leach out of plastic products quite easily during use, enter the environment and can damage endocrine system of mammalians, affect their development and male reproductive system [1]. The aim of this study was to determine the genotoxic effects of di-(2-ethylhexyl)-phthalate (DEHP) and di-butyl-phthalate (DBP) in concentrations found in Lithuanian wastewater on second-generation rats. This experiment was approved by the Ethical Committee of State Food and Veterinary Service of the Republic of Lithuania (2022-08/09, No G2-221).

The 36 female *Wistar* strain rats, approximately 1 month old, were divided into 6 groups, each group containing 6 rats. These rats were bred from an earlier part of the project – the first generation of rats, which were also exposed to the same doses of phthalates. Every day, along with their food, the rats were fed a biscuit with a drop of phthalates dissolved in oil. The first group was the control group, which received a biscuit with only oil. The second and third groups were exposed to DEHP at doses of 200 µg/kg/d (DEHP_2) and 1000 µg/kg/d (DEHP_10). The fourth and fifth groups received DBP doses of 100 µg/kg/d (DBP_1) and 500 µg/kg/d (DBP_5). The sixth group received a mixture (DEHP+DBP) of DEHP 200 µg/kg/d and DBP 100 µg/kg/d. After 2 months, the rats were euthanized and their bone marrow preparations were prepared for micronucleus assay. The micronucleus test was performed by counting 2000 PCE cells from each sample, including cells with micronuclei [2].

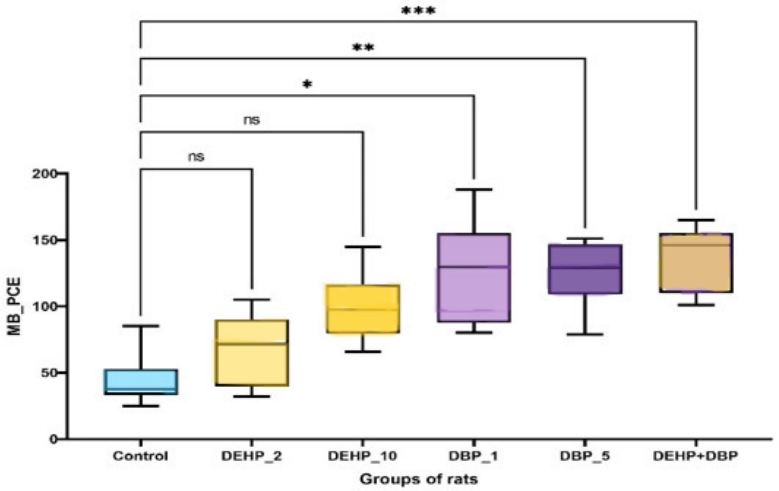

Figure 1. Distribution of polychromatic erythrocytes with micronuclei (MB_PCE) in the studied groups. The asterisk indicates statistically significant results: * - p < 0,05; **- p < 0,01; *** - p < 0,001. Statistical analysis was performed using Kruskal - Wallis post hoc Dunn test.

The results (Fig. 1) showed significant increase of polychromatic erythrocytes with micronuclei (MB_PCE) in the groups affected by both DBP doses (DBP_1 and DBP_5) and mixture (DEHP+DBP). This suggests that even low concentrations of DBP found in Lithuanian waters have a genotoxic effect. Meanwhile, the genotoxic effect of low doses of DEHP, observed in generation I [3], disappears, which most likely reflects increased levels of chromosome repair and adaptive capabilities of the organisms. Conclusion: DBP is more genotoxic than DEHP, therefore the use of even low doses of DBP in plastics should be banned and wastewater should be cleaned of phthalates.

Acknowledgments: This project was financed by the Science Promotion Fund for Scientific projects of VU, Agreement No MSF-JM-18/2022.

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A19

THE EFFECT OF THE POLYSTYRENE DIET ON THE FITNESS OF *TENEBRIO MOLITOR* (L.) OF VARIOUS LARVAL STAGES

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Tenebrio molitor, a grain store pest, is an insect that has found various uses. *T. molitor* larvae are not only a nutritious source of food for animals and humans, but have been used as a model organism in various research fields. Insect larvae exhibit plastic chewing behavior, which inspired the idea of using *T. molitor* as a biodegradation agent. Special interest was taken in expanded polystyrene (PS) biodegradation, as expanded polystyrene due to its low density is easy for insects to chew, takes up a lot of space in landfills, has high recycling cost and limited profitability. Also, polystyrene does not degrade easily, it stays in the environment from decades to centuries, or even longer, if it is not exposed to direct sunlight [1] or mechanical abrasion. Scientists have performed many biodegradation tests, but the results still are inconclusive. [2, 3].

The aim of this study was to evaluate the effect of a polystyrene diet on the different larval stages of *T. molitor*. It was important to determine the survival and fitness of *T. molitor* at different larval stages after polystyrene ingestion. For that purpose, insects were distributed into four diet groups: oatmeal, oatmeal and polystyrene, polystyrene only, and starvation. Weight of larvae was recorded and dead larvae were removed every two days during the 30 days of the experiment. Surviving individuals were collected for protein content evaluation and biomarker enzymes activity assay. The enzymes glutathione S-transferase (GST) and acetylcholine esterase (AChE) were used as biomarkers. Activity changes of these enzymes can indicate negative impact on the organism (AChE activity change can indicate neurotoxic effects on the organism, and the GST activity change can indicate elevated oxidative stress and detoxification processes [4]). Protein concentration was determined by Badford [5] method. Acethylcholine esterase (AChE) activity was determined by Ellman method adapted for microplates [6]. Glutathione S transferase (GST) activity was determined by Habig et al. [7] method adapted for microplates.

The data has shown that polystyrene consumption is not lethal for *T. molitor* larvae, but it affects fitness. The mortality rate of the larvae does not depend on the chosen diet. Larval survival did not differ significantly between starved larvae and those fed only polystyrene, as well as survival did not differ significantly between larvae fed polystyrene supplemented oatmeal diet and those fed an oatmeal diet only in any age group. The same trends held for the larval weight gain. In most age groups (except for the youngest) polystyrene and oatmeal fed larvae accumulated less protein than those fed oatmeal diet only. In all age groups except for the youngest AChE activity is elevated in groups that consumed polystyrene. GST activity change did vary in different age groups. There was no significant difference in activity in the youngest larvae group, middle aged larvae showed increased GST activity in polystyrene consuming group and the oldest larvae showed highest activity in starved group.

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A20

THE IMPACT OF SALINIZATION ON THE INVASION SUCCESS OF THE CYANOBACTERIUM *CHRYSOSPORUM BERGII* IN FRESHWATER ECOSYSTEMS

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Chrysosporum bergii is a cyanobacterium belonging to the Nostocales group, which is native to the saline Aral Sea, but also occurs in freshwater ecosystems outside its native range. The spread of this cyanobacterium to the northern regions of Europe is due to changing environmental factors such as global warming and salinization of freshwater. Salinization of freshwater ecosystems is one of the current problems associated with human activities leading to changes in biodiversity. The changes in ecosystems create conditions for the establishment and development of halotolerant alien cyanobacteria. The invasion of alien cyanobacteria can lead to a deterioration in water quality and changes in the structure of the local community, altering the nutrient cycling and displacing native aquatic plants. The aim of this study is to determine the influence of freshwater salinization on the establishment of the cyanobacterium *Chrysosporum bergii* and the resulting changes in the structure of the native phytoplankton community.

The establishment of *Chrysosporus bergii,* cyanobacteria species alien in European freshwater, was investigated in a microcosm experiment with the native spring community under controlled laboratory conditions. The duration of the experiment was thirteen days. In this experiment, two treatment groups were formed: one containing the native phytoplankton community alone and the other containing the native phytoplankton community with the addition of alien cyanobacterium *C. bergii.* Each group was subjected to four salinity levels (freshwater (0 g/L NaCl) and three brackish water treatments (0.2, 1 and 5 g/L NaCl)). The growth and establishment of *C. bergii* were monitored at the beginning and end of the experiment, as well as the taxonomic composition and biomass of the phytoplankton community. The phytoplankton samples were analyzed using a Nageotte counting chamber (0.5 mm^3) under a light microscope (Motic B3 Professional Series) following LeGresley, McDermott [1].

The analysis of the phytoplankton samples revealed the presence of four different taxonomic groups in all treatments: Bacillariophyta, Ochrophyta, Cyanophyta and Chlorophyta. When comparing the different taxonomic groups under freshwater and brackish water conditions, a change in phytoplankton biomass was observed. Green algae (Chlorophyta) grew similarly at all salinity levels. However, Ochrophyta were only able to develop and thrive in non-saline and low-salinity (0.2 g/L NaCl) environments. While diatoms (Bacillariophyta) developed successfully at all salinity levels and generally outcompeted other phytoplankton species, *Chrysosporum bergii* became the dominant species at the highest salinity level (5 g/L NaCl). So, it can be concluded that non-native cyanobacterium *C. bergii* is able to adapt and thrive in a saline environment and displace other phytoplankton species to become a dominant species in the ecosystem.

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A21

OBLIGATE SAPROXYLS OF GENUS *LIPSOTHRIX* (DIPTERA, LIMONIIDAE) IN DEAD, PARTIALLY SUBMERGED WOOD

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The genus *Lipsothrix* Loew, 1873, belongs to the order Diptera and the family Limoniidae. These insects are obligate saproxyls because their larvae develop in dead, partially submerged wood, primarily in small, clean streams. There are 35 known species of *Lipsothrix* worldwide, with four recorded species in Lithuania: *Lipsothrix errans* Walker, 1848; *Lipsothrix remota* Walker, 1848; *Lipsothrix nobilis* Loew, 1873; and *Lipsothrix ecucullata* Edwards, 1938. No research has been conducted in Lithuania regarding the diversity of *Lipsothrix* species in dead, partially submerged wood. The main aim of this study is to identify *Lipsothrix* species from dead, partially submerged wood in Lithuania using both morphological and molecular methods.

Material was collected by hand from dead, partially submerged wood in May, June, September, and October 2024. Tools used for collection included measuring tapes, a knife, tweezers, and small jars filled with 70% alcohol. Morphological methods were employed to identify larvae and adults. Microscopic preparations of the larvae's head capsule and terminal segment were photographed using a Leica DM6 B microscopic camera. For molecular identification, techniques such as DNA extraction, polymerase chain reaction (PCR), DNA electrophoresis, and purification of PCR products were utilized. Samples were sent for sequencing to BaseClear B.V. in Leiden, Netherlands. The DNA sequence fragments were then analyzed using BioEdit and MEGA software.

A total of 1,136 individuals of the *Lipsothrix* genus were collected — 1,127 larvae and 9 pupae. The head capsule and terminal segment of the larvae were described. *Lipsothrix errans* and *Lipsothrix remota* were identified through morphological and molecular methods. Notably, the larval stage of *L. remota* was previously unknown to science. It was confirmed that both species can coexist at the same site; however, further research is needed.

Two species: *L. errans* and *L. remota* were identified from four occurring *Lipsothrix* species in Lithuania.

Figure 1. General larvae view of genus *Lipsothrix* (by Virginija Podėnienė).

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A22

Great tit (*Parus major*) nestlings have longer telomeres in old-growth forests than in young forests

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Modification and deterioration of old-growth forests by industrial forestry have seriously threatened species diversity worldwide. The loss of natural habitats increases the concentration of circulating glucocorticoids and incurs chronic stress in animals, influencing the immune system, growth, survival, and lifespan of animals inhabiting such areas [1].

In this study, we tested whether great tit (*Parus major*) nestlings grown in old-growth unmanaged coniferous forests have longer telomeres than great tit nestlings developing in young managed coniferous forests.

This study showed that the patches of young managed coniferous forests had lower larval biomass than old-growth forests. Since insect larvae are the preferred food for great tit nestlings, the shortage of food may divert energy resources away from growth, which can show up as physiological stress, often raising the heterophil/lymphocyte (H/L) ratio. The H/L ratio revealed a significant difference in stress levels, being the highest in great tit nestlings developing in young-managed pine forests. We also found that the development of great tit nestlings in young managed forests had significantly shorter telomeres than in old-growth forests. Although nestling survival did not differ between the habitats, nestlings growing up in old-growth forests had greater telomere lengths, which can positively affect their lifespan.

Our results suggest that the forest habitats affected by industrial forestry may represent ecological traps as the development of young birds in deteriorated environments can affect the age structure of populations.

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A23

VARIABILITY IN THE BODY ELEMENTAL COMPOSITION AND REPRODUCTIVE OUTPUT OF THE BUFF-TAILED BUMBLEBEE

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Free-living organisms encounter various stressors in their environments, and the quality of these habitats often impacts their development and life history traits. The rising pressures from agricultural intensification have been shown to affect the diversity and abundance of insect pollinators, and it may also influence their elemental makeup. We examined reproductive success, body concentrations of carbon (C) and nitrogen (N), and the C/N ratio, each serving as indicators of stress, in the buff-tailed bumblebee (*Bombus terrestris*). Bumblebee colonies were placed in oilseed rape fields and in semi-natural ancient apple orchards. After the flowering season, colonies were removed and frozen. Their reproductive output was assessed by counting hatched and unhatched cells. Individual bumblebees were dried and homogenized to obtain their whole-body C and N mass percentage using element analyzer. We then compared how these metrics differ between bumblebee castes, sexes and their field of origin. The reproductive success was significantly greater in oilseed rape fields than in apple orchards, while the C/N ratio of queens and workers, which indicates physiological stress, was lower in apple orchards, where bumblebees exhibited a notably higher body nitrogen concentration. We concluded that the more productive environment of oilseed rape fields provides bumblebees with greater opportunities to enhance their fitness than the more natural setting of old apple orchards, albeit at the cost of physiological stress, as demonstrated by the significantly elevated C/N ratio found in bumblebees living in oilseed rape fields.

A24

Serotonin-dependent repression of glucose metabolism by induced predator stress in fruit flies *Drosophila melanogaster*

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Predation can have both lethal and non-lethal effects on prey. The non-lethal effects of predation can instil changes in prey life history, behaviour, morphology and physiology, causing adaptive evolution. The chronic stress caused by sustained predation on prey is comparable to chronic stress conditions in humans. Conditions like anxiety, depression, and post-traumatic stress syndrome have also been implicated in the development of metabolic disorders such as obesity and diabetes.

Drosophila flies were reared in incubators at 23 ± 1°C. This study used the wild strains *Oregon-R* and w^{1118} of *D. melanogaster* obtained from the Bloomington Drosophila Stock Centre (IN, U.S.A.). The experimental groups were grown with pirate otter-spiders (*Pirata piraticus*). Flies remained together with spiders for their egg and larval stages. Adult flies were collected within 5–7 h after the imaginal eclosion for biochemical analyses or used for behavioural assays within 2–3 days after eclosion. To measure the rate of feeding, food supplemented with blue dye (Blue FCF dye) was fed to flies. The amount was quantified spectrophotometrically from homogenate. Starvation tolerance measurements. In the chronic starvation tolerance test, flies were kept on 1% agar in tubes containing 10 individuals. Survival was monitored every 3 h. Death was determined as the last activity time point from the final recorded activity for each fly. In the acute starvation tolerance test, flies were starved on deionized water-soaked filter paper.

To assess whether a diabetes-like phenotype has any adaptive value, we tested the survival of *Drosophila* under conditions of direct predation by spiders. We used 10 experimental and 10 control groups, each consisting of 10 male flies. We placed each group in a plastic container (20 cm width, 10 cm depth, 10 cm height) for 12 h during daylight time. Each jar contained one pirate otter-spider and one vial with *Drosophila* food. Surviving flies were counted at the end of the experiment. Each spider was used only once.

To perform behavioural trials, we used sterile Petri dishes moulded from clear polystyrene (60 × 15 mm) as novel arenas to record individual flies' locomotor activity. Only one fly was aspirated into the arena for each test. The locomotor activity of six flies was recorded with the resolution of 1920 × 1080 pixels at 5 frames per second simultaneously by a video-tracking system using the Logitech HD Pro Webcam C920 (Logitech Inc., Newark, CA, USA). Distance moved with the temporal bin width of 1 min as the most important locomotor activity parameter was extracted offline from the recorded video files using EthoVision. The distances moved were used to calculate the speed.

Western analyses: batches of flies were homogenized in western lysis buffer supplemented with protease and phosphatase inhibitor cocktails. Lysates were incubated on ice for 15 min and then centrifuged. Supernatant protein concentrations were measured using the Bradford assay (Thermo no. 1856209), and 70 µg aliquots were loaded onto precast Bio-Rad Criterion AnyKD gradient gels. Gels were run in ProSieve EX running buffer. Proteins were transferred to Amersham Protran nitrocellulose membrane (no. 10600020) in ProSieve EX transfer buffer. Membranes were incubated in 5% BSA in 1× TBS/0.05% Tween for 1 h for blocking, after which they were incubated in the same buffer with primary antibodies. Antibodies and dilutions used were: Akt 1 : 5000 (Cell Signaling no. 9272), phospho-Akt 1 : 5000 (Cell Signaling no. 4054), ACC 1 : 5000 (Cell Signaling no. 3676), HRP-conjugated anti-rabbit 1 : 10 000 (PI-1000-1).

After washing membranes three times for 15 min with 1× TBS/0.05% Tween, they were incubated with anti-rabbit secondary antibody. After an additional three rounds of washing, results were visualized with the BioRad ChemiDoc XR detection system. The signal was quantified, and the data were analysed with ImageQuant software.

Metabolite analyses: we detected total glucose, free glucose, glycogen, trehalose, total glycerol, free glycerol, triglycerides, ATP, pyruvate. Respiration exchange ratio was calculated as the ratio of CO_2 produced and O_2 used.

In this study, we found that predator stress induced during larval development in fruit flies *Drosophila melanogaster* impairs carbohydrate metabolism by systemic inhibition of Akt protein kinase, which is a central regulator of glucose uptake. However, *Drosophila* grown with predators survived better under direct spider predation in the adult phase. Administration of metformin and 5-hydroxytryptophan (5-HTP), a precursor of the neurotransmitter serotonin, reversed these effects.

Our results demonstrate a direct link between predator stress and metabolic impairment, suggesting that a diabetes-like biochemical phenotype may be adaptive in terms of survival and reproductive success. We provide a novel animal model to explore the mechanisms responsible for the onset of these metabolic disorders, which are highly prevalent in human populations [1].

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A25

Bet-hedging strategy in *Drosophila melanogaster*: serotoninergic modulation of phototactic variability

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Bet-hedging, a strategy for coping with environmental uncertainty, may explain the diverse light preferences observed in *Drosophila melanogaster*. To investigate this, we examined the phototactic behavior of flies from stable tropical and variable boreal climates, using a custom-designed instrument enabling rapid, simultaneous quantification of individual fly responses. Flies were reared on food supplemented with either 5-HTP or αMW to manipulate serotonin levels. We then compared the phototactic variability between the two fly populations and analyzed the effects of altered serotonin levels on this behavior. Our findings reveal greater phototactic variability in the boreal population and suggest that serotonin plays a key role in mediating bet-hedging strategies in response to unpredictable environments [1].

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A26

Bumblebee infestation and interspecific competition with honeybees intensify near honeybee hives

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Bumblebees are essential pollinators in temperate regions of the Northern Hemisphere. However, niche overlap and competition with honeybees can pose significant stressors, reducing nutrient acquisition and facilitating the spillover of pathogens and parasites. One such parasite is the bumblebee wax moth (*Aphomia sociella*), whose larvae infest bumblebee and wasp nests, as well as weakened commercial honeybee hives. Using *Bombus terrestris* colonies as experimental models, we investigated the effects of competitive pressure (proximity to honeybee hives) and parasitic *A. sociella* infestation on bumblebee immunity, measured by encapsulation response in young queens and workers. We hypothesized that both factors would weaken immune function, with the strongest immune suppression occurring in infested nests closest to honeybee hives. Our findings revealed increased infestation rates, lower reproductive output, and weaker encapsulation responses in bumblebee colonies near honeybee hives. These results offer insights into the ecology of *A. sociella* infestations in environments where honeybees and bumblebees coexist. The observed reduction in immune response among bumblebees in close proximity to honeybee hives is particularly concerning, as it may increase their susceptibility to additional parasites and pathogens.

A27

ECOPLATE™ AS A TOOL FOR SCREENING THE SUBSTRATES STIMULATING PLASTIC-DEGRADERS

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The efficiency of plastic biodegradation is influenced by plastic properties (e.g. types, glass transition temperature, surface hydrophobicity, types of additives), organism species (e.g., bacteria, fungi), and abiotic environmental factors (e.g. temperature, oxygen content, solar radiation, physical abrasion) [1]. The impact of various co-substrates, e.g., Tween 40, Tween 80, and glycogen on microplastic breakdown has been demonstrated earlier [2,3,4].

Ecoplate™ (Biolog, USA) are used to evaluate the metabolic activity of microbial communities and their biodegradation potential, revealing dominant groups of target substrates [5,6]. This study was aimed at estimating the shift in the community-level physiological profile of two microbial communities, which were derived from landfill and wastewater treatment plant (WWTP) and exposed to microplastics with particle size ≤300 µm. Two types of plastic, i.e., polyethylene terephthalate (PET) and polyethylene (PE) were used. Among 31 substrates in EcoPlate™, glycogen, D-cellobiose, glucose-1-phosphate, and alpha-ketobutyric acid promoted the growth of microbial community under the selective pressure of PET or PE (Table 1). Additionally, our data showed, that glycogen consumption by WWTP microbial community was dependent on the level of chemical oxygen demand tested in the range 500-1500 mg/L. Other substrates have also stimulated the microbial growth in the presence of plastic; however, this effect was plastic-specific or/and dependent on the period of incubation. In particular, landfill microbial community exposed to PET, was stimulated by D-galactonic acid-gamma-lactone, 4-hydroxy benzoic acid, while WWTP microbial community – by alpha-cyclodextrin, D-cellulose, and itaconic acid, respectively.

Table 1. The substrates of Biolog EcoPlate™ stimulating the growth of microbial communities in the presence of microplastic. The stimulation effect is expressed as a ratio [growth with plastic/growth without plastic]. Incubation in EcoPlate™ was performed at 30 °C for 24h.

Substrate	Ratio [growth with plastic/growth without plastic]			
	Landfill		WWTP	
	PET	PE	PET	PE
Glycogen	1.05	0.67	2.25	3.97
D-cellobiose	1.32	1.13	5.19	1.15
Glucose-1-phosphate	4.25	0.48	7.70	0.84
Alpha-ketobutyric acid	9.62	1.65	1.98	0.49

The findings related to specific responses of microbial activity to carbon source in the presence of microplastic bridge a knowledge gap in the mechanisms of plastic degradation by complex microbial communities derived from landfills and activated sludge from WWTP.

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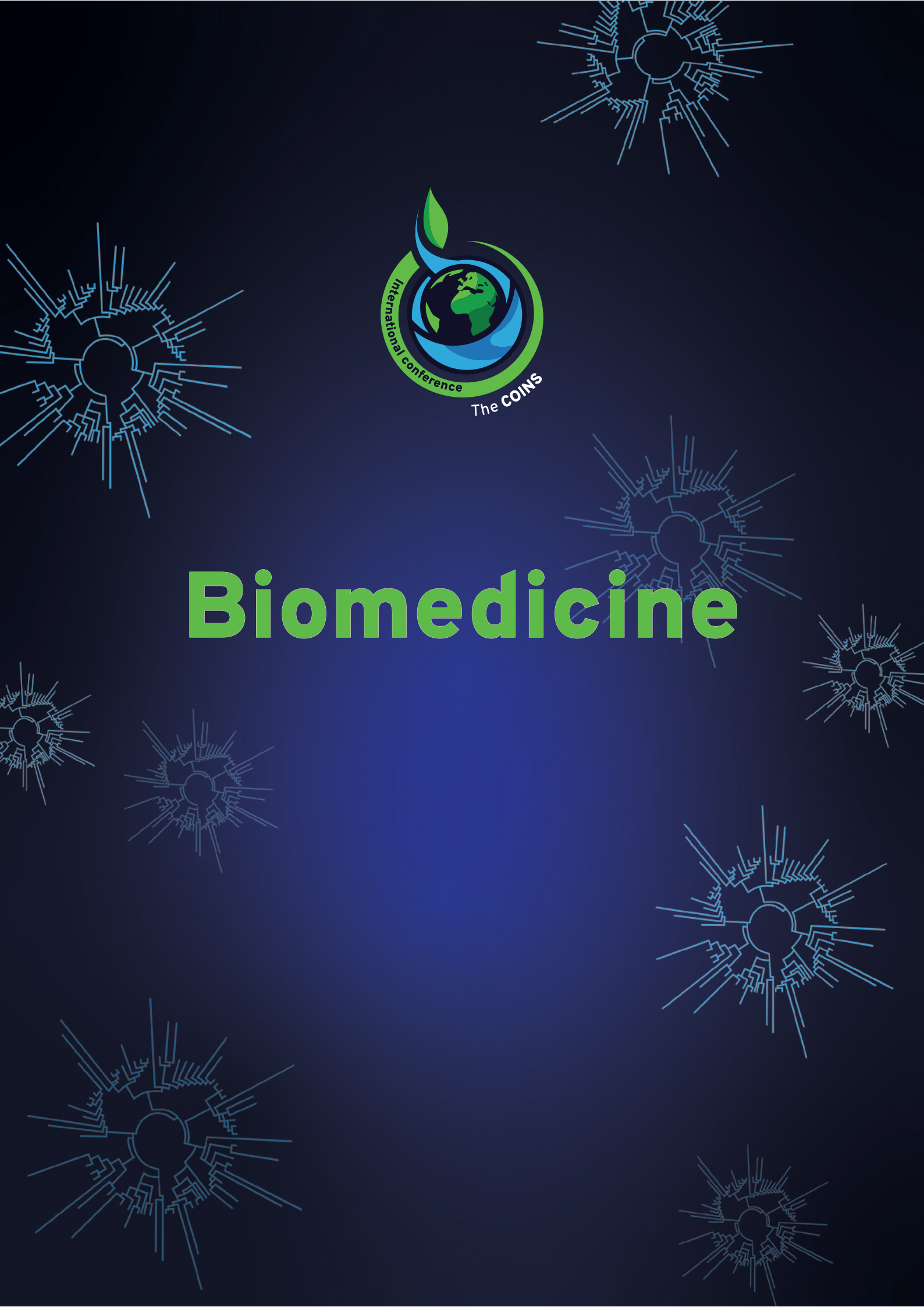

Biomedicine

B1

MORTALITY RISK IN MAJOR CAUSES OF DEATHS AMONG CHORNOBYL LIQUIDATORS: EXAMING THE IMPACT OF THE ARRIVAL PERIOD TO THE DISASTER ZONE

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Introduction. After the Chernobyl NPP accident, workers, known as liquidators, were brought in to address the aftermath of the disaster. During the period of 1986-1987 alone, approximately 240,000 liquidators were called to the Chornobyl zone, which included both direct work at the damaged reactor and in the 30 km zone surrounding the NPP. [1]. Chornobyl liquidators faced acute, high-dose exposures in an uncontrolled emergency. Immediately after the accident, the construction of a protective sarcophagus began, which lasted until November 1986. With the completion of the sarcophagus, the radiation released into the environment from the damaged reactor decreased. Additionally, the largest cleanup efforts to mitigate the consequences of the accident were carried out in the first months following the disaster.

Methods and materials. Our study analyzed data of 523 Chornobyl NPP liquidators with a total person-years of follow-up of 11,372.09. At the beginning of the follow-up, the average age of the liquidators was 39.0 years. By the end of the follow-up their average age was 60.7 years, indicating that the study covered a significant portion of their lives, particularly as they entered higher cancer risk years. Our study included only men due to the insufficient number of women.

The follow-up period begins on the date of the cytogenic test (1991-1994, 2020-2021) and continues until the earliest of the following events: the date of death or the end of follow-up on December 31, 2002. The dates of the death were acquired from the State Register of Death Cases and Their Causes. Standardized mortality ratios (SMR) were determined by comparing the observed number of deaths to the expected number. The expected numbers were determined by multiplying the person-years at risk – stratified by 5-year age groups and 1-year calendar periods – by the corresponding mortality rates in Lithuania. To calculate the 95% confidence intervals (CI) for the SMRs, we assumed that the observed case numbers followed Poisson distribution.

We categorized liquidators based on their arrival time in the Chornobyl NPP accident zone:

1. From date of the accident until 1986-10-25 (until the protective sarcophagus was built),
2. 1986-10-26 to 1987-04-25, and
3. All who arrived after 1987-04-25.

Results. When comparing mortality data by major causes of deaths among Chornobyl liquidators who arrived in the disaster zone at different periods, a statistically significant increase in overall mortality risk was observed among those who arrived after April 25, 1987 (SMR 1.25, 95% CI 1.03–1.53).

In this group, there was also a significantly increased mortality risk due to alcohol poisoning (SMR 2.75, 95% CI 1.14–6.61). Additionally, the risk of suicide was nearly three times higher, with an SMR of 3.56 (95% CI 1.97–6.42).

Meanwhile, mortality due to malignant neoplasms (SMR 1.34) and cardiovascular diseases (SMR 1.17) was higher among those who arrived in later periods, particularly after 1987. Other causes of death, such as infectious or respiratory diseases, also showed an increased risk at certain arrival dates, though not as pronounced as external causes.

Conclusions. Our study's findings, particularly the increased mortality from alcohol poisoning and suicides, reinforce that mental health disorders were a major public health issue after the Chernobyl NPP accident. Liquidators faced uncertainty about their health, societal stigmatization, and concerns about future generations, leading to long-term psychological consequences. Persistent anxiety and unexplained symptoms remain prevalent [2].

The designation of "Chernobyl victims" and state-provided aid may have further impacted their mental health. The excess mortality from suicides and alcohol poisoning highlights the lasting psychological burden on liquidators, underscoring the need for continued attention to their mental well-being [3]. The increased mortality risk from cancer and cardiovascular diseases may be linked to both radiation exposure and other risk factors. Further detailed studies are needed to assess these contributing factors.

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B2

INVESTIGATION OF CONNEXIN 43 SENOTHERAPEUTIC AGENTS EFFECTS ON CHONDROCYTE PHENOTYPE IN VITRO

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Human articular cartilage is known to have poor self-regeneration capacity following injury or damage, leading tissue to progressive degeneration and diseases such as osteoarthritis (OA). Unfortunately, traditional treatment methods are often ineffective in stopping inflammatory tissue degeneration and progression of OA [1]. Senotherapeutic peptides targeting gap junction channels forming proteins, such as connexin 43 (Cx43), introduce a promising approach to anti-inflammatory effects and promoting tissue regeneration [2]. However, despite growing interest in Cx43, only few studies have demonstrated different compounds effect on Cx43 and articular cartilage regeneration [3].

The aim of this study is to evaluate the effects of connexin 43 senotherapeutic agents on chondrocyte phenotype using 3D human articular cartilage cell culture models.

Human cartilage explants were isolated from OA patients after knee joint replacement surgery in compliance with all bioethical requirements. Explants were incubated for 3 and 7 days in chondrogenic medium without growth factors with 12 µM and 6 µM senotherapeutic agents TUB1-Arg and TUB1-PEG6 added. Additionally, a known inflammatory cytokine interleukin-1β (IL-1β) was added to stimulate inflammatory conditions on articular cartilage tissue. Gene expression of *GJA1* gene (Cx43 coding gene) and cartilage typical genes as *COL1A1*, *COL2A2*, *COL10A1*, *SOX9*, *ACAN* were analysed after incubation with mentioned agents using RT-qPCR assay.

Results revealed that agent TUB1-Arg 12 µM downregulated *COL2A1*, *COL10A1* and *ACAN* gene expression after 3 days of incubation, while prominently upregulated *COL10A1* gene expression after 7 days of incubation, compared to untreated samples. Meanwhile, 12 µM of TUB1-PEG6 agent upregulated *COL2A1* and *COL10A1* gene expression after 7 days of incubation, compared to untreated samples. Compared to 12 µM, 6 µM of TUB1-Arg had minor effect and 6 µM of TUB1-PEG6 had a more prominent effect on gene expression of *COL1A1, COL2A1, COL10A1*. Results suggest, that 12 µM of TUB1-Arg and TUB1-PEG6 agents tend to downregulate *GJA1* gene expression in chondrocytes after 7 days of incubation, compared to samples stimulated with agents and IL-1β added. Meanwhile, samples stimulated only with IL-1β, demonstrated increased *GJA1* expression. This suggests that both agents may have a specific effect on cell-cell communication, considering the function of Cx43. Both agents 12 µM upregulated *SOX9* gene expression after 7 days of incubation. Considering that *SOX9* gene is important in regulating collagen type II synthesis, this result may imply that both agents indirectly affect *COL2A1* gene expression.

To conclude, our findings suggest that senotherapeutic agents targeting Cx43 could be a novel approach to treating OA, since increased Cx43 expression is a known factor relating to disease progression and articular cartilage inflammation and degeneration.

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B3

UNRAVELING FLASH RADIOTHERAPY: miRNA PATERNS AND CELL VIABILITY IN HEALTHY AND CANCEROUS 3D SPHEROIDS

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Radiotherapy (RT) is currently used in approximately 50% of all cancer patients [1]. While highly effective, its clinical potential is limited by toxicity to healthy cells, which restricts the doses that can be safely administered to the patients [2]. To address this issue, research has focused on studying the unique radiobiological responses observed at ultrahigh dose rates, known as FLASH-RT [3]. Recent studies have shown that FLASH-RT reduces damage to healthy tissues while maintaining tumor control, making it a promising alternative to conventional RT (CONV-RT) [4]. However, the molecular mechanisms underlying the distinct responses to FLASH and CONV irradiation remain insufficiently understood. Therefore, the aim of this study was to compare the effects of FLASH-RT and CONV-RT on miRNAs expression and cell viability in prostate cancer and normal colon 3D spheroids.

All samples were irradiated using a clinical linear accelerator modified to achieve the required dose rates for FLASH-RT. Prostate cancer (C4-2) and normal colon (CRL-1541) cell spheroids were irradiated with 17 Gy doses. MiRNA-21-5p, miRNA-29a-3p, and miRNA-222-3p levels were measured at 12-, 24-, 48-, and 72-hour post-RT using reverse transcription quantitative PCR. Results were normalized to exogenous control cel-miRNA-39-3p, and miRNAs expression was determined relative to untreated controls using the $2^{-\Delta\Delta Ct}$ method. Cell viability was assessed on days 1, 4, 7, and 11 post-RT using the XTT assay, with results expressed as a percentage relative to untreated controls.

At 24 hours post-RT, there were no differences in miRNAs expression between CONV-RT and FLASH-RT in either cell line. By 72 hours, CRL-1541 cells showed significant downregulation of the studied miRNAs following CONV-RT compared to FLASH-RT, while no significant differences were found between two treatments in C4-2 cells. Additionally, CONV-RT significantly reduced cell viability compared to FLASH-RT on day one post-RT in CRL-1541 cells, whereas no significant differences in FLASH-RT and CONV-RT treatment effects were observed in C4-2 cells.

Distinct miRNA expression patterns and cell viability outcomes suggest differing mechanisms of FLASH-RT and CONV-RT in healthy and cancerous cell lines. Notably, miRNAs expression at 72 hours post-RT and cell viability on day one post-RT reveal that while FLASH-RT affects cancer cells similarly to CONV-RT, it shows unique protective effects on normal cells at these time points. These findings suggest that FLASH-RT may selectively spare healthy cells, highlighting the need for further investigation into the underlying processes involved in its effect.

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B4

SYNTHESIS AND INVESTIGATION OF *N*-(2,4-DIFLUOROPHENYL)-β-ALANINE DERIVATIVES

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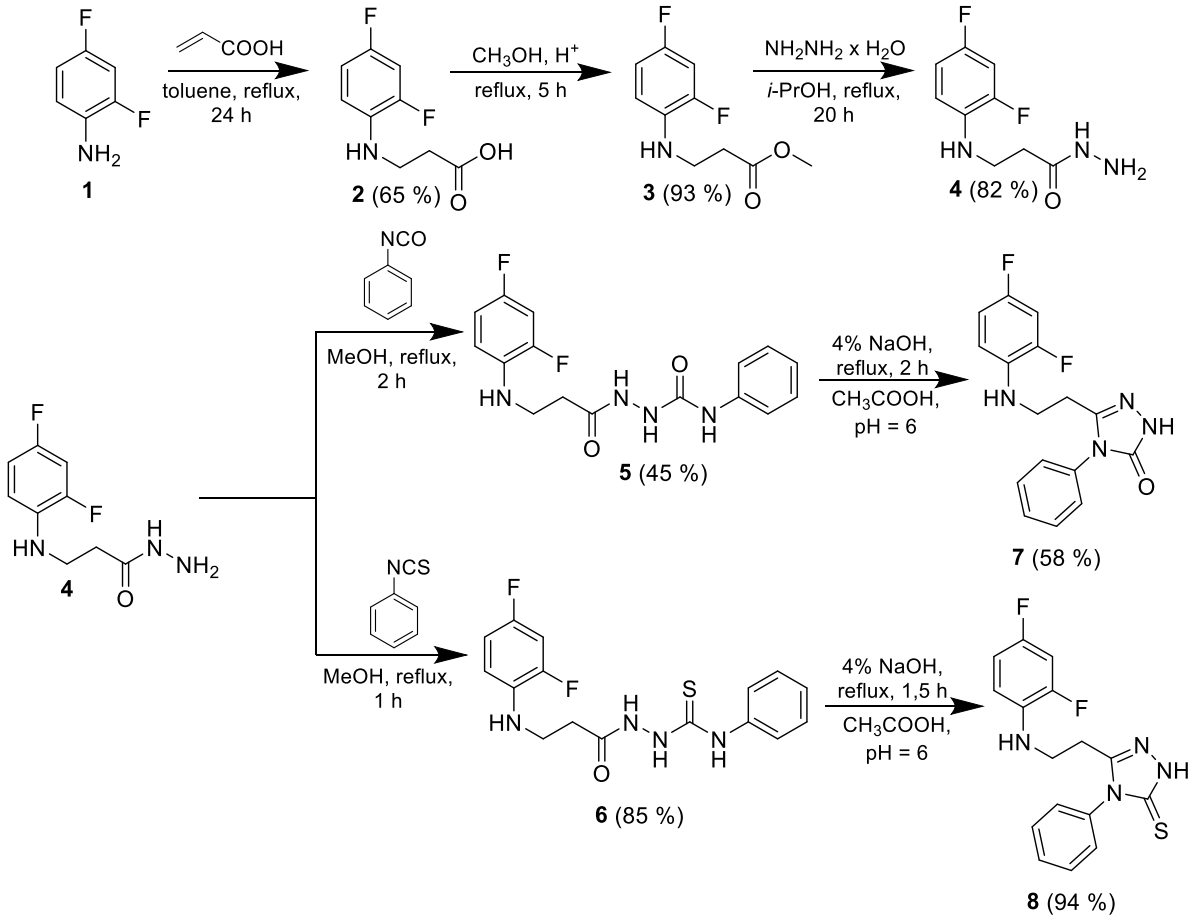
Thiosemicarbazide functional group is not only used to synthesize biologically active heterocyclic derivatives, this fragment containing compounds also exhibit antioxidant, antimicrobial and anti-inflammatory effects. Anticancer activity of thiosemicarbazides is being studied more widely, as cytotoxicity against HOP-92 and SK-MEL-2 cell lines *in vitro* were proven [1]. Triazolone ring containing compound Doravirine is one of the most promising non-nucleoside reverse transcriptase inhibitor (NNRTI) used in HIV treatment. Heterocyclic ring of this structure helps form hydrogen bonds with the target, therefore, based on SAR data, triazolone fragment plays crucial role in biological activity of therapeutic compounds [2].

The starting compound 3-((2,4-difluorophenyl)amino)propanoic acid (**2**) was prepared by the reaction of 2,4-difluoroaniline (**1**) with acrylic acid in toluene. Methyl 3-((2,4-difluorophenyl)amino)propanoate (**3**) was synthesized by the *Fischer* esterification reaction. Obtained methyl propanoate **3** was transformed into propanehydrazide **4**, by the reaction of hydrazine monohydrate in refluxing isopropanol. In the further step of the synthesis, reactions with phenyliso(thio)cyanate in refluxing methanol were investigated, semicarbazide **5** and thiosemicarbazide **6** were synthesized. Afterwards these compounds were cyclized using 4% NaOH solution into compounds **7** and **8** containing triazolone and triazolethione moieties, respectively (Scheme 1).

The structure of the compounds has been proven by ^{1}H NMR, ^{13}C NMR, FT-IR spectroscopy and elemental analysis. Investigation of the biological activity of the synthesized compounds are planned.

Scheme 1. Synthesis of 3-((2,4-difluorophenyl)amino)propanoic acid derivatives



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B5

MACROPHAGE-DERIVED HYBRID NANOPARTICLES FOR TARGETED DOXORUBICIN DELIVERY TO GLIOBLASTOMA

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Glioblastoma (GBM), an exceptionally aggressive form of brain cancer, typically presents a median survival rate of less than 2 years [1]. The blood-brain barrier (BBB) greatly restricts drug penetration, significantly contributing to ineffective treatment. Consequently, various nanoparticles (NPs) have been investigated for their potential to enhance the delivery of therapeutics to the brain. Hybridization of synthetic liposomes and natural exosomes has emerged as a promising strategy to enhance drug delivery across the BBB, improve NP biocompatibility, and optimize tumor targeting [2]. Our study focuses on synthesizing and characterizing hybrid NPs for doxorubicin delivery to GBM cells.

Two liposome formulations, F1 and F2, were created via thin layer evaporation, with specific lipid molar ratios: 6:3:1 for DPPC:CHOL:DSPEmPEG2000 in F1 and 7:4:6:1 for DPPC:DPPS:CHOL:DSPEmPEG2000 in F2. Lipid films were hydrated with PBS, $(NH_4)_2SO_4$ solution, or M_0 macrophage-derived exosomes in PBS, followed by extrusion through membrane filters. DOX was trapped using a pH gradient and remote loading, with entrapment efficiency (EE%) and release determined by fluorescence. Drug release was evaluated in HEPES with 10% FBS at pH 7.4 and 5.5 for 72 hours. Dynamic light scattering determined size, polydispersity index (PdI), and zeta potential (ZP). The stability of empty, DOX-loaded, and hybrid NPs at 4°C was assessed weekly for a month. The short-term stability of NPs in DMEM with 10% FBS at different pH levels was evaluated under stirring at 37°C for 72 hours.

The average size of F1, F2, M_0-F1, and M_0-F2 was < 150 nm; DOX-loaded liposomes increased by up to 20 nm. The PdI of empty and loaded NPs was < 0.2. ZP values were -19.4 ± 1.9 mV (F1), -25.7 ± 1.5 mV (F2), -36.4 ± 3.9 mV (M_0-F1), and -24.6 ± 2.4 mV (M_0-F2), while DOX loading altered ZP by 3 to 6 mV. EE% of F2 reached up to 62%, while that of F1 was only 25%. Additionally, DOX was released faster into the medium under acidic pH conditions, resembling the GBM environment. Liposomes remained stable in DMEM with serum under stressful conditions for 72 hours. NPs remained stable at 4°C for 4 weeks, except DOX-F1, which exhibited an increase in PdI up to 0.3 after 17 days.

In conclusion, initial findings suggest that the F2 liposomal formulation may be more suitable for fusion with exosomes due to its higher drug encapsulation efficiency and stability. However, more research is necessary to understand pharmacokinetics, potential surface modifications, and transport to GBM cell lines.

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B6

DEVELOPMENT OF HYBRID LIPID-POLYMERIC NANOPARTICLES FOR ISCHEMIC HEART DISEASE APPLICATIONS

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Partial or complete obstruction of the coronary arteries can lead to ischemic heart disease (IHD), also known as coronary heart disease (CHD) [1]. Insufficient heart muscle blood supply causes multiple symptoms and serious complications or even death. Prolonged ischemia results in myocardial necrosis, which is subsequently followed by scarring of the myocardium. This permanently affects the function of the myocardium. IHD is the leading cause of human morbidity and mortality worldwide [2]. Therefore, early detection of ischemia and prompt, effective treatment are essential to minimize the risk of further complications. Nanoparticles have become a promising tool in managing IHD, providing new opportunities for targeted drug delivery [3], imaging [4] and tissue regeneration [5]. Studies show that nanoparticles are promising not only in the visualization of damaged myocardium but also in therapy. In vivo studies have shown that incorporating cardiological drugs into nanoparticles can enhance their solubility, leading to improved absorption and distribution in the bloodstream and target tissues [6]. Additionally, nanoparticles can be engineered for gradual drug release, helping maintain therapeutic levels in the ischemic myocardium, reducing dosing frequency, and enhancing treatment efficacy [7]. This study aimed to optimize hybrid lipid-polymeric nanoparticles (HLPN) by adjusting various lipid-to-polymer ratios (1:10, 1:5, 1:3, 2:5, 1:2, 3:5, 2:3, 3:4, 4:5, 1:1). After synthesis, the most effective formulations, selected based on their physicochemical properties, were loaded with curcumin.

Poly(lactic-co-glycolic acid), cholesterol and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] were used for the synthesis of HLPN. The dynamic light scattering method was used to assess the nanoparticles' size, polydispersity index (PdI) and zeta potential. The colloidal stability of the selected HLPN was evaluated in a cell growth medium over 72 hours. Curcumin entrapment in HLPN was analyzed using fluorescence spectroscopy, and the entrapment efficiency was determined from the measured values.

Following synthesis, nanoparticles with sizes up to 150 nm were selected. Four different formulations (1:2, 1:3, 1:1, 2:3) were chosen for further experiments. Stability tests revealed that the 1:1 formulation exhibited variations in PdI, while the 2:3 formulation showed a significant increase in particle size. The most stable formulations were 1:2 and 1:3. After incorporating curcumin into the 1:2 and 1:3 nanoparticles, it was found that the 1:3 formulation, with a higher polymer content, exhibited a greater curcumin entrapment efficiency of 65%, compared to 54% for the 1:2 formulation.

In conclusion, the 1:3 nanoparticle formulation demonstrated the highest suitability for further studies, exhibiting improved stability and curcumin entrapment efficiency.

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B7

EMPLOYEES' OPINION ABOUT THE 4-DAY WORKWEEK AND ITS RELATION WITH MENTAL WELL-BEING

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The four-day workweek is gaining increasing attention and popularity worldwide, as organizations seek innovative solutions to enhance employee well-being and motivation while fostering a better work-life balance [1][2]. Recent years have seen a significant focus on the implementation of a four-day workweek, which is regarded as a strategy to improve working conditions and boost employee engagement. Companies in economically advanced countries such as Australia, the United States, and Japan have adopted and maintained the four-day workweek, although this practice is more common among smaller enterprises with fewer than 100 employees [3]. Research has demonstrated that the four-day workweek positively influences factors such as social interactions, happiness, stress management, mental and physical health, personal time, motivation, and environmental sustainability by reducing office electricity use, fuel consumption and CO_2 emissions [3][5]. Although some comprehensive studies explore productivity and business performance alongside health outcomes, research specifically examining the relationship between a four-day workweek and employee health remains limited, particularly in the context of Lithuania [4][6].

The aim of the study is to evaluate the opinions of employees at Company X in Lithuania regarding the four-day workweek and determine its relationship with well-being

A cross-sectional prevalence study was conducted with employees of Company X, the largest company in Lithuania to adopt a four-day workweek since January 2021. The target sample size was calculated to be 216 respondents from a pool of 490 eligible employees. Data were collected using a custom questionnaire developed to assess the relationship between the four-day workweek and employee health. The survey included 16 questions on demographics, work experiences and original WHO well-being questionnaire. The questionnaire was distributed through multiple channels, including company emails, internal news platforms, and printed forms for non-digital employees. A total of 221 completed responses were collected and analysed using Microsoft Excel and R Commander for statistical evaluation.

Statistical analysis revealed that 97% of respondents positively evaluated the four-day workweek, with 99% expressing a willingness to continue under this schedule long-term and 95 % of employees take pride in their workplace due to the opportunity to work a four-day workweek. The majority of employees, 74,2%, report that their workload, which is distributed across four working days, is considered to be of medium intensity. Additionally, nearly 60% of employees perceive the pace of work to be moderate. The overall level of emotional well-being reached 77 out of 100 points. It was observed that the fewer hours an employee works, the higher their level of emotional well-being.

To conclude, the four-day workweek is highly regarded by employees, with 97% evaluating it positively and 99% willing to continue long-term. Most employees find the workload and work pace moderate, and overall emotional well-being score is high. Additionally, fewer working hours are related with higher levels of emotional well-being.

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B8

THE ROLE OF TIANEPTINE IN REDUCING OBSESSIVE FOOD AND BODY IMAGE THOUGHTS IN ANOREXIA NERVOSA: A PROMISING THERAPEUTIC APPROACH

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Anorexia Nervosa (AN) is a severe psychiatric disorder characterized by a persistent restriction of food intake, a strong fear of gaining weight, and a distorted body image, leading to a significantly low body weight. [1] Psychotherapy and dietary management are central to AN treatment, but pharmacological options are limited, particularly for core symptoms like obsessive thoughts about food and body image. Most medications focus on managing comorbid conditions like anxiety and depression, but do not effectively address the cognitive distortions central to AN. [5] Tianeptine, a selective serotonin reuptake enhancer (SSRE), is an atypical antidepressant that has shown promise in treating psychiatric disorders, including depression and anxiety. Unlike conventional selective serotonin reuptake inhibitors (SSRIs), tianeptine enhances serotonin reuptake, which may offer a more balanced approach to serotonin regulation. [2] Recent research also suggests that tianeptine interacts with the mu-type opioid receptor (MOR), contributing to its therapeutic effects. [3] Additionally, its modulatory effects on dopamine and glutamate receptors could provide a comprehensive approach to addressing the multifaceted psychological and neurobiological components of AN. [4]

Two patients with severe anorexia nervosa were selected based on their lack of response to previous treatments, including multiple antidepressants and medications. Both were prescribed tianeptine alongside psychotherapy targeting food-related obsessions and distorted body image. Both patients came from dysfunctional family backgrounds, which were considered in treatment. Both patients also experienced suicidal ideation prior to starting tianeptine treatment. After starting tianeptine, significant improvements were noted in their obsessive thoughts and anxiety about food and body image, despite their low body weight. Additionally, two more patients have recently been started on tianeptine, and their progress is being monitored as part of an ongoing study. The research continues, with the aim of evaluating tianeptine's long-term effects and its potential as a treatment for anorexia nervosa.

These cases suggest tianeptine as a novel pharmacological approach for anorexia nervosa, particularly in alleviating resistant obsessive thoughts and anxiety. Its unique pharmacodynamics—enhancing serotonin reuptake and modulating μ-opioid receptors—set it apart from traditional SSRIs and may offer advantages in treating the psychological aspects of AN. Tianeptine's modulation of serotonin without causing overload, along with its potential neuroplastic effects, may contribute to its efficacy. [4]; [7] Additionally, its influence on opioid receptors can regulate mood and stress. [3] Traditional SSRIs are often less effective in patients with malnutrition or those with disrupted tryptophan metabolism, but tianeptine's receptor-based mechanism bypasses this limitation, making it potentially more effective in malnourished individuals. [6]

These initial results suggest that tianeptine may benefit not only the patients in these cases but also others with anorexia nervosa. Ongoing studies are assessing its continued efficacy, safety, long-term effects, and potential in combination with other therapies to optimize treatment for patients unresponsive to traditional interventions.

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B9

DEVELOPMENT OF CARDIAC HYPERTROPHY MODEL ON 3D SCAFFOLDS

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Introduction. Cardiomyocyte hypertrophy is characterized by morphological and structural declines of the heart tissue, making it one of the significant risk factors of cardiovascular diseases. Identifying the hallmarks of ageing cardiomyocytes is critical for developing therapeutic approaches to treat heart diseases [1]. The transformed human cardiac cell line AC16 is widely used to study cardiomyocyte biology and disease mechanisms. In vitro, various chemical stressors are used to induce senescence in these cells, including the cancer drug doxorubicin, which is known for its cardiac toxicity [2]. Although 2D cell cultivation models are commonly used, 3D cell cultures more accurately replicate the architecture of in vivo tissues, retaining their phenotypic and functional characteristics and thus providing a more physiologically relevant environment for evaluating biological responses [3]. This study aims to evaluate the morphological and functional changes in AC16 cardiomyocytes following senescence induction with doxorubicin in 3D polyvinylidene fluoride (PVDF) scaffolds and PVDF scaffolds incorporating graphene oxide (PVDF+GO).

Methods. The methods were first applied in 2D AC16 models and later extended to 3D constructs on PVDF and PVDF+GO scaffolds. Cell senescence and hypertrophy was induced by low dose of doxorubicin (0.05 µM). Cell proliferation was measured by Alamar Blue following cell cycle analysis by flow cytometry. Calcein AM staining was used to analyze cell size and DAPI staining for nuclear size. PVDF and PVDF+GO scaffolds with aligned fibers were fabricated by electrospinning. Biocompatibility of scaffolds was analysed by Live/Dead staining. Statistical analysis was performed using RStudio 2023.9.1.494, Student's t-test was used for comparisons between two groups, while two-way ANOVA followed by Tukey's post hoc test was employed for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

Results. Following the treatment with doxorubicin, morphological and functional changes, specific to hypertrophy, were observed in AC16 cells in 2D culture. Size of cell and nucleus was increased significantly. On account of cell viability, 0.05 µM doxorubicin reduced proliferation compared to control, cell cycle was arrested at G2/M phase suggesting that cell division has been inhibited, which is a hallmark of senescence. PVDF and PVDF+GO scaffolds were biocompatible and cells on scaffolds showed similar response to doxorubicin treatment – cell size and nucleus size increased significantly compared to control without changes between different scaffolds. Cell proliferation was also reduced by doxorubicin on both types of scaffolds, however, with significantly lower proliferation levels determined on PVDF+GO scaffold after 3 days of 0.05 µM doxorubicin treatment compared to PVDF under the same conditions.

Conclusions. Our results showed that the hypertrophic model induced in AC16 cells by 0.05 µM doxorubicin treatment can be transferred to a more realistic and biomimetic 3D format using PVDF and PVDF+GO scaffolds. These constructs can serve as an advanced model of hypertrophy for drug and biomarker development.

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B10

NEUROMUSCULAR ADAPTATIONS AND POWER RESPONSE TO EXERCISE UNDER THE INFLUENCE OF PSYCHO-EMOTIONAL STATE IN OVERWEIGHT WOMEN

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BACKGROUND: Resistance training is known to enhance muscle function, but its effectiveness may be influenced by external factors such as sleep quality and psycho-emotional state. Body composition changes are well-documented with training, but neuromuscular adaptations are crucial for strength, power, and muscle efficiency improvements. Poor sleep and high anxiety levels can impair recovery, motor unit recruitment, and overall training adaptations, potentially limiting power gains. This study aims to evaluate the relationship between body composition remodeling, muscular power gains, and psycho-emotional state in metabolically healthy overweight women after a 12-week exercise intervention.

METHODS: Thirty-five metabolically healthy overweight women (BMI 32 kg/m^2 ± 4.2, age 27-58, mean 41 years) participated in a 12-week exercise program comprising in total 150 minutes of aerobic sessions (60-70% max HR) every week and two 45-55 minutes of bodyweight resistance sessions weekly. Power were assessed via (1) 10-second maximal work on an ergometer, and (2) leg power via 5 repetitions on an isokinetic dynamometer. Body composition parameters, including BMI (kg/m2), weight (kg), water content (L), muscle and skeletal muscle mass (kg), and mineral content (kg), were evaluated using a body composition analyzer. Sleep quality, sleep duration, and psycho-emotional status were assessed using validated international questionnaires, including the *Hospital Anxiety and Depression Scale (HAD)* and *Pittsburgh Sleep Quality Index (PSQI)*. Statistical analysis involved descriptive statistics and Pearson's correlation using SPSS.

RESULTS: Significant correlations were observed among all body composition in overweight women following the 12-week exercise intervention, including decreases in BMI (0.8±1.7 kg/m^2, $p<0.01$), body weight (1.3±3.8 kg, $p<0.01$), total body water (0.4±1.1 L, $p<0.01$), muscle mass (0.5±1.4 kg, $p<0.01$), skeletal muscle mass (0.3±0.8 kg, $p<0.01$), and mineral mass (0.1±0.2 kg, $p<0.01$). However, power changes showed more associations with psycho-emotional factors and sleep quality than with body composition changes. Increased sleep latency score (1.1±0.9) was linked to lower gains in 10s maximal muscle work (-37.6W±78, $p<0.05$) and left leg extensor peak torque (4.7kJ±14, $p<0.01$), while higher sleep disturbance score (1.1±0.5) and Pittsburgh total score (5.3±2.9) correlated with reduced right leg extensor peak torque improvements (-2.8kJ±10, $p<0.05$). Conversely, higher HAD score (12±6) correlated with greater increases in body weight ($p<0.01$) and mineral mass ($p<0.01$), suggesting a link between anxiety and post-training adaptations.

CONCLUSIONS: The findings indicate that power gains in overweight women following a 12-week exercise intervention had more associations with neuromuscular adaptations rather than body composition changes. Increased sleep latency and disturbance were linked to reduced power development. Additionally, higher anxiety levels (HAD score) were associated with increased body weight and mineral mass, suggesting a complex interaction between psycho-emotional factors and training adaptations. These results highlight the need to consider sleep quality and anxiety when optimizing power training outcomes, especially in sedentary overweight populations.

B11

SYNTHESIS AND ALKYLATION OF 1-(NAPHTHALEN-1-YL)-4-PHENYL-1*H*-IMIDAZOLE-2-THIOL

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Over the years, imidazole based derivatives have been proven to be antibacterial, anti-inflammatory, antifungal, antioxidant, anticancer[1]. Likewise, the 1-naphtylamine fragment bearing compounds, due to their unique chemical structure, also acts as anticancer and antimicrobial agents[2,3].

The starting compound **2** was prepared by the reaction of 1- naphthylamine **(1)** and 2-bromo-1-phenylethan-1-one at room temperature. 2-(Naphthalen-1-ylamino)-1-phenylethan-1-one oxime **(3)** was obtained from aminoketone **2** and hydroxylamine hydrochloride reaction at reflux in the 2-propanol. Compound **4** was yielded by the reaction between aminoketone **2** and KSCN in 10% HCl at reflux.

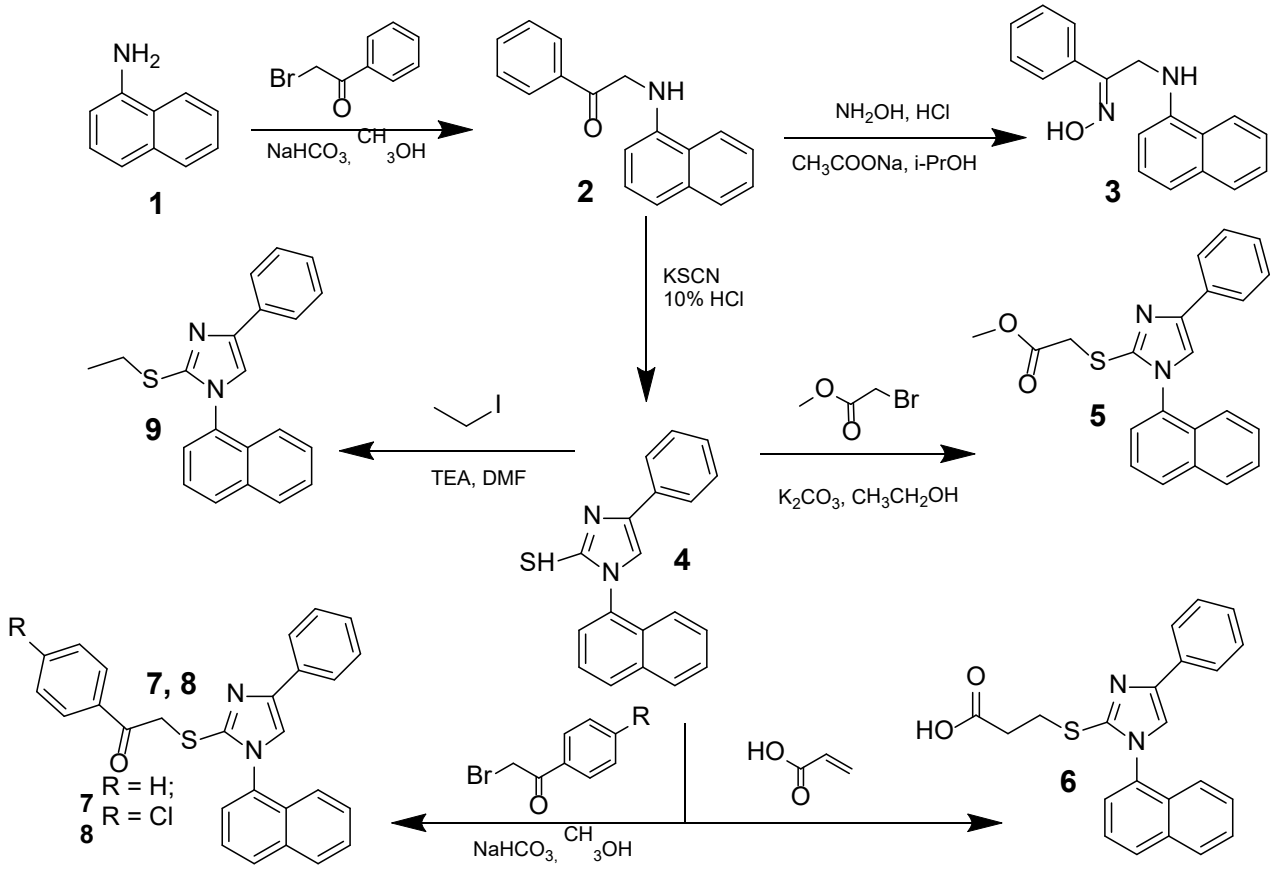

Figure 1. Synthesis of compounds **2–9**.

Alkylation reactions of the synthesized compound **4** with various alkylating agents were performed and it was found that in all cases only S-alkylated derivatives **5-9** were formed.

The structure of the synthesized compounds was characterized by spectral data (IR, NMR spectra, and elemental analysis).

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B12

SYNTHESIS AND INVESTIGATION OF CONDENSED SYSTEMS WITH PYRAZOLE SCAFFOLD

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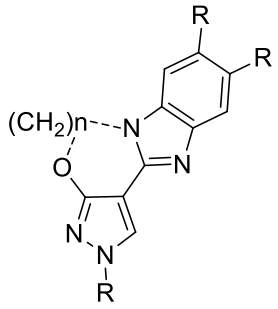
Pyrazole derivatives are widely studied due to their broad biological activities, such as anticancer, antioxidant, antimicrobial, antidepressant, antiviral and various other activities [1]. Meanwhile, benzimidazole derivatives, like compounds containing a pyrazole fragment, also exhibit a diverse spectrum of biological activities and are widely used in the production of pharmaceuticals, such as Omeprazole or Pimozide [2]. Combining these two bioactive fragments allows new, potentially biologically active compounds to be created, which could be applied in the developing new pharmaceuticals. Recent studies have shown, novel pyrazole-benzimidazole derivatives, has exceptional anti-proliferative activity and could significantly induce cancer cell cycle arrest and apoptosis [3]. Other identified biological activities in pyrazole-benzimidazole compounds are analgesic, antimicrobial, antioxidant and antiulcer [4].

The aim of this work is to synthesize new cyclic potential active pyrazole derivatives by applying alkylation and cyclocondensation reactions.

A multi-step synthesis obtained the starting compound 3-hydroxy-1-phenyl-1*H*-pyrazole-4-carbaldehyde. To synthesize the carbaldehyde, a benzyl protecting group was first attached to the hydroxy group. An alkylation reaction of 1-phenyl-1*H*-pyrazol-3-ol was carried out using benzyl bromide as an alkylating agent and sodium hydride as a base. In the next step, 3-(benzyloxy)-1-phenyl-1*H*-pyrazole, an aldehyde group, was introduced into the pyrazole ring using a Vilsmeier-Hack reaction. After obtaining 3-(benzyloxy)-1-phenyl-1*H*-pyrazole-4-carbaldehyde, the benzyl group was removed in a reaction using trifluoroacetic acid in toluene. The intermediate pyrazole derivative was synthesized in good yield. The desired products were obtained by amination reaction and then by cyclisation reaction (Figure 1). The resulting functionalized cyclic pyrazole derivatives were investigated for their biological activity.

Figure 1. Condensed pyrazole derivatives



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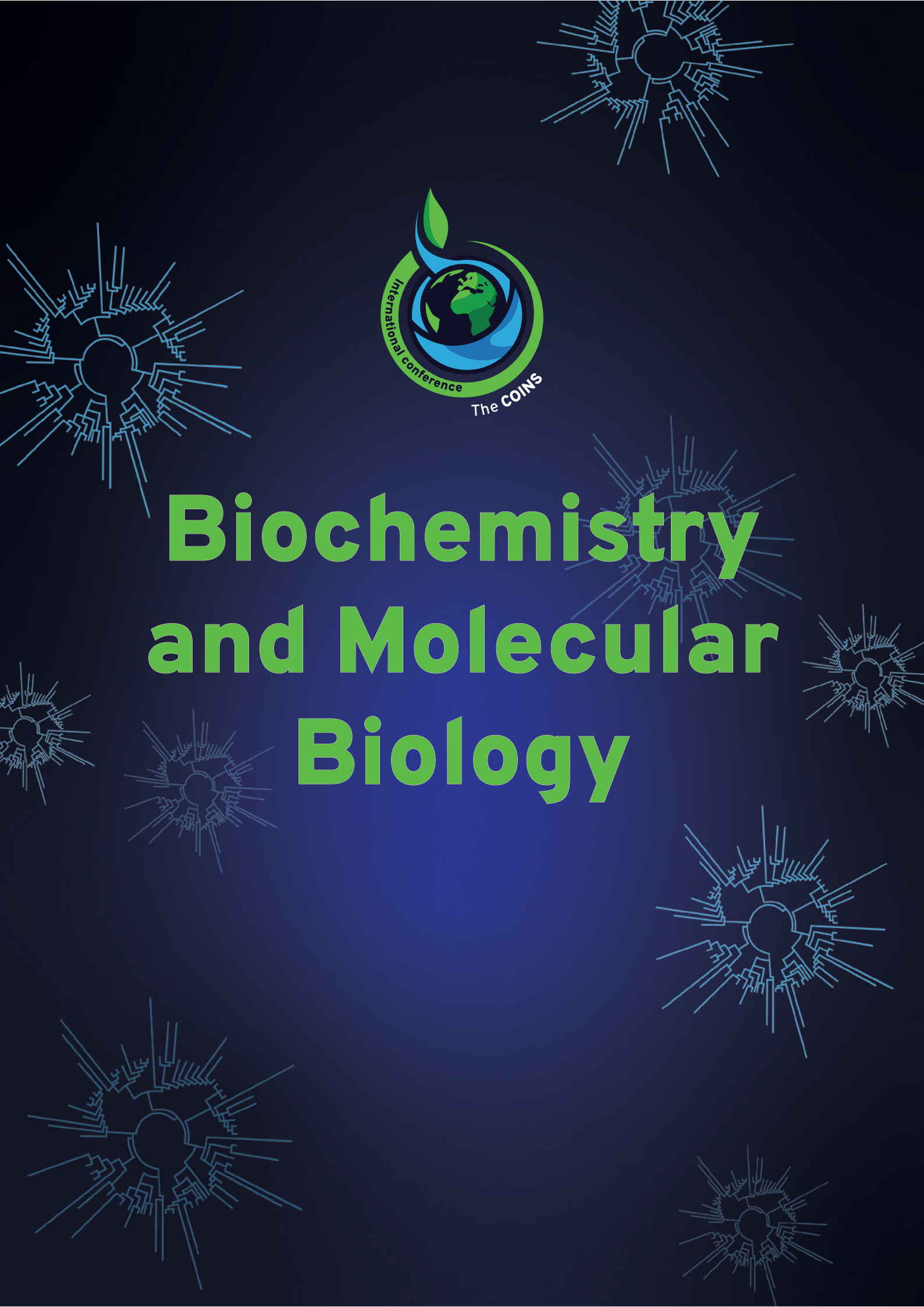

Biochemistry and Molecular Biology

C1

Determination of the viability of *Sarcocystis spp*. oocysts by real-time PCR after treatment with propidium monoazide

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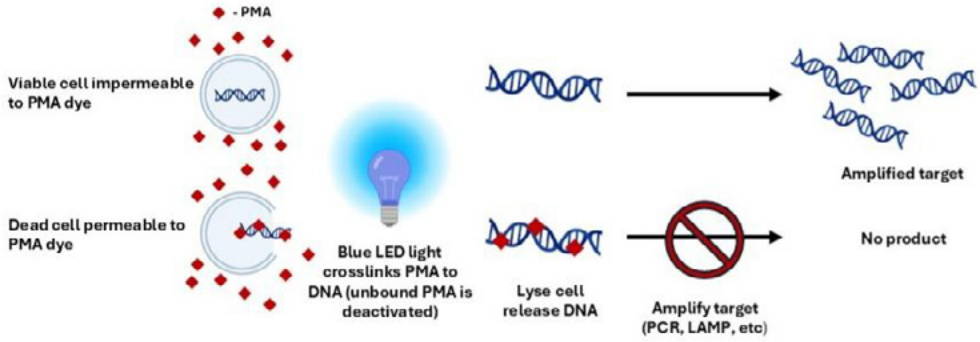
Sarcocystis spp. are protozoan parasites with a two-host lifecycle, wherein sarcocysts develop within the muscles of intermediate hosts. At the same time, sporocysts form in the intestines of definitive hosts and are subsequently excreted into the environment. Intermediate hosts can acquire infection with these protozoa through ingesting food or water contaminated with sporocysts. Infected animals may develop acute infections with symptoms such as rapid weight loss, anemia, fever, or in severe cases mortality. The detection of viable Sarcocystis sporocysts in environmental samples represents a significant challenge due to their low abundance and inability to culture them. Quantitative PCR (qPCR) is a highly sensitive technic for detecting low concentrations of DNA, however, it cannot differentiate between DNA originating from viable and non-viable cells. This study aimed to evaluate the suitability of propidium monoazide (PMA), a dye that binds to DNA from non-viable cells and inhibits its amplification, for assessing the viability of *Sarcocystis* sporocysts.

In this study, environmental samples were collected from two ponds in the Vilnius district. Water samples were filtered and subsequently concentrated onto a membrane before being washed with sterile water. Then, samples were treated with PMA dye, followed by photoactivation and genomic DNA (gDNA) extraction. PMA dye was not applied to controls. To assess the viability of *Sarcocystis* spp., a qPCR assay targeting the COX1 gene was performed using species-specific primers.

Figure 1. Differentiation of viable and non-viable cells using PMA. Created with BioRender.com



A qPCR-based viability assay performed on water samples, both treated and untreated with PMA dye, revealed that the amplification curve for the PMA-treated sample appeared at Ct23, while the untreated sample's curve emerged at Ct 29. This result contradicts theoretical expectations, as PMA dye is intended to bind to DNA from non-viable cells, inhibiting its amplification. In contrast, DNA from the untreated sample should amplify more efficiently, as no inhibitory agent is present to interfere with the PCR reaction allowing DNA from both living and dead cells to be amplified. The experiment was repeated three times with different *Sarcocystis* species, consistently showing the same unexpected trend of earlier amplification in the PMA-treated samples. Increasing the concentration of PMA dye and extending the incubation period did not affect the outcome.

It was concluded that further research is required to identify the factors preventing PMA dye from binding to Sarcocystis DNA and to evaluate its suitability for assessing the viability of *Sarcocystis* spp. in environmental samples.

C2

BERBERINE, A NATURAL INHIBITOR OF PCSK9, AS ADJUVANT IN CANCER THERAPY

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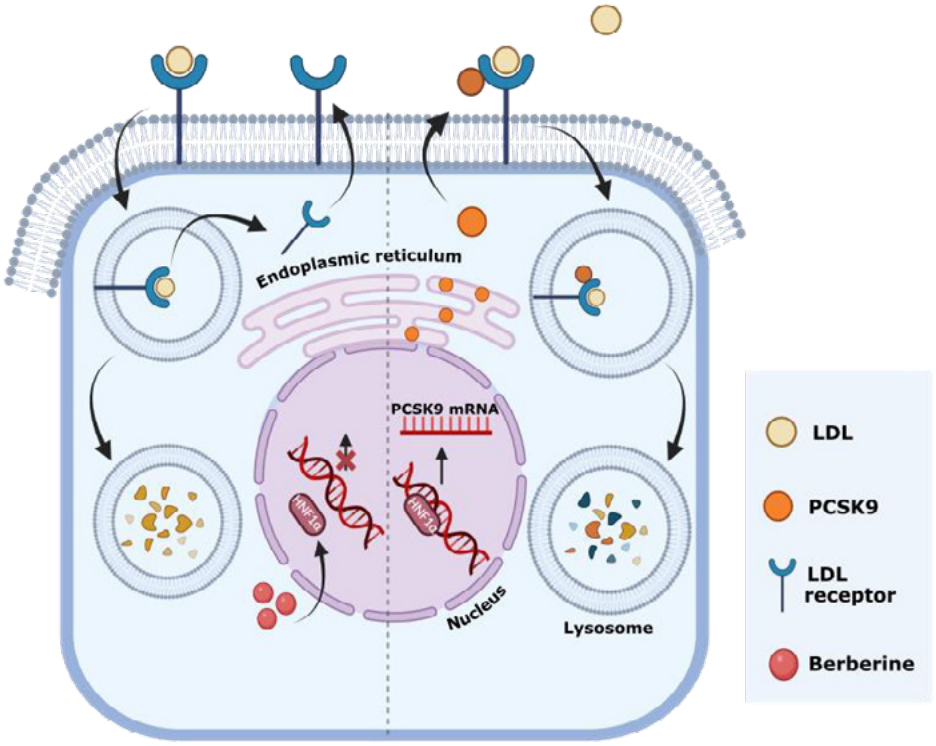
Berberine, a bioactive compound derived from various plants including *Berberis* species, has gained attention for its potential as an adjuvant in cancer therapy. It is reported as an inhibitor of PCSK9, a gene involved in lipid metabolism and lately connected to cancer due to its contribution to immune evasion, regulation of apoptosis, and cell proliferation. In our study, we selected a panel of different tumor cell lines and tested their sensitivity to berberine treatment: MTT and LDH assay revealed that A431 cells, expressing high levels of PCSK9, are more sensitive to berberine cytotoxic effects. In this cell line, berberine treatment induces a strong downregulation of PCSK9 levels (PCR analysis) and upregulation of LDLR protein levels (immunofluorescence). To evaluate the cytotoxic potential of berberine, we tested several chemotherapeutic drugs, alone or in combination with berberine, to confirm the presence of synergistic effects.

Figure 1. Berberine role in regulating PCSK9 synthesis.



C3

THE ROLE OF CAPSULAR POLYSACCHARIDES IN *ACINETOBACTER BAUMANNII* RESISTANCE TO ANTIBIOTICS

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The morbidity of infections caused by multidrug-resistant bacteria is increasing and has reached epidemic levels in some regions. These bacterial infections are often hard or impossible to treat with available antibiotics. The World Health Organization (WHO) compiled a list of priority pathogens to encourage the development of new antibiotics [1]. *Acinetobacter baumannii* is one of these multidrug-resistant opportunistic bacteria. Although *A. baumannii* causes various infections with a mortality rate of up to 44%, its virulence mechanisms are not fully understood [2, 3]. The polysaccharide capsule is one of the virulence factors of *A. baumannii* which protects it from desiccation, the host immune system, and antibiotics [4]. Therefore, understanding the role of capsular polysaccharides in *A. baumannii* antibiotic resistance is crucial.

This study aimed to evaluate the growth of *A. baumannii* wild-type isolate and its capsule-deficient mutant (Δ*galU*) under exposure to different classes of antibiotics. Bacterial biofilms were exposed to antibiotics and incubated for 24 h at 37°C. After incubation, biofilms were separated, diluted, and inoculated onto agar plates. After incubation for 18 h at 37°C, colony-forming units (CFUs) were counted.

The results showed a statistically significant difference ($p < 0.05$) in CFUs comparing *A. baumannii* wild-type isolate and Δ*galU* mutant when exposed to colistin and tetracycline. However, no statistically significant difference ($p > 0.05$) in CFU counts was observed between strains when exposed to gentamicin. When exposed to colistin, the wild-type isolate produced 23.96% fewer CFUs than the Δ*galU* mutant (Cohen's $d = 1.95$, large effect). In contrast, exposure to tetracycline resulted in the wild-type isolate producing 5.08% more CFUs than the Δ*galU* (Cohen's $d = 1.07$, large effect).

The results highlight the multifaceted role of capsular polysaccharides in *A. baumannii* survival under antibiotic-induced stress. These findings may contribute to the discovery of new treatment methods for *A. baumannii* infections.

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C4

SELECTION OF ANALYTICAL METHOD TO INVESTIGATE BIODEGRADATION OF POLYURETHANE

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Polyurethane (PU) ranks as the 6th most produced plastic in the world. It is used in various industries such as automotive, construction, electronics, etc. At the start of 2025, the global PU market was valued at 87.68 billion USD, and it is expected to reach 113.84 billion USD by 2030 at an annual growth rate of 5.36 % [1]. Due to high resistance to degradation and lack of effective recycling methods, only 29.7 % of PU waste is recycled, while the rest end up in landfills (30.8 %) or are incinerated (39.5 %).

PU is recycled either chemically or mechanically. Chemical recycling produces raw components for the resynthesis of PU but, at the same time, is very expensive and can result in more pollution, leading to a higher carbon footprint. Mechanical recycling of PU is cheaper than chemical recycling, but received products are of poor quality and have limited applicability [2]. An alternative approach that requires less energy and produces fewer harmful byproducts is biodegradation. PU could be broken down into its primary components by utilising specific enzymes such as lipases, esterases, and urethanases, as well as microorganisms like bacteria or fungi. This approach could enhance recycling efficiency while promoting environmental sustainability and a circular economy [3].

This study aimed to select an analytical method to investigate and evaluate the biodegradation of PU. To achieve this, two linear PUs were synthesised as reference compounds. Initially, both synthesised PU were chemically hydrolysed using NaOH to serve as the reference reaction and to ensure complete hydrolysis. The hydrolysis products were analysed exploring possibilities of ATR-FTIR and GC, two of the most commonly used methods, to assess the chemical degradation of PU. Both analysis methods were further applied to evaluate enzymatic and microbial degradation of PU. Based on the obtained analysis results, GC was selected as a more suitable analytical tool to investigate enzymatic PU degradation. Wider elaboration of the results will be presented during the poster session.

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C5

In vivo Expression Studies of a Methylation-Sensitive CRISPR-Cas9

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CRISPR (clustered regularly interspaced short palindromic repeats) and Cas (CRISPR-associated) proteins provide adaptive immunity in bacteria and archaea by integrating foreign DNA into the CRISPR locus.[1] This sequence is transcribed into a guide RNA (gRNA), which directs a Cas nuclease to recognize and cleave target DNA adjacent to a PAM (protospacer adjacent motif). [2], [3] The programmability of gRNA enables precise genome editing, with applications in diagnostics, agriculture, and therapeutics. [4] However, off-target effects limit broader use, prompting research into nucleases with enhanced specificity and improved delivery strategies. [5]

CRISPR-Cas specificity can be influenced by epigenetic modifications. While the widely used SpCas9 (*Streptococcus pyogenes* Cas9) is not directly affected by methylation on the target or PAM sequence, its activity can be hindered by DNA condensation in highly methylated regions, making certain genomic sites less accessible. [6], [7] In contrast, AceCas9 from *Acidothermus cellulolyticus* is inherently methylation-sensitive. Its activity depends on the methylation state of its PAM sequence (5'-NNNCC-3'), where methylation of the first but not the second cytosine disrupts cleavage. [8] Although AceCas9's sensitivity does not align with the predominant ^{5m}CpG modification in mammalian cells, it could be valuable for detecting epigenetic changes in non-^{5m}CpG regions of some stem and plant cells. [9], [10] However, its *in vivo* editing efficiency and expression in mammalian cells remain unknown.

To address this, we designed a mammalian expression vector encoding AceCas9 with nuclear localization sequences (NLS) and a TwinStrep tag. Following transfection into mammalian cells, Western blot analysis confirmed successful expression, and genome-editing activity was assessed by targeting the UBE3A gene. These findings provide insights into the suitability of AceCas9 as a potential tool for methylation-sensitive genome editing in mammalian cells.

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C6

APPLICATION OF MICROFLUIDIC TECHNOLOGY TO α-SYNUCLEIN LIQUID-LIQUID PHASE SEPARATION STUDY

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α-Synuclein (α-Syn) is an intrinsically disordered protein associated with the pathogenesis of neurodegenerative diseases, including Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. α-Syn aggregates into amyloid fibrils, a major component of Lewy bodies, which is a marker for neuronal degeneration [1]. The aggregation of α-Syn can be initiated through liquid-liquid phase separation (LLPS) – a biophysical process involved in the formation of membraneless organelles [2]. Imitating intracellular environment requires precise control, therefore, our goal is to apply microfluidic technology for an in-depth LLPS study of α-Syn aggregation.

For this study we constructed and purified α-Syn with four fluorescent protein tags – eGFP, mCherry, mOrange, mCerulean. Initially, fluorescence microscopy was used to observe the LLPS process of each fluorescently tagged protein with wild type α-Syn. Then, we applied microfluidic technology that enables the study of rapidly generated subnanoliter volume droplets within microchannel systems [3]. We formed droplets containing α-Syn fibrils mixed with α-Syn monomer, suspended in a buffer solution, as well as with the molecular crowder polyethylene glycol (PEG), which induces LLPS. The resulting droplets were then analyzed using fluorescence microscopy.

Our study demonstrates that applying microfluidic technology to generate droplets containing α-Syn fibrils and monomers in the presence of PEG concentrates protein aggregates at the center of the droplets, compared to mixtures without a crowding agent. After separating aggregates from the droplets and using them as a seed for aggregation, the fibrils formed under LLPS conditions showed weaker aggregation compared to control and α-Syn fibrils formed without PEG in droplets. In the further steps, we will investigate the structures of α-Syn fibrils and optimize the application of microfluidic technology.

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C7

PRODUCTION OF MUTANT *ARTEMISIA VULGARIS* ALLERGEN COMPONENT ART V 3 VARIANTS

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Allergy is an overreaction of the immune system to molecules that are harmless to most people. Sources of allergens can vary and may include pollen, dust mites, certain foods, and pharmaceuticals. The most common allergens are proteins. The human body produces antigen-specific immunoglobulin E (sIgE) in response to allergens, leading to allergy symptoms such as sneezing, coughing, skin rashes, and runny noses [1]. Over one-fifth of the population suffers from allergic diseases such as allergic rhinitis and allergic asthma, which place a significant strain on healthcare systems worldwide. Symptom control can be achieved by allergen avoidance and pharmaceuticals. However, allergen-specific immunotherapy (ASIT) can achieve long-lasting disease-modifying effects [2]. Hypoallergens are molecules that are less likely to induce an allergen-specific IgE response than natural allergen extracts and recombinant allergens. Due to this property, using hypoallergens in ASIT reduces the risk of adverse effects such as allergic symptoms or anaphylactic shock [3].

Airborne pollen is a common trigger of respiratory allergies in a changing global climate. Pollen allergy disorders are affecting many people globally [4]. *Artemisia vulgaris*, also known as common mugwort, is a highly allergenic plant that produces large amounts of wind-borne pollen. *Artemisia vulgaris* pollen is one of the main causes of allergic reactions in Europe. It affects roughly 10% to 15% of patients suffering from pollinosis during the flowering period of late summer and fall [5]. The non-specific lipid transfer protein Art v 3 is one of the six allergen components of *Artemisia vulgaris*. Although the allergen component Art v 3 was approved in 2003, its IgE epitopes have not been identified. Its reactivity with sIgE from *Artemisia vulgaris* allergic patients varies from 22% to 70% [6].

The aim of this study was to synthesize and purify mutant variants of the *Artemisia vulgaris* allergen component Art v 3 under native conditions. These mutants contain amino acid substitutions at specific target sites, potentially involved in IgE-binding epitope formation. Ten mutant variants of allergen component Art v 3 were synthesized in *E. coli* and purified by Ni^{2+} affinity chromatography using buffer solutions containing varying concentrations of imidazole. Antigenicity assays of the purified mutants will provide essential findings on their potential for use in allergen-specific immunotherapy.

The research is funded by the Research Council of Lithuania (LMTLT), agreement No S-MIP-24-41.

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C8

STUDY OF SPLIT pAGOS AND THEIR ASSOCIATED HEPN EFFECTORS' ACTIVITY IN *E. COLI*

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As genome editing continues to advance, the scientific community remains dedicated to refining the tools that drive this progress. Currently, CRISPR-Cas systems remain at the forefront of genome editing technology, with minor research focusing on their association with the HEPN domain and its interactions. However, recent advancements in Argonaute protein research indicate that the HEPN domain also plays a crucial role in the functionality of certain Argonaute systems.

This study investigates the possible interaction of prokaryotic Argonautes (pAgos) with a HEPN domain-containing effector protein and its impact on Argonaute protein functionality and effects on *E. coli*. In pAgos, the catalytic center resides within the PIWI domain, where the conserved DEDX tetrad mediates the cleavage of target nucleic acids [1]. In the Argonaute-HEPN systems examined, the PIWI domain is inactive, and the HEPN domain functions as the primary effector responsible for cleavage. By studying several homologous Argonaute–HEPN systems, we seek to investigate the role and mechanism of pAgo-HEPN interaction in bacterial defense. This research not only establishes a foundation for further investigations but also holds substantial potential for programmable DNA targeting in biotechnology [2].

Exploring bacterial defense systems not only deepens our understanding of the complex mechanisms by which bacteria protect themselves from foreign DNA but also paves the way for advancements in biotechnology and genome editing tools.

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C9

DEVELOPMENT OF A MICROFLUIDICS-BASED ULTRA-HIGH THROUGHPUT PLATFORM FOR THE ISOLATION OF SINGLE DNA MOLECULES

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Multiple displacement amplification (MDA) of single DNA molecules isolated in water-in-oil droplets results in condensed DNA microparticles (DNAps) containing 10^4-10^5 copies of a clonally amplified gene. These microparticles form due to interactions between Mg^{2+} ions from the reaction buffer, the amplified DNA, and pyrophosphate, a byproduct of the reaction [1,2]. DNAps could be used in the development of a microfluidics-based method for *in vitro* directed evolution. The method would involve preparing a gene library, synthesizing DNAps from single DNA molecules, expressing DNAps *in vitro* and using fluorescence-activated droplet sorting to select enzyme variants with enhanced catalytic activity.

Our research team has already developed a method for DNAp synthesis and *in vitro* expression using regular microfluidic chips. However, the throughput of this method–approximately 10^6 droplets per hour–is relatively low for broader directed evolution applications. Also, the MDA reaction may begin outside the droplets and the Phi29 polymerase used in the reaction could lose its initial activity. To prevent these issues, we need a method allowing to generate droplets at a much higher throughput.

In this work, we aimed to validate the design of a microfluidic chip as well as flow parameters to achieve an encapsulation throughput of 10^6 single DNA molecules per minute. For that purpose, we used a step emulsification chip, whose geometry enables a throughput exceeding 10^4 droplets per second [3], minimizing the time the reaction mixture remains outside the droplets. To balance the efficient isolation of single DNA molecules in droplets with reagent cost considerations, we selected 4 pL droplets. Unlike in regular microfluidic chips, droplet size in step emulsification chips does not depend on the flow rate of the oil phase. To achieve the desired 4 pL droplet volume, we tested a silicone oil that interacts with the chip, causing it to swell and narrowing the chip channels. By using a low-viscosity silicone oil and a step emulsification chip at an encapsulation rate of 10^6 single DNA molecules per minute, we generated a monodisperse emulsion with water-in-oil droplets of approximately 4 pL in volume. The ultra-high-throughput droplet generation that we achieved should facilitate directed evolution experiments that rely on screening large number of enzyme variants *in vitro*.

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C10

Structural Investigation of a Bacterial Anti-Phage System

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Bacteriophages (phages) pose a constant threat to bacteria, driving the evolution of diverse anti-phage defense mechanisms. Among these defense strategies, recently identified anti-phage systems involve protein components of eukaryotic ubiquitination pathways. Structural characterization of these proteins is essential for elucidating their role in bacterial immunity and phage evasion strategies.

In this study, we focus on the structural and mechanistic characterization of key proteins from a bacterial anti-phage system. Using recombinant expression strategies and a multi-step purification protocol involving affinity and size-exclusion chromatography, we successfully produce protein samples for further investigation. These purified proteins will undergo structural analysis to determine their three-dimensional architecture, in particular cryo-electron microscopy (cryo-EM) for high-resolution visualization of protein complexes. Greater knowledge of bacterial anti-phage systems could help enhance the stability of bacterial cultures for fermentation and probiotic production.

C11

MIR-126-5P AND MIR-543 IN PARKINSON'S DISEASE: INSIGHTS INTO DIAGNOSIS AND SYMPTOM CORRELATION

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Parkinson's disease (PD) is the second most common neurodegenerative disorder globally, predominantly affecting people over 50 and characterized by symptoms like tremor and bradykinesia due to the degeneration of dopaminergic neurons. During PD progression, extracellular vesicles (EVs) carry biomolecules that reflect the disease state and transfer their cargo to recipient cells, potentially worsening the condition. MiRNAs within EVs may help distinguish disease stages, evaluate progression, and guide individualized treatment options. This study aimed to evaluate the expression levels of EV miRNAs in the blood serum of PD patients to differentiate between healthy individuals and PD patients with varying disease severity.

EV miRNAs were isolated from blood serum, transcribed into cDNA, and quantified using RT-PCR. miRNA expression profiles were analyzed in 20 healthy controls (HC) and 88 Parkinson's disease (PD) patients. We compared HC and PD groups and examined associations between miRNA expression and symptom severity, including bradykinesia, tremor, rigidity, dyskinesia, gait disturbances, nocturnal akinesia, the "on-off" phenomenon, orthostatic hypotension, as well as disease onset and duration. Long-term miRNA expression changes were also evaluated in patients undergoing gamma knife surgery (n=5) and deep brain stimulation (n=9) as treatments for PD. Statistical analyses were performed using GraphPad Prism 8.

We identified significant differences in EV miR-126-5p and miR-543 expression among patient groups. ROC analysis demonstrated that the combination of miR-126-5p and miR-543 effectively differentiated PD patients from healthy controls, with an AUC of 0.982 ($p<0.001$). In PD patients, miR-126-5p was upregulated, whereas miR-543 was downregulated compared to healthy controls. Moreover, increased miR-126-5p expression correlated with greater severity of bradykinesia, orthostatic hypotension, and cognitive impairment ($p<0.05$). miR-543 expression was elevated in patients with more severe nocturnal akinesia but decreased in those with orthostatic hypotension ($p<0.05$). Disease onset and duration had no significant effect on miR-126-5p or miR-543 expression. Regarding surgical treatments, gamma knife surgery did not alter miRNA expression, while deep brain stimulation significantly increased miR-543 levels ($p<0.05$), suggesting a potential link between higher miR-543 expression and improved disease outcomes.

In conclusion, the work demonstrates the importance of miRNA molecules in the context of PD. More detailed miRNA studies could be valuable in clinical practice for early PD diagnosis, enabling timely treatment and tracking disease progression.

C12

INTRACELLULAR LOCALIZATION AND FUNCTIONAL DYNAMICS OF TOTIVIRUSES IN *SACCHAROMYCES CEREVISIAE*

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Saccharomyces cerevisiae is extensively used across a diverse range of industrial and scientific disciplines. Various strains of *S. cerevisiae* serve as natural hosts for some of the viruses belonging to the Totiviridae family, including the M satellite virus and its associated helper virus ScV-LA and ScV-LBC. These viral entities play significant role in yeast-virus interactions, influencing host phenotypes and evolutionary dynamics of yeast populations. For instance, the coexistence of the M satellite virus and L-A virus is a key determinant of the yeast biocidal activity. It is commonly asserted that L-A (or L-BC) alone has no significant impact on the host cell. However, emerging research suggests that the molecular mechanisms underlying yeast-virus interactions are highly intricate. A deeper, systematic exploration could provide valuable insights into virology and host-pathogen dynamics while also unlocking potential applications in biotechnology.

Recent research has identifiedAa diverse range of biocidal yeast strains with the most extensively characterized K1, K2 and K28 *S. cerevisiae* killer systems. The double-stranded genomic RNA (dsRNA) of the M satellite virus encodes a precursor toxin that underlies the biocidal activity of these strains. While genetic architecture of the M satellites is largely conserved, the encoded toxin regions exhibit high sequence variability and are non-homologous between different strains. Notably, the N-terminal of virus-encoded preprotoxins display functional divergence. To investigate their function, we transformed the BY4741::SEC61-GFP yeast strain with plasmids encoding the N-terminal fragments of K1 or K2 proteins, fused at the C-terminus to mCherry protein. The transformed yeast cells were analyzed using fluorescenceamicroscopy and the deep lawn assay method. Our findingsd suggest that they are involved in intracellular preprotoxin translocation, toxin maturation and even the development of immunity mechanisms within host cells. Another aspect of our research focuses on the localization dynamics of totiviruses within *S. cerevisiae*. It has been previously established that ScV-LA replication occurs exclusively in the cytoplasm, while the localization of ScV-LBC remained unexplored. However, transformation of the BY4741::HTA2-GFP strain with a plasmid encoding gag protein fused to mCherry, followed by fluorescence microscopy analysis, revealed the presence of both ScV-LA and ScV-LBC within specificr cellular compartments. These findingsg challenge the traditional understanding of their intracellular distribution, suggesting alternative mechanisms of propagation and persistence.

C13

INVESTIGATION OF THE CYTOTOXIC PROPERTIES OF CAPSULAR POLYSACCHARIDES FROM *ACINETOBACTER BAUMANNII*

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Gram-negative bacterium *Acinetobacter baumannii* is an opportunistic pathogen, which causes hospital-acquired infections worldwide. The rapid spread and multidrug resistance of this pathogen have contributed to its proliferation in healthcare facilities, particularly in intensive care units. Therefore, this pathogen poses an increasing threat to immunosuppressed patients. One of the main virulence factors is the capsular polysaccharides (CPS) covering the surface of the bacterium [1]. CPS increase resistance to desiccation, disinfectants, antimicrobials, and antibiotics as well as host immune responses. CPS can protect the pathogen itself from lysozyme-initiated killing and initiate the death in innate immune cells – macrophages. The aim of this study was to determine the cytotoxicity of purified CPS.

Methods: CPS from clinical *A. baumannii* strain were purified by gelfiltration method. CPS purity was tested by Bradford assay and by running CPS samples on agarose and SDS-PAGE gels. CPS concentration was determined by Anthrone reaction based on the colour change measuring optical density at 620 nm wavelength. After the determination of CPS concentration, J774 murine macrophages were exposed to purified CPS and their viability was determined by MTT assay. Also, the viability of J774 macrophages was determined by MTT after exposure to CPS co-purified with outer membrane vesicles (OMVs).

Results: CPS were purified without any remaining of nucleic acids, proteins, and most importantly without lipooligosaccharide. CPS fractions demonstrated a 2-19 mg/ml range of concentrations. Cell viability assay demonstrated that CPS fractions 1-6 exhibited similar cytotoxicity to J774 macrophages and reduced their viability by up to 40%. Whereas CPS fractions 7-8 showed increased cytotoxicity to J774 macrophages and reduced their viability by up to 20%. Studies of CPS co-purified with OMVs demonstrated that OMVs produced by wild-type *A. baumannii* and a non-capsulated mutant exhibited similar cytotoxicity to J774 macrophages. Those results indicate that CPS produced by *A. baumannii* were cytotoxic to J774 macrophages and induced their death. Further research is needed to investigate how CPS from *A. baumannii* regulate the expression of pro-inflammatory cytokines in macrophages.

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C14

SYNTHESIS OF 2-PYRROLIDINONE-AZOLE DERIVATIVES AND EVALUATION OF THEIR BIOLOGICAL ACTIVITY *IN SILICO*

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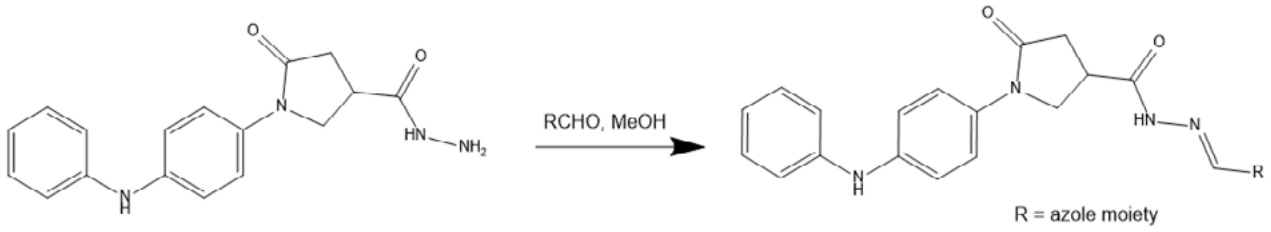
Antimicrobial resistance (AMR) is an exceptional worldwide challenge for contemporary medicine and public health [1], in which microorganisms adapt to withstand the effects of antimicrobial agents, rendering previous treatments ineffective. AMR is responsible for increased morbidity, mortality, and healthcare costs, corresponding to nearly 700,000 deaths worldwide every year, which is estimated to increase to up to 10 million deaths per year by 2050. In 2014, the World Health Organization (WHO) included AMR among the top ten global health threats, a position that remains the same almost ten years later.

AMR's emergence and dissemination is accelerated by the inappropriate use of antimicrobial agents in agriculture, veterinary and human health, poor infection control practices, inadequate sanitation, and improper food handling. At its core, AMR is driven by genetic changes within microbial populations, enabling them to survive and proliferate in the presence of antimicrobial drugs. Since the pace of discovering novel drugs has drastically slowed in recent decades, insufficient development and availability of new antimicrobial agents for the treatment of life-threatening infections caused by resistant pathogens is behind the growing demand [2], making the synthesis of such compounds and analysis of their properties one of the most important research areas in medicinal chemistry. Therefore, this work has aimed to contribute to the solution of the antimicrobial resistance problem.

Azole-based derivatives have driven the interest of medicinal chemists because they have demonstrated notable pharmacological activities [3], including antibacterial and antifungal ones. Hydrazones are important intermediate compounds in the synthesis of pharmacological substances [4], especially in the development of prodrugs, which is an important aspect of the pharmaceutical industry, as it provides the opportunity to improve the efficacy of drugs, reduce side effects and/or improve their bioavailability.

A set of compounds bearing 2-pyrrolidinone and various azole moieties linked via hydrazone fragment were synthesized from 5-oxo-1-(4-(phenylamino)phenyl)pyrrolidine-3-carbohydrazide and the corresponding aldehydes in methanol (Fig. 1).

Figure 1. Synthesis of target hydrazones bearing 2-pyrrolidinone and azole moieties



The primary screening of the biological activity of the target compounds was carried out *in silico* to assess their druggability. By analyzing their predicted properties according to ADMET, all synthesized compounds are characterized by good absorption, compliance with Lipinski's rule of five, high toxicity and insubstantial blood-brain barrier crossing ability. The predicted toxicity of these compounds is an area of interest and requires further investigation, therefore it will include *in vitro* screening of the synthesized compounds against a set of selected pathogens.

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C15

BIOSYNTHESIS OF SARS-COV-2 NUCLEOCAPSID PROTEIN IN THE MAMMALIAM CELL EXPRESION SYSTEM

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Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which causes mild to moderate upper respiratory tract disease, has four major structural proteins – spike, envelope, membrane and nucleocapsid (N), which are necessary for its survival and circulation. N protein is an important protein in the virus life cycle as it binds to the virus RNA and packages it into a ribonucleoprotein complex. It also participates in the transcription and translation of viral RNA, and it is involved in the assembly of the viral particle [1]. However, scientists have discovered other functions of the N protein, such as activating inflammatory processes or inhibiting the cell's defense systems, leading to a more severe form of the disease. One of these functions is the activation of complement, which is an immune surveillance system that quickly responds to infection [2]. The complement system operates in the extracellular space where the N protein binds directly to and activates MASP-2 kinase, leading to the hyperactivation of the complement [3]. It has also been discovered that the N protein binds to the cell membrane surface proteins heparin and heparan sulphate and thus remains in the extracellular space [4]. Still, it is not known how and to what extent this protein affects cell viability.

The aim of this study was to produce recombinant secreted SARS-CoV-2 N protein in a mammalian expression system and to purify it from a cell growth medium. Despite *E. coli* and yeast being cost-effective and high-yield recombinant protein expression systems, the use of mammalian cells ensures proper post-translational modifications, correct protein folding, and functional integrity of the SARS-CoV-2 N protein. Post-translational phosphorylation is crucial for regulating the N protein's activity; therefore, the mammalian cell expression system was chosen. Synthetic DNA fragment coding N protein gene together with secretion peptide and hexahistide tag was cloned to three different mammalian expression vectors (pFUSE-hIgG1, pLV-EF1a-EGFP, pcDNA3) and transfected into HEK293/T17 cells. An enzyme-linked immunosorbent assay using previously generated N protein-specific MAbs was performed to confirm the synthesis and secretion of N protein. The results showed that only cells carrying the pLV-EF1a-EGFP-N and pcDNA3-N constructs were able to synthesize the N protein and secrete it into the growth medium. Next, target cell selection was carried out to obtain a stable HEK293/T17 cell line secreting the N protein. This was only achieved with cells carrying the pLV-EF1a-EGFP-N construct, with clones 1D7, 2B2 and 2B8 finally obtained. The growth medium of these cell lines was collected and purified by immobilized metal ion affinity chromatography using the AKTA start™ chromatography system. The results of the purification were analyzed by SDS-PAGE and Western Blot, which showed that the N protein was purified, however the yield of the protein was not high, and the sample was not pure.

Further studies will optimize the purification conditions to achieve higher protein purity and yield. Studies will also be carried out to determine whether the protein has an inflammatory effect on lung cells and how much it affects the viability of these cells.

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C16

THERMOSTABILITY STUDY OF AI-GENERATED *BOS TAURUS* TERMINAL DEOXYNUCLEOTIDE TRANSFERASE VARIANTS

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Terminal deoxynucleotidyl transferase (TdT) is a unique, template-independent DNA polymerase extensively utilized in molecular biology for applications such as DNA ends labeling and enzymatic de novo DNA synthesis [1]. TdT is mesophilic polymerase with an unfolding T_m around 40 °C [2]. At lower temperatures its activity can be hindered by the formation of secondary structures in the DNA substrate, such as hairpins or loops, which impede the enzyme's access to the 3' end of nucleic acids [3]. TdT with enhanced thermal stability is particularly beneficial when working with double-stranded DNA (dsDNA) as a substrate. The increased temperature can also facilitate the melting both of dsDNA and hairpins, making them more accessible for the TdT enzyme to act upon [2].

This study aimed to characterize AI-generated *Bos taurus* TdT variants with improved thermostability, specifically targeting functionality at elevated temperatures.

To achieve this*, Escherichia coli* KRX strain was transformed with plasmids containing AI-generated TdT gene variants for protein synthesis. Purification was performed using a two-step chromatography approach. Enzyme activity was assessed by incubating the purified TdT variants with nucleotides at various temperatures, followed by denaturing urea polyacrylamide gel electrophoresis to analyse nucleotide incorporation.

All of the tested TdT variants maintained substrate specificity and enzymatic activity comparable to that of the wild-type enzyme when extending the 3’-end of the primer with canonical nucleotides. Among the tested variants, TdT-P15 exhibited an enhanced thermostability, retaining enzymatic activity at 70°C, whereas the TdT-WT enzyme showed diminished activity at this temperature.

The successful development of a thermostable TdT variant highlights the potential of AI-driven protein engineering for enzyme optimization. Further studies will explore its structural basis and expand its biotechnological applications.

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C17

INSIGHTS INTO RESISTANCE MECHANISMS TO PHOTODYNAMIC THERAPY-INDUCED OXIDATIVE STRESS IN OPPORTUNISTIC PATHOGEN *STENOTROPHOMONAS MALTOPHILIA*

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Antimicrobial resistance is one of the most pressing global health challenges today [1]. *Stenotrophomonas maltophilia* is an emerging opportunistic pathogen responsible for highly lethal infections, particularly among immunocompromised patients. *S. maltophilia* resistance to a broad spectrum of antibiotics and ability to form biofilms complicates treatment of infections, necessitating the development of alternative antimicrobial strategies [2]. One perspective method is antimicrobial photodynamic therapy (aPDT), which involves a photosensitizer, molecular oxygen and light to generate reactive oxygen species, inducing oxidative stress and ultimately killing bacterial cells [3]. A key bacterial defense mechanism against oxidative stress involves antioxidant enzymes such as superoxide dismutases, catalases and peroxidases [4]. However, there is no published data about the role of these enzymes in *S. maltophilia* resistance to aPDT.

The aim of this study was to evaluate the role of manganese/iron-dependent superoxide dismutase (Fe_Mn_SOD) in the *S. maltophilia* resistance to aPDT-induced oxidative stress. First, we analysed the viability of two multidrug-resistant biofilm forming clinical isolates of *S. maltophilia* (SM3 and SM21) after chlorophyllin-based aPDT (Chl-aPDT) using 402 nm light at different doses. The SM3 isolate was found to be more susceptible to Chl-aPDT than the SM21 isolate. Given this difference in sensitivity, we sought to identify the molecular mechanisms underlying this variation. Previous studies by our colleagues revealed that SM21 isolate possesses an additional *Fe_Mn_sod* gene, which is absent in SM3 isolate. To investigate the function of this gene, we generated two new isolates: SM3_pBAD_sod (SM3 transformed with pBAD vector carrying the *Fe_Mn_sod* gene from SM21) and SM3_pBAD (SM3 with an empty control plasmid). The impact of Fe_Mn_SOD on oxidative stress resistance will be evaluated by comparing the viability of these isolates after Chl-aPDT. Additionally, we will examine *Fe_Mn_sod* gene expression in the SM21 isolate after Chl-aPDT using the quantitative polymerase chain reaction. Finally, we will quantify superoxide ion production after aPDT exposure. Further details of the project and preliminary results will be presented during the poster session.

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C18

MICROBIAL DEGRADATION OF α-RIBAZOLE PHOSPHATE: EXPLORING THE FATE OF VITAMIN B12 LOWER LIGANDS

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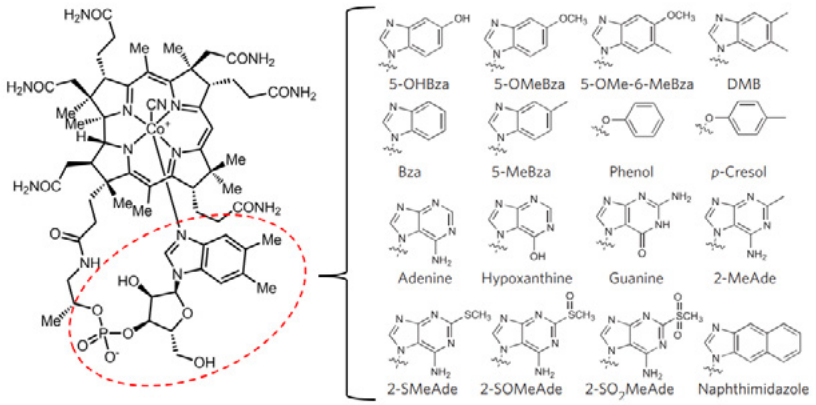
Corrinoids, such as vitamin B12, are the most complex organometallic cofactors utilized across all domains of life to catalyze crucial biochemical reactions, including methyl group transfer, carbon skeleton rearrangement, and reductive dehalogenation. Complete corrinoids (cobamides) consist of an upper Coβ ligand, a central cobalt-containing corrin ring, and a lower Coα base, which is part of the nucleotide loop linked to the corrin ring. For instance, vitamin B12 (cyanocobalamin) features an artificial cyano group as its upper Coβ ligand and 5,6-dimethylbenzimidazole (DMB) as the lower base[1]. In nature, the nucleoside base of cobamides varies depending on the microorganism that synthesizes them (Fig. 1) and while the biosynthesis and function of different lower ligands is well understood, little is known about their fate once they are no longer needed.

In this study, we aimed to identify catabolic pathways for α-ribazole phosphate (αRP), the predominant lower ligand of vitamin B12. To achieve this, we screened various surface soil and water samples for αRP degradation activity. In total five samples were selected for further investigation and from them we successfully isolated several microorganisms belonging to the *Ochrobactrum* and *Agromyces* genera that can remove DMB from αRP. Ongoing research is now focused on identifying the enzymes responsible for hydrolyzing the α-glycosidic bond in αRP.

In summary, his study explores the catabolism of α-ribazole phosphate, identifying microorganisms capable of degrading it and working to uncover the enzymes responsible for hydrolyzing its α-glycosidic bond.

Figure 1. Structure of vitamin B12 and variations in its lower ligand.



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C19

THE CHARACTERIZATION OF PRO-INFLAMMATORY S100A4 PROTEIN AGGREGATION PROPERTIES

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Relevance of the research:

S100A4 belongs to the S100 pro-inflammatory protein family, consisting of 30 calcium-binding members [1]. The S100 family induces inflammation signal pathways for other cells and mediates immune cell migration [2]. However, they can also be detrimental and facilitate neurodegeneration or cancer progression. For example, S100A4 is known for accelerating tumor metastasis and can form insoluble protein aggregates -amyloid fibrils [3]. Similar to other S100 proteins, S100A4 is a dimer, with each monomer possessing two EF-hand calcium-binding motives that control its conformation, but there is little information about its aggregation properties. This research aims to elucidate the aggregation characteristics of S100A4 protein and identify under which conditions, mainly different pH levels and oxidative stress, it is more prone to aggregate. These findings could be beneficial for a better understanding of neurodegeneration and cancer metastasis.

Methodology:

S100A4 protein was purified using nickel metal affinity and size exclusion chromatography. Briefly, BL21 Star™ (DE3) E. coli cells were transformed with a pET28b plasmid containing 6xHis-SUMO-S100A4 gene. Cells were grown until 0.6 optical density and protein production was induced with 0.1 mM IPTG. Harvested cells were lysed using sonication, and the soluble medium was separated via centrifugation. Then, the protein was applied to the Ni-NTA column, and eluted fractions were cleaved with SUMO protease Senp1 domain. After the second Ni-NTA purification, S100A4 was concentrated and purified by gel filtration. The aggregation assay was followed with amyloid-specific dye ThT, and the aggregates were visualized using atomic force microscope (AFM) [4].

Results:

We observed that S100A4 did not aggregate under neutral pH conditions, but amyloid fibril formation was induced by lowering pH, potentially due to the destabilization of the protein. Additionally, aggregation was induced with an addition of hydrogen peroxide up to 5% concentration, while higher peroxide concentration inhibited aggregation. AFM images confirmed the presence of worm-like fibrils at the acidic pH and longer fibrils in hydrogen peroxide samples. In the next research steps, we will investigate structural changes and liquid-liquid phase separation properties of S100A4 protein.

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C20

Calcium-mediated amyloid co-aggregation of S100A1 and S100A8 proteins

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The S100 protein family consists of structurally similar calcium-binding proteins that play diverse roles in regulating calcium homeostasis, cell growth, differentiation, cytoskeleton dynamics and response to inflammation[1]. In recent years, several members have been found to play significant roles in neurodegeneration by facilitating neuroinflammatory signalling and forming amyloid fibrils[2]. Among these proteins, S100A9 has been well-characterized, while the roles of S100A1 and S100A8 remain unclear.

S100A1 and S100A8 are expressed in the cerebral cortex[3] and share structural similarities[1]. Both proteins have active roles in Alzheimer's disease (AD) and interact with TLR and RAGE receptors[4], which play a crucial role in the AD cascade for transmitting neuroinflammation signals. Given S100A's ability to form heterodimers with other S100 proteins[2], we aimed to elucidate a potential S100A1/A8 complex formation.

Aggregation kinetics of S100A1/A8 was explored with the amyloid-specific Thioflavin T fluorescence assay, followed by visualization of the samples using Atomic Force (AFM), Transmission Electron (TEM) and Total Internal Reflection Fluorescence (TIRF) microscopies. A S100A1/A8 heterodimer formation was investigated via Electron Paramagnetic Resonance Spectroscopy (EPR).

Our research revealed that S100A1/A8 aggregation into fibrillar structures is a calcium-dependent process, inhibited at higher calcium concentrations. AFM and TEM imaging confirmed that the interaction between these proteins led to the formation of worm-like fibrils. Using TIRF microscopy, we observed co-localization of fluorescently tagged S100A1 and S100A8 within aggregates. However, EPR did not detect a stable complex, suggesting that interactions between these proteins may be transient or occur within larger, higher-order structures. These findings add to understanding S100 protein aggregation dynamics and offer valuable insights into their potential relevance in various diseases.

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C21

METABOLIC LABELING OF INDIVIDUAL DNA METHYLOMES

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S-adenosyl-L-methionine (AdoMet) serves as a donor of methyl group that is used by DNA methyltransferases for DNA, RNA, and protein modification, making it essential for epigenetic regulation and enzymatic activity. Within living cells, AdoMet is produced from L-methionine (Met) and ATP by a family of methionine adenosyltransferases (MAT). For MTases-directed site-specific alkylation, synthetic AdoMet analogs with an extended side chain were developed for various biotechnological applications. However, AdoMet and its analogues cannot permeate the cell membrane. Thus, to conduct in vivo experiments, AdoMet analogues must be synthesized inside the cell. Previously, we demonstrated a chromosomally expressed MAT2A-DNMT cascade in mouse embryonic cells that permits selective chemical tracking of individual DNMT-specific methylomes upon exposure to cell-permeable methionine analogue, S-(6-azidohex-2-ynyl)-L-homocysteine (N_3-Met) in the presence of physiological levels of Met [1]. Our next objective was to apply this technology to human cells. Therefore, the goal of our research is to investigate catalytic activities of human wt hMAT2A and mutant hMAT2A in the presence of N_3-Met *in vitro*. Vectors of the human MAT2A catalytic mutant were genetically engineered, and wt hMAT2A, hMAT2A mutant were successfully purified from E. coli cells. By performing two-step cascade reactions with M. TaqI methyltransferase N_3-Met was added to investigate time-course DNA modification which demonstrated that in the presence of the N_3-Met, better DNA modification results were produced using mutant hMAT2A in comparison to wt hMAT2A. By performing two-step cascade reactions with M. TaqI methyltransferase MAT2B's regulatory effects on MAT2A activity were investigated, but no catalytic difference was observed. To identify if Ado-6-azide is synthesized in the presence of L-Met, high-performance liquid chromatography-mass spectrometry/MS (HPLC–MS/MS) was performed. The results showed that MAT2A mutant can synthesize Ado-6-azide, from N_3-Met and ATP, in the presence of the L-Met.

In conclusion, acquired results show that MAT2A- DNMT technology could be applied in human cells to label individual DNA methylomes.

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C22

HAPLOTYPE PROFILING OF TNF GENE VARIANTS IN LARYNGEAL CANCER

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Squamous cell carcinoma (SCC) is the most common histology type of laryngeal cancer [1]. The overall survival rate for laryngeal SCC is still low, especially in elderly and co-morbid patients, even with advancements in diagnostic procedures [2]. This information promotes more investigation into possible tissue- or blood-based biomarkers for the early diagnosis of laryngeal SCC [3]. Nonetheless, it is well recognized that inflammatory mechanisms, such as angiogenesis, control the growth of solid tumors [4]. Mast cells that proliferate close to blood arteries and release mediators that encourage angiogenesis and inhibit the immune system—such as histamine, tumor necrosis factor (*TNF*), vascular endothelial growth factors, and interleukins—help tumors grow [5]. Single nucleotide variants (SNVs) in the *TNF* promoter region have been thoroughly investigated for their role in cancer. As a result, the SNVs could affect the risk of developing cancer, which might be linked to an adjacent gene in tight-linkage disequilibrium or changed TNF production [6]. In this study we investigated the role of *TNF* variant haplotypes in the development of laryngeal SCC.

This research aims to reveal the associations between haplotypes of *TNF* and laryngeal SCC patients.

A total of 190 patients with LSCC and 190 healthy individuals were enrolled in the study. DNA was isolated from the venous blood by the salting out method. Genotyping of *TNF* (rs361525, rs1800629, and rs1800630) SNVs was performed by real-time polymerase chain reaction (RT-PCR). Statistical data analysis was conducted using "The IBM SPSS Statistics 30.0.0.0 " program. Haplotype analysis was performed in the laryngeal SCC and control groups online SNPStats software [7].

Haplotype analysis was performed and pairwise linkage disequilibrium (LD) between studied variants was observed. The analysis of the haplotypes showed that individuals carrying rs1800630, rs1800629, and rs361525 haplotype C-A-G had decreased risks of laryngeal SCC (OR = 0.64, 95% CI: 0.41–0.99; p = 0.048).

In conclusion, we found that the haplotype rs1800630C-rs1800629A-rs361525G may play a protective role in laryngeal SCC patients in the Lithuanian population.

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C23

N4BP1 RNASE UTILIZES TANDEM KH DOMAINS TO INTERACT WITH EDC4 AND MRNA DECAPPING FACTORS IN P-BODIES

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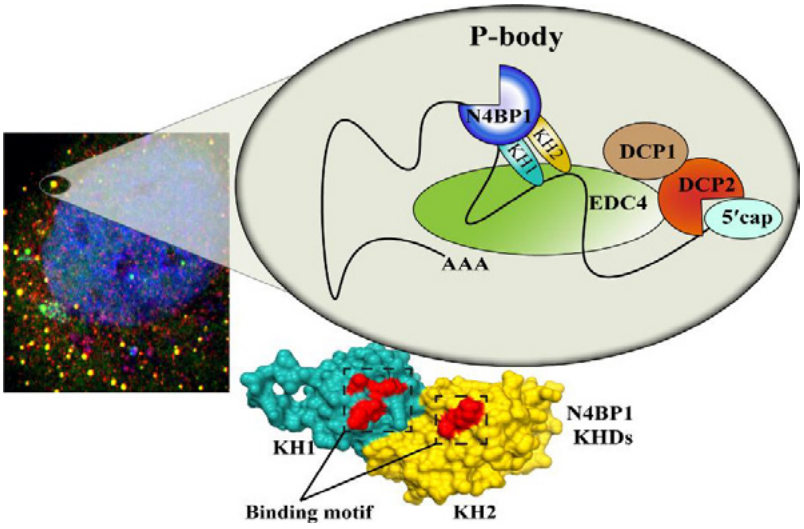
N4BP1 (*Nedd4 Binding Protein 1*) is a ribonuclease composed of two K-homology (*KH*) domains, a Ubiquitin Associated (*UBA*) domain, a NYN (*N4BP1, YacP-like Nuclease*) domain, and a CoCUN (*Cousin of CUBAN*) domain. It plays an important role in modulating immunological responses [1], NFκB signal transduction [2], and viral mRNA degradation [3].

In this study, we demonstrate that N4BP1 interacts with EDC4, a key scaffolding protein of the mRNA decapping complex, as well as other proteins associated with 5'-cap hydrolysis in processing (P) bodies, including DCP1A, DCP2, and XRN1. To elucidate the molecular basis of the N4BP1-EDC4 interaction, we identified the two tandem KH domains (KH-1 and KH-2) as essential for this interaction. Mutational analysis and domain deletion studies revealed that the interaction between N4BP1 and EDC4 was disrupted upon deletion of the KH domains, mutations in KH domains GxxG loops or RNase treatment, implicating an RNA-mediated mechanism. Moreover, we showed that N4BP1's interaction with EDC4 is necessary for the association with other decapping factors. RNA sequencing after N4BP1 overexpression revealed nearly 500 differentially expressed genes, mostly associated with blood vessels development, cell adhesion and differentiation. Moreover, we identified several transcripts regulated by N4BP1 that could potentially be influenced by its ribonuclease activity.

In conclusion, our findings highlight the critical role of RNA in mediating the interaction between N4BP1 and EDC4. This study provides valuable insights into N4BP1's new functions within RNA-protein complexes and its involvement in mRNA decay pathways.

Figure 1. N4BP1 associates with decapping factors in P-bodies using tandem KH domains.



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C24

PUTATIVE TTV CAPSID PROTEIN SYNTHESIS

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Torque teno virus (TTV) is a part of human virome. TTV is under active investigation as a marker of immunocompetence. After a solid organ transplant (SOT), patients are treated with immunosuppressants to reduce the immune response. However, if immunosuppression is not optimal, the risk of infection or organ rejection is higher, and the patient's health could be at risk. Therefore, a reliable molecular marker of immunosuppression is needed. Previous studies in this area have reported that TTV levels are elevated in the blood of SOT patients, making it a potential marker for monitoring the level of immunosuppression [1].

TTV is a small, non-enveloped, single-stranded DNA virus of the *Anelloviridae* family. The TTV genome consists of at least 6 open reading frames (ORFs), of which ORF1 is the longest [2]. It encodes the putative TTV capsid protein ORF1, which exposes antigenic peptides in its C-terminus and is recognized by antibodies [3]. The aim of this study is to achieve the bacterial synthesis of recombinant putative TTV capsid protein. A gene sequence was selected and the gene encoding the required protein was acquired based on the epitopes identified in the literature. The gene construct was chosen for its ability to allow gene expression in bacteria, followed by potentially soluble protein synthesis [3][4]. A gene expression vector was selected, and protein synthesis was confirmed by Western blot.

In conclusion, the synthesis of a putative TTV capsid protein has been achieved. Future plans include protein purification, mice immunization, and the development of monoclonal antibodies specific to this protein as universal tools for detection of TTV in patient samples.

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C25

Uncovering New Prokaryotic Antiviral Defense Systems: PD-T4-1, PD-T7-1, Azaca and Mokosh I

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The ongoing evolutionary battle between bacteria and bacteriophages has led to the emergence of diverse prokaryotic antiviral defense systems. Historically, research focused on well-known defense mechanisms like restriction-modification (RM), abortive infection (Abi) systems and the widely-recognized CRISPR-Cas system. However, recent advancements in exploring the vast bacterial pangenome, the collective genetic pool of a species, are revealing a hidden arsenal of previously unknown defense systems clustered in "defense islands" [1, 2].

In this study, we explore the molecular mechanisms and functional optimization of four novel defense systems: PD-T4-1, PD-T7-1, Azaca and Mokosh I. Our research starts by testing whether the defence systems are able to protect against bacteriophages. We have tested the defence systems against bacteriophages from the BASEL collection and have shown that they are active and able to defend against some of the bacteriophages in the collection. We were also able to isolate several bacteriophage mutants (escapers). In future studies, the genomic DNA information isolated from these bacteriophage mutants will provide a more detailed understanding of the defence systems we are studying. Subsequently, we purified wild-type and mutant proteins from the Azaca, PD-T4-1, and PD-T7-1 systems and conducted nuclease activity assays using various DNA substrates. These experiments revealed the specificities and efficiencies of the nucleases, highlighting key functional domains and residues. Finally, we performed optimization studies to determine the optimal conditions for the nuclease activity of the wild-type proteins, enhancing our understanding of their biochemical properties and potential applications.

This work not only advances our knowledge of prokaryotic antiviral defense mechanisms but also lays the groundwork for the development of novel biotechnological tools and therapeutic strategies.

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C26

INTERACTION BETWEEN SMALL PROTEIN OF PHAGE VpaE1 AND RIBOSOMAL PROTEIN S1

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All viruses require a viable host cell for their propagation. The intracellular resources, including proteins of the essential functions, are necessary for the production of new viral particles. Although some viruses encode their own proteins for genome replication and transcription, all of them depend on host translation apparatus for the synthesis of viral proteins. Viruses, including bacteriophages (phages), use a variety of mechanisms to manipulate host ribosomes. Frequently, the labile proteins of this nucleoprotein complex are the targets for chemical modifications or modifications by interacting viral proteins [1, 2]. In this study, we detected interaction between small protein of the *Felixounavirus* genus phage VpaE1 and *Escherichia coli* ribosomal protein S1 – the multifunctional protein, which is essential for the initiation of translation [3].

The in vivo interaction was detected by cloning, overexpression and purification of the His-tagged felixounaviral protein VpaE_gp09, which co-purified together with unknown protein of the host cell. The proteomics analysis of the in-gel digested protein band identified several host proteins of similar molecular mass including protein S1 among them.

The in vitro interaction analyzed by pull-down assay of the recombinant proteins VpaE1_gp09 and S1 was less obvious indicating the need of other methods for confirmation of this interaction. To test the essentiality and possible function of VpaE1_gp09 for viral infection, a deletion mutant of VpaE1 was constructed by replacing this gene with the molecular marker *trxA,* which is most efficient for felixonaviruses [4]. The deletion mutant was viable under standard laboratory conditions showing that this protein is non-essential for phage viability.

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C27

STEADY-STATE KINETICS OF FERREDOXIN:NADP$^+$ OXIDOREDUCTASE FROM *CHLOROBACULUM TEPIDUM*: REACTIONS WITH NONPHYSIOLOGICAL OXIDANTS

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Ferredoxin:NADP$^+$ oxidoreductases (FNR, EC 1.18.1.2) are flavin-containing enzymes classified as dehydrogenases and electron transferases, utilizing a non-covalently bound FAD as a prosthetic group. These enzymes are found in plastids, mitochondria, and bacteria, where they play a key role in electron transfer, particularly in mediating two-electron and one-electron transitions. FNR-catalyzed two-electron transfer from two equivalents of reduced ferredoxin to NADP$^+$ is the final step of the electron transfer during photosynthesis, while electron transfer in the opposite direction plays a role in nitrogen fixation and isoprenoid biosynthesis.

A recently identified subclass of glutathione reductase-type FNRs includes the thioredoxin reductase-type FNR, found in *Bacillus subtilis*, *Rhodopseudomonas palustris*, and *Chlorobaculum tepidum*. The TrxR-type FNRs exhibit a homodimeric structure and their NADP(H)-binding domain is inserted between the two segments of an FAD-binding domain. *C. tepidum* is a thermophilic anaerobe that performs anoxygenic photosynthesis using sulfur compounds as electron donors. Its type I photoreaction center, homologous to photosystem I in chloroplasts, allows for direct photoreduction of ferredoxin (Fd), enabling FNR to catalyze NADP$^+$ reduction to NADPH [1-3].

In this study, we analyzed the kinetics, redox properties, and structural-functional characteristics of *C. tepidum* FNR (*Ct*FNR). The oxidation mechanism of *Ct*FNR by various nonphysiological electron acceptors, including nitroaromatic compounds, quinones, and aromatic N-oxides, was investigated. Quinone reductase and nitroreductase reactions proceed via a *ping-pong* mechanism. The considerable variation in the observed reaction rates for the enzyme-catalyzed oxidation of NADPH in the presence of different oxidants suggests that the oxidative half-reaction is the rate-limiting step. The k_{cat}/K_m values for different quinones and aromatic *N*-oxides for *Ct*FNR increase with their single-electron reduction potential, following a parabolic trend, whereas for nitroaromatics this increase is linear. Moreover, the reactivity of nitroaromatics is lower, thus indicating that the reactivity is determined by the energetics of the compounds and not their structural features. The single-electron flux in the case of reduction of nitroaromatic compounds trinitrotoluene and *p*-nitrobenzaldehyde is approximately 80–85 % and reaches 65 % in the case of 1,4-benzoquinone. The enzymatic reaction is inhibited by the reaction product NADP$^+$ acting competitively towards NADPH (K_{is} = 33.8 ± 4.0 μM) and uncompetitively towards the quinone (K_{ii} = 447 ± 54 μM). The results obtained are generally similar to those observed with other TrxR-type FNRs, namely *B. subtilis* and *R. palustris* [4,5].

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C28

DEVELOPMENT OF A SYNTHETIC ANTIBODY FOR THE DETECTION OF RECOMBINANT Sup35NM AGGREGATES

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Conformation-specific antibodies are crucial for diagnosing and treating diseases caused by protein misfolding, such as Parkinson's and prion diseases. These diseases are often incurable and fatal, making conformation-specific antibodies an attractive approach for diagnosis and drug development. VHH antibodies are smaller and more stable [1], potentially offering advantages over traditional antibodies. Since there is no commercially available antibody targeting the NM region of the Sup35 protein, modified VHH antibodies could enhance specificity and affinity for detecting misfolded proteins.

The goal was to design a synthetic antibody, NbGY, capable of recognizing Sup35NM aggregates and evaluate the NbGY and Nb484 synthetic antibodies along with Sup35NM protein biosynthesis in *E. coli* cells.

A PCR reaction was performed to amplify Sup35NM-6xHis, Nb484 and NbGY fragments. The pET21c(+) plasmid and DNA fragments of Nb484, NbGY and Sup35NM-6xHis were digested with the appropriate restriction endonucleases and then ligated. The ligation mixtures were used for the transformation of *E. coli* DH5α cells, followed by transformation into *E. coli* BL21(DE3) cells. Protein synthesis was analyzed via immunoblotting.

The designs of Nb484 and NbGY antibodies were successfully produced. The amplification of Nb484, NbGY and Sup35NM-6xHis sequences was successful. Plasmids containing the sequences of Sup35NM-6xHis, Nb484 and NbGY were successfully transformed into *E. coli* DH5α and *E. coli* BL21(DE3), resulting in fragments of the expected lengths. Western blot analysis confirmed the presence of proteins with sizes consistent with the theoretical protein sizes of Sup35NM-6xHis, NbGY and Nb484. The results will be discussed in more detail during the poster session.

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C29

EXPANDING THE ARSENAL: FIRST PEEK AT A NOVEL TYPE III CRISPR-CAS EFFECTOR

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Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) loci and CRISPR-associated proteins (Cas) protect prokaryotes from bacteriophages by specifically targeting viral nucleic acids. Among these systems, type III CRISPR-Cas systems offer an additional layer of defense through a unique signaling pathway. When the interference complex of the type III CRISPR-Cas system detects a foreign transcript, the Cas10 subunit synthesizes cyclic oligoadenylates. These signaling molecules bind to the sensory domains of ancillary effector proteins, activating their effector domains. Once activated, effectors can hydrolyze RNA or DNA, inhibit translation, or disrupt cell membrane integrity, thereby interrupting the development of viral infection or eliminating infected cells [1], [2].

Recent systematic analyses of previously uncharacterized genes in CRISPR-Cas operons have uncovered a broader set of effector proteins [3] with predicted biochemical activities not previously linked to CRISPR-Cas systems. However, the precise roles of these novel effectors in type III CRISPR-Cas-mediated immunity remain poorly understood.

Here, we present the initial studies on novel type III CRISPR-Cas effectors. The impact of activation of putative effectors on bacterial survival was assessed in a heterologous *E. coli* host using a plasmid-based system for cyclic oligoadenylate production as described in [1]. Subsequently, functionally active effector proteins were purified utilizing affinity and ion exchange chromatography methods for further biochemical and structural analysis.

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C30

WHOLE-GENOME ASSOCIATION WITH SARCOPENIA AND FRAILTY IN THE LITHUANIAN ELDERLY

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Sarcopenia and frailty are geriatric syndromes that significantly contribute to morbidity and mortality in the elderly [1, 2]. These conditions are closely linked, as both are characterized by a decline in physical capacity and skeletal muscle mass [3]. Although extensive research has explored age-related reductions in muscle mass and physical function, the exact pathogenesis of sarcopenia and frailty remains unclear. However, growing evidence suggests that both genetic and environmental factors play a crucial role in their development [4]. This study aimed to evaluate and identify genetic variants associated with sarcopenia and frailty in the elderly through a large-scale whole genome association analysis.

A total of 192 Lithuanian older adults (42 men, 150 women, mean age 82.2±7.6 years) participated in the study: 57 individuals with sarcopenia andfrailty (17 men, 40 women, mean age 85.3±6.7 years), and 135 healthy controls without sarcopenia and frailty (25 men, 110 women, mean age 80.2±7.5 years). Phenotypic data were collected through questionnaires, scales, and physiological tests. Sarcopenia was assessed using EWGSOP criteria, while frailty was determined by Fried's criteria (weakness, low walking speed, low physical activity, weight loss, exhaustion). Select phenotypes (lean body mass, walking pace, grip strength) were used for a genome-wide association study (GWAS). DNA was extracted from peripheral blood leukocytes using the PureLink™ Genomic DNA Mini Kit. Genotyping of 700,000 SNPs per participant was conducted with the Infinium Global Screening Array24 v3.0 Kit. Data quality control was performed using GenomeStudio, PLINK, and KING. SNPs were analyzed with a genome-wide significance threshold of $<5\times10^{-8}$ and a suggestive threshold of 1.0×10^{-5}. GWAS was conducted using PLINK v1.07, with annotation via ANNOVAR and VEP tools.

GWAS results showed no statistically significant association between SNPs and sarcopenia and frailty status. However, two SNPs—rs75652203 (ENSR00001362624, regulatory region variant) and rs17102732 (intergenic variant)—were significantly associated with grip strength (p = 8.134e-12 and 3.373e-09; Bonferroni-corrected p = 1.416e-06 and 0.000587, respectively). Additionally, four suggestive SNPs (rs3744589 [ACACA, intronic], rs850577 [RN7SL509P, upstream], rs10043030 [intergenic], and rs8066532 [ENSR00001599387, regulatory]) were linked to grip strength, while two suggestive SNPs (rs60001950 [intergenic] and rs10903128 [ENSR00000250793, regulatory]) were associated with walking pace.

In conclusion, we found significant association of two SNPs (rs75652203, rs17102732) with grip strength in patients with sarcopenia and frailty. Additionally, four SNPs showed suggestive association with grip strength, and two SNPs with walking pace. These findings point to specific genetic loci that might play a role in key aspects of sarcopenia and frailty, calling for further research to better understand their biological significance.

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C31

Biochemical characterization of short prokaryotic Argonaute SPARDA containing nuclease effector domain

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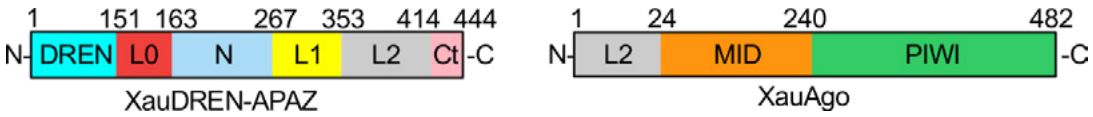
Prokaryotic Argonautes (pAgos) are divided into long and short Agos. The majority (~60%) are short pAgos containing only MID and catalytically inactive PIWI domains (Fig. 1). Short pAgos have a guide-mediated target recognition function, while various effector domains (Sir2, TIR, DREN, fig. 1) of their associated APAZ proteins execute enzymatic reactions to kill the host cell, thus, preventing spread of the invader [1-4]. It has been demonstrated that short prokaryotic Argonaute associated with DNase/RNase effector nuclease (DREN-APAZ) (SPARDA) proteins using RNA guides (gRNA) recognize their DNA targets (tDNA), leading to nonspecific collateral cleavage of DNA and RNA [4-5]. However, the detailed SPARDA activation mechanism remains unknown.

Here we present the *in vitro* study of previously uncharacterized SPARDA systems. Firstly, we determined the specificity of XauSPARDA for gRNA and tDNA with a dsDNA substrate. Then we analysed collateral cleavage activity of XauSPARDA with various substrates. Next, we purified 4 different active site mutants and measured their DNase activity. Finally, by varying different bases in guide RNA and target DNA sequences we determined key base pair positions responsible for activating enzymatic activity.

Figure 1. Schematic representation of XauSPARDA domain organization.



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C32

STUDY OF TYPE IV-A2 CRISPR-CAS SYSTEMS

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CRISPR-Cas systems assure adaptive immunity against invading mobile genetic elements in bacteria and archaea. Among them, type IV-A CRISPR-Cas systems stand out due to their distinct mechanism. Type IV-A systems lack the typical DNA/RNA nuclease activity, instead, these multisubunit systems employ a helicase DinG, which unwinds the DNA rather than cleaving it, thus causing transcriptional interference [1][2]. While subtypes IV-A1 and IV-A3 have been thoroughly characterized through structural and biochemical studies, subtype IV-A2 remains largely unexplored. This is mainly because type IV-A2 systems diverge from other subtypes with the absence of a Cas8 homologous protein, which in type IV-A1 and A3 systems, together with Cas5, is responsible for protospacer adjacent motif (PAM) recognition [1][3][4]. However, in our study using a bioinformatic guilt-by-association approach, it was discovered that CRISPR-Cas type IV-A2 systems co-occur with a small, conserved gene near the *cas* operon, which is transcribed in the opposite direction. Investigating the function of the protein encoded by this gene will not only deepen the understanding of type IV-A2 mechanisms but also lead to their potential applications in gene editing in the future. Here, we present the preparatory studies laying the groundwork for the structural and functional characterization of one of type IV-A2 systems.

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C33

MOLECULAR CHANGES IN miR146a-5p INHIBITED SH-SY5Y NEURONAL CELLS

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This study investigates the molecular changes occurring in SH-SY5Y neuronal cells with suppressed anti-neuroinflammatory microRNA miR146a-5p. MicroRNA-146a (miR-146a) is known for its role in the innate immune response and is abundant in both mouse and human brains, expressed in both microglia and neurons [1].

To examine this, SH-SY5Y neuroblastoma cells were differentiated into a more mature neural phenotype using a specialized differentiation medium consisting of BrainPhys™ neuronal medium supplemented with Penicillin/Streptomycin, serum- and antioxidant-free B-27™ supplement, 50 ng/ml BDNF, and 10 µM retinoic acid. Following differentiation, a mirVana inhibitor was applied to suppress miR146a-5p activity. The results demonstrated that miR146a-5p plays a crucial role in SH-SY5Y cell neurodifferentiation, as RT-qPCR analysis revealed increased expression of *NTRK2*, *BDNF*, *SYP1*, and *NCAM1* in differentiated cells, while miR146a-5p inhibition significantly reduced their expression. These findings suggest that miR146a-5p regulates key genes involved in neuronal survival, synaptic function, and differentiation.

Beyond neurodifferentiation, miR146a-5p inhibition also influenced mitochondrial function. RT-qPCR analysis showed a reduction in *UCP1* expression, suggesting alterations in mitochondria metabolic efficiency. Additionally, Seahorse Mito Stress analysis revealed an increase in oxygen consumption rates (OCR) in miR146a-5p-inhibited cells compared to the experimental control, indicating potential shifts in oxidative phosphorylation and mitochondrial respiratory capacity. These results suggest that miR146a-5p suppression may enhance mitochondrial activity, potentially as a compensatory response to metabolic stress.

In conclusion, these findings indicate that miR146a-5p is a significant regulator in SH-SY5Y cells, impacting both neuronal differentiation and mitochondrial function. Our ongoing research will further investigate how different molecular changes induced by miR146a-5p inhibition affect neuroinflammatory pathways and contribute to neuronal resilience.

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C34

STUDIES OF HUMAN DT-DIAPHORASE (NQO1) REACTIONS WITH QUINONES AND NITROAROMATIC COMPOUNDS

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Various factors, including hypoxia, intense metabolism, xenobiotics, and immune responses, can induce oxidative stress. To protect against the consequences of oxidative stress, cells deploy various mechanisms, one of which involves NAD(P)H:quinone dehydrogenase 1 (NQO1). It serves as a primary enzyme in responding to oxidative stress [1]. At its active site, it has a cofactor FAD which carries out the reduction of quinones by transferring two electrons from NAD(P)H via hydride ions. This process ensures that upon the formation of redox-active quinones, they are converted to hydroxyquinones without forming unstable radicals- semiquinones [2].

It has been observed that NQO1 enzyme expression significantly increases in cancerous cells, making it a potential target for anticancer therapy. Several therapeutic strategies include increasing NQO1 expression to stabilize anticancer proteins and synthesizing specific NQO1-activating prodrugs. Currently, quinone derivatives that may inhibit tumor growth upon activation by the NQO1 enzyme are being extensively studied, and several have shown anticancer activity in trials. The action of these drugs relies on the formation of unstable hydroquinones after activation by NQO1, which subsequently cause DNA or other structural damage [3]. Quinones may also be used as probes for achieving specific prodrug activation [4]. Additionally, attempts to inhibit this enzyme in cancer are underway, though solely inhibitory therapy has not yet shown significant anticancer effects, thus prompting exploration of combining this treatment approach with other therapeutic agents.

We employed steady-state kinetics to obtain the mechanism by which human NQO1 reduces quinones into semiquinones. Our study demonstrated that NQO1 interacts with quinone compounds through a ping-pong mechanism. To determine if there is a connection between the single-electron reduction potential (E^1_7) and reactivity, we compared enzymatic effciency- k_{cat}/K_m and turnover number k_{cat} with E^1_7. It was noted that as the E^1_7 increases, the reactivity of the quinones also increases, although we observe exceptions to this trend, which we believe are related to steric factors, as these compounds have a larger Van der Waals volume. The reduction of nitroaromatic compound tetryl in the presence of NADPH regeneration system is accompanied by the formation of *N*-methylpicramide, which indicates that the reaction proceeds with the involvement of single-electron transfer.

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C35

Development of primary cell cultures and solid tumour samples technologies derived from human bladder cancer cells and their possible clinical application

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Introduction: Bladder cancer (BlCa) is the ninth most frequent cancer worldwide, with approximately 614 000 new cases and 221 000 deaths reported in 2022 [1]. BlCa shows a high tendency for recurrence, here's why from an economic point of view, BlCa is the most expensive form of all cancers, considering the time from the initial diagnosis to patient death, due to short-intervalled follow-up examinations [2].

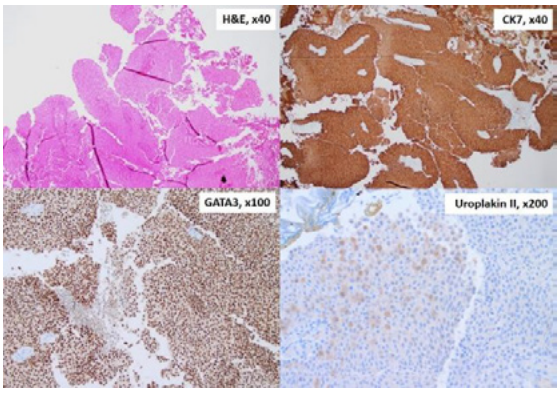
Objective: 1. Primary cell culture development, which will reflect the closest primary cancer cell heterogeneity. 2. Microscopic and immunohistochemical evaluation of solid tumor samples. 3. Evaluation of the methylation level of the retrotransposon LINE-1, which is important to assess the role of hypo/ hypermethylation in the development of BlCa. 4. Analysis of primary cell cultures and solid tumor samples and their possible application in a clinical practice.

Materials and methods: 1. Tumor samples were obtained from human urinary bladder, transferred to a 24-well plate and cultured. 2. Solid tumor samples were evaluated by an experienced pathologist for histology and immunohistochemical markers for CK7, GATA3 and uroplakin II. 3. DNA was extracted from solid tumor pieces and bisulfite conversion treatment was performed. 4. Pyrosequencing was performed to assess the methylation level of the retrotransposon LINE-1.

Results: 1. More than percentage 50 of our cultivated cell cultures show great growth results. 2. Histological interpretation of solid tumor was performed extremely quickly and we were able to know what we are growing in cell culture. 3. 100 percentage of solid tumors were positive for CK7, GATA3 and majority - for Uroplakin II immunostains (Fig. 1). 4. Pyrosequencing shows that all samples were in hypomethylation state. 5. Statistical analysis of two groups of patients: muscle invasive bladder cancer (recurrence/remission) and non-muscle invasive bladder cancer
(recurrence/remission) revealed that for methylation status it is more important the state of cancer renewal or not renewal.

Conclusions: Early pathologist findings help researchers know what cell cultures they are growing and what to expect. Based on microscopic and immunohistochemical evaluation, we are able to grow epithelial and urothelium origin cancer cells in primary cell cultures that are close to the primary tumor state. Hypomethylation status focuses more on the state of cancer recurrence or remission, wherefore it might be clinically relevant to measure the methylation state when the cancer is not yet recurrent. The cell and solid tumor models we are creating will provide doctors more references while choosing the therapy and monitoring ways.

Figure 1. Sample S23-993: Example of comprehensive pathology evaluation - non-invasive high grade (G2) papillary urothelial carcinoma, pTa.



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C36

REGULATION OF LARGE TERMINASE SUBUNIT NUCLEASE ACTIVITY BY THE PORTAL PROTEIN IN NOVEL JUMBO PHAGE KLEB27-3

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The study of bacteriophages, viruses that infect bacteria, has greatly advanced molecular biology and biotechnology. Among these, jumbo phages, with genomes over 200 kilobases, stand out for their genomic complexity and partial independence from host machinery. Jumbo phages have a broad host range, infecting diverse bacterial species, including multidrug-resistant pathogens [1]. They play key roles in microbial ecosystems by regulating pathogenic populations, making them valuable for environmental and clinical applications. Given the growing threat of antibiotic resistance, jumbo phages hold promise as therapeutic agents.

Within the *Podoviridae* family, the jumbo phage KLEB 27-3, a double-stranded DNA (dsDNA) virus, provides an intriguing model for studying viral assembly and genome packaging [2]. A critical step in this process is the packaging of viral genomes into preformed capsids, facilitated by the terminase complex (TerC) and regulated by the portal protein. The large terminase subunit performs ATP-dependent genome translocation and cleavage, yet how the portal protein regulates the large subunit's nuclease function remains unclear.

This study investigates the portal protein of KLEB 27-3 aiming to identify amino acid residues responsible for regulating TerL nuclease activity. Using AlphaFold3 structural modeling, mutagenesis, and DNA curtains it aims to uncover the molecular mechanisms governing this regulation, offering insights into jumbo phage biology and genome packaging dynamics.

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C37

THE INTERACTION OF S100A9 PROTEIN WITH MEMBRANES AND ITS IMPACT ON MEMBRANE INTEGRITY IN THE CONTEXT OF NEUROINFLAMMATION AND NEURODEGENERATIVE DISEASES

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The plasma membrane is essential for neuronal integrity, and disruptions in lipid composition contribute to neurodegenerative diseases such as Alzheimer's disease (AD). S100A9 has been specifically linked to neurodegeneration, with its expression elevated in microglial cells in AD, promoting neuroinflammation and disease progression [1]. However, the mechanisms by which inflammation-related proteins interact with the membrane and compromise its integrity remain largely unknown.

To address this, we employed Atomic Force Microscopy (AFM) and Electrochemical Impedance Spectroscopy (EIS) to investigate how S100A9 affects membrane properties and disrupts lipid bilayer integrity upon interaction. In this study, we examined the interaction of S100A9 with model membranes of two distinct compositions: (1) DOPS/DOPE/DOPC/Cholesterol, which includes the negatively charged phospholipid DOPS, and (2) DOPC/Cholesterol, a neutral membrane variant. Our results demonstrate that S100A9 selectively interacts with the negatively charged membrane, leading to notable disruption.

These findings provide important insights into how S100A9 may contribute to membrane integrity loss in neuroinflammatory conditions, advancing our understanding of its role in neurodegenerative diseases progressnion.

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C38

INFLUENCE OF THE PGLX PROTEIN MUTATIONS ON THE BREX PROTECTION

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The ongoing co-evolution and co-adaptation of bacteria and bacteriophages has led to the development of antiviral defense systems that are encoded in regions of the bacterial genome called defense islands [1]. One of the bacterial antiviral defense systems is BREX (Bacteriophage Exclusion). It is present in about 10 % of prokaryotic genomes. This system inhibits bacteriophage replication by an unknown mechanism of action [2].

Type 1 BREX system consists of a cassette of 6 protein-coding genes: *brxA, brxB, brxC, pglZ, brxL, pglX*. The *pglX* gene encodes the m6A DNA methyltransferase, which methylates specific sequences in the host genome to distinguish itself from foreign DNA [2]. Bacteriophages can evade BREX protection by encoding protein inhibitors. The Ocr protein encoded by the T7 bacteriophage binds specifically to the PglX protein in a way that mimics the double-stranded helix of DNA and blocks BREX protection [3].

In this study, we analysed the influence of PglX mutations on BREX activity and its inhibition. A phage interference assay was used to determine whether BREX system with PglX mutants can inhibit bacteriophage infection. The interaction of PglX mutant proteins with the Ocr repressor and DNA substrates containing BREX recognition sequences was investigated by differential scanning fluorimetry and biolayer interferometry.

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C39

CHARACTERIZATION OF A NOVEL PROKARYOTIC ANTIPHAGE DEFENSE SYSTEM – PD-T7-5

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The long ongoing fight between bacteria and bacteriophages has led to bacteria evolving and/or acquiring diverse defense mechanisms. Many of these have become irreplaceable tools in molecular biology and biochemistry. CRISPR-Cas systems for genome editing and restriction-modification systems (RM) for manipulating nucleic acids are all derived from prokaryotic antiviral defense systems[1]. However, there is always a need for new tools to be discovered. Fortunately, recent bioinformatics approaches have identified “defense islands” (DIs), genomic regions enriched in defense-related genes, leading to the identification of numerous novel and uncharacterized defense systems[2, 3].

This study aims to characterize the novel prokaryotic antiviral defense system - PD-T7-5, discovered in the *E.coli* UMB0934 strain. To better understand the underlying mechanism of PD-T7-5, site-specific mutagenesis targeting the predicted active center has been carried out. Following mutagenesis, Western blotting was used to confirm the expression of the mutant defense system. At the same time, phage plaque assays were employed to determine the effect of these mutations on the system’s ability to protect bacteria from bacteriophage infection *in vivo*. The purified mutant protein was also subjected to biochemical assays to investigate its nucleolytic activity *in vitro*. Crystallization screening was conducted to determine the crystallization conditions, with the long-term goal of solving the PD-T7-5 structure.

This work contributes to a deeper understanding of prokaryotic antiphage defense and its potential applications in biotechnology.

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C40

FUNCTIONAL CHARACTERIZATION OF THE APEA ANTIVIRAL PROTEIN THROUGH BACTERIOPHAGE SENSITIVITY AND KEY RESIDUE ANALYSIS

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The intense coevolutionary arms race between bacteria and phages has resulted in an unexpectedly wide and diverse array of antiphage defense systems [1]. While well-characterized antiviral systems such as CRISPR-Cas and restriction-modification directly target phage genetic material, abortive infection (Abi) systems function by activating a toxic protein during late phases of infection, causing programmed death of an infected cell to halt phage proliferation and protect the rest of the bacterial colony [2]. A widely used component of numerous Abi system proteins is the HEPN (Higher Eukaryotes and Prokaryotes Nucleotide-binding) domain, which possesses endoribonuclease activity and is also associated with a variety of bacterial immune responses [3].

Our research focuses on one such Abi system Gao_Ape which encodes a single protein ApeA with a HEPN domain variant HEPN_apea as its key functional feature. It was previously known that this system defends against classic T phages [4] but the molecular mechanisms were not yet clear. To characterize this defense system five more homologous ApeA proteins were selected for analysis. *In vivo* experiments were conducted to evaluate the system's ability to protect bacteria against a diverse collection of phages, revealing that the homologs exhibit varying levels of effectiveness against different groups of phages.

ApeA protein contains a ligand pocket, whose importance in activation is being analyzed. Activation triggers HEPN domains to cleave host tRNA anticodon loops and induce abortive infection mechanisms. To better understand the molecular basis of the activation of ApeA we focused on two homologs that provided the strongest defense against phages. These homologs were subjected to targeted mutagenesis, where specific amino acids predicted to be essential for enzymatic function and activation were substituted. This approach allowed us to identify critical residues involved in the activation of the protein, showing how certain mutations reduce the protein's toxicity, as well as its protective effect against phage infection. Our results provide important insights toward a complete understanding of the mechanism of this defense system.

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C41

ENZYMATIC ANALYSIS OF PUTATIVE β-LACTAMASE PROTEINS FROM OPPORTUNISTIC PATHOGEN *STENOTROPHOMONAS MALTOPHILIA*

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Rapidly emerging bacterial resistance to antibiotics has become one of the main challenges of the 21st century, affecting millions of people by making bacterial infections less treatable and more deadly. Clinically significant multidrug-resistant pathogens pose a particular challenge by showing resistance to many commonly used antibiotics. Multidrug-resistant *Stenotrophomonas maltophilia* is an opportunistic, Gram-negative pathogen that is relatively understudied yet rapidly acquiring resistance worldwide, leading to serious and difficult to treat infections [1].

S. maltophilia exhibits resistance to commonly used antibiotics, including aminoglycosides, fluoroquinolones, vancomycin, macrolides, and, most notably, β-lactams, which are among the most clinically important antibiotics [2]. Although *S. maltophilia* resistance to β-lactam antibiotics is well-documented, only two β-lactamases, L1 (a metallo-β-lactamase) and L2 (a class A serine β-lactamase), have been thoroughly studied and characterized as enzymes that confer resistance by degrading β-lactam antibiotics [3]. Our analysis of β-lactam resistant *S. maltophilia* SM3 isolate identified 10 additional proteins homologous to known β-lactamases, suggesting that these proteins may play a role in β-lactam antibiotic resistance.

The aim of this study was to evaluate the β-lactamase specific enzymatic activity of 10 proteins from *S. maltophilia* potentially conferring resistance to β-lactam class antibiotics . This was achieved by first performing a bioinformatic analysis of putative β-lactamases using the Beta-Lactamase DataBase [4]. Secondly, the genes encoding proteins potentially involved in β-lactam resistance were cloned into an expression plasmid, and their functional activity was evaluated in an *Escherichia coli* expression strain using the nitrocefin assay, which specifically detects β-lactamase activity.

The bioinformatic analysis revealed that all examined proteins shared homology with known β-lactamases, with amino acid sequence identities ranging from 24.8% to 29.5%, which is relatively sufficient for further functional analysis [5]. The methodology for assessing β-lactamase enzymatic activity using the nitrocefin test was successfully validated with the well-characterized L1 β-lactamase and applied to two of the homologous proteins. The functional activity of the remaining putative β-lactamases will be evaluated using the same approach with nitrocefin testing.

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C42

FROM DNA CONSTRUCTION TO PROTEIN IN 1 DAY

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Protein production plays a critical role in various research domains, such as drug discovery and biotechnology. The growing demand for customized proteins with specific properties has been addressed by significant advancements in molecular biology, revolutionizing the field of protein engineering. Although these advancements empower researchers to precisely modify proteins, including their sequence, purification tags, secretion signals, and performance attributes, the methods usually rely on live cells, involve labor-intensive, multi-day workflows, and have limitations in terms of throughput and time efficiency.

To overcome these limitations, we designed an *in vitro* workflow for streamlined protein expression, enabling researchers to generate customized proteins with enhanced speed and efficiency. This workflow combines the ordering of gene-of-interest as GeneArt™ Strings™ DNA Fragment with GeneArt™ Gibson Assembly™, or self-circularization, rolling circle amplification (RCA), and cell-free protein expression (CFPE) to generate an array of proteins for subsequent functional tests. While Gibson Assembly or self-circularization provides a standardized and efficient approach for gene assembly into a circular form, RCA offers an excellent method to rapidly amplify those assembled genes for CFPE. This cell-free system reduces the experimental timeline from ~3 days to ~1 day by replacing time-consuming steps of bacterial transformation, liquid culture, and plasmid purification, which are necessary for traditional cloning workflows, with a fast and efficient RCA reaction (Fig. 1).

Our innovative workflow holds great potential for its widespread adoption in laboratories focused on high-throughput and time-sensitive protein engineering projects, such as drug discovery and biotechnology, helping enhance the speed and efficiency of testing multiple protein designs, including toxic ones.

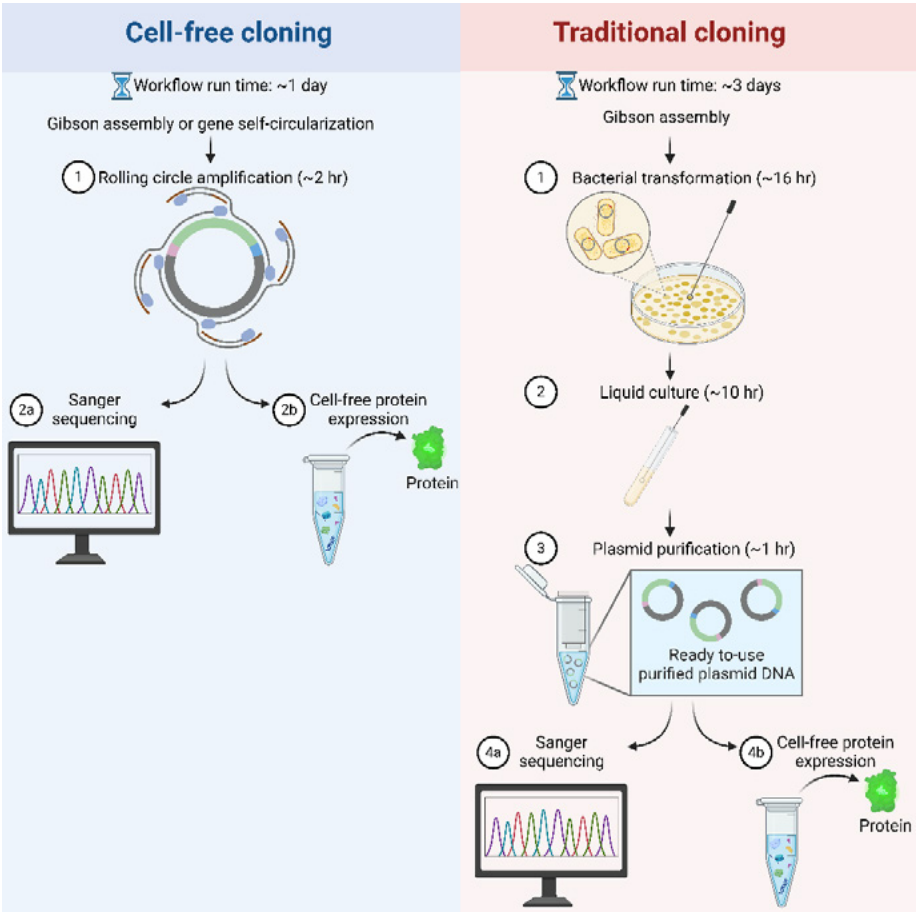

Figure 1. Comparison of the total reaction time using cell-free cloning versus the traditional cloning approach. Created with BioRender.com.

C43

SINGLE-CELL ISOLATION IN SEMI-PERMEABLE CAPSULES: MARKER GENE DETECTION AND 3D CELL CULTIVATION

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Single-cell isolation from heterogeneous populations enables precise characterization of individual cells. Although high-throughput microfluidic techniques are widely used [1], many conventional methods struggle to support multi-step biochemical reactions while preserving genetic integrity. To overcome these limitations, we utilize semi-permeable microcapsules (Fig. 1A), which retain genetic material while allowing the diffusion of small molecules, such as primers or buffer components [2], [3]. This method presents a cost-effective approach for studying biological systems.

We employed capsule-based technology for two distinct purposes: single-cell marker gene analysis (Fig. 1B) and 3D cell cultivation. For marker gene detection, we tested both mild and harsh cell lysis, with DNase treatment included in the latter before cDNA synthesis, PCR, and fluorescent *in situ* hybridization (*FISH*). So far, detection has been limited to housekeeping genes, with further optimization needed for other targets. Separately, we explored the use of capsules for long-term cell growth, where suspension cells (K562) proliferated for 18 days, gradually expanding the capsule as they grew (Fig. 1C), after which the capsule shell could be removed to release the spheroid. Similarly, adhesive cells (HeLa) remained attached to the capsule's inner surface over extended periods, indicating a supportive microenvironment (Fig. 1D).

These findings highlight semi-permeable capsule technology as a promising approach for single-cell marker gene detection and controlled growth in a 3D microenvironment, with broader applications in spheroid formation, drug screening, and high-throughput single-cell studies.

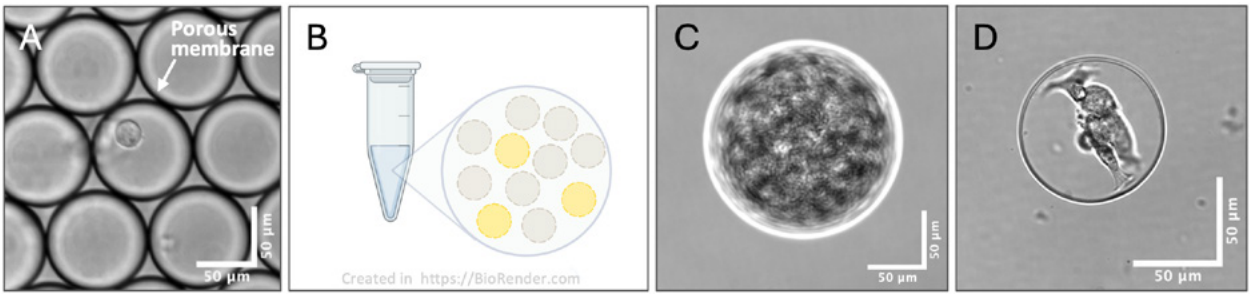

Figure 1. (A) Bright-field image of a semi-permeable capsule hosting a single cell. (B) Schematic representation of fluorescent capsule detection in a suspension mix for marker gene detection. (C) Single-cell-derived spheroid of suspension cells (K562) after 18 days of growth. (D) Single-cell-derived adherent cells (HeLa) after 4 days of growth.

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C44

ADAPTATION OF CROFT-SEQ FOR OFF-TARGET DETECTION OF VARIOUS GENOME EDITING TOOLS

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Programmable genome editing nucleases, such as CRISPR-Cas9, have attracted significant scientific interest due to their immense potential for gene editing and the treatment of human genetic diseases. The successful development of these tools is crucial for both fundamental and clinical applications to ensure that genome editing tools are reliable and safe. Because of their ability to easily target specific DNA sites, these nucleases enable precise and rapid genome editing. Although these nucleases are designed to cut their intended target site (on-target), they often tend to also cut DNA sequences similar to the target site (off-targets). This can lead to harmful gene mutations or chromosomal rearrangements, potentially causing cancerous transformations or even cell death. To address these issues, a variety of methods have been developed to detect off-target sites of Cas9 nucleases both *in vitro* and *in vivo* [1]. However, the vast majority of these methods are specifically designed to detect double-strand DNA breaks caused by genome editing nucleases like CRISPR-Cas9, which leave "blunt" DNA ends after cutting. Only a few methods have been developed and adapted for other genome editing tools (non-Cas9), such as Cas12 or base editors, for detecting off-targets *in vitro* [2, 3]. However, these methods are often expensive, complex and have relatively low sensitivity. Therefore, the ability to detect off-target sequences of various genome editing tools would enable the improvement and selection of more suitable and safer tools for genome editing.

Recently, we have developed a sensitive, simple, fast, and cost-effective CRISPR-Cas9 off-target detection method called CROFT-Seq [4], which, in many aspects, rivals or even outperforms other well-known *in vitro* off-target detection methods, such as SITE-Seq [5] and CIRCLE-Seq [6]. In this research, we will aim to employ and modify existing CROFT-Seq method by incorporating additional enzymes and experimental stages for detecting off-target sites of various genome editing tools, such as Cas12 and base editors. Additionally, we will modify the bioinformatics algorithm of the CROFT-Seq off-target site analysis to make it suitable for identifying off-target sequences of different genome editing tools. Furthermore, we will perform genome editing of HEK293T cells *in vivo* and validate wether potential off-target sites identified *in vitro* are also cleaved during genome editing in living cells. Comparing *in vitro* off-target sequences with those validated *in vivo* will allow us to draw significant conclusions regarding the accuracy and sensitivity of the developed methods. With the advancement of the robotics industry, there is a growing demand for adapting off-target detection methods to robotic systems. Therefore, as part of this project, we will also aim to adapt the modified CROFT-Seq methods for use with the Opentrons OT-2 robotic system.

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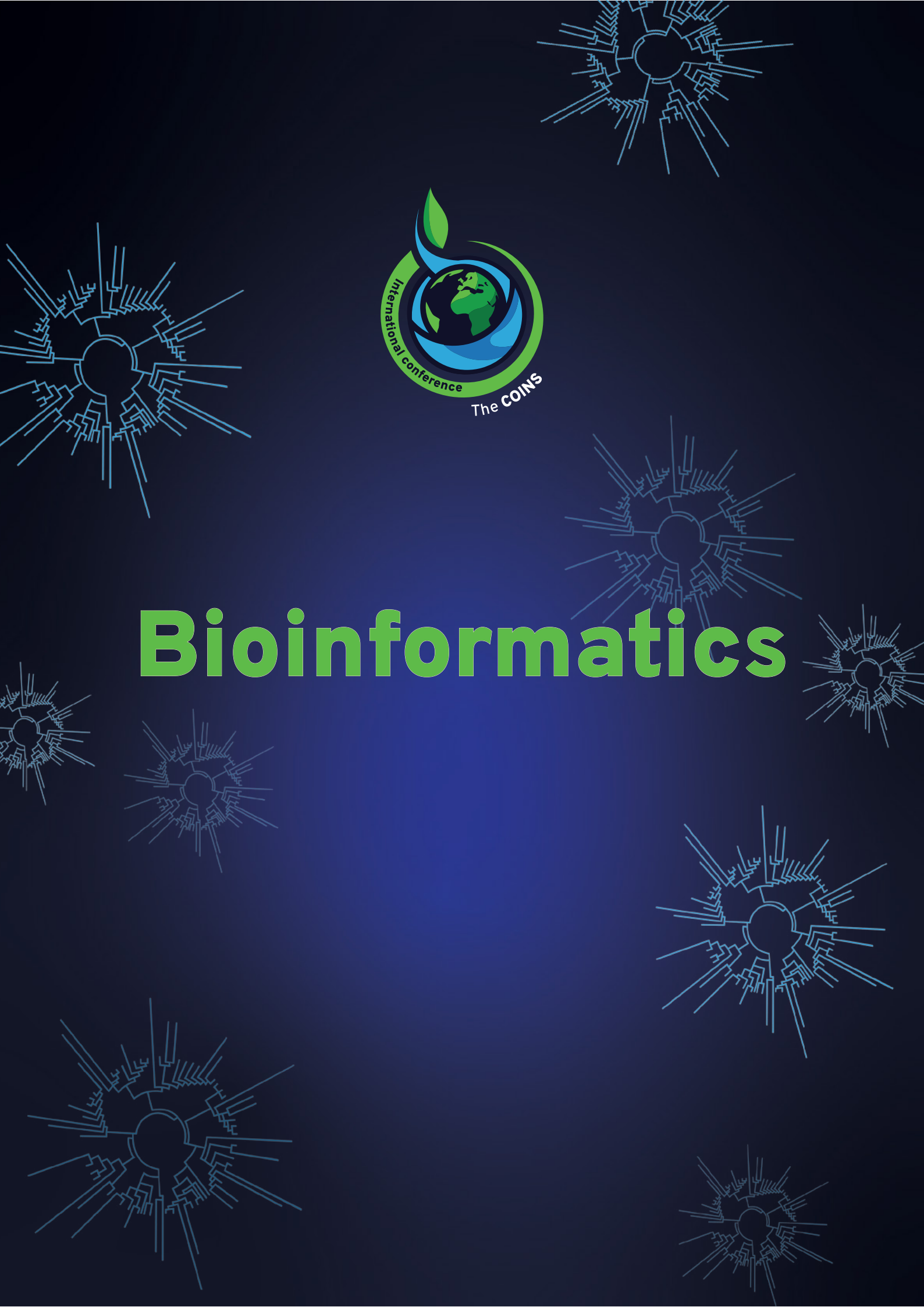

Bioinformatics

D1

ARTIFICIAL INTELLIGENCE FOR ASSISTING THE RECONSTRUCTION OF FULL OCCLUSION FOR PATIENTS WITH DENTAL HARD TISSUE DEFECTS, TEMPOROMANDIBULAR JOINT DISORDERS AND JAW ABNORMALITIES

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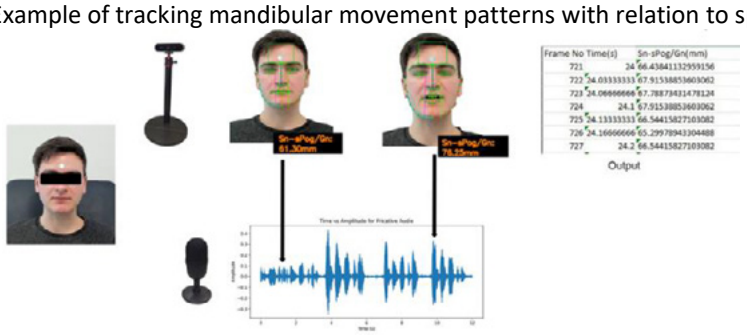
From "Hey Siri" to complex algorithms powering self-driving vehicles, artificial intelligence (AI) is rapidly integrating into nearly every aspect of modern life. There has been a dramatic increase in medical research around AI in recent years. Prosthodontics, the branch of dentistry dedicated to restoring and replacing missing teeth and oral tissues, continues to be at the forefront of innovation in dentistry. The convergence of AI's growing capabilities and the multifaceted nature of prosthodontics creates exciting possibilities for advancements in diagnosis, treatment planning, and prosthesis design.

Full mouth reconstruction presents a complex prosthodontic challenge, often involving overlapping issues such as dental tissue defects, temporomandibular joint disorders (TMDs), and jaw abnormalities [1]. Traditionally, TMJ analysis, crucial for these cases, relied on invasive and radiation-exposing methods. However, advancements in non-invasive digital imaging, including cone beam computed tomography (CBCT), magnetic resonance imaging (MRI), and, more recently, intraoral scanners (IOS) for tracking jaw motion [2], have revolutionized diagnostics. This digital data, coupled with the integration of mandibular movement records (Fig. 1) into computer-aided design (CAD) workflows [3], creates an ideal environment for the application of artificial intelligence. AI has the potential to significantly aid dentists in full mouth reconstruction by analyzing complex datasets, assisting in treatment planning and prosthesis design, ultimately leading to improved patient outcomes.

Figure 1. Example of tracking mandibular movement patterns with relation to speech [4].



This research is focused on answering the question: “How can AI assist in critical intervention to reconstruct the whole occlusion for patients with dental hard tissue defects, TMJ disorders, and jaw abnormalities?” While existing literature explores AI applications across prosthodontics, this study will focus on this particularly demanding area. The research is going to cover diagnostic data acquisition and processing, analytic techniques, and the development of AI-driven predictions of TMJ function to assist dental professionals in full occlusion reconstruction.

The review will follow a strict PRISM methodology for relevance and bias. The article search is going to include four main databases: PubMed/Medline, EBSCO, Web of Science, and Scopus. Only peer-reviewed articles, case studies, systematic reviews and meta-analyses published over 2019-2025 are going to be included. Risk biases of the selected studies are going to be assessed using the Quadas-2 methodology.

Although AI is revolutionizing prosthodontics, full mouth reconstruction remains a complex undertaking. This research will explore how AI can improve the accuracy and efficiency of this challenging procedure, enabling dentists to deliver more precise treatments with fewer errors.

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D2

ANALYSIS OF THE DIVERSITY OF THE T7 SSB PROTEIN FAMILY

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ssDNA-binding (SSB) proteins temporarily coat and protect ssDNA intermediates during DNA replication, recombination, translation and repair [1]. Bacteriophage T7, like other viruses that encode their own DNA metabolism proteins, has SSB, which is the product of gene 2.5 (gp2.5). The T7 SSB protein family is highly diverse at the sequence level, although their structures share common features. It includes SSB proteins from T7 phage, NCLDV (nucleocytoplasmic large DNA viruses), and herpes viruses [3]. In 2023, herpesvirus-related Mirusviruses were discovered, and a large proportion of their genes are homologous to genes from giant eukaryotic DNA viruses, however, no SSB proteins were found during the initial analysis of their genomes [2].

The diversity of T7 SSB family proteins was studied in more detail more than ten years ago [3]. During this time, protein sequence and structure databases have grown significantly, and it has become possible to model structures whose quality is on par with experimentally solved ones. This work aimed to investigate the sequence and structural diversity of the T7 SSB protein family.

To check the presence of SSBs in Mirus viruses, their proteome was modeled with Colabfold and clustered together with other characterized SSBs. Three Mirus proteins (83, 203 and 223) showed the highest similarity to T7 SSB proteins.

To check the structural diversity of T7 SSB proteins, a search was performed with Foldseek against the Big Fantastic Virus Database (BFVD) database. The following query structures were used for the queries: 1je5, 1urj, 7yeq, mirus 203 and mirus 223, VpaE1 gp103, E. coli SSB, T4 SSB, N4 SSB, poxvirus SSB, mitochondrial SSB (6cqm). The significant hits and query structures were compared using the Dali program. Similarity scores were used to group the structures with the CLANS program.

The results highlighted the diversity of T7 sequences and the lack of sequence conservation. The T7 SSB structures are divided into three overlapping groups. The mirusvirus SSBs are structurally similar to the T7 SSBs and link the herpesvirus SSBs to other proteins in this family. In addition, we identified VpaE1 gp103 as a divergent member of the T7 SSB family and showed experimentally that it binds ssDNA and that its C-terminus is essential for its function.

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D3

EVOLUTIONARY AND FUNCTIONAL INSIGHTS INTO A CAS7-LIKE PHAGE PROTEIN AND ITS ASSOCIATION WITH TRANSPOSON ELEMENTS

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CRISPR-Cas systems are adaptive immune systems found in bacteria and archaea. The interference in class 1 systems is mediated by Cas effector nucleases that assemble into multi-subunit complexes. The Cascade complex plays a critical role in target recognition and DNA binding, with Cas7 acting as a key subunit that binds CRISPR RNA (crRNA) to facilitate this process [1]. Beyond their canonical role in immunity, CRISPR-associated proteins have been linked to transposon-associated systems, facilitating RNA-guided genome rearrangements [2]. Transposons and recombinases contribute significantly to genome evolution by enabling horizontal gene transfer and structural modifications [3]. In this context, phage-encoded homologs of CRISPR-Cas proteins may have diverged to acquire novel functions in mobile genetic elements.

We identified a similar phage protein (gp87) that exhibits high structural similarity to Cas7 proteins despite minimal sequence conservation and the absence of the active site. It was found that it is co-expressed with an adjacent gene encoding gp86 protein alongside non-coding RNAs (ncRNAs) in its flanking region, which shares similarities with CRISPR-like spacer-repeat regions, and it binds explicitly to one of these ncRNAs. Using the in-silico approach, we found homologous ncRNA sequences flanking serine recombinase genes in bacterial genomes, which are majorly involved in genome rearrangements. Gp87-gp86 adjacent encoding was also identified not only within the closest related phages but bacterial genomes as well. In some cases, gp86 was found to be substituted with distinct but structurally similar winged-HTH domain containing proteins.

To better understand the function of this protein, we analyzed the genomic context of gp87 using computational approach. Using sensitive searches against the GTDB database, we identified 1,222 homologs in bacterial genomes and extracted their genomic neighborhoods, including the encoded proteins and ncRNAs located within two positions upstream and downstream. To analyze the diversity of the neighbor proteins, we modeled their 3D structures using ESMFold and conducted all-to-all structural comparisons. Subsequent hierarchical clustering allowed us to identify the most common encoded patterns in the environments.

This analysis revealed four major types of proteins associated with the environment of the gp87 protein: (1) DNA Repair Helicase proteins, (2) gp86-like proteins, (3) HTH-transcription regulators, and (4) tyrosine recombinase. Additionally, we identified distinct genomic environments of this protein where it is encoded adjacent to one or more proteins from these four major groups in different combinations. The presence of gp87 in these distinct environments highlights the functional diversification of gp87 protein and also supports our hypothesis that gp87 functions in RNA-guided transposition. However, further analysis of the ncRNA environment is required to provide deeper insights into its role.

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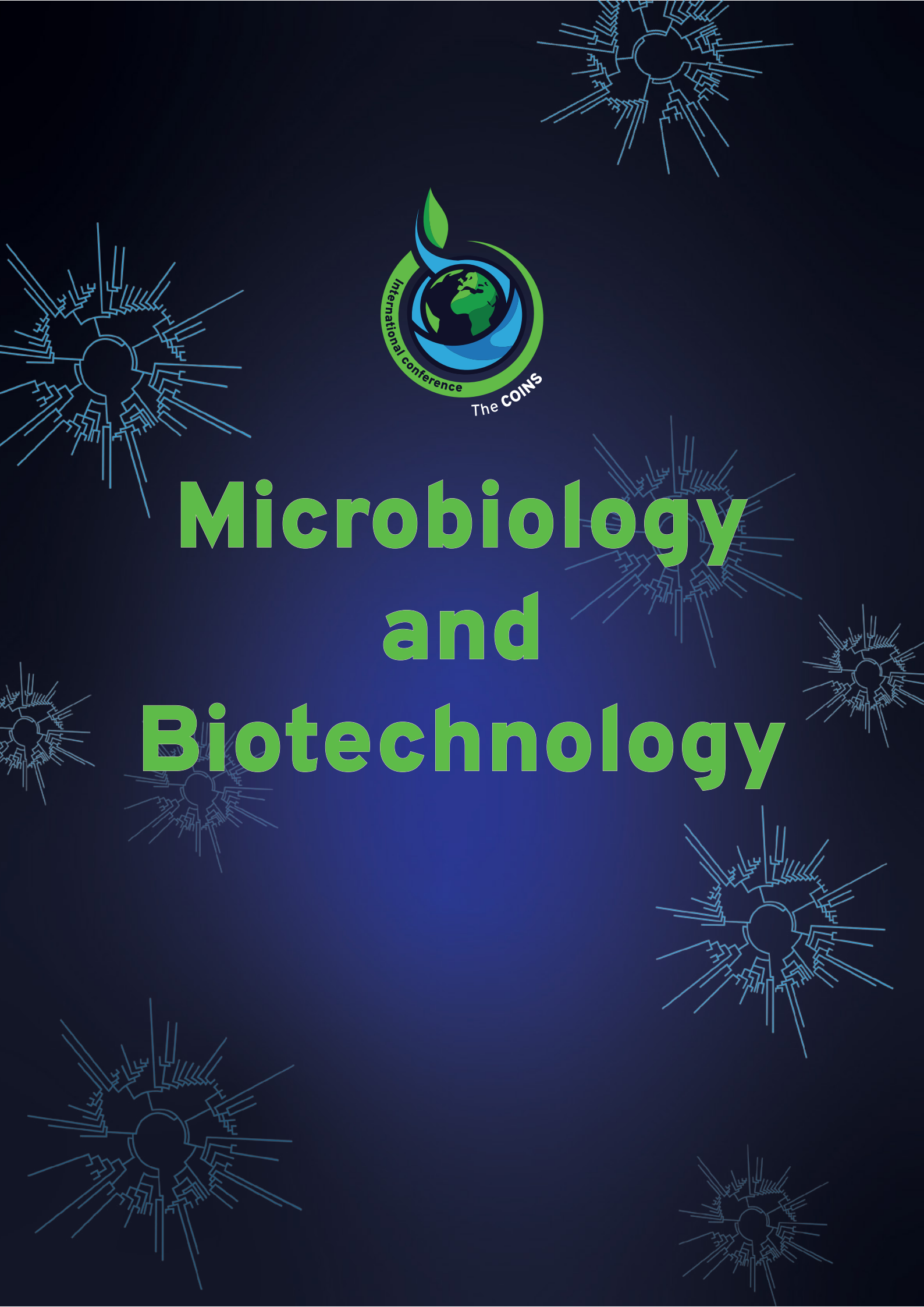

Microbiology and Biotechnology

E1

ANTIBACTERIAL POTENTIAL OF ESSENTIAL OILS AND HYDROLATES FOR HONEYCOMB HEALTH MAINTENANCE

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Plant essential oils (EOs) are hydrophobic liquid concentrates of secondary metabolites extracted from almost all plant parts. Hydrolates are formed when EOs are steam distilled. While EOs are highly concentrated, hydrolates are milder but still contain bioactive compounds exhibiting antibacterial properties. Despite differences in composition, both typically include terpenes, terpenoids, alcohols, or phenols. Different bacteria are found in honeycombs. Some can cause honeybee diseases such as foulbrood, septicemia, and gut infections, while others play beneficial roles, supporting gut health, enhancing nutrient absorption, and producing antimicrobial compounds that help protect against pathogens. The aim of this study was to evaluate the antimicrobial efficacy of various plant hydrolates and essential oils against five bacterial strains, focusing on identifying those that inhibit pathogens without harming beneficial bacteria.

In this study, honeycomb bacteria were identified using molecular methods such as PCR, restriction analysis, and sequencing. Bacterial cultures on solid media were exposed to EO vapors, and antibacterial activity was assessed by measuring growth inhibition zones. Hydrolates were mixed with bacterial cultures, and survived colony counts were used to evaluate their effects.

Four species were detected on honeycombs: *Bacillus licheniformis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Priestia megaterium*. For analysis of the antimicrobial efficacy of EOs the bacteria from *Bacillus*, *Pseudomonas*, *Streptococcus and Lactobacillus* genera were selected. Nine EOs were tested, including mint (*Mentha arvensis*), lemon balm (*Melissa officinalis*), lemon tea tree (*Leptospermum petersonii*), various thyme chemotypes (*Thymus vulgaris, T. zygis*), a thyme-mint blend, linden flower (*Tilia cordata*), and carrot seed (*Daucus carota*). Additionally, seven hydrolates were used: aniseed lofant (*Lophanthus anisatus*), lemongrass (*Cymbopogon citratus*), wormwood (*Artemisia absinthium*), medicinal chamomile (*Chamomilla recutita*), lavender (*Lavandula angustifolia, L. x angustifolia*), and calendula (*Calendula officinalis*). Evaporation tests showed that the thyme-mint blend and all thyme EOs had the strongest antimicrobial effects, while linden flower and carrot seed had no impact on any bacterial cultures. Lemon balm EO uniquely inhibited pathogenic bacteria while not affect *Lactobacillus* spp., whereas other EOs inhibit bacteria with different specificity. Among hydrolates, lavender had the strongest antibacterial properties, while medicinal chamomile was the weakest. However, no hydrolate selectively targeted pathogens while preserving *Lactobacillus.*

This study highlights the potential of essential oils and hydrolates as natural antimicrobial agents in beekeeping. Further research is needed to explore how essential oils and hydrolates interact with beeswax and how honeybees respond to their use in hives.

E2

EXPRESSING A *LYSINIBACILLUS* SP. URETHANASE FOR ENZYMATIC DEGRADATION OF POLYURETHANE

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Polyurethane (PU) is a polymer with a wide range of applications, including the manufacture of furniture, clothing, and various automotive and construction products. However, over time, PU products inevitably deteriorate, resulting in waste, that is either sent to landfills or incinerated. These conventional waste management methods are not sustainable due to the non-biodegradable nature of PU and the harmful effects of the resulting combustion by-products on human health and the environment. An alternative approach to address this issue is mechanical, chemical, or thermochemical recycling. However, the drawbacks of these methods are the inferior quality of recycled products, harsh reaction conditions, and toxic by-products [1].

Enzymatic PU treatment has the potential to address these issues. The majority of known PU-degrading enzymes are esterases, lipases, and cutinases, which usually target the ester bond, which is commonly found only in polyester PU. However, in other types of PU this bond might not be present. Consequently, the urethane bond, which connects the monomers of PU, emerges as a more viable target for enzymatic PU recycling. The cleavage of the urethane bond can be performed by urethanases (EC 3.5.1.75), enzymes that have predominantly been researched for their role in the degradation of ethyl carbamate, a compound present in fermented food and beverages such as soy sauce, bread, and wine [2]. The potential of harnessing these enzymes for PU degradation is almost unexplored, with only two studies published on this topic so far [3, 4].

The focus of this study was a urethanase (UTH) extracted from *Lysinibacillus* sp. (GenBank: QCW12854.1). The objective was to obtain an active and soluble UTH and apply it for PU degradation. We investigated the influence of fusion partners, *Escherichia coli* expression strains, and expression conditions for the synthesis of UTH. Subsequently, the obtained enzyme was purified, and optimal enzymatic activity and stability conditions were determined. Finally, urethanase was applied for the degradation of PU. The results will be discussed in more detail during the poster session.

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E3

RELEASE STUDIES FROM K2 TOXIN AND NISIN-LOADED FUCOIDAN PARTICLES

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Nisin – cationic antimicrobial peptide that has activity against gram-positive bacteria [1]. *Saccharomyces cerevisiae* K2 toxin disrupts the structural and functional integrity of plasma membrane of the yeasts [2]. Both of these compounds are of protein origin and because of that antimicrobial activity could be reduced by environmental stress, proteolysis, and undesirable interactions with food components [1]. To overcome this issue, proteins could be encapsulated and protected. By encapsulating nisin and K2 toxin together, we obtain particles with activity against gram-positive bacteria and yeasts. Encapsulation not only protects antimicrobial activity, but also provides controlled release which plays a key role in improving the shelf life of foods and ensuring food safety [1]. In this study, fucoidan was used for encapsulation.

Fucoidan is an anionic, sulfated polysaccharide. It is produced by brown seaweed and has antioxidant, antithrombotic, antitumor and anti-inflammatory activities [3]. These properties of fucoidan could also provide additional beneficial properties for food products.

The *in vitro* release from the particles was analyzed by using dialysis against 25 mM phosphate buffer solution at different pH values (2; 6 and 8) and at different NaCl concentrations (0; 0.1; 0.2 and 0.5M) at 37ºC. The diffusion of free nisin at 0.5M NaCl was used as a control. The results show that the release after 192 hours at pH 2 was about 55–85% and was similar at all NaCl concentrations. At pH 6 release was approximately 50–75% for the particles at all NaCl concentrations. In comparison, 100% of the nisin at 0.5M NaCl was released after 48 hours at both pH values. At pH 8, release after 196 hours was approximately 45–70% at all NaCl concentrations. 100% of free nisin was detected after 196 hours. This means, that at lower pH encapsulated compounds are released faster than at higher pH. Also, at higher pH values the influence of ionic strength is evident. At pH 6 with NaCl, the release is about 50–60% and in the absence of NaCl, release was about 70%. At pH 8 with 0.5M NaCl the release is only about 45% and without NaCl it is about 65%. Research shows that, hydrophobic forces increase at higher ionic strengths [4]. This could mean that at higher pH hydrophobic forces are not less important for encapsulation that electrostatic forces. This could be the reason why release is slower at higher NaCl concentrations than in the absent of NaCl.

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E4

NISIN-ULVAN PARTICLES: RELEASE STUDIES

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Nisin (molecular weight of 3.5 kDa) produced by *Lactococcus lactis* is a well-known food preservative due to its good antimicrobial activity and ability against gram-positive bacteria and other spoilage organisms. However, its stability can be limited by external factors such as temperature extremes, pH changes, and enzymes [1]. To mitigate these drawbacks, biopolymer encapsulation has been proposed as a means to modulate its stability and release. The release of antimicrobial material is a crucial factor in evaluating the quality of particles for food.

One of the biopolymers successfully used for nisin delivery is ulvan - a natural polysaccharide isolated from green algae (Ulva species) which is highly biocompatible and biodegradable. Well-encapsulated nisin-ulvan systems could be used for food preservation. At the same time, they could improve the stability and activity of antimicrobial agents [2].

The aim of this work is to prepare particles based on ulvan and evaluate how nisin release depends on pH, temperature and ionic strength. Nisin-loaded ulvan particles were prepared by the complexation method with a final ulvan concentration of 0.4 mg/mL and a nisin concentration of 0.2 mg/mL. Nisin release from nisin-loaded particles was evaluated at different temperatures (20 °C and 37 °C), pH (2, 6, 8), and ionic strengths (0, 0.1, 0.2, 0.5, and 1.5 M) using the dialysis method. Dialysis was performed with a 6-8 kDa membrane for 140 hours to collect released nisin. The amount of released nisin at a given time point was determined by the BCA (Bicinchoninic Acid) method.

The obtained results show that the release rate of nisin increase at higher temperatures. Regardless of temperature, the release is slowest at pH 8. In addition, the ionic strength was shown to be an additional variable in the release kinetics.

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E5

B-CAROTENE-2-HYDROXYPROPYL-B-CYCLODEXTRIN COMPLEXES COATED WITH PECTIN: ANTIOXIDANT ACTIVITY STUDIES

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Beta-carotene is a secondary metabolite synthesized primarily by plants. It is also known as a red-orange organic pigment that belongs to the non-oxidized compounds known as carotenoids. This pigment is an essential molecule for the human body and health, and for this reason, it is widely used in the food, pharmaceutical, and cosmetic industries [1]. However, as a pigment, β-carotene is unstable and sensitive to environmental changes such as pH, temperature, oxygen, light, and its hydrophobic nature, which makes it insoluble in water, complicates its bioavailability and application in various industries. To overcome these limitations, various delivery systems are being developed. To increase the stability and solubility of β-carotene, cyclodextrins - cyclic oligosaccharides with a non-polar inner cavity - can be used as carriers into which the hydrophobic β-carotene can be incorporated. In this work, modified 2-hydroxypropyl-β-cyclodextrin was used as an alternative because it has better water solubility than other cyclodextrins. In order to increase the stability of the complexes, the obtained inclusion complexes were additionally coated with biopolymers, i.e., different types of pectins [2], [3].

β-Carotene is known for its antioxidant activity, which quenches free radicals and singlet oxygen, thereby slowing or inhibiting oxidation processes caused by molecular oxygen or reactive oxygen species for the most important function - protection of lipids and proteins [4]. The aim of this work was to prepare three-component particles – β-carotene/2-hydroxypropyl-β-cyclodextrin complexes coated with pectin – and to determine their antioxidant activity using different methods under different conditions.

Water-soluble β-carotene three-component particles were formed by adding three types of pectin solutions (pectin acid, low- or high-esterification pectin) to the β-carotene/2-hydroxypropyl-β-cyclodextrin inclusion complexes at different pH values (4, 7). The obtained samples were stored under various conditions: at 4°C in the dark, at room temperature in the dark and light, as well as at 37°C, 60°C, and 95°C in the dark, and their antioxidant activity was measured at appropriate time intervals. Three electron transfer methods based on different principles were used to measure antioxidant activity: 2,2'-casino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP).

The changes in antioxidant activity observed by all three methods showed an uneven and insignificant change in activity during the first two weeks at temperatures up to 37 °C. After 3 weeks or more, a decrease in antioxidant activity was observed, which was greatest when the samples were kept in the light. At higher temperatures, the antioxidant activity of the samples decreased more significantly and was highest during the first 30 minutes at 95 °C. In this case, the additional coating with LMP and HMP pectin helped to maintain a slightly better antioxidant stability of the samples.

In conclusion, the additional coating of the inclusion complexes or the type and pH of pectin used for coating did not have a very significant effect, but in most cases, the uncoated β-carotene-2-hydroxypropyl-β-cyclodextrin complex showed slightly lower antioxidant activity values than the additionally coated complexes.

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E6

INDUCTION OF APOPTOSIS AND PYROPTOSIS IN MACROPHAGES BY BACTERIAL POLYSACCHARIDE CAPSULES DURING INFECTION

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Gram-negative opportunistic bacteria *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* are among the major causes of nosocomial infections. Their ability to infect the host is linked to multiple virulence factors, such as polysaccharide capsules. Bacterial polysaccharides are known to mask highly immunogenic antigens and interfere with the antibacterial response by host immunity. When the antibacterial response is activated, macrophages are one of the first immune cells to be involved. Besides their known role in phagocytosis, macrophages can undergo inflammatory or non-inflammatory cell death in response to immunogens or infection-caused stress. Apoptosis, a non-inflammatory form of cell death, benefits bacteria through the absence of direct immune activation. In contrast, pro-inflammatory immune cell death forms, such as pyroptosis, effectively reduce the spread of infection. Despite a known role in immune evasion, the impact of *A. baumannii* and *S. maltophilia* polysaccharide capsules on macrophage cell death has not been studied. This study aimed to test how capsular polysaccharides of *A. baumannii* and *S. maltophilia* affect induction of apoptosis and pyroptosis in macrophages during infection.

We performed in vitro infections using J774 macrophages. We used clinical strains of *A. baumannii* (AB52) and *S. maltophilia* (SM21), as well as their non-capsulated mutants, AB52Δ*galU* and SM21Δkaps. The levels of apoptosis markers (pro-Casp3 and Casp3) and pyroptosis markers (pro-IL-1β and IL-1β) were measured by Western blot analysis. We analyzed the response in macrophages induced by capsulated and non-capsulated bacteria.

In infected macrophages, the relative levels of Casp3 were higher compared to pro-Casp3, while the opposite result was observed in non-infected macrophages. Infection with the capsulated AB52 isolate led to significantly higher Casp3 levels in macrophages compared to the AB52Δ*galU* mutant. However, we did not observe significant differences in macrophages infected with *S. maltophilia*. Further results demonstrated no significant differences in the relative amounts of pro-IL-1β and IL-1β comparing macrophages infected with capsulated and non-capsulated bacteria.

Our findings indicate that *A. baumannii* capsular polysaccharides activate caspase-3 in J774 macrophages. In contrast, the *S. maltophilia* capsule does not induce the production and activation of caspase-3. Furthermore, *A. baumannii* and *S. maltophilia* polysaccharide capsules do not significantly affect the relative levels of pro-IL-1β in J774 macrophages.

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E7

MICROGRAVITY AND TELOMERE LENGTH MODULATE YEAST RESISTANCE TO GLYCOLIC ACID: IMPLICATIONS FOR SPACE BIOTECHNOLOGY AND COSMETIC APPLICATIONS

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Humans living under Earth's gravitational conditions experience significant physiological changes during space travel due to microgravity—a state in which the effects of gravity are greatly reduced. Microgravity has been shown to cause early osteoporosis, skeletal muscle atrophy, and cardiovascular dysfunction [1]. One of the most affected systems is the immune system: microgravity suppresses lymphocyte proliferation, activation, motility, and cytotoxic function, making astronauts more susceptible to infections [2][3].

Additionally, spaceflight induces changes in telomere length: in microgravity, telomeres tend to lengthen, but upon return to normal gravity, they become even shorter than before the space mission. This phenomenon may contribute to cellular aging and genomic instability [4]. Telomeres are tandem DNA sequence repeats located at the ends of chromosomes, protecting genetic integrity from degradation during replication. Telomere length abnormalities are associated with age-related diseases and premature aging syndromes [5][6].

In this study, *Saccharomyces cerevisiae* yeast, one of the most commonly used model eukaryotic systems for investigating cellular aging mechanisms, was used. The aim was to determine how microgravity affects the resistance of yeast strains with different telomere lengths to glycolic acid, a common cosmetic ingredient. Minimum inhibitory concentrations (MICs) were determined using a drop assay, followed by growth analysis under both gravity conditions.

After performing the droplet test, it was observed that induced microgravity led to an increased sensitivity to cosmetic acid by 1.4 and 1.2 times in yeast strains with normal and short telomeres, respectively, while it had no significant effect on the strain with long telomeres. Additionally, differences in resistance to glycolic acid were observed among the strains—under normal gravity conditions, the strain with long telomeres was the most sensitive, whereas the strain with normal telomeres was the most resistant.

Analysis of the growth curves showed that exposure to glycolic acid resulted in a lower final optical density (OD_{600}) in all tested samples compared to the control group, regardless of telomere length or gravity conditions. An extension of the lag phase was also observed: for strains with long and short telomeres, the lag phase increased by 1.6 times, while for the strain with short telomeres, it increased by 1.5 times. This indicates that glycolic acid leads to extended adaptation in growth medium. When 0,5 MIC and 0,25 MIC of glycolic acid were added to the medium, a statistically significant effect of microgravity was observed only in the strain with short telomeres. In this strain, microgravity conditions led to a higher OD_{600} than normal gravity, suggesting better survival or faster recovery from glycolic acid stress.

This study suggests that microgravity influences yeast susceptibility to glycolic acid, particularly in strains with normal and short telomeres. The observed reduction in final biomass accumulation indicates that glycolic acid exposure may disrupt cellular growth dynamics. Notably, the enhanced survival of the short telomere strain under microgravity suggests a potential adaptation or stress response mechanism. Further research is needed to investigate the molecular pathways involved and determine whether similar patterns occur in other eukaryotic models during prolonged microgravity exposure.

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E8

RECOMBINANT MICROBIAL CARBONIC ANHYDRASE PROTEIN PRODUCTION

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Carbon dioxide (CO_2) levels in our atmosphere are rising, mainly due to human activities such as burning fossil fuels like coal, oil, and natural gas. As the most common greenhouse gas, CO_2 plays a significant role in global climate change. Interestingly, while carbon dioxide is often seen as merely a waste product, it has the potential to be transformed into valuable compounds. However, finding effective and sustainable ways to convert CO_2 presents a significant challenge.

Carbonic anhydrases (CAs) are enzymes that catalyse the reversible hydration of carbon dioxide and ($CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$). These enzymes are crucial for many processes, such as regulating pH and transporting CO_2 and bicarbonate in the body. So far, scientists have identified eight different classes of carbonic anhydrases, labelled with Greek letters: α, β, γ, δ, ζ, η, θ, and ι. Among these, the α-class CAs in mammals have been the focus of extensive research due to their association with various diseases like glaucoma and cancer [1].

Recently, however, there has been growing interest in CAs from other classes for their possible uses in industry. β-Carbonic anhydrases (β-CAs) have gained attention because they offer advantages over α-CAs, such as higher stability at elevated temperatures and resistance to harsh chemical environments. This stability is crucial for industrial processes that often experience extreme temperatures and varying pH levels. Additionally, β-CAs are found in many microorganisms, providing a broader range of options for enzyme discovery and use [2-4].

In our study, we aim to produce recombinant β-CAs from bacteria and fungi, and we plan to develop a method for employing these enzymes to synthesise cyclic carbonates (Fig. 1). We will present more details about our research during the poster session.

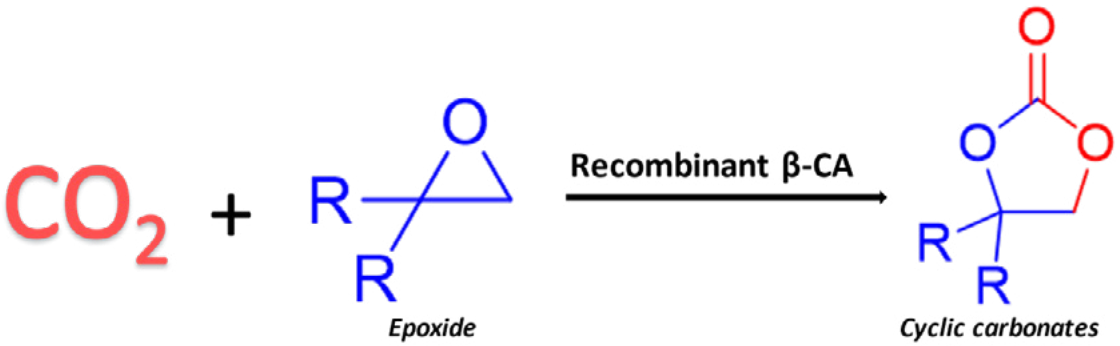

Figure 1. Schematic representation of enzymatic synthesis of cyclic carbonates.

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E9

Search, Purification and Analysis of Pyrethroids-degrading and Other Biotechnologically Applicable Lipolytic Enzymes

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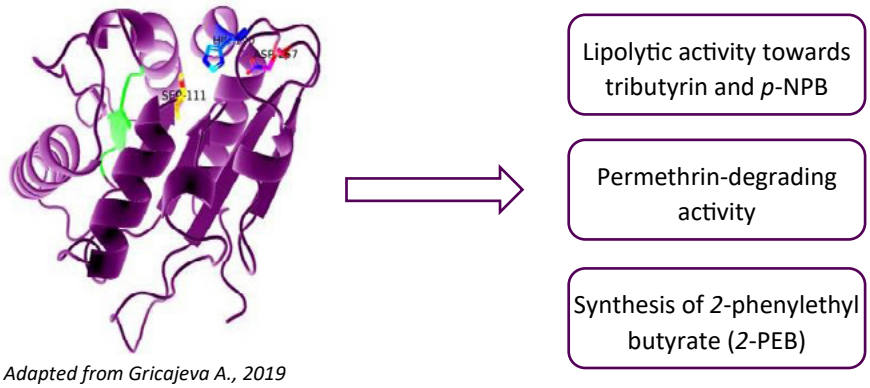
Pyrethroids are synthetic pesticides widely used for pest control in agricultural and public settings, but their high insecticidal potency, persistence, and slow degradation can harm non-target organisms and contaminate natural habitats [1]. Bacteria produce different lipolytic enzymes - carboxylesterases and true lipases which are multifunctional biological catalysts and have been the subject of research for a long time. Although lipolytic enzymes are mainly known as enzymes catalyzing the hydrolysis (and other transformations) of ester-bonded lipid substrates, it has been discovered more recently that they are also capable of breaking down the ester linkages of pyrethroids and can thus serve as an efficient and eco-friendly method for their removal [1,2].

In this study, plant growing substrate samples from two Lithuanian farmlands were used to isolate pyrethroid-degrading bacteria through enrichment culture with pyrethroid (permethrin) as the sole carbon source. Isolated cultures were selected and identified employing 16S rDNA analysis. After identification of target bacteria, 7 bacterial cultures belonging to the *Pseudomonas* spp. (II1; II3; II10; IIB; IIC), *Rhodanobacter* sp. (II14), *Priestia* sp. (III3) genus, were selected for further study based on their lipolytic and pyrethroid-degrading activity (Fig. 1). The lipolytic enzymes located in extracellular media were salted out using ammonium sulphate and gel filtrated. Intracellular proteins were derived from bacteria via sonication. Lipolytic activity of both partially purified protein fractions was determined using *p*-NPB assay.

Partially purified proteins from the extracellular medium of *Pseudomonas* spp. (II1; II3; IIB) and *Rhodanobacter* sp. (II14), as well as intracellular proteins of *Pseudomonas* sp. (II10) and *Rhodanobacter* sp. (II14), formed hydrolysis zones in permethrin (tested by agar diffusion method). To determine the size of target proteins a zymography was used. Zymography analysis showed that all tested protein fractions formed tributyrin hydrolysis zones, *Pseudomonas* sp. (IIB) total cell protein (TCP) fraction was active toward both tributyrin and permethrin in the zymography gels.

Enzymes, partially purified from the extracellular and cell's intracellular fractions, were used for the synthesis of *2*-phenylethyl butyrate (*2*-PEB). This method was chosen to evaluate the ability of the obtained protein solutions to perform esterification reactions, resulting in the production of *2*-PEB, an ester widely used in the fragrance and flavor industry (Fig. 1).

Figure 1. A simplistic protein model with functional annotations.



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E10

INVESTIGATION OF *PSEUDOMONAS AERUGINOSA* LIPOXYGENASE EXPRESSION IN *PICHIA PASTORIS*

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In the industrial sector, there is an increasing reliance on biocatalysis to enhance sustainability and efficiency. The use of enzymes in biocatalysis provides a greener alternative to traditional chemical methods, facilitating highly selective reactions under mild conditions. A significant area of potential for this approach is the transformation of fatty acids into valuable compounds, including biofuels, polyols and flavour compounds. In this context, lipoxygenases (LOX) have been identified as vital function catalysts in the peroxidation of polyunsaturated fatty acids. While the majority of research has focused on human and plant LOX, the potential of bacterial lipoxygenases, which has only recently been discovered, remains largely unexplored [1]. Among these, *Pseudomonas aeruginosa* lipoxygenase (PaLOX) is a notable example of resilient enzyme, maintaining activity at elevated temperatures and efficiently peroxidizing a broad range of substrates, including linoleic, linolenic, arachidonic, and oleic acids [2].

The aim of this study is to develop an expression system for extracellular PaLOX secretion in the *Pichia pastoris. P. pastoris* is a highly advantageous expression system due to its ability to achieve high cell densities and protein yields, as well as low concentrations of secreted endogenous proteins, which simplifies protein purification [3]. The *palox* gene was cloned into the shuttle vector pPic9K, which ensures integration of the gene into the yeast genome, determines controlled protein expression by methanol-inducible AOX1 promoter and contains α-factor secretion signal for protein secretion. The transformation of these recombinant plasmids into *P. pastoris* GS115 was followed by a screening process and subsequent cultivation in BMMY medium to induce expression. The verification of lipoxygenase expression was conducted through the use of SDS-PAGE and lipoxygenase activity assay techniques. A comprehensive presentation of results will be showcased at the poster session.

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E11

SCREENING AND CHARACTERIZATION OF PSYCHROPHILIC PROTEOLYTIC BACTERIA ISOLATED FROM KRUBERA-VORONJA CAVE

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The interest in enzymes from psychrophilic bacteria has increased because of their wide application in biotechnology and better activity at lower temperatures compared to mesophilic enzymes. One of them are proteolytic enzymes, which are used in different sectors such as food, textile and detergent industries [1].

The aim of this study was to select and characterize bacteria with proteolytic properties from Krubera-Voronja cave, which is located in West Caucasus. 100 isolates were selected from different cave sites and all samples were tested on their ability to hydrolyze gelatin and casein by detecting hydrolysis zones under psychrophilic conditions. 10 isolates with the best proteolytic ability on both media were selected and identified using 16S rRNA phylogenetic analysis. One isolate with the lowest percentage similarity of 16S rRNA gene was selected for full genome sequencing.

35 out of 100 samples showed proteolytic activity on selective media. Genotyping was performed using BOX- and GTG-PCR methods on 10 selected isolates. Results showed that all isolates are different strains and were later identified using 16S rRNA gene. It was identified that strains belonged to *Bacillus* (VR14 TSA 30-6, VR10 ACT 30-1), *Pseudomonas* (VR12 SCNA 30-4, VR4` HT 30-3), *Stenotrophomonas* (VR14 ISP 4-1), *Pseudarthrobacter* (VR1 H2O-30, VR12 SCNA 30-2, VR6` ACT 30-2), *Glutamicibacter* (VR10 SCNA 30-6), *Micrococcus* (VR7 ACT 30-8) and *Arthrobacter* (VR12 SCNA 30-2) genera. VR4` HT 30-3 isolate had the lowest (93.09%) 16S rRNA gene similarity using NCBI database and it was selected for the whole genome sequencing using Nanopore and Illumina methods. Isolate was identified as *Pseudomonas mandelii* after using whole genome and ANIb, dDDH, TCS bioiformatic tools. A potential secreted gelatin hydrolyzing enzyme was identified as M48 family metalloprotase.

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E12

HIGH-VALUE BIOPRODUCTS FROM THE BIOMASS OF CYANOBACTERIA AND MICROALGAE

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One of the recently relevant areas of scientific research are high value-added products obtained from cyanobacteria and microalgae biomass. These bioproducts can be used for human food supplements, functional feed for animals, cosmetic and pharmaceutical industries. The aim of the work was to investigate valuable bioproducts in the biomass of cyanobacteria and algae biomass. Pigments, proteins, lipids and carbohydrates were studied in the wild biomass dominated by *Aphanizomenon flos-aquae* or *Microcystis* spp. cyanobacteria, and in the biomass of cyanobacteria *Aphanizomenon flos-aquae*, *Nodularia spumigena*, *Sphaerospermopsis aphanizomenoides*, *Arthrospira platensis* and green algae *Scenedesmus acutus*, *Desmodesmus quadricauda*, *Coelastrum microporum* isolates. The content of phycobiliproteins, chlorophyll-a and carotenoids was determined according to the spectrophotometric method described in [1, 2] by measuring the optical density of the extracts at different wavelengths. The modified Bradford method [3] with the reagent Commassie G 250 [4] was used to quantify the proteins. The lipids were determined gravimetrically after extraction with a mixture of methanol, chloroform and a salt solution in a ratio of 2:1:0.8 [5]. The carbohydrates were determined according to the established methodology [6] using the MBTH reagent. To ensure the reliability of the results, three replicates were performed for each sample. The protein content in the biomass of cyanobacteria and green algae was the highest among all bioproducts studied and comprised 13.0–58.6% of the freeze-dried biomass. The amount of phycobiliproteins was significantly higher compared to other investigated pigments (chlorophyll-a, carotenoids). The highest total amount of phycocyanin, allophycocyanin and phycoerythrin (223.75 mg/g ± 33.31 mg/g) was found in the biomass of *Aphanizomenon flos-aquae* isolate. The content of lipids and carbohydrates was 1.4-2 times higher in the biomass of green algae isolates compared to the biomass of cyanobacteria. The study revealed that the wild biomass of cyanobacteria is a valuable resource rich in phycobiliproteins and proteins, but its value depends on the dominant species. *Aphanizomenon flos-aquae* accumulated higher amounts of valuable bioproducts compared to biomass dominated by cyanobacteria of the genus *Microcystis*.

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E13

ELECTROGENICITY OF MEDICALLY RELEVANT BACTERIAL BIOFILM

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Biofilms are involved in a wide variety of microbial infections in the body. The National Institutes of Health (NIH) revealed that biofilms contribute to 60-80% of microbial infections, leading to more extended hospital stays, higher costs, and increased morbidity [1]. Electrochemical techniques can provide quick results, often within minutes to hours, compared to the days required by traditional culture methods [2]. This rapid detection is crucial for managing infections caused by biofilm-forming pathogens, enabling healthcare providers to initiate appropriate treatments more swiftly [3]. The aim of the study is to determine electrogenicity of Gram-positive *S. heamolyticus*, *E. faecalis* and Gram-negative *P. aeruginosa*, *P. mirabilis* bacteria's biofilms.

Biofilm growth was monitored using open circuit potential (OCP), chronoamperometry and optical density (OD) measurements. Potentiometric assessments involved a two-electrode setup with a carbon cloth (CC), and an Ag/AgCl 3M KCl (+205 mV vs SHE) served as a working and reference electrode (Fig. 1). This setup allows continuous tracking of bioanode potential and reflects the main stages of biofilm development. Once the biofilm is formed, the OCP changes from ~100-50 mV (CC in LB media) to –200 - –300 mV (CC in a bacterial solution containing 10^6 CFU mL^{-1} cells) within 5 hours (Fig. 2), suggesting that all tested bacteria can establish a bioelectrochemical coupling with the electrodes. We assign the development of negative potentials mainly to the mediated electron transfer (MET) in microbial biofilms, which connects the metabolic activity of bacteria to the conducting material. Amperometric investigations further verified the ability of electrogenic bacteria to transfer electrons to the surfaces of electrically conducting material. Various extracellular electron transfer (EET) mechanisms, including DET and MET, are involved in this process. In line with OCP and OD measurements, the current-time dependence reflects the EET at different biofilm formation or bacterial growth stages, including the lag, log, and stationary phases, respectively.

Figure 1. Open circuit potential measurement scheme

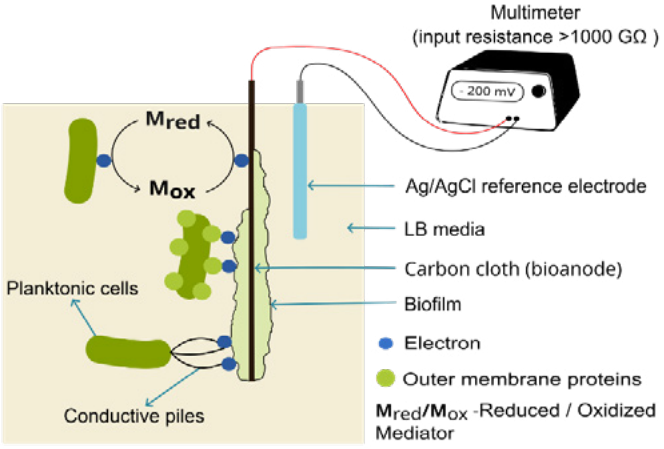
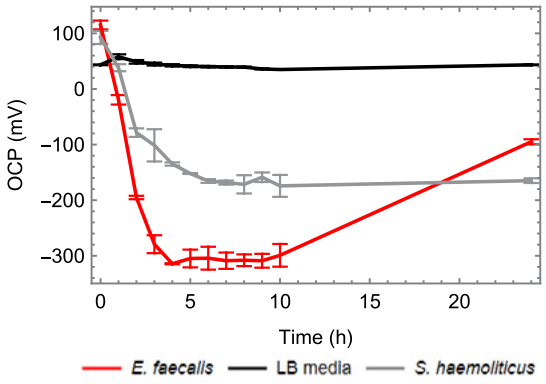


Figure 2. Example of OCP changes with bacteria's



This study reveals that *E. faecalis* exhibited the most negative OCP (-303 mV) and highest current density (435 nA/cm^2), while *S. haemolyticus* showed minimal electroactivity with an OCP of -165 mV and current density of 120 nA/cm^2. Gram-negative bacteria displayed stable OCP and sustained currents, indicating consistent bioelectrochemical activity. These findings support the use of electrochemical methods for the rapid detection and characterization of biofilm-associated infections.

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E14

SYNTHESIS OF NOROVIRUS-LIKE PARTICLES IN YEAST *S. CEREVISIAE*, *K. LACTIS* AND *K. MARXIANUS*

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Virus-like particles (VLPs) are multimeric nanostructures that self-assemble and resemble the morphology of native viruses. They mimic the form and size of the virus but lack its genomic material, meaning they cannot replicate and cause infections. VLPs can serve as delivery systems for various antigens, which may be presented on their surface or encapsulated within their structure. They can potentially be used in allergen-specific immunotherapy, where they are being studied as carriers for allergenic molecules. VLPs provide a promising platform for facilitating the uptake, recognition, and processing of antigens by the immune system. They can diffuse through the 200 nm pores of lymphatic vessels and due to their small size, VLPs can drain into the lymphatic system, allowing them to efficiently travel from the injection site to the draining lymph nodes. Moreover, virus-like particles are easily taken up and actively transported by antigen-presenting cells (APCs) [1]. The uptake of antigens by APCs strongly depends on features like size, surface structure, form, hydrophobicity, hydrophilicity, and charge distribution. These features contribute to optimal interaction with the MHC-II receptor. The properties of VLPs are particularly favorable for promoting uptake by APCs [2].

VLPs can be produced using various expression systems, including bacteria, yeast, plants, and insect or mammalian cells. Yeast has proven to be an effective platform for VLP production, due to its ability to efficiently synthesize heterologous proteins and its cost-effective, scalable, and simple cultivation process [3]. *Saccharomyces cerevisiae* and *Pichia pastoris* are the most commonly used yeasts for producing VLPs. Still, apart from them, other yeast species, including *Kluyveromyces lactis* and *Kluyveromyces marxianus* have also been successfully used for VLP expression and purification [4]. All of these yeast species are classified as Generally Recognized As Safe (GRAS) and have the Qualified Presumption of Safety (QPS) status, making them suitable for therapeutic product production [5, 6].

In the present study, Norovirus-like particles (NoV VLPs) were studied. NoV is a single-stranded RNA virus with a genome of approximately 7,500 nucleotides, consisting of three open reading frames (ORFs). ORF2 encodes the major capsid protein VP1 (~58 kDa), which assembles into VLPs measuring either 38-40 nm or 23 nm in diameter. Previous analysis of NoV VLPs revealed that their structural properties are potentially suitable for presenting specific antigens in a nanoparticle structure [7]. During this study, NoV VLPs were produced in three different yeast expression systems – *S. cerevisiae*, *K. lactis*, and *K. marxianus*. The VLPs were partially purified by ultracentrifugation in a CsCl gradient and visualized using Transmission Electron Microscopy (TEM). Yields of recombinant VP1 protein produced in different yeast species were determined and the most suitable yeast expression system was selected for future experiments. The research is funded by the Research Council of Lithuania (LMTLT), agreement No S-MIP-24-41.

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E15

PREDICTION OF COVID-19 DISEASE COURSE BASED ON BLOOD BIOMARKERS

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Freely circulating DNA (cell-free DNA (cfDNA)) are DNA fragments released to blood after the cells death or from active secretion. During COVID-19 infection, due to mass cell death from various organs, an increase in cfDNA is observed in blood plasma samples [1]. For this reason, cfDNA as a biomarker test has the potential to be applied in clinical practices to better predict the course, outcome and to plan treatment strategy of COVID-19 disease.

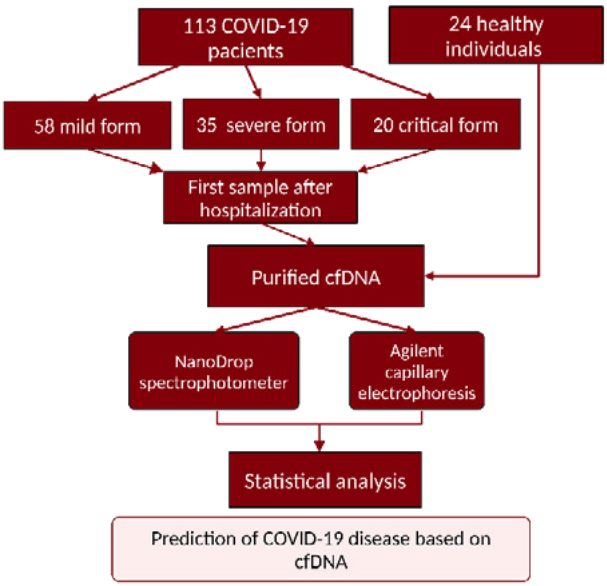
The aim of this study is to study the application of cfDNA in clinical practice to predict the course and outcome of the COVID-19 disease. Firstly, we assessed the possibility of purifying cfDNA from blood serum samples. Then we measured cfDNA concentrations in COVID-19, non-sick groups and the different COVID-19 subgroups of patients. Finally, we analyzed the prognostic value of cfDNA for the course and outcome of the COVID-19 disease.

First of all, permission was obtained from the Vilnius Regional Ethics Committee for Biomedical Research to conduct this study (permit number: 2023/6-1521-982). A total of 137 blood serum samples were obtained from the Vilnius Santaros Klinikos Biobank (Fig. 1). cfDNA from serum samples was extracted using QIAamp Circulating Nucleic Acid Kit. Afterwards NanoDrop and Agilent capillary electrophoresis was used to measure extracted cfDNA concentrations. NanoDrop was used because it is readily available and does not require additional reagents and Agilent capillary electrophoresis because it also shows the size distribution of extracted cfDNA samples.

A primary data analysis showed that there was a statistically significant difference in cfDNA concentrations between healthy group and COVID-19 group samples ($p < 0.001$). Also, a statistically significant difference was calculated between different subgroups of the COVID-19 disease (mild form of the disease, severe form of the disease and critical form of the disease). Afterwards the prognostic value of cfDNA for COVID-19 disease stages has been estimated. It has been established that based on the concentration of cfDNA, it is possible to determine with sufficient accuracy the critical form of the disease (critical concentrations of cfDNA: NanoDrop – 35.75 ng/µL and Agilent – 5.82 ng/µL)., moderately well, severe form of the disease (severe cfDNA concentrations: NanoDrop – 17.75, Agilent – 2.67 ng/µL), but the assessment of cfDNA concentrations is not suitable for predicting a mild form of the disease.

From this study we can see that cfDNA can be extracted from blood serum samples, and that it can be used to predict critical and severe forms of COVID-19 disease. This study will be expanded by analyzing other nucleic acids found in serum samples.

Figure 1. The course and methods of the study.



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E16

DISASSEMBLY OF TDV1 VIRUS-LIKE PARTICLES

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Virus-like particles (VLPs) are nanostructures composed of structural proteins from viruses. The key difference between VLPs and viruses is the absence of genetic material in VLPs, despite assembling from the very same structural proteins. Due to their ability to invoke a long-lasting immune response several VLP-based antiviral vaccines are commercially available against agents such as human papillomavirus and hepatitis B virus (Kheirvari et al., 2023). VLPs have an empty inner cavity that can be used to encapsulate nucleic acids, proteins or other small chemical compounds. Recently VLPs have received attention for their successful use in gene therapy, imaging and as drug nanocarriers (Nooraei et al., 2021).

The TdV1 is a native virus of yeast *Torulaspora delbrueckii* Td70. TdV1 virus belongs to the *Cryspovirus* genus of the family *Partitiviridae*, previously these viruses have only been found in protozoa hosts. The double stranded RNA (dsRNA) genome of TdV1 encodes only two proteins: a coat protein (CP) and an RNA-dependent RNA polymerase (RdRp). The VLPs that self-assemble from the TdV1 virus are composed of 120 copies of the CP, they are spherical and 30 nm in size (Bartininkaitė, 2021). Considering that TdV1 VLPs are made of protein, they are biodegradable and their small size and spherical shape allows for easier clearance from the body compared to rod shaped nanoparticles (Zhao et al., 2017).

The aim of this study was to synthesize TdV1 VLPs and determine conditions for the disassembly of the purified particles. To perform this task, pFX7 plasmid encoding TdV1 CP sequence was used to synthesize TdV1 VLPs in *Saccharomyces cerevisiae* strain BY4741-S2. Capsid protein expression was induced for 48 hours with 3 % galactose. The assembled VLPs were extracted after yeast cell lysis and then purified using ultracentrifugation through sucrose cushion and cesium chloride solution. After purification, TdV1 CP were obtained with the yield of 0.28 mg of protein per gram of biomass. Finally, the purified VLPs were incubated under sixteen different conditions consisting of different concentrations of salts, protein denaturing and reducing agents. VLPs were incubated overnight at a final concentration of 0.5 mg/ml to evaluate disassembly conditions. The samples were observed using DLS to determine the average size of VLPs. Subsequently, samples with a significant variation from the average size of assembled VLPs were additionally analyzed with TEM microscopy to confirm disassembly.

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E17

THE EFFECT OF SILVER NANOPARTICLES ON VARIOUS MICROORGANISMS

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The objective of this study was to evaluate the toxicological effects of silver nanoparticles (Ag NPs) on various microorganisms under in vitro conditions. Silver nanoparticles were synthesized via chemical methods, specifically the Lee-Meisel and Creighton methods [1].

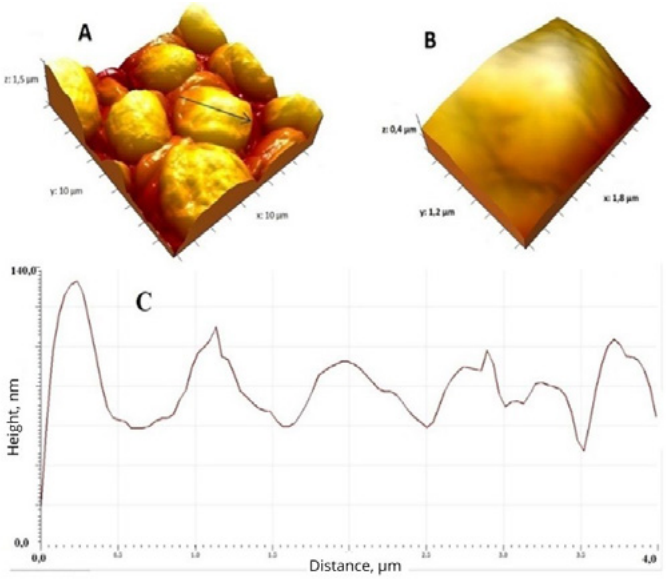
The structural properties of the synthesized nanoparticles were characterized using UV-Vis spectroscopy, atomic force microscopy (AFM), and transmission electron microscopy (TEM). The Ag NPs synthesized using the Lee-Meisel method exhibited a spherical morphology with a size distribution ranging from 4 to 80 nm and a surface plasmon resonance (SPR) peak at 428 nm. In contrast, the Creighton method produced spherical nanoparticles with a size range of 5 to 160 nm and an SPR peak at 394 nm. For comparison, commercially available Ag NPs displayed a spherical shape, with a size range of 10 to 150 nm and an SPR peak at 411 nm [2].

To assess the biological effects of Ag NPs, yeast cells were cultured in liquid YEPD medium in the presence of silver nanoparticles and $AgNO_3$, followed by AFM analysis. The AFM data indicated that yeast cells exposed to Lee-Meisel-synthesized Ag NPs exhibited the most pronounced structural alterations, particularly affecting cell wall elasticity.

Furthermore, yeast cells treated with silver nanoparticles were plated onto agarized medium to evaluate the impact of Ag NPs on yeast colony formation. The results demonstrated that laboratory-synthesized Ag NPs exerted the strongest inhibitory effect on yeast growth.

To investigate the viability of bacterial cells following Ag NP exposure, the agar diffusion method was used.

Figure 1. A Topographic study of unaffected S. cerevisiae yeast cells by AFM:
A – 3D image of yeast cells, 10μm×10μm scanning area;
B – 3D image of yeast cells, 1.8μm×1.2μm scanning area;
C – Roughness profile of the cross-section at the location marked with an arrow.



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E18

GENETIC TRAITS OF *ENTEROCOCCUS* SP.: RESISTANCE, VIRULENCE, PHENOTYPIC ACITVITY AND BIOFILM FORMATION IN CLINICAL ISOLATES

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Enterococcus sp. are a facultative anaerobic gram-positive opportunistic pathogens responsible for healthcare-associated infections. Clinical facilities provide a favorable environment for bacteria to express antimicrobial substances, develop resistance to antibiotics, such as vancomycin, and form biofilms on various surfaces and instruments. Addressing these issues is critical in the development of novel strategies to mitigate the challenges associated with opportunistic pathogens. [1] [2] Hence, this study aimed to characterize vancomycin-resistant *Enterococcus* sp. and identify the essential genes associated with vancomycin antibiotic resistance, virulence and biofilm production, as well as to characterize phenotypic attributes and biofilm formation capabilities.

The study involved the collection of 61 isolates from Lithuanian hospitals between January and November 2024. The multiplex PCR genetic characterization method was used to confirm enterococci species and detect vancomycin resistance (*vanA*, *vanB*, *vanC1/2*, *vanD*, *vanE*, and *vanG*) and virulence genes (*esp, gelE, agg, asa1, cylA, hyl, efaA, ace, acm* and *scm*). Phenotypical characterization of *Enterococcus* sp. isolates was performed using Chrome Azurol S assay, Gelatinase activity assay, Protease activity assay, Lipase activity assay and Bacteriocin activity assay. Biofilm formation by enterococci was determined using the microtiter plate method. Six distinct vancomycin resistance genes were identified using multiplex PCR genetic characterization: *vanA*, *vanB*, *vanC1/2*, *vanD*, *vanE*, and *vanG*. Among these, *vanA* gene, which is responsible for the resistance phenotype due to its alteration of the peptidoglycan precursor, was the most dominant gene and was identified in 26 isolates (42.63%). Moreover, all tested virulence genes were identified, including *acm, scm, ace, efaA* adhesins coding genes, *gelE* and *hyl* enzyme genes, *esp* surface protein gene, *cylA* toxin gene and *asa1*, *agg* aggregation substance genes. The most common virulence gene was *acm,* which was found in 60 isolates (98.4%). 61 isolates (100%), using Chrome Azurol S assay and Lipase activity assay methods, demonstrated varying halo zones, indicating their ability to produce siderophores and lipases. Gelatinase activity assay identified in 10 (16.4%) isolates, which produced gelatinase. The protease activity assay identified 30 (49.2%) isolates, that were protease positive. Bacteriocin activity assays against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were negative for all 61 *Enterococcus* sp. isolates. The microtiter plate method revealed that 44 isolates (72.13%) could produce biofilm, where 37 (60.66%) isolates had weak biofilm formation capabilities, moderate biofilm formation type was identified in six (9.84%) isolates and one (1.64%) isolate expressed strong biofilm formation, whereas 17 isolates did not form any biofilm.

The research emphasized the significant role of *Enterococcus* sp. as opportunistic pathogens in healthcare-associated infections, examining their genetic and functional characteristics that contribute to antibiotic resistance, virulence, biofilm formation, and overall pathogenesis.

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E19

SEARCH AND ANALYSIS OF PYRETHROID-DEGRADING MICROBIAL LIPOLYTIC ENZYMES

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Pyrethroids are ester-containing synthetic compounds derived from natural pyrethrins. They are widely used as insecticides in agriculture, animal husbandry, and homes, but their persistence poses risks to non-target organisms and natural habitats [1;2]. Microbial lipolytic enzymes (e.g., carboxylesterases EC 3.1.1.1, lipases EC 3.1.1.3) offer potential for pyrethroid degradation due to their broad substrate specificity, thermo-, pH and other attractive characteristics can be a new and effective means of removing pyrethroids from the environment. Bacteria such as *Bacillus* sp., *Pseudomonas* sp., *Serratia* sp., *Micrococcus* sp., *Sphingomonas* sp., and *Staphylococcus* sp. produce lipolytic enzymes capable of breaking ester bonds in compounds like cypermethrin, permethrin, and deltamethrin [1].

In this study, plant growing substrate samples from three Lithuanian farmlands were investigated using conventional methods to identify pyrethroid-degrading bacteria. To isolate bacteria capable of degrading pyrethroids, an enrichment culture was used with permethrin as the sole carbon source. The selected bacterial isolates were identified through 16S rRNA gene sequencing and phylogenetic analysis. After identifying the target bacteria, 12 cultures from the genera *Pseudomonas, Staphylococcus, Micrococcus, Bacillus, Peribacillus* and *Paenibacillus* sp. were assessed based on the hydrolysis zones around their colonies in solid minimal salts media supplemented with permethrin. Further, target proteins were purified from both intracellular and extracellular fractions of the most active bacterial strains and analyzed using zymographic analysis. For the latter, tributyrin and permethrin were used as substrates. Lipolytic activity of partially purified enzymes was also tested using para-nitrophenyl butyrate (*p*-NPB) substrate.

In four bacterial strains, which according to the phylogenetic analysis most likely belong to *Pseudomonas knackmussii, Staphylococcus warneri*, *Micrococcus aloeverae* and *Solibacillus* sp., 55-70 kDa lipolytic enzymes active towards tributyrin and permethrin in the zymogram gels, were determined. In the partially purified fractions of other studied bacterial strains enzymes (20-100 kDa) active towards only the tributyrin were determined.

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E20

MOLECULAR EPIDEMIOLOGY STUDIES OF CLINICAL *KLEBSIELLA PNEUMONIAE* ISOLATES

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Klebsiella pneumoniae functions as a significant gram-negative pathogen that exploits opportunistic conditions to cause diverse manifestations, including infections of the urinary system, hepatic tissue and lungs. Its capacity to evade the host immune response and develop resistance mechanisms has facilitated its persistence in both healthcare and community settings. Over time, the extensive use of antibiotics has driven the adaptation of *K. pneumoniae*, leading to the emergence of multidrug-resistant strains with enhanced virulence and pathogenicity, making infections more difficult to treat and control. In the antibiotic era that followed, it emerged as a significant cause of hospital-acquired infections and a risk factor for severe community-acquired infections [1].

The aim of this study was to perform molecular epidemiological studies using 20 clinical isolates of *K. pneumoniae*. Polymerase chain reaction (PCR) was performed to determine the antibiotic resistance of each strain. Antibiotic-resistant carbapenemases were detected using PCR with primers targeting OXA-48, KPC, NDM, VIM, and IMP carbapenemases, as previously described [2], [3], [4]. The study also examined the phylogenetic relationships of selected *K. pneumoniae* clinical isolates (2, 5, 7, 8, 10, 15, and 20) by analyzing the *rpoB* gene, which was amplified by PCR. The sequences of the selected clinical isolates were analyzed using Sanger sequencing. This analysis also included reference sequences of *rpoB* gene from several closely related species. The resulting PCR products were visualized by electrophoresis on 1% agarose gel. This study also involved genome analysis of a selected isolate using different typing schemes, such as Multilocus sequence typing (MLST), Core genome multilocus sequence typing (cgMLST), and virulence typing.

The analysis of carbapenemase production demonstrated the absence of these enzymes in the evaluated *K. pneumoniae* clinical isolates. Species identification was confirmed by phylogenetic assessment of the *rpoB* gene sequence. Based on its molecular characteristics, isolate 203 was selected for a comprehensive genomic analysis. MLST categorized this clinical isolate as sequence type ST45, corresponding to the Kp1 phylogenetic lineage within the clonal group CG45. Genetic analysis revealed the presence of resistance determinants associated with quinolone (*gyrA, parC, parE, qnrB)* and β-lactam antibiotics (bla_{AmpC}, bla_{OKP_A}, bla_{OKP_B}, bla_{OKP_C}, bla_{OKP_D}, and bla_{SHV}). Furthermore, virulence factor screening revealed the presence of a yersiniabactin siderophore system, specifically corresponding to lineage ytb10, with the integrative conjugative element classified as ICEKp4.

This study focused on molecular epidemiological studies that contribute to the clinical investigation of *K. pneumoniae*. It aids in analyzing the diversity of pathogens in Lithuania and comparing their genetic characteristics with those of pathogens from other countries.

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E21

SYNTHESIS OF VB_ECOS_NBD2 BACTERIOPHAGE-ORIGINATED POLYTUBES IN *KLUYVEROMYCES LACTIS*

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Virus-like particles (VLPs) or virus-based nanoparticles (VNPs) are protein structures morphologically similar to viruses. Nevertheless, they are non-infectious due to the absence of viral genetic material that could be used for virus replication [1, 2]. The application of these particles is highly extensive, making them the subject of numerous scientific studies in recent years.

gp39 is a tail tube protein encoded by the *g39* gene of the bacteriophage vB_EcoS_NBD2. Previous studies have shown that the tubes formed by these proteins expressed in *S. cerevisiae* are immunogenic, resistant to extreme environmental conditions such as pH and temperature changes, and stable in the presence of denaturing agents. Moreover, they can self-assemble and form nanostructures even without other phage proteins [3]. All these properties make them an attractive biomedical research subject. To ensure that the synthesized nanostructure proteins have eukaryotic-specific post-translational modifications and can be applied in medicine, yeast expression systems are beneficial. Nevertheless, the main drawback of this system is the hyperglycosylation of proteins by yeast, which can affect protein functionality. Different yeast strains with a glycosylation pattern more similar to that of the human organism are used [3] such as *Kluyveromyces lactis* to address this issue.

This study analyzed the synthesis of vB_EcoS_NBD2 bacteriophage-originated polytubes in *K. lactis*. For this purpose, the gene encoding the gp39 protein was cloned into the yeast expression vector pKDA1Kan-GAL7. Recombinant protein synthesis experiments were carried out using the yeast *K. lactis*. The results of this experiment provide significant findings about this yeast expression system's suitability for tail protein tube production.

The research is funded by the Research Council of Lithuania (LMTLT), under agreement No. S-MIP-24-41.

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E22

REFINING *HALOBACTERIUM SALINARUM* GROWTH MEDIUM FOR IMPROVED BIOMASS PRODUCTION

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Halobacterium salinarum is an extremely halophilic archaeon with diverse industrial applications. It produces bacterioruberin, a potent antioxidant carotenoid with applications in pharmaceuticals, cosmetics, and food industries [1]. Additionally, *H. salinarum* synthesizes bacteriorhodopsin, a light-driven proton pump widely studied for its use in bio-optical applications, including holographic data storage and artificial retina development [2]. However, commercial-scale cultivation remains costly due to expensive medium components. This study aims to develop a more cost-effective growth medium by eliminating non-essential ingredients and optimizing key salts from a standard halophilic archaea cultivation media DSM 372 [3].

Firstly, the removal of $FeCl_2$ and $MnCl_2$ was investigated as these metal salts are not only expensive but also pose toxicity risks to operators handling the medium. By confirming that these components are unnecessary for *H. salinarum* growth, we demonstrate safer and more economical formulation. Secondly, NaCl and $MgSO_4$ were chosen for optimization because they are the most critical components for osmotic balance and cellular function in *H. salinarum*. Their concentrations directly influence cell viability, metabolic activity, and overall biomass yield. Using Response Surface Methodology (RSM) to fine-tune their levels allows for a systematic and precise approach to improving growth conditions while reducing excess resource consumption.

Growth curve analyses were performed to evaluate the impact of removing $FeCl_2$ and $MnCl_2$ on *H. salinarum* biomass production. Using Central Composite Design in an RSM framework an experiment determining the optimal NaCl and $MgSO_4$ concentrations was created and then executed by cultivating all the designed *H. salinarum* medium combinations. The model was validated statistically to identify conditions yielding the highest growth.

Excluding $FeCl_2$ and $MnCl_2$ did not adversely impact *H. salinarum* cultivation, confirming these metals as unnecessary, thereby reducing overall medium expenses without sacrificing cell density. RSM analysis revealed that the ideal concentrations of NaCl and $MgSO_4$—20.60% and 1.97%, respectively — maximize growth, thus optimizing the existing medium for industrial scale applications.

This optimization strategy presents a cost-effective method for *H. salinarum* cultivation. By removing unnecessary metallic salts and fine-tuning key growth components, we enable scalable production of high-value biomolecules such as bacterioruberin and bacteriorhodopsin. These findings hold significant promise for broader industrial use of *H. salinarum* and other halophilic archaea in biotechnology and related fields.

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E23

MICROBIAL CONTAMINATION ANALYSIS IN USED EYESHADOWS AND EYELINERS

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In modern times, various cosmetic products, including eye cosmetics such as eyeliners and eyeshadows, are widely used to enhance self-esteem and appearance. Microbial contamination of cosmetics is a significant public health concern and a source of worry for consumers [1]. Many cosmetic products contain ingredients that promote the growth of microorganisms, making them prone to contamination by pathogenic bacteria and fungi [2]. Microbial contamination in cosmetics can lead to product deterioration, and when the microorganisms are pathogenic, they pose a significant health risk to consumers [3]. Several studies have indicated that the most commonly found microorganisms in eye cosmetics are *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli*, and *Bacillus* species, along with other bacteria that are often present [4].

The aim of this study was to evaluate the microbial contamination of the used eyeshadows and eyeliners. The study examined 45 samples, consisting of 30 eyeshadows and 15 eyeliners, selected based on their expiration date (no older than two years). All samples were enriched in thioglycollate broth before being transferred to selective media for further analysis. Of the 30 eyeshadow samples, *Bacillus spp.* were identified in 80% (24/30) of the samples, and *S. aureus* was identified in 13.3% (4/30) of the samples. The presence of *S. aureus* was confirmed through *S. aureus* DNase test and plasma coagulase tests. Additionally, plasma-coagulase-negative *Staphylococcus spp.* was found in 40% (12/30) of the eyeshadow samples. In the 15 eyeliner samples, *Bacillus spp*. were found in 40% (6/15), and *S. aureus* was found in 13.3% (2/15) of the samples, along with 33.3% (5/15) plasma-coagulase-negative *Staphylococcus spp.*. Other microorganisms were also detected in the samples, though they were not identified.

The results indicate that eye cosmetic products are frequently contaminated with harmful microorganisms, such as Bacillus spp. and Staphylococcus aureus, which can pose significant health risks to users if applied improperly. These findings highlight the necessity of strictly adhering to proper storage and hygiene protocols for cosmetic products. Additionally, it is recommended that consumers avoid using products past their expiration date, while manufacturers should implement improved hygiene practices during production and storage to minimize contamination risks.

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E24

IDENTIFICATION OF BACTERIA AND FUNGI IN MASCARA SAMPLES

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Cosmetic and personal care products are an integral part of our everyday routine making it imperative that these products remain safe for use on humans [1]. Cosmetic products are not required to be sterile, but they must be free from harmful microorganisms (such as *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*), and the total aerobic microbial count should be kept low. The presence of pathogenic organisms and high microbial levels in cosmetic products can lead to spoilage [2]. This type of products may become contaminated either during production or through consumer usage. This contamination can result undesirable changes in the product's texture, fragrance, or appearance. Moreover, the microorganisms present could be harmful, posing potential health risk to user. While regulations and the adoption of Good Manufacturing Practices (GMP) have improved microbiological safety, contaminated cosmetics are still being found, and in some cases, this has led to severe health issues for users [3].

The aim of this study was to evaluate the microbial contamination of used mascaras. A total of 30 mascaras samples were examined, selected based on their expiration date (no older than two years). All samples were enriched in thioglycolate broth before being transferred to selective media for further analysis. Of the 30 mascaras samples, *Bacillus spp.* bacteria were identified in 66,6% (20/30) of samples. To see if cosmetic products are contaminated with *Staphylococcus spp,* we used Mannitol salt agar. There was a significant amount of plasma negative *Staphylococcus spp*. 73,3% (22/30), which were detected with plasma coagulase test. Other microorganisms were also detected, though they were not identified.

This study shows significant microbial contamination in mascaras, with *Bacillus spp*. and plasma negative *Staphylococcus spp.* These results emphasize the critical need for proper storage and hygiene practices to minimize contamination risks in cosmetic products. Improper handling, such as sharing products or incorrect storage conditions, can further contribute to microbial growth.

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E25

INVESTIGATION OF BIOFILM AND POLYSACCHARIDE CAPSULE FORMATION OF CLINICAL *ACINETOBACTER BAUMANNII* STRAINS

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Acinetobacter baumannii is a Gram-negative opportunistic pathogen that has recently emerged as a global threat associated with high morbidity and mortality [1]. The adaptive mechanisms of *A. baumannii*, such as biofilms and polysaccharide capsules, have a major impact on the spread of antibiotic resistance. Insights into the diversity of biofilm-forming and capsule-producing *A. baumannii* clinical strains may help improve infection control measures. The aim of this study was to determine the ability of clinical *A. baumannii* strains to form biofilms and the polysaccharide capsule, also to identify a possible relationship between these two factors.

A total of 59 clinical isolates (n = 59) of *A. baumannii*, collected at Vilnius University Hospital Santaros Clinics in 2022-2023, were examined. Biofilm formation of clinical *A. baumannii* strains was studied using the crystal violet staining method; capsule formation was determined using the Percoll gradient method. To quantify biofilm formation, the optical density absorbance of planktonic cultures was measured at a wavelength of 600 nm. The optical density absorbance of the biofilms stained with crystal violet was measured at 580 nm. To normalize the results obtained between the different growth rates of the isolates, the $OD_{580/600}$ ratio was calculated. Capsule production was assessed by the arrangement of cells at a given density of Percoll gradient.

58 isolates out of 59 (98.3%) showed the ability to form biofilms and only one isolate (1.7%) did not form a biofilm. That isolate also showed the lowest OD_{600} values compared to the rest of the isolates. The $OD_{580/600}$ ratio ranged from 0 to 3.28. The average value of $OD_{580/600}$ among all tested *A. baumannii* isolates was 1.92. Most isolates (98.3%) were able to produce the polysaccharide capsule. The isolate demonstrating a capsule-negative phenotype had the lowest $OD_{580/600}$ ratio, forming a weak biofilm.

The high prevalence of biofilm and capsule formation among clinical isolates of *A. baumannii* emphasizes their critical role in bacterial survival in clinical settings. The correlation between low biofilm formation ability and lack of capsule suggests that capsule formation may contribute to biofilm stability. However, there was the capsule-producing isolate that lacked biofilm formation ability. Further research is needed to understand the interaction between biofilm formation and capsule production in *A. baumannii* isolates.

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E26

INVESTIGATION OF THE TOTAL LIPASE ACTIVITY IN IDENTIFIED MICROORGANISMS CAPABLE OF DEGRADING OIL PRODUCTS

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Petroleum is the most widely used fossil fuel worldwide. However, its extraction and transportation often lead to oil spills, which degrade soil structure and quality, posing risks to plants, human health, and the environment [1]. Due to the health hazards and social consequences of petroleum hydrocarbon contamination, scientists are actively developing remediation strategies to restore and manage affected areas [2]. Currently, traditional approaches such as chemical and physical remediation are insufficient for effectively resolving pollution problems. Bioremediation utilizing microorganisms is a promising treatment technology for contaminated sites due to its ease of use, cost-effectiveness, and effectiveness in preventing secondary contamination [3]. Despite various bioremediation studies, there is limited research on microbial degradation of oil-contaminated wooden railway sleepers. These sleepers, were preserved with creosote, during their use, absorbed additional toxic compounds. Such chemicals can migrate into the soil and water, posing risks to ecosystems and human health.

In this study, microorganisms capable of degrading wooden railway sleepers contaminated with oil hydrocarbons were investigated. The selected bacterial strains were isolated and identified from microorganisms growing in a medium containing oil-contaminated wooden railway sleepers. These bacterial strains were tested for their ability to degrade oil products by measuring their lipase activity using a tributyrin substrate. The five species that showed positive results were further analyzed for their biosurfactant properties. All microorganisms exhibited similar results: the drop collapse and oil spread tests were positive, assessing drop collapsing time and spread efficiency in a Petri dish, while the 24-hour emulsification test was negative, the strains' supernatants failed to maintain a stable emulsion with diesel. The bacterial strains were grown at 30°C, 37°C, and 42°C in a tributyrin medium to determine the optimal temperature for maximum total lipase activity. The results showed that 30°C was the optimal temperature, as the strains, isolated from outdoor environments, demonstrated the highest total lipase activity compared to the other temperatures. Further characterization of the isolated strains is currently in progress.

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E27

CHEMICAL SYTHESIS AND CATABOLISM OF MODIFIED PYRIMIDINE DERIVATIVES

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Pyrimidine derivatives play an important role in biological processes as essential components of nucleic acids, cofactors and various metabolic intermediates. Major cellular source of modified nucleosides is tRNA, where >170 modified nucleotide species are found. Uracil is one of the most chemically modified nucleobases in tRNA, with its derivatives accounting for about 40 % of all tRNA modifications. The modifications are important for tRNA metabolism, structure, stability, localisation and transport. The study of modified pyrimidine derivatives has attracted considerable attention due to their importance in medicinal chemistry. Chemical modifications of uracil and cytosine have led to the development of anticancer drugs, such as 5-fluorouracil and azacitidine, which act by blocking tumour regeneration. As a pyrimidine analogue, 5-fluorouracil is metabolised intracellularly to a number of active metabolites including 5-fluoro-2'-deoxyuridine 5'-monophosphate. Such active metabolites interfere with RNA synthesis [1]. Despite the huge progress in discovery of genes that introduce chemical modifications into tRNA and investigation of their role in cell physiology and diseases, little is known about the metabolism of modified nucleotides and corresponding heterocyclic bases.

In this study, methylated pyrimidine heterocyclic bases, such as 2- and 4-methylthiouracil, N^4-methylcytosine, N^4,N^4-dimethylcytosine, *N*3-methyluracil, N^4-methyl-2sulfonyluracil and others, were synthesized and purified using column chromatography. The structure and the purity of synthesized compounds were proved by UV/Vis, NMR spectroscopy, TLC and HPLC analysis. The synthesized methylated compounds and some other commercially available modified pyrimidines were used as substrates to select enzymes involved in the catabolism of the modified heterocyclic bases. For this purpose, uracil auxotrophy-based selection systems were used, which are similar to previously described [2]. These systems allow the selection of enzymes that convert modified pyrimidines, respectively, into unmodified derivatives. It was found that some tested methylated pyrimidines, such as N^4-methylcytosine, *N*3-methyluracil, support the growth of uracil auxotrophs, whereas other tested modified derivatives did not support the growth of *E. coli*. Further investigations on these phenomena are under way.

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E28

Optimization of bacterial cellulose production by *Komagataeibacter xylinus* LMG 1515

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Over the last few decades, the increasing focus on environmental sustainability has led to more research into bio-based materials that offer improved performance and cost-effectiveness. Bacterial cellulose (BC), a renewable and biodegradable nanostructured biopolymer, has gained significant scientific interest due to its high purity and distinct physicochemical characteristics, such as high crystallinity, outstanding mechanical strength, and biocompatibility [1]. These attributes make BC highly versatile, with applications in biomedical and pharmaceutical fields, cosmetics, food industries, and beyond [2, 3]. The aim of this study was to optimize bacterial cellulose production to achieve maximum yield using different growth parameters.

This study evaluated the effects of carbon source, cultivation time, and medium volume on bacterial cellulose (BC) production by *Komagataeibacter xylinus* LMG 1515. Specifically, it investigated the impact of different carbon sources (glucose, mannitol, sucrose, and glycerol), varying cultivation times (1 day agitated at 250 rpm followed by 7 days static, 9 days static, and 14 days static), and different medium volumes (10%, 20%, 25%, and 50% of the total flask volume). The methods used to assess BC production included optical density (OD), colony-forming units (CFU), wet cell weight (WCW), dry cell weight (DCW), cellulose production rate and water holding capacity to assess the material's water retention ability. These parameters were applied across all experimental conditions to determine optimal factors for BC production.

This study demonstrated that mannitol and glycerol were the most effective carbon sources, yielding the highest bacterial cellulose (BC) production, with dry weights of 8.8 ± 4.0 g/L and 8.9 ± 0.1 g/L, respectively. Among the varying cultivation times, the 14-day static condition resulted in the highest BC yield compared to the shorter static and agitated conditions, with a mean BC yield of 19.0 ± 8.15 g/L. In terms of medium volume, the highest BC production was observed when the medium filled 20% of the total flask volume, which produced 14.66 ± 8.92 g/L.

The optimized conditions from previous experiments were applied to evaluate the use of damaged carbonated beverages from company UAB “Sentonis” as an alternative, more sustainable carbon source for *Komagataeibacter xylinus* cellulose production. Three different beverages were tested: an energy drink, an energy-flavored carbonated soft drink, and a kvass. As the beverages initially had a low pH, it was adjusted to create a more suitable environment for bacterial growth. While no growth was observed in the energy drink, the energy-flavored carbonated soft drink exhibited moderate bacterial growth yielding 1.31 ± 0.90 g/L. In comparison, the kvass resulted in the highest BC production, with a yield of 2.39 ± 1.63 g/L.

This study presents an analysis of the factors influencing bacterial cellulose production by *Komagataeibacter xylinus* LMG 1515. The results indicate that mannitol and glycerol are the most effective carbon sources, a cultivation time of 14 days in static conditions is optimal, and a medium volume of 20% flask volume yields the highest BC production. Additionally, while the use of damaged carbonated beverages as an alternative carbon source offers a promising route for sustainable BC production, the yield remains substantially lower compared to optimized conditions.

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E29

BACTERIAL OUTER MEMBRANE VESICLES AS A DRUG DELIVERY TOOL AGAINST *ACINETOBACTER BAUMANNII*

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Gram-negative bacteria *Acinetobacter baumannii* has emerged as a prevalent cause of nosocomial infections. A key factor contributing to the rapid spread of *A. baumannii* as a pathogen in hospital-related settings is the formation of biofilms. Additionally, *A. baumannii* exhibits a remarkable ability to acquire antibiotic resistance. The World Health Organization (WHO) currently classifies *A. baumannii* as a pathogen of critical priority for new antimicrobial development [1].

One promising strategy to combat antibiotic resistance is the development of novel drug delivery mechanisms. For example, biodegradable nanocarriers have been investigated to improve drug delivery by reducing the exposure of the microbes to sub-lethal doses, lowering minimal inhibitory concentrations, and potentially mitigating dose-dependent toxic effects [2]. Outer membrane vesicles (OMVs), naturally shed by Gram-negative bacteria, represent a promising lipid-based nanocarrier for this purpose. As bacteria primarily use OMVs for intercellular transport, their structural similarity to bacterial membranes could aid in the efficient transfer of antibiotic substances [3].

In this study, we compared two *A. baumannii* strains (AB52 and ABV15) in terms of their growth characteristics and biofilm formation. Additionally, we evaluated whether OMVs could enhance the efficacy of antibiotics against these strains. Ampicillin, a common β-lactam antibiotic, was used against strain ABV15, while gentamicin, a widely prescribed aminoglycoside, was used against strain AB52 due to its β-lactam resistance. Antibiotics were loaded into OMVs using sonication, a method that destabilises OMV membranes, allowing antibiotic molecules to penetrate the vesicle lumen. The effects of OMV-mediated antibiotic delivery were assessed by measuring optical density of the growth medium after *A. baumannii* reached the stationary phase. Since viable biofilms release planktonic cells into the medium, changes in turbidity served as an indicator of bacterial dissemination and treatment efficacy. Additional details of the project, along with preliminary results, will be shared during the poster session.

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E30

Identification of Pathogenesis Mechanisms and Biofilm Formation Factors in Clinical Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant pathogen capable of forming biofilms, which enhance its resistance to antibiotics and host immune responses [1]. Pathogenesis mechanisms and biofilm formation are regulated by various genes, including *pvl, eta, etb, sea, sec, spa* and *bap* [2,3]. The research focused on the prevalence of key pathogenicity genes, including those coding for exotoxins and enterotoxins, as well as the presence of genes associated with biofilm formation. The aim of this study was to evaluate the relationship between biofilm formation strength and the presence of pathogenesis and biofilm-associated genes in clinical MRSA isolates. Understanding the prevalence of these genes in clinical isolates can provide insights into the development of MRSA biofilms and their potential impact on pathogenicity.

This study analyzed the presence of *mecC*, *pvl* genes, exotoxin-encoding genes *eta* and *etb*, enterotoxin genes *sec* and *sea*, and biofilm-associated genes *spa* and *bap* in 95 clinical MRSA isolates from two hospitals in Vilnius using PCR. Isolates were categorized as strong or moderate biofilm formers based on a quantitative biofilm assay using microtiter plates method. The optical density of stained biofilms was measured spectrophotometrically to determine biofilm formation strength. The most frequent genes were identified, and their distribution among the biofilm-forming groups was statistically assessed.

All MRSA isolates demonstrated the ability to form biofilms. 72% produced strong biofilm and 28% produced moderate biofilms. The *mecC* gene, which is associated with methicillin resistance, was not detected in this study. The most prevalent gene among the isolates was the *pvl* gene, detected in 79.4% of isolates, while the exotoxin genes *eta* and *etb* were absent in all isolates. The enterotoxin gene *sec* was present in only a small percentage (3.16%) of isolates, and the *sea* gene was not detected. Biofilm formation did not correlate with the presence of specific pathogenicity genes, as statistical analysis showed no significant differences in gene prevalence between the biofilm-producing groups ($p > 0.05$).

Furthermore, all the isolates contained the spa gene, which is associated with biofilm formation. This gene demonstrated polymorphism based on its size and was categorized into three main categories: <300 bp, 300–400 bp, and >400 bp. The majority of isolates (78.95%) had a spa gene size in the 300–400 bp range, whereas only a few isolates (2.11%) had a gene size greater than 400 bp. No statistically significant differences were observed between the isolates with moderate and strong biofilm-forming capabilities. Additionally, the bap gene, which is involved in biofilm formation, was not detected in any isolate.

These findings contribute to our understanding of the genetic factors underlying biofilm formation and pathogenicity in MRSA isolates, highlighting the complexity of virulence mechanisms, and the need for further research to identify additional factors that may influence biofilm development and resistance profiles in MRSA strains.

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E31

LIPOLYTIC ENZYMES FOR POLYLACTIC ACID AND POLYBUTYLENE SUCCINATE FILM DEGRADATION

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In today's world, plastic is an indispensable part of both industrial applications and everyday life. Consequently, management of plastic waste has emerged as a globally recognized problem yet to be resolved. While plastic production has more than doubled over the last two decades, 22% of produced plastic end up mismanaged [1]. Recognizing this problem, efforts could be shifted towards biodegradable plastics such as polybutylene succinate (PBS) or their integration with other commonly used plastics, such as polylactic acid (PLA) and polycaprolactone (PCL). Although biodegradable plastics could mean a great step towards more efficient plastic management, the challenge of degrading them sufficiently remains. Plastic degrading enzymes, produced by microorganisms, are a promising tool in finding not only effective, but also environmentally friendly solution to this problem.

This study focuses on four lipolytic biocatalysts: GD-95RM lipase [2], GDEst-95 esterase [3], GDEst-lip chimeric enzyme [3] and Cut+SP cutinase [4] and their abilities to degrade plastic polymers [2]. Enzymes mentioned above catalyze ester bond hydrolysis, which form the backbone of polyester plastics like PLA and PBS. Plastic degrading abilities were examined applying the following procedure. Both PLA and PBS films were made by dissolving plastic granules in chloroform until homogeneous solution was reached and dried fully to form thin uniform films [5]. Prepared films were fully submerged in a buffer solution containing each of the enzymes for 72 hours. Films were weighed before and after exposure to enzymes to evaluate weight changes as an indicator of degradation. The weight loss of films was calculated using the following equation [6]:

$$w_l = ((w_{initial} - w_{dry})/w_{initial}) \times 100\ [\%],$$

where w_l is the weight loss, $w_{initial}$ – weight before biodegradation, and w_{dry} is, in our case, weight of the dried samples after degradation.

The experiment was performed for each of the four enzymes separately maintaining the same conditions. In addition, PLA film degradation was examined using different organic solvents – 25% ethanol and 25% methanol solutions, to evaluate their effect on enzymatic activity.

After enzymatic exposure, no significant weight changes were observed in PLA samples, indicating that there has not been any enzymatic degradation or it has been insignificant. However, enzymatic degradation was detected in PBS samples treated with each of the four enzymes. The highest weight loss of 16% occurring using Cut+SP cutinase.

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E32

CYANOPHAGE-INDUCED TRANSCRIPTIONAL CHANGES IN *RAPHIDIOPSIS RACIBORSKII*

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Raphidiopsis raciborskii is a nitrogen-fixing freshwater cyanobacterium that recently gained attention due to its high invasiveness potential, toxicity, and harmful blooms, which can negatively impact biodiversity, ecosystem functions, and water quality. Despite its ecological significance, factors controlling this species' occurrence and bloom dynamics remain poorly understood. Cyanophages, viruses that infect cyanobacteria, may play a crucial role in microbial population and community dynamics through host lysis, horizontal gene transfer, and metabolic reprogramming [1,2]. Understanding these interactions in freshwater ecosystems is essential for advancing our knowledge of cyanophage ecology and may provide insights into the mechanisms by which viruses regulate cyanobacterial activity, growth, and transcription. Investigations into transcriptional regulation of host cyanobacteria by viral infection are also crucial for the assessment and development of phage-based technologies for the mitigation of harmful algal blooms.

To investigate the transcriptional response of *R. raciborskii* to cyanophage infection as well as to establish a transcriptional scheme for cyanophage, we used *R. raciborskii* strain KLL07 and its lytic cyanophage Cr-LKS4 as a model virus-host system. In this study, control and infection treatments were established by adding either filter-sterilized growth medium or cyanophage suspensions at the multiplicity of infection of three (MOI = 3) in four biological replicates to unialgal yet non-axenic cultures. The experiment lasted 120 hours, with samples collected every 4-8 hours for cell and phage abundance measurements, DNA and RNA sequencing, and proteomic analysis.

We will present results on cyanobacterial and cyanophage dynamics during the infection experiment along with differential gene expression analysis during different infection stages (adsorption, DNA replication and lysis) of *R. raciborskii*. Additionally, changes in *R. raciborskii* microbiome composition induced by the cyanophage-mediated lysis of the photosynthetic host will be presented. These findings will contribute to a better understanding of the ecological significance of freshwater cyanophages and their impact on cyanobacteria growth and succession.

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E33

CHARACTERIZATION OF WILD-TYPE AND MUTANT VARIANTS OF *STREPTOMYCES SCIABIEI* 87.22 CUTINASE

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Cutinases are versatile enzymes from the α/β-hydrolase superfamily, known for their ability to hydrolyze ester bonds in natural and synthetic polymers. Their relatively small size and lack of a lid domain grant them broad substrate specificity, making them promising candidates for biotechnological applications, including the degradation of polyethylene terephthalate (PET), one of the most widely used plastics [1, 2]. While PET possesses desirable properties for manufacturing, its accumulation in the environment has become a major concern, as it is highly resistant to natural degradation processes [3]. However, for the effective application of cutinases in PET degradation, a deeper understanding of their stability, activity under different conditions, and resistance to harsh industrial environments is essential.

This study focuses on the characterization of the wild-type *Streptomyces scabiei* 87.22 cutinase and three site-directed mutants: Asp94Ala, Gln88Ala and Ser123Ala. The mutant variants were created using site-directed mutagenesis, cloned into pET-21c(+) vector, expressed in *Escherichia coli* BL21 (DE3) cells and purified via immobilized metal affinity chromatography (IMAC). The enzymatic properties of both the wild-type and mutant cutinases were assessed in terms of temperature and pH stability, substrate specificity and resistance to organic solvents using synthetic *p*-NP esters as substrates.

The obtained results revealed that the Gln88Ala mutation led to a complete loss of enzymatic activity, while the Asp94Ala mutation enhanced the thermostability of the enzyme. Furthermore, the Ser123Ala mutation increased the enzyme's activity at high pH and improved its performance towards long-chain esters. These findings contribute to a better understanding of the structure-function relationship in *S. scabiei* 87.22 cutinase and offer valuable insights for the development of more robust biocatalysts for industrial and environmental applications, including polymer degradation.

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E34

SURFACE MODIFICATION OF ELECTROSPUN POLY(3-HYDROXYBUTIRATE-CO-3-HYDROXYVALERATE) (PHBV) SCAFFOLDS USING FUNGAL HYDROPHOBINS

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Hydrophobins are a family of small (<20 kDa) globular proteins synthesized exclusively by filamentous fungi. They possess the unique ability to self-assemble at hydrophilic-hydrophobic interfaces and alter properties of surfaces. Hydrophobins have applications in the field of electrochemistry, where they help to avoid aggregation of nanoparticles, in medicine, where they are used for surface modification of invasive instruments to lower traction between the instrument and tissues, and in tissue engineering, where they are used for improving surface wettability and cell adhesion of electrospun scaffolds [1].

PHBV has great properties that can be of use in biomedical research – biocompatibility, biodegradability, piezoelectricity and optical activity. However, it still possesses some properties that are considered as drawbacks – high crystallinity, brittleness and hydrophobicity [2]. This study aims to assess the proliferation promoting differences between unmodified and hydrophobin-modified scaffolds.

In this study we used a hydrophobin, AfRodA, of *Aspergillus fumigatus* that was fused with histidine and SUMO tags. The pSMART-I-*rodA* plasmid in which *rodA* is under control of T7lac promoter, was transformed into *Escherichia coli* BL21 (DE3) and induced using IPTG. Purification was performed via Ni^{2+} affinity chromatography, followed by dialysis with GSH/GSSH (10 mM / 1 mM) in sodium acetate buffer, pH 5, to ensure proper protein folding and activity. To prepare the protein solution for experiments involving eukaryotic cells, the buffer solution was changed to PBS buffer via desalting. Fifteen percent PHBV scaffolds were produced by using electrospinning at voltage of 19 kV with solution flow rate of 1 ml/min. For evaluation of mechanical properties of produced scaffolds, tensile strength tests were performed. To assess surface modification affects, scaffolds of 0,32 cm^2 area were treated with a 50 µg/ml solution of recombinant AfRodA at 4 ^oC. Growth of AC16 cardiomyocyte cell cultures in DMEM/F12 medium were evaluated comparing the treated and untreated scaffolds. It was obtained that the treated scaffolds promoted 70% to 78% greater cell growth compared to untreated scaffolds after 5^{th} to 10^{th} days of growth.

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E35

A *rep5*-BASED VECTOR FOR GENETIC ENGINEERING OF *PARAGEOBACILLUS THERMOGLUCOSIDASIUS*

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Parageobacillus thermoglucosidasius is a Gram-positive, facultatively anaerobic or aerobic, thermophilic bacteria that is characterized by growth at high temperatures and the use of various substrates for the synthesis of beneficial compounds such as enzymes, biofuels, volatile substances, and others [1]. On the other hand, to fully exploit the potential of *P. thermoglucosidasius*, efficient tools of genetic engineering are required. The plasmid replicons are one of the main tools in the genetic engineering of bacteria because they control the stability and the copy number of plasmids in the cells. The currently used vectors designed for *Parageobacillus* genus bacteria are based on two different plasmid replicons: repBST1 and repB. These replicons belong to two different incompatible replication systems, but unfortunately, they are not very stable, especially without selective pressure [2, 3]. For these reasons, the main goal of this work is to expand the set of existing vectors by creating new vectors with a minimal *rep5* replicon [4].

To achieve this, the target gene *rep5* sequence was amplified from the cryptic pGTG5 plasmid using specific primers with inserted restriction sites. The pGTG5 plasmid was used because it is the smallest plasmid identified in bacteria belonging to the genus *Geobacillus* (= *Parageobacillus*) [4]. The resulting DNA fragment was cloned into the pG2K vector in place of the *repB* replicon using restriction and ligation reactions. The recombinant plasmid was transformed into *Escherichia coli* DH5α electrocompetent cells by electroporation. The transformants were selected for kanamycin resistance and confirmed by restriction analysis and sequencing. The confirmed construct was transferred into *P. thermoglucosidasius* DSM 2542^T cells to evaluate its stability and efficacy in thermophilic bacteria.

The resulting plasmid is a ~3.8 kb shuttle vector containing CoIE1 (origin of replication for *E. coli*), rep5 (replication initiation protein, 244 aa; origin of replication for *Parageobacillus* sp.), *kanR* (kanamycin resistance gene) and MCS (multiple cloning site). The size of the designed plasmid is small enough to confer high transformation efficiency. Additionally, the rep5 protein of the new family of RC (rolling-circle) plasmids guarantees a new incompatibility group [4].

To conclude, the *rep5* replicon-based vector will expand the choice of vectors for *P. thermoglucosidasius*. This will create new opportunities for further advancements in using *Parageobacillus* sp. not only in scientific research but also in the industrial sector.

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E36

N, O-METHYLATED PURINES: SYNTHESIS, CHARACTERIZATION AND CATABOLISM

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Ribonucleic acid plays a vital role in various cellular processes; each type of RNA is modified by unique chemical modifications that are underlying their functions. One of the most modified RNA species is tRNA, which contains over 170 modified nucleotide species. Transfer RNA contain adenine and guanine methylated purine derivatives that play crucial roles in its metabolism, structure, stability, localization and transport. Ongoing studies are exploring their potential implications in diseases such as mitochondrial disorders, neurological obesity, diabetes and cancer, with aim of developing novel therapeutic strategies [1]. Despite significant progress in understanding the genes responsible for making chemical modifications to tRNA, not much is known about metabolic pathways of these modified nucleotides and their corresponding heterocyclic bases.

During this project, *N,O*-methylated purine heterocyclic bases adenine and guanine were synthesized and purified by flash column chromatography. The purity and structure of synthesized compounds were determined by UV/Vis, NMR spectroscopy, TLC and HPLC-MS analysis. The synthesized methylated compounds and some other commercially available modified purines were used as substrates to select enzymes involved in the catabolism of the modified heterocyclic bases. For this purpose, purine auxotrophy-based selection systems were used, which are similar to previously described one [2]. These systems allow the selection of enzymes that convert modified purines into respective unmodified ones. Specifically, *Escherichia coli* strains with *purH, purA, guaA* or *guaB* mutations were used to evaluate the enzymatic conversion of methylated purines. It was found that certain tested modified purines, such as N^6-methylguanine, O^6-methylguanine, 6-chloroguanine, 2-chloroadenine supported the growth of purine auxotrophs, whereas other tested modified derivatives did not support the growth of *E. coli*. These phenomena are currently under investigation.

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E37

EXPLORING CARBON SOURCES FOR *HALOBACTERIUM SALINARUM: GROWTH OPTIMISATION AND BACTERIORUBERIN EXTRACTION*

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Halophilic archaea are extremophilic microorganisms that achieve optimal growth in culture media with high salt concentrations (from 4 M). Among the biomolecules or metabolites of biotechnological interest synthesised by haloarchaea, small proteins and peptides, enzyme bioplastics, carotenoids, and nanoparticles can be highlighted. The carotenoids found in haloarchaea have garnered significant attention in various industries due to their versatility, serving as antioxidants, anticancer agents, antimicrobials, anti-inflammatory compounds, and food colourants, thus offering numerous biotechnological and biomedical applications. The major carotenoids produced by haloarchaea are bacterioruberin and its derivatives monoanhydro- and bisanhydro-bacterioruberin [1,2].

A cost-effective strategy for optimizing the cultivation of *Halobacterium salinarum* was developed in this study, aiming to maximize bacterioruberin production and cell biomass. To achieve the study objectives, growth curve analysis was implemented to assess the effects of different carbon sources on the growth rate of *H. salinarum*. Growth dynamics of *H. salinarum* were investigated across various carbon sources, including galacto-oligosaccharides (GOS), glucose, galactose, and glycerol, with each component tested at concentrations of 0.5%, 1%, and 2% in separate flasks. Growth dynamics were monitored at 12-hour intervals, and growth curves were plotted. Upon entry into the stationary phase of growth, wet biomass was quantified to evaluate biomass production efficiency. Furthermore, bacterioruberin was extracted from cultures grown in each medium as stated by Kholany et al. using solid-liquid extraction, with subsequent centrifugation to recover the supernatant [3]. The purity of bacterioruberin was evaluated using thin-layer chromatography (TLC) using a solvent mixture of acetone and dichloromethane (1:1), and absorption was measured at 494 nm wavelength spectrophotometrically.

The growth curves demonstrated that archaea exhibited the fastest growth in media containing either glycerol or galacto-oligosaccharides (GOS), highlighting these as superior carbon sources compared to glucose and galactose. During the exponential phase, archaea achieved the highest growth rates when cultivated with GOS. TLC analysis separated the carotenoids into three bands, identified as bacterioruberin, monoanhydrobacterioruberin, and 2-isopentyl-3,4-dehydrorhodopin, with no significant variation between the tested media.

In this study, the impact of various carbon sources, including GOS, glucose, galactose, and glycerol, on the growth dynamics and carotenoid biosynthesis in *H. salinarum* was investigated. The obtained results proposed that *H. salinarum* exhibited the highest proliferation rates in media containing glycerol or GOS, with GOS supporting the fastest growth during the exponential phase while also serving as a cost-effective and sustainable alternative for cultivation. These findings suggested that specific carbon sources could be strategically selected to enhance the efficiency and scalability of carotenoid production in industrial settings.

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E38

STUDY OF XYLOSE TRANSPORT IN MODIFIED *OGATAEA POLYMORPHA* YEAST DURING ALCOHOLIC FERMENTATION

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Growing concerns about the depletion of fossil fuels and their impact on the environment are driving the search for alternative energy sources. Bioethanol, produced from renewable materials, is a promising alternative fuel. When burned, it releases less nitrogen oxides and particulate matter into the environment. In particular, second-generation bioethanol, which is produced from lignocellulosic biomass derived from agricultural and forestry waste, makes it a more sustainable option as it uses raw materials that are not food-based [1].

Xylose, which is the second most abundant monosaccharide after glucose and the most abundant pentose sugar, is a key component of the hemicelluloses. In fact, more than half of the world's agricultural plant biomass consists of lignocellulosic crop residues [2]. The utilisation of xylose can increase the bioethanol yield from feedstocks, and maximising xylose uptake by yeast cells is crucial to improving its use in lignocellulosic bioethanol production.

Ogataea polymorpha, a thermotolerant methylotrophic yeast, has the ability to ferment xylose at elevated temperatures. However, a challenge for efficient lignocellulosic ethanol production is the transport of xylose into yeast cells in the presence of both glucose and xylose. In xylose-utilising wild-type yeast cultures, xylose consumption does not begin until after glucose has been depleted, resulting in longer fermentation times and incomplete conversion of the sugars from the lignocellulose hydrolysates [3]. This problem arises because the transporters that are responsible for the uptake of xylose have a higher affinity for glucose than for xylose. Furthermore, some potential xylose transporters are removed from yeast plasma membranes at low glucose levels, so making modifications to increase their stability are essential [4]. For this reason, the transporters found in *Saccharomyces cerevisiae* Gal2 and Hxt7 or *O. polymorpha* Hxt1 are considered to be potential xylose carriers and mutagenesis could improve the uptake of xylose and its utilisation in alcoholic fermentation. The aim of this research project is to investigate the importance of the *O. polymorpha* Hxt1 transporter and heterologous modifications of the *S. cerevisiae* Gal2 or Hxt7 transporters in *O. polymorpha* yeast, in particular for high temperature alcoholic fermentation. The rate of glucose and xylose uptake into cells can be determined by recording the activity of energy metabolism (respiration, glycolysis, ATP synthesis).

In our experiments, the respiration and glycolysis of *O. polymorpha* cells were evaluated under different environmental conditions, such as reduced or increased sugar concentrations, at different temperatures, in salt buffers and/or in the growth media. The results of the experiments showed that at 40-50°C, dissolved oxygen consumption was higher and media acidification was greater. It was also found that a high concentration of one of the sugars (glucose or xylose) inhibited the entry of the other sugar into the cells of *O. polymorpha*.

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E39

DETERMINATION OF ACTION OF SMALL REGULATORY RNA SLLM1238 INVOLVED IN STRESS RESPONSE IN LACTIC ACID BACTERIA *LACTOCOCCUS LACTIS*

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Lactococcus lactis are Gram-positive, immobile, facultatively anaerobic, non-spore-forming lactic acid bacteria that have become an important model organism due to their industrial role as primary components of dairy starter cultures and their impact on public health due to their probiotic properties [1]. In order to maintain and improve these properties it is important to understand how these microorganisms adapt to environmental changes, especially when subjected to stress conditions caused by temperature changes, nutrient availability or other stress inducing factors.

Small regulatory RNAs (sRNAs) are amongst the most abundant gene-regulating RNAs, serving as key post-transcriptional regulators essential for bacterial adaptation to environmental changes [2]. These sRNAs regulate gene expression by base pairing with target mRNAs influencing its translation or by directly interacting with proteins and affecting their activity [3]. Gaining deeper insight into the role of regulatory RNAs in controlling stress responses and metabolic processes in *Lactococcus lactis* could potentially lead to innovative practical industrial applications [4]. In the Department of Biological DNA Modification, it was shown that transcription of several small regulatory RNAs is observed in a region of the *L. lactis* genome, which we have named the sLLM1238 system.

The aim of this study is to determine the mechanism of action of *L. lactis* sLLM1238 sRNAs. Firstly, we determined different forms of sRNAs by Northern blotting. Afterwards, to facilitate overexpression of sRNAs, we created a vector with a sLLM1238 gene and an empty one for control and electroporated them into *L. lactis*. In order to see expression changes of genes possibly regulated by sLLM1238, we performed total RNA sequencing analysis of *L. lactis* electroporated with these vectors. Furthermore, we analysed how *L. lactis* with and without overexpression of sRNAs responds when grown on agar plates with different concentrations of lysozyme. In addition, we extracted total RNA samples from *L. lactis* subjected to lysozyme in order to observe sRNA expression changes.

The obtained results will help to understand the mechanisms of action of sRNAs in *L. lactis* and their role in gene regulation as well as response to environment changes.

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E40

INVESTIGATION OF NON-CANONICAL GENOME ORGANIZATION POLYOMA VIRUS PUTATIVE VP1 STRUCTURAL PROTEIN EXPRESSION AND ASSEMBLY INTO VIRUS-LIKE PARTICLES

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Polyoma viruses (PyVs) are small circular DNA viruses that typically encode early and late genes in different directions in the genome. A year ago, new PyV-like sequences which encode early and late genes in the same direction were discovered in the metagenomic sequencing data from various species [1]. Such non-canonical genome organization PyV DNA sequence was also discovered in our department by sequencing *Apodemus agrarius* mice kidney samples. The sequence of this PyV genome is very distant from other known mammalian PyVs and it forms a distinct monophyletic group with the PyV-like sequences identified in the same study mentioned above [1]. Together, these sequences form a monophyletic group with scorpion PyVs [2], suggesting that the PyV sequenced from *A. agrarius* samples could possibly be an arthropod virus.

Currently, it has not yet been determined what organisms can be infected by these non-canonical genome organization PyVs, so it is important to determine whether *A. agrarius* mice are the true host of this virus. Studying it could provide very important insights into how PyVs have evolved.

Therefore the first step of our study was to investigate the expression of a recombinant putative VP1 structural protein in *S. cerevisiae* yeast and to assess the ability of these proteins to assemble into virus-like particles (VLPs). These VLPs can later be applied for testing *A. agrarius* mice blood serum samples for the presence of antibodies against PyV we have detected, which could indicate whether it is indeed capable of infecting these mice.

After determining the sequence of the putative VP1 gene in the PyV genome, it was observed that it encodes a protein of about 512 amino acids (a.a.) in length, although the experimentally determined PyV VP1 proteins of *Mus musculus* mice are only about 380 a.a. The C-terminus of the protein it encodes was predicted to contain additional alpha helices, whose structure corresponds to the previously discovered hypothetical Cah domain encoded by certain PyV VP1 genes [1] and whose function and existence in VP1 proteins has not yet been determined. Due to the unusual length of the putative VP1 gene, in silico analysis of the splice sites was performed which led to the selection of gene splice variant whose splicing sites overlap with the additional alpha helices encoded at the end of the gene. Following the construction of yeast expression plasmids with the selected VP1 gene variants, their protein expression and assembly into VLPs in *S. cerevisiae* yeast were assessed by SDS-PAGE. To investigate VP1 assembly into VLPs, samples of the soluble fraction of yeast cell lysates were ultracentrifuged through a 30 % sucrose layer, through which the VLP particles can diffuse due to their density, sediment and thus be concentrated and analysed with the collected protein samples [3].

Obtained SDS-PAGE results showed that the synthesis of the recombinant putative VP1 protein of the non-canonical genomic organisation PyV was not detected in *S. cerevisiae* yeast, either by expression of the full-length VP1 gene or by its predicted splice variants. In order to further investigate putative VP1 protein assembly into VLPs, additional studies are being performed.

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E41

DEVELOPMENT OF THE BACTERIAL AMIDOHYDROLASE YQFB TO IMPROVE ITS BIOTHERAPEUTIC APPLICATIONS

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Amidohydrolases are a large class of enzymes catalyzing the hydrolysis of a wide range of substrates bearing amide or ester functional groups at the carbon and phosphorus centers. Due to their functionality, amidohydrolases are applicable in various fields, starting from research assaying their role in metabolic pathways, such as the metabolism of certain amino acids and nucleotides, and ending with the usage of these enzymes in chemical synthesis, food, and cosmetic industry [1]. Nevertheless, a remaining conundrum regarding the application of amidohydrolases in biotherapy is the targeted delivery of these enzymes to malignant cells or affected tissues. Therefore, the aim of this study was to develop modified variants of YqfB amidohydrolase which possess alterations in the amino acid sequence not interfering with its catalytic activity, but providing improved biotherapeutic applications.

Firstly, targeted mutagenesis of the sequence encoding YqfB amidohydrolase was performed by PCR using synthetic primers. Next, the mutant sequences were transformed into an *Escherichia coli* strain (*HMS174ΔpyrFΔyqfB*), in which the protein expression was later induced using IPTG at 20 °C for 22 hours. The bacterial biomass was collected and lysed. The final steps included the collection of the soluble protein extract via centrifugation and the verification of enzyme activity by thin-layer chromatography using the soluble protein extract and N^4-acetylcytosine as a substrate.

The results showed that modifications that were tested in the amino acid sequence of YqfB did not interfere with its enzymatic activity towards N^4-acetylcytosine. This not only indicated sites in the protein's amino acid sequence amenable to modification without compromising enzymatic activity, but also opened up the possibility of more efficient application of modified YqfB variants in biotherapy, which requires further research.

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E42

pmrB GENE DELETION IN *ACINETOBACTER BAUMANNII* AND ITS ROLE IN COLISTIN RESISTANCE

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Acinetobacter baumannii is an aerobic, Gram-negative opportunistic pathogen that primarily affects immunocompromised individuals. Decades ago, infections caused by *A. baumannii* were effectively treated with standard antibiotics such as aminoglycosides, β-lactams, and fluoroquinolones. However, the bacterium has developed multiple resistance mechanisms, rendering these drugs ineffective and necessitating the use of alternative treatments [1]. For example, colistin, which disrupts the bacterial outer membrane, is now considered the antibiotic of last resort against multidrug-resistant *A. baumannii* infections [2]. Unfortunately, colistin resistance is becoming increasingly common, mainly due to mutations in genes involved in lipid A synthesis, such as *pmrB* and *lpxACD* [3]. These mutations alter lipid A structure, reducing colistin's effectiveness.

A study of *A. baumannii* isolates collected at Vilnius University Hospital Santaros Klinikos between 2022 and 2023 revealed that 81% of tested isolates were resistant to colistin. In this research, potential colistin resistance genes (*lpxACD* and *pmrB*) which had been previously identified in the literature, were analyzed. Gene sequences from the collected isolates were compared, revealing non-synonymous mutations in different genes across various isolates. Protein pattern analysis showed no significant visual changes in LpxC and LpxD, whereas the PmrB pattern exhibited clear alterations.

Since mutations in the *pmrB* gene could affect protein folding, it was decided to test how the absence of this gene would affect the isolates. To further investigate the role of *pmrB* gene in colistin resistance, primers were designed for targeted deletion of this gene in a colistin-sensitive isolate. The upstream and downstream regions of the *pmrB* deletion, along with a gentamicin resistance cassette for transformant selection, were amplified via PCR and assembled. The resulting construct was transformed into *Escherichia coli* to generate a deletion plasmid. After purification, electroporation into a susceptible *A. baumannii* isolate was attempted, although no successful transformants have been obtained so far. Future work will focus on obtaining transformants with a deleted *pmrB* gene and assessing colistin susceptibility changes. Once successful deletions are achieved, the transformation of both sensitive and resistant isolates will be performed to evaluate whether the deletion leads to increased resistance or restored sensitivity to colistin.

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E43

THE GROWTH OF *C. ALBICANS* YEAST UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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Candida albicans is one of the most important opportunistic pathogens that cause thousands of superficial or disseminated and fatal infections in immunocompromised individuals yearly. The ability of these cells to change their morphology from single yeast cells to pseudohyphae and hyphae increases virulence and helps to infect the host [1]. Hyphal forms facilitate immune system evasion and can have a higher expression of efflux pumps which increases resistance to drugs [2]. To make the treatment against *Candida* pathogens more effective, learning more about physiological changes in cells during the formation of hyphae forms is necessary.

This study aimed to evaluate the effects of the medium composition and the aeration rate on *C. albicans* wild-type yeast growth using a personal bioreactor RTS-1 (Biosan). This bioreactor allows the cells to grow, rotating the tube with the yeast suspension in the bioreactor and changing the direction of the rotation at defined time intervals. Such type of agitation helps to ensure efficient aeration and online monitoring of the optical density (OD) of the cell suspension. The experiments were performed in aerobic and/or anaerobic conditions, under different rotation/aeration rates, using two types of medium - YPD or RPMI. It was detected that in YPD medium cell cultures grow to higher optical densities than in RPMI medium. Using a slower rotation/aeration the growth of the culture was approaching the growth at anaerobic conditions. It was observed that cells grown in the YPD medium do not form pseudohyphae, but in the RPMI medium, the real pseudohyphae are formed. The medium's volume also affected cell morphology: after 24 hours of incubation in 25 ml of RPMI medium pseudohyphae only started to form, but in 10 ml volume of this medium real pseudohyphae were observed. Moreover, the rotation rate affected the efficiency of the most popular antifungal compound fluconazole. Using the highest rotation rate (2000 rpm), the cells continued to grow and reached the highest levels of OD in the presence of 128 µg/ml fluconazole, but at lower aeration rates, the same concentration of this drug highly suppressed the growth of *C. albicans* cells.

These findings deepen our understanding of *C. albicans* culture growth and morphology changes which, in turn, will help fight the multidrug resistance of these yeast.

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E44

APPLICATION OF ENZYMATIC ASSAYS FOR CHARACTERIZATION OF PLASTIC-DEGRADERS

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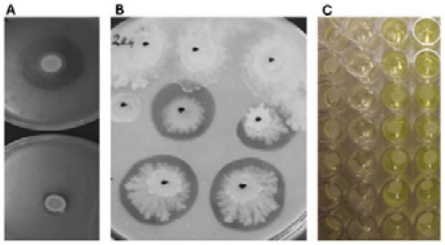
Biodegradation of plastic by microorganisms is based on enzymatic activity. Hydrolytic enzymes like phosphatases, esterases, lipases, proteases/peptidases, and others are essential in this process [1,2]. The present study was focused on characterization of bacterial consortium and pure isolates by their hydrolytic activity after incubation with microplastic. Bacteria with a plastic-degrading potential were isolated from landfill.

The screening of non-specific esterase activity of microbial isolates was carried out through agar plate-based screening methodology. Tributyrin (TBT; short- chain triglyceride) and Impranil®-DLN assays were performed as described earlier [3]. The use of TBT will detect the activities of lipolytic enzymes (for instance, esterases, true lipases and phospholipases). Impranil-DLN® emulsion was reported as a substrate for polyurethanase screening [4]. Zones of clearance formed around the bacterial colonies indicate the production of catalytically active enzymes able to degrade the added substrate (Fig.1A, B). We hypothesized that this activity may correlate with the bacteria's potential for plastic degradation.

Enzymatic activity of planktonic cells and biofilm on polyethylene terephthalate (PET) and polyethylene (PE) granules after 42-days incubation was evaluated by performing fluorescein diacetate (FDA) hydrolysis [5], with some modifications. The FDA hydrolysis assay is based on the hydrolysis of fluorescein diacetate by metabolically active bacteria producing fluorescein, which can be measured photometrically at λ=492 nm. Among the enzymes involved in FDA hydrolysis are: lipases, proteases and esterases. Recently, FDA hydrolysis has been used to characterize microbial responses to microplastics [6]. The experiments were carried out in 96-well microplates at 30 °C (Fig.1C).

Figure 1. Quantification of microbial enzymatic activity related to plastic degradation. Agar-based plate screening method with pure cultures of *Bacillus* spp.: A – tributyrin assay; B – Impranil®-DLN assay; C – fluorescein diacetate hydrolysis activity of biofilm.



The results have revealed a non-specific esterase activity, which varied depending on strain and medium composition. Comparative testing detected the highest activity in *Bacillus altitudinis* and *B. licheniformis*. In turn, the FDA hydrolysis activity of biofilm greatly varied in replicates within the same treatment type, while the testing of planktonic cells showed a high reproducibility.

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E45

SINGLE-CELL RT-PCR FROM PFA-FIXED BACTERIAL SAMPLES

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Transcriptomics studies enhance our understanding of organisms, their regulatory mechanisms and their responses to changing environmental conditions. However, studies at the population level do not take into account the heterogeneity inherent in the population. Therefore, single-cell analysis is conducted to observe emerging differences. Despite significant progress in the transcriptomic analysis of eukaryotic cells, the analysis of prokaryotic cells remains relatively constrained [1]. This is due to several challenges: prokaryotic messenger RNA (mRNA) molecules lack polyadenylation, the number of mRNA molecules within cells is low, and prokaryotic cells exhibit extreme resistance to lysis. Additionally, mRNA molecules have a short lifespan, often lasting only a few minutes [2]. Therefore, to avoid the risk of altering the RNA profile, it is critical to ensure optimal cell fixation and RNA stability before initiating the workflow.

In this work, it was tried to optimize the fixation of the microbiological samples with the aim to achieve highest RNA stability. One of the optimization steps involved selecting fixation reagent among formaldehyde, glutaraldehyde and paraformaldehyde (PFA) and determining its optimal concentration. The following reactions aimed to determine the most effective fixation duration and the compatibility of fixed cells with different buffer solutions. To ensure that the fixation process is compatible with subsequent multi-step enzymatic reactions, fixed cells were isolated in semi-permeable capsules, followed be reverse transcription (RT) and polymerase chain reaction (PCR), which are the primary enzymatic reactions used to analyze cell transcription profiles.

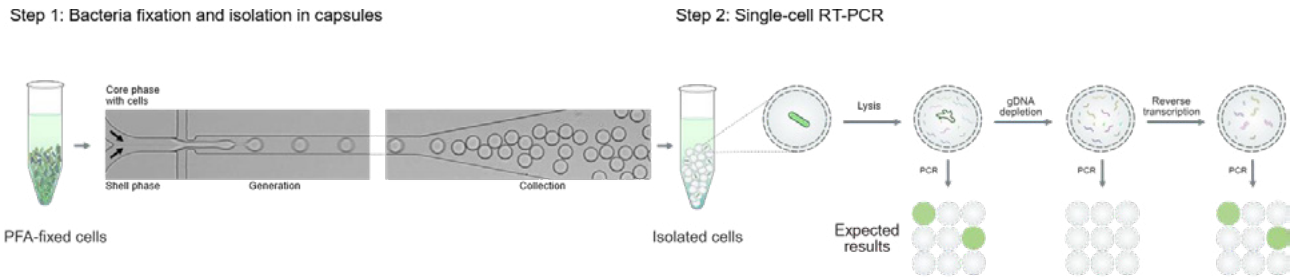

Figure 1. Experimental workflow to test RT-PCR from fixed bacterial samples.

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E46

Applications of Prussian Blue in bio-analytical and bio-energy systems

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Iron hexacyanoferrate (Prussian blue (PB)) is an inorganic, electrochromic compound, which is selective for several monovalent ions (Cs+, Rb+, K+ and NH4+). These ions (Cs+, Rb+, K+ and NH4+) are incorporated in the crystal lattice of PB when PB is electrochemically reduced in Cs+, Rb+, K+ or NH4+ ions containing solution [1]. Moreover, the concentration of PB reduction promoting ions (Cs+, Rb+, K+ and NH4+) affects the reduction potential of PB. For this reason, PB can be used as a signal transducer in optical and electrochemical analytical and energy systems, such as electrochemical ion sensors and yeast – based bio-cells [2, 3]. Thus, Prussian Blue is often used as a redox mediator and signal transducer in biosensors, for example urea and glucose biosensors [4, 5] and Prussian Blue – modified yeast – based bio-cell [3].

In this research work, applications of Prussian Blue as redox mediator, are demonstrated. Prussian Blue was used in formation of glass/FTO/PB/enzyme type electrodes, for the analysis of glucose and urea. This study introduces the glass/FTO/PB/GOx electrode for optical glucose sensing and the glass/FTO/PB/urease electrode for electrochemical sensing of urea by using electrochemical impedance spectroscopy. Moreover, a method for Baker's yeast modification with Prussian Blue is introduced and a construction and principle of operation of PB-modified yeast – based bio-cell (fig.1) is demonstrated.

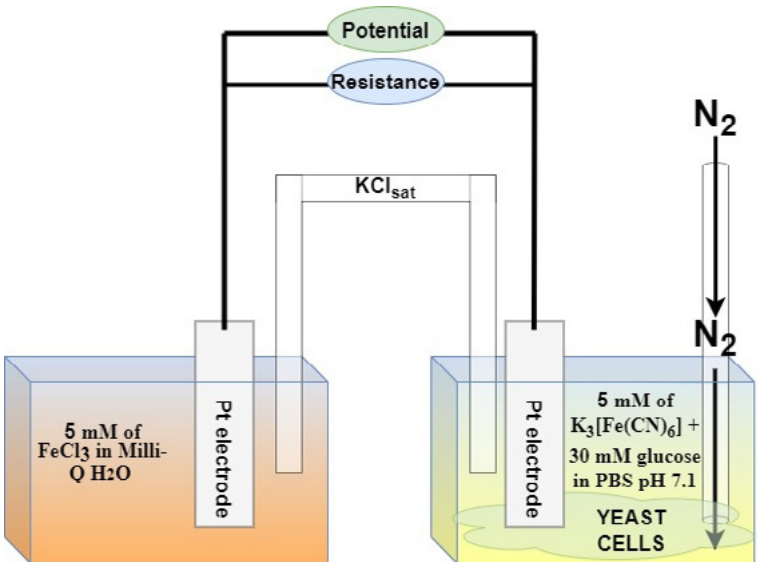

Figure 1. A scheme of PB-modified yeast – based bio-cell.

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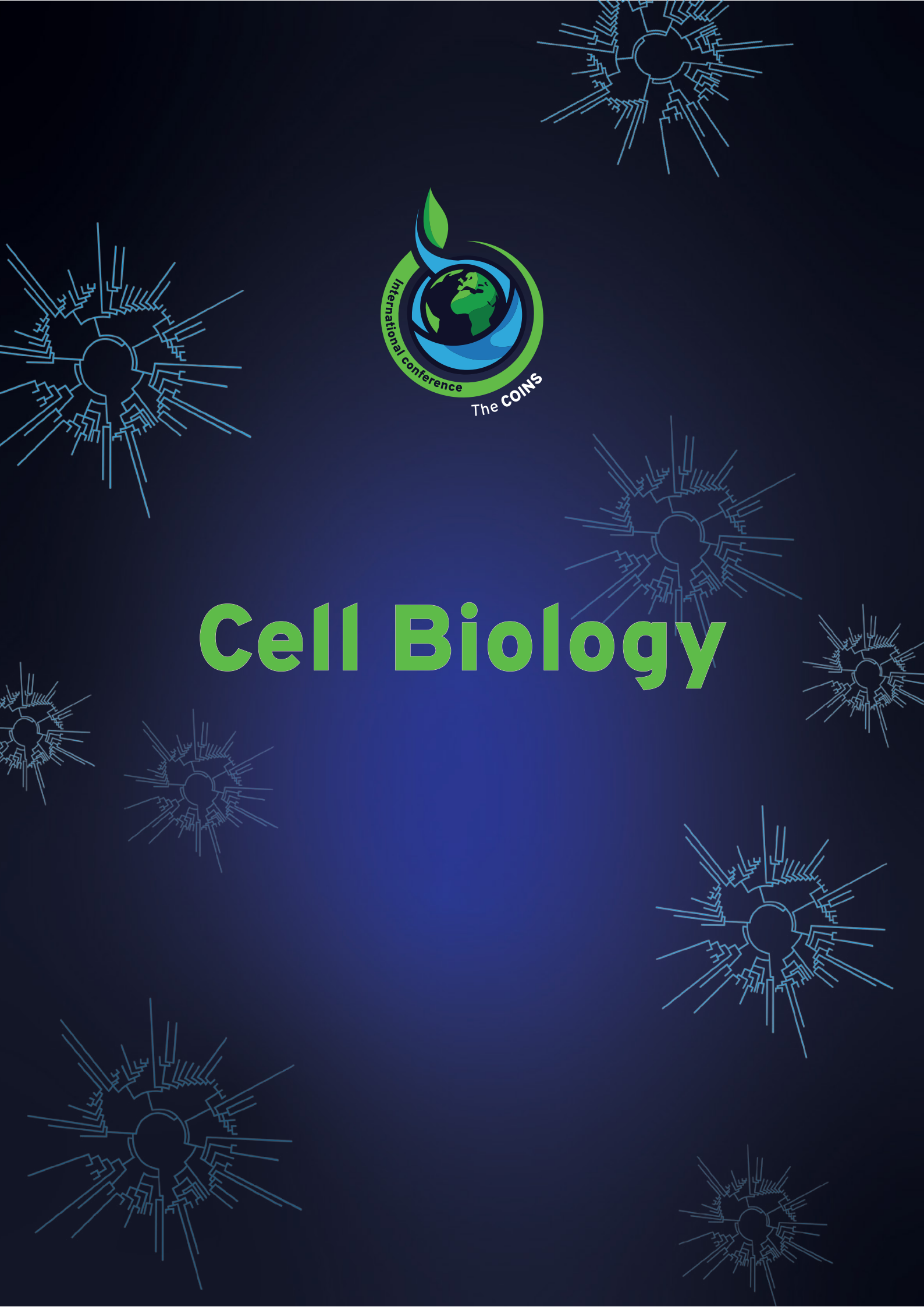

Cell Biology

F1

PI3K/AKT/mTOR SIGNALING PATHWAY INHIBITORS INDUCE APOPTOSIS IN HUMAN ENDOMETRIAL CANCER CELLS

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Endometrial cancer is the 6th most common cancer in women, with Lithuania and Poland leading in Europe [1]. Common risk factors of endometrial cancer are older age, obesity, hyperglycemia, diabetes and certain genetic disorders, but why Lithuania and Poland have the highest rates of endometrial cancer remains unknown. Surgery is usually the first-line treatment for endometrial cancer, if there is any risk of cancer recurrence, chemotherapy is applied afterward. However, the most common medicines used for endometrial cancer such as paclitaxel, carboplatin, and cisplatin have many downsides, including their non-specificity. As an alternative to classical chemotherapy, targeted therapies are continuously being developed and actively investigated [2]. These compounds are also widely applied in personalized medicine [3].

In this study, we aimed to explore the effects of different clinically approved targeted therapy compounds - PI3K/AKT/mTOR and receptor tyrosine kinase inhibitors, regulators of cell cycle, and others - on endometrial cancer cell lines, established in our laboratory from the tumor tissue of Lithuanian endometrial cancer patients. Firstly, we determined the most effective targeted therapy drugs by measuring endometrial cancer cell viability after treatment. We investigated apoptosis induction after treatment with the most cytotoxic compounds by fluorescent microscopy and flow cytometry. Results revealed that in the endometrial cancer cells, apoptosis was induced after exposure to nine of the tested compounds, however, PI3K/AKT/mTOR inhibitors such as MK-2206, apitolisib and samotolisib had the strongest pro-apoptotic activity.

This work was funded by Vilnius University Science Promotion Fund (project no. MSF-JM-17/2022) and Future Biomedicine Fund (project no. (1.78) SU-442).

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F2

THREE-DIMENSIONAL (3D) *IN VITRO* MODELS OF ENDOMETRIAL CANCER FOR PRECLINICAL DRUG TESTING

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Endometrial cancer is the most common gynecologic malignancy and a significant contributor to morbidity and mortality in females [1]. It is broadly categorized into two main subtypes, Type I and Type II, based on clinicopathologic and molecular characteristics. Type II tumors are typically non-endometrioid histology, estrogen-independent, and commonly associated with endometrial atrophy. Increased risk of endometrial cancer (EC) is associated with increased age, certain ethnicities, higher BMI, endogenous or exogenous estrogen exposure, early menarche, late menopause, lower parity, metabolic syndrome, family history and genetic predisposition [2]. Surgery is the primary treatment for localized early-stage disease. Chemotherapy is primarily beneficial in treating serous or advanced-stage endometrial cancers, which tend to recur distally. Carboplatin and paclitaxel are recommended as first-line agents for endometrial cancer when chemotherapy is indicated. However, new targeted therapy agents are constantly under development [3]. Nowadays, there are several preclinical models in endometrial cancer to evaluate drug efficacy and predict patient outcomes. Traditional 2D monolayer cell cultures have long been the standard model for cancer research. However, their ability to accurately mimic the complex molecular mechanisms of tumors *in vivo* is limited. The emergence of 3D cell culture models has provided a more physiologically relevant alternative, better capturing key tumor characteristics such as cellular heterogeneity, interactions with the extracellular matrix (ECM), hypoxic microenvironments, and ECM-dependent signaling pathways [3]. Additionally, 3D cultures exhibit more realistic growth kinetics, drug responses, gene expression profiles, and epigenetic patterns, making them a valuable tool for cancer biology studies.

In this study, 3D cultures were grown from novel endometrial cancer cell lines, previously derived in our laboratory from the tumor tissue of Lithuanian endometrial cancer patients. The effect of the drugs was assessed by changes in spheroid or aggregate size and metabolism after treatment. Cell viability after various targeted therapy compounds was evaluated using the XTT method. Different clinically approved targeted therapy compounds such as PI3K/AKT/mTOR and receptor tyrosine kinase inhibitors, cell cycle, proliferation and survival regulators were investigated. Our results revealed that the most effective compounds were nuclear transport inhibitor selinexor, PI3K/AKT/mTOR inhibitors such as sapanisertib, samotolisib, apitolisib, MK-2206 as well as proliferation regulator selumetinib.

This work was funded by Vilnius University Science Promotion Fund (project no. MSF-JM-17/2022), Future Biomedicine Fund (project no. (1.78) SU-442) and Research Council of Lithuania (project no. S-ST-24-101).

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F3

Exploring the Cell Entry Potential of Wuhan Mosquito Virus 6 Glycoprotein

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The prevalence of segmented RNA viruses in a variety of organisms became possible because of sequencing and bioinformatics breakthroughs. Consequently, viruses of Thogotovirus and Quaranjavirus, lesser-known genera of the Orthomyxoviridae family, were more frequently observed not only in invertebrates but also in vertebrates. These observations have sparked numerous studies focused on the zoonotic potential of new orthomyxoviruses to cause an epidemic. Our research object is the surface glycoprotein of a quaranjavirus-like Wuhan mosquito virus 6 (WuMV-6), which is detectable in mosquito metagenomics data worldwide.

The goal of this study is to experimentally test the membrane fusion capabilities of WuMV-6 glycoprotein gp64, the sequence of which was determined using bioinformatics tools. Frist viral infection's step - cell entry - can be tested by producing pseudotyped lentiviral vectors with WuMV-6 gp64 integrated into their envelope and infecting cells with them in vitro. Successful WuMV-6 infection can only occur if gp64 binds with cell surface receptors. We used pseudoviruses, each bearing one of three WuMV-6 gp64 sequence variants, SINUV gp64, AcMNPV gp64, or VSV G.

Synthesis of C terminal hemagglutinin-tagged viral glycoproteins in mammalian HEK293T/17 cells was confirmed, although the efficiency of each glycoprotein's synthesis differed. Pseudoviruses coated with previously mentioned glycoproteins and carrying RNA with reporter gene nanoluciferase (nLuc) were produced by HEK293T. Additionally, VSV pseudoviruses with EGFP gene transcripts were synthesized, during which the nLuc protein was also synthesized. SINUV and WuMV-6 pseudoviruses were coated with their respective gp64 but SINUV gp64 was incorporated more than WuMV-6 gp64. The quantification of pseudoviruses was determined with TaqMan qPCR. *Culex tarsalis* mosquito (natural WuMV-6 host) cells and chicken mononuclear blood cells were infected with pseudoviruses. Infection efficiency was evaluated by the strength of chemiluminescence from infected cells. According to nLuc analysis, mosquito and chicken blood cells were infected with SINUV pseudoviruses. However, variation in the infection capability of pseudoviruses produced at separate times was noticed, highlighting the importance of quality checks. Nonetheless WuMV-6 gp64 successfully mediated pseudovirus entry into *Cx. tarsalis* cells in vitro. Cells infected with VSV pseudoviruses carrying EGFP RNA still generated a chemiluminescence signal. Due to nLuc background, these results should be tested by another method, such as producing antibiotic resistance gene RNA transporting pseudoviruses.

F4

THE IMPACT OF *L. CASEI* SUPERNATANT ON ENDOMETRIUM STROMAL CELL DECIDUALIZATION

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Infertility has been recognised as a worldwide health issue with the rate of infertility increasing in recent years. Various factors can cause female infertility most of which fall in the category of female reproductive system disorders, however the molecular mechanisms related to infertility are often unknown. A growing number of studies show that there is a high correlation between the composition of female reproductive organ microbiota and infertility. Recent studies revealed that the most abundant bacteria genus in the female reproductive organs is *Lactobacillus*, and that the bacteria of this genus can have a positive impact on the function of female reproductive system organs [1].

The human endometrium - inner layer of the uterus - is a dynamic tissue which experiences cyclic changes like growth and shedding [2]. It is composed of four cell populations: epithelial cells, stromal cells, immune cells and endothelial cells [3]. Endometrium decidualization refers to a process that occurs during every menstrual cycle when endometrium stromal cells (EnSCs) differentiate into specialised secretory cells. During decidualization, EnSCs go through functional, morphological and genetic changes in order to form the decidual lining into which the blastocyst implants. Improper decidualization response can be a determinant of several pregnancy complications, not only in early but also in advanced gestation [4, 5]. This demonstrates the importance of decidualization for female reproductive health.

In this study, we aimed to determine the effect of the supernatant of *Lacticaseibacillus casei* on decidualized and non-decidualized EnSCs by studying cell viability and changes in gene expression. EnSCs were isolated from endometrial tissue obtained from female patients undergoing *in vitro fertilization* (IVF). EnSC decidualization was induced with 8-bromo-cAMP and MPA and cells were treated with 5X, 20X, 50X and 100X *L. casei* supernatant dilutions for 72 hours. To assess EnSC viability, propidium iodide in conjunction with Annexin V were used to evaluate viable, apoptotic and necrotic EnSC populations with flow cytometry. Using RT-qPCR, changes in *IGFBP1* and *PRL* gene expression of non-differentiated and decidualized EnSCs treated with the supernatant of *L. casei* were analysed.

In both pregnant and non-pregnant patient cell lines it was shown that the 5X *L. casei* supernatant dilution significantly reduced EnSC viability – over 90 % of the cells fell in the category of late apoptotic or necrotic cells. However, higher dilutions such as 20X, 50X and 100X showed no significant difference in cell populations compared to control EnSCs, the ones that had not been treated with the supernatant of *L. casei*. RT-qPCR results revealed that EnSC decidualization was successfully induced, as visible by the increase of the expression of *IGFBP1* and *PRL* genes in cells after the induction of decidualization. Furthermore, the highest gene expression levels of these genes were reached in cells treated with 20X dilution of the supernatant of *L. casei*, showing the possible significance of the metabolites of *L. casei* for gene expression regulation during decidualization.

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F5

CONSTRUCTION OF ENDOMETRIAL ORGANOIDS

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As the inner lining of the uterus, the human endometrium dynamically changes under ovarian hormones to prepare the tissue and its microenvironment for trophoblast implantation, nutritional support of the conceptus, and successful pregnancy [1]. Impaired function of endometrium often leads to various gynecological problems and the main obstacle in their studies is the absence of ideal model systems. Recently, organoids, the term describing 3D cell arrangements, have been gaining more and more attention since, in comparison to commonly used 2D monolayer culturing methods, they more closely resemble organ or tissue *in vivo* biology, reproduce many aspects of its functions and have genetic as well as phenotypic stability properties that are useful for long-term expansion [2,3].

In this work, we aimed to construct endometrial organoids from patient-derived endometrium samples and an immortalized endometrial cell line. Firstly, endometrial cells were extracted from *ex vivo* tissue samples and characterized morphologically and by cell surface markers (CD13, CD15, CD90, etc.) using flow cytometry analysis. For organoid construction, patient-derived endometrial epithelial cell clusters were cultured in a commercially available cell basement membrane solution that polymerizes to mimic extracellular matrix conditions. Additionally, immortalized endometrial adenocarcinoma cell line KLE organoids were formed under similar culturing conditions.

Characterization of endometrial cells, extracted from *ex vivo* tissue samples, revealed two cell types, indicated by their unique morphology and surface marker phenotypes – stromal (69% of which were $CD13^+$, 58% – $CD90^+$, and were negative for CD15) and epithelial (16% of which were $CD15^+$, 32% – $CD13^+$, 11% – $CD90^+$) – the latter being used for organoid construction. We explored variations in organoid-forming conditions such as cell seeding density. Our cultivation results show that constructed endometrial organoids conform to typical morphological organoid characteristics: epithelial cells cultured in an extracellular matrix form 3D spherical organoid architecture and maintain their structure even after passaging. Conversely, KLE cells assemble in more disorganized spherical and multicellular 3D structures.

Overall, our study demonstrates that patient-derived endometrial epithelial cells and KLE cells can be utilized for the construction of organoids that model human endometrium, providing a platform to research implantation and other endometrial physiology aspects.

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F6

The Modulation of Notch Signaling Activity in Endometrial Cancer Cells

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Endometrial cancer (EC) is the sixth most common malignancy among women in the world, with the incidence rising over the past years. A high prevalence of EC is observed in the developed countries of North America and Europe and is linked to increased age, obesity, late menopause, estrogen exposure, and others [1]. In addition to surgery, chemotherapy is a standard adjuvant therapy approach applied to treat EC. Nevertheless, due to commonly acquired chemoresistance and toxicity to non-malignant cells, more effective targeted treatment options are currently being developed [2].

It was found that some signaling pathways are related to the incidence and development of endometrial cancer. One of them is the Notch signaling pathway - a highly conserved signaling mechanism implicated in key cellular processes such as proliferation, survival, and differentiation. Abnormal Notch signaling is linked to many diseases, including various types of cancer, EC being one of them. However, due to limited and conflicting data, the understanding of the role of this pathway in EC cells remains lacking [3, 4].

To better grasp the importance of Notch signaling in EC cells, we analyzed the effect of Notch inhibition and activation on the viability of four EC cell lines: CRL-230315C, CRL-230315Dif, and CRL-230407, which are derived from endometrial tumors of Lithuanian women, as well as commercial cell line KLE. To block Notch signaling two inhibitors were employed: nirogacestat, a γ-secretase inhibitor, and IMR-1, an inhibitor of NICD ternary complex formation. Additionally, a probable Notch activator, ataluren, an inhibitor of early translation termination due to nonsense mutations, was used. We further assessed the impact of Notch inhibitors on KLE cell cycle progression, apoptosis, motility and susceptibility to chemotherapeutic drugs cisplatin and paclitaxel. We found that Notch activity modulators have varying effects on the viability of studied EC cell lines. Additional experiments with KLE cells showed that the inhibitors of the Notch signaling pathway induce apoptosis, but have no effect on their cycle, migration and sensitivity to chemotherapy.

Our findings suggest the potential role of the Notch signaling pathway as a therapeutic target in some cases of EC. Nevertheless, further studies are required to gain a deeper understanding of the mechanisms underlying the carcinogenesis of endometrium in relation to Notch signaling.

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F7

STUDY OF COLORECTAL CANCER CELLS WITH DIFFERENT SENSITIVITIES TO CHEMOTERAPEUTIC DRUGS AFTER EXPOSURE TO TARGETED THERAPY COMPOUNDS

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A few decades ago, colorectal cancer was rarely diagnosed. Today it is the third most frequently diagnosed cancer, and the second most common cause of cancer-related deaths worldwide [1]. Many chemotherapy drugs are used to treat this cancer, but in many cases the effectiveness of the drug treatment is impaired by the tumour's innate or acquired resistance to chemotherapy treatment. Tumour tolerance to chemotherapy can be associated with a reduced drug uptake, drug efflux, genetic or epigenetic changes, an enhanced repair of DNA damage and inhibition of cell death [2].

To study the innate resistance of cells to chemotherapy, the colorectal cancer cell line DLD-1 was first cloned by seeding the cells one at a time in separate plates and waiting for individual clonal populations to form. From the 26 clones produced, those with a different sensitivity to chemotherapeutic drugs oxaliplatin and cisplatin compared to the parental line were selected. Clones were treated with targeted therapy compounds, that are clinically approved for colorectal cancer treatment. Cell sensitivity to targeted therapy compounds was assayed by crystal violet method. Results showed that the Z clone is hypersensitive to the VEGFR inhibitors sorafenib, nintedanib and lenvatinib, while the K clone has an increased resistance to sorafenib and nintedanib, in addition, clone K has an increased resistance to the mTORC1/2 inhibitors samotolisib, apitolisib, sapanisertib and rapamycin, and the Akt inhibitor MK-2206 compared to the parental DLD-1 line.

In conclusion, our results indicate that intratumor heterogeneity of colorectal cancer stems from alterations in VEGFR and mTOR/Akt signaling pathways.

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F8

THE ENGINEERING OF HUMAN CELL SPHEROID 3D CULTURE MODEL IN THE HYDROGELS

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Cancer remains one of the most important health problems in the world, causing the death of several million people every year. According to statistics published by the World Health Organization, cancer is the second most common cause of death worldwide, after cardiovascular diseases. Although cancer treatment depends on its type, stage, and the patient's health condition, the most commonly used cancer treatment methods currently include surgical removal of the cancer, radiation therapy, chemo and biological therapy. These treatment methods may not be effective at all if cancer is not diagnosed at an early enough stage. They also often cause a number of side effects, such as reduced immunity, damage to healthy organs, fatigue, etc. Scientists are looking for new, more effective methods for treating cancer. Before testing the effects of developed anticancer drugs on humans, detailed laboratory studies must be conducted to ensure that the drugs are not harmful to human health. Studies in cell cultures are one of the standard research methods used to study the effects of substances without directly testing them on humans.

The aim of our work was to create a unique 3D spheroid culture model using the hanging drop method and human 293T fibroblasts, endothelial cells (EC), glioblastoma U87 cancer cells and 3D hydrogels.

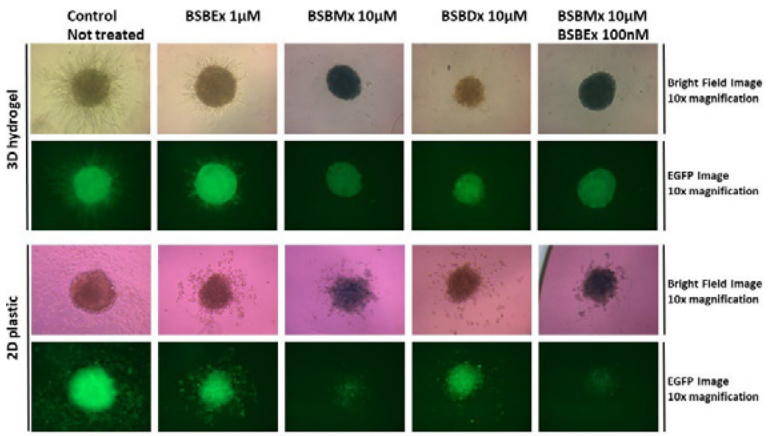
The main tasks of the work presented here were as follows : a) to create spheroids with different cell numbers in hanging drops, b) to place formed cell spheroids on a 2D plastic surface or in 3D hydrogels, c) to analyze the effect of three different chemical drugs on the viability of single U87 cells and cell spheroids and d) to photograph cells/cell spheroids and to analyze the obtained results.

The human cells, which were genetically modified by introducing the GFP gene (Green fluorescent protein) of the jellyfish *Aequorea Victoria*, were grown under sterile conditions in the plastic dishes supplemented with complete serum containing DMEM medium in CO2 incubator at +37C. When such cells were illuminated with the blue light and observed through a fluorescence microscope, they had green fluorescence. By using the enzyme trypsin with EDTA, we detached cells from the plastic surface. After counting in a Neubauer chamber, we placed 300, 1000, 3000 and 10000 cells in hanging drops with medium containing methylcellulose. We observed that 293T and EC cells formed stable spheroids in less than 24 hours. Meanwhile, U87 cells formed compact spheroids only after 72 hours. We found that spheroids with different cell numbers also differed in size as observed under a microscope. When the spheroids were placed on a plastic surface in the complete nutrient medium, the cells migrated from the spheroids onto the plastic surface and formed a cell monolayer. Meanwhile, when spheroids were placed in 3D hydrogels in serum-free medium, cells did not migrate, but in serum-containing medium, they began to migrate from the spheroids into the 3D hydrogels and the spheroids appeared as if they had “long protrusions”. The most intense migration was observed after 48-72 hours. We also exposed single U87-EGFP cells seeded in 96-well plates and their spheroids grown in 2D and 3D cultures to three chemotherapeutic agents and measured cell viability using the MTT method. Furthermore, we evaluated changes in cell morphology and GFP fluorescence under the microscope. We found that low concentrations of chemotherapeutic agents (10-100nM) had little effect on cell morphology or viability. However, higher concentrations (1-10μM) inhibited cell proliferation, migration and induced cell death. Single cells exposed to higher drug concentrations started to round up and detach from the plastic surface. When analyzing 3D spheroids, we noticed that dying cells lose intense green fluorescence and stop moving in 3D hydrogels. It is also interesting that combinations of chemotherapeutic agents killed tumor cells much more effectively than single drugs.

In conclusion, we have created a unique in vitro 3D cell culture model, which partially recapitulates the behavior of cells in the body and is suitable for high-througput novel drug discovery and for the evaluation of drug cytotoxicity and synergistic effectiveness.

This work will also be presented in the VGTU Lyceum Engineering Education Practice Program.

Figure 1. U87-EGFP glioblastoma cell spheroids in 3D hydrogels and on 2D plastic surface, treated with various chemotherapy drugs for three days and visualized by fluorescent microscopy.



F9

UNLOCKING POTENTIAL: TRANSFORMING DENTAL PULP MESENCHYMAL STEM CELLS INTO NEURAL STEM CELLS

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Glioblastomas (GBMs) are the most aggressive and lethal primary brain tumors, characterized by their rapid growth, invasive nature, and resistance to conventional therapies. Despite significant advancements in understanding their molecular and cellular mechanisms, patient survival rates remain dismally low [1]. A growing body of evidence suggests that glioblastoma stem cells (GSCs) – a subpopulation of tumor cells with self-renewal and tumor-initiating capabilities – play a critical role in tumor progression, therapy resistance, and recurrence [2]. As such, they represent a vital target for therapeutic intervention. Importantly, GSCs share striking similarities with neural stem cells (NSCs), including overlapping molecular markers, signaling pathways, and plasticity. These shared characteristics not only complicate the identification and targeting of GSCs but also underscore the importance of including NSCs in analyses to distinguish between normal and tumor-associated stem cell populations. Furthermore, NSCs contribute to the tumor microenvironment through their potential recruitment and interaction with GBM cells, influencing tumor behavior and treatment response [3]. By incorporating NSCs into glioblastoma research, we can refine our understanding of the unique and shared features of GSCs and NSCs, paving the way for more precise therapeutic strategies aimed at eradicating tumor-initiating cells while preserving normal brain function [3,4]. This integrative approach is essential for advancing the field of glioblastoma biology and improving clinical outcomes for patients. However, sourcing NSCs often presents ethical and technical challenges [5]. Dedifferentiating somatic cells like human dental pulp mesenchymal stem cells (hDPSCs) into NSCs represents a groundbreaking approach, leveraging the accessibility and multipotency of hDPSCs while bypassing these constraints. The reprogramming approach is regarded as safer and is in accordance with current EU regulations [6].

Consequently, we aim that dental pulp-derived mesenchymal stem cells have the potential to dedifferentiate into NSCs, exhibiting characteristics comparable to those of brain-derived NSCs.

The research was done in Lithuanian University of Health Sciences, Neurosience institute, Molecular neurooncology laboratory and was approved by the Kaunas region Bioethics Committee. In total, four different cell lines derived from human dental pulp were previously validated as mesenchymal stem cells. For the induction of MSCs to dedifferentiate into NSCs were used DMEM/F12 conditioning media supplemented with B27, N2, and bFGF and EGF factors. After the initial 8 hours of dedifferentiation, some of the non-adherent and spheroid-forming cells were transferred to a low-adhesive plastic dish, where dedifferentiation continued in neurospheres form. For gene expression studies, cells were collected after 8 hours, 1 and 2 weeks of dedifferentiation. RNA was extracted from cell pellets using TRIzol reagent and subsequently converted into cDNA with the High-Capacity RNA-to-cDNA kit. The expression levels of neural stem cell-specific markers were measured using RT-qPCR. The differences in expression were calculated using the 2^-dCT method which revieled mRNA changes in the genes *CDK5R1, MAP2* and *MSI1*, compared to the control.

The study revealed that dental pulp MSCs have the ability to dedifferentiate into neural stem cells, however, the efficiency of this dedifferentiation was significantly lower compared to the neural stem cell-specific molecular markers observed in comercial neural stem cells.

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F10

LINE-1 PROMOTER METHYLATION PATTERNS AS A PREDICTIVE MARKER FOR PRECANCEROUS AND CANCEROUS COLORECTAL LESIONS, ANALYZED SEPARATELY BY GENDER

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Colorectal cancer ranks as one of the most prevalent cancer types, occupying the third position in terms of incidence and the second position in cancer-related mortality, both globally and in Lithuania [1]. The initial indication of colorectal cancer is hyperplastic polyps that develop on the intestinal lining. Due to novel mutations and alterations in genome methylation patterns, conventional adenomas or serrated lesions may emerge. Serrated lesions have higher potential malignancy and distinguishing them from tubular adenoma is complicated [2]. Research is actively focused on understanding the differences between non-malignant polyps and cancer, aiming to discover genetic, biological, and epigenetic markers that could enable the early detection of polyps before they progress to adenocarcinoma. Recent studies indicate that the pathways of colorectal cancer development may differ between men and women, as well as overall methylation in healthy tissue [3]. LINE-1 retrotransposon serves as a key epigenetic marker, facilitating the assessment of overall genome methylation patterns. The expression of LINE-1 retrotransposon and hypomethylation of its promoter is linked to genomic instability, providing insights into the potential risk for cancer progression [4]. The changes in LINE-1 promoter methylation can serve as an early biomarker for distinguishing potential malignancies.

The current study evaluated LINE-1 promoter methylation in tubular adenomas, serrated lesions, adenocarcinomas, and adjacent healthy tissue separately for male and female cohorts.

Samples of adenocarcinoma (men: n=9; women: n=11), tubular adenoma (men: n=18; women: n=13), serrated adenoma (men: n=7; women: n=9), and their corresponding adjacent normal tissue were collected (NCI). Quantitative analysis of LINE-1 promoter methylation at three CpG islands in bisulfite-converted DNA was performed using the pyrosequencing method (Qiagen). Methylation levels of three CpG islands: 328 (1 CpG), 321 (2 CpG), and 318 (3 CpG) (X58075.1), as well as their average methylation, were assessed in both male and female groups separately.

The results indicate that methylation patterns in tubular adenoma, adenocarcinoma, and serrated adenoma statistically significantly differ between male and female cohorts. Also, the differences in healthy tissue's methylation levels indicate possible early epigenetic changes that may lead to malignancies. The methylation status of the LINE-1 promoter may serve as an epigenetic marker for diagnosing precancerous and cancerous lesions in the colon. In evaluating methylation, gender must be considered for the most accurate diagnosis.

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F11

DISSECTING MECHANISMS OF ASTROGLIAL CELL DEVELOPMENT IN THE MODEL *C. ELEGANS*

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Harmony within the nervous system's functionality begins with the precise development of neurons and glial cells. Interactions of glia and neurons foster faithful circuit formation, vital for proper circuit function and behavior. Malfunction in glia development disturbs efficient connectivity between neurons, which results in compromised circuit function [1,2]. Therefore, it is essential to understand the mechanism of glia formation and potential factors implicated in their development, in order to comprehend how they drive the integrity of circuit architecture. While glia have recently become recognized as being morphologically complex, molecularly diverse, and essential for neural circuits, their development remains understudied. While some genes are known to be important for glia development, their role is not known yet. *Caenorhabditis elegans* offers a powerful setting to study glia biology due to stereotypical nervous system structure, easy genetical manipulation, mapped connectivity and transparent body, allowing relatively simple quantitative imaging of glial cellular features. Moreover, *C. elegans* presents specific glial cells with architecture, functionality and molecular content analogous to vertebrate glia [3,4]. These *C. elegans* astroglial cells project non-branching processes in early developmental stages for brain assembly and later ramify their membranes, to also ensheathe synapses [1]. Additionally, they express transcription factors and functional molecules with homologs enriched in vertebrate astroglia. Thus, studying genes implicated in development of *C. elegans* astroglial cells may have an impact in understanding conserved astroglia mechanisms beyond *C. elegans*.

To identify genes involved in *C. elegans* astroglia morphogenesis, we study animals mutated for genes with mouse homologs implicated in glial biology, or we perform unbiased mutagenesis screens to isolate new mutants. The role of these genes in *C. elegans* astroglial cell development has not been studied before and remains unknown. To investigate the molecular mechanisms at play, we analyze glial cell architecture using advanced genetics, quantitative *in-vivo* imaging and a toolkit of fluorescent reporters of (sub)cellular astroglia that we generated. Our research implicates conserved transcription factors, cell adhesion and extracellular molecules in glial cell development. We will report our updates on methodologies we establish, as well as the characterization of genes implicated in glia cell architecture.

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F12

EFFECTS OF HYPOXIA-REOXYGENATION ON HL-1 CARDIOMYOCYTE VIABILITY AND INTRACELLULAR MECHANISMS

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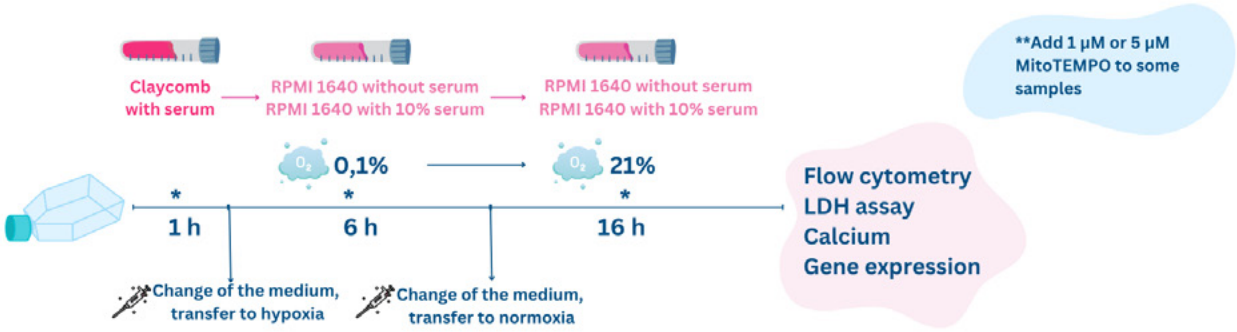
Globally, ischemic heart disease claims 9.14 million lives each year [1]. This condition impairs the delivery of oxygen and nutrients to the heart due to a reduction or complete blockage of blood flow in the coronary arteries [2]. When ischemia persists, surgical intervention is used to restore blood flow, triggering a process called reperfusion. However, the sudden reintroduction of oxygen to cardiomyocytes leads to oxidative stress, inflammation, and irreversible myocardial damage. In laboratory research, the hypoxia-reoxygenation model allows controlled investigation of these processes [3]. Therefore, this study aimed to develop an HL-1 cardiomyocyte hypoxia-reoxygenation model and evaluate the protective effects of mitochondrial antioxidant MitoTEMPO.

This study examined the impact of hypoxia-reoxygenation and the mitochondrial antioxidant MitoTEMPO on HL-1 cardiomyocyte viability, calcium levels, and gene expression. Hypoxia-reoxygenation was induced using 1% and 0.1% oxygen concentrations to determine which condition caused the most severe effects on HL-1 cardiomyocytes. The study also assessed the protective role of MitoTEMPO by analyzing its influence on LDH activity, early apoptosis, calcium levels, and the expression of genes linked to hypoxia (*Hif1a*) and calcium homeostasis (*Cacna1c*, *Atp2a2*, *Scl8a1*, *Ryr2*).

Figure 1. Scheme for studying the effects of MitoTEMPO during hypoxia-reoxygenation conditions



The results indicated that the most suitable hypoxia-reoxygenation model involved acute 0.1% hypoxia combined with serum-free RPMI 1640 medium. Under these conditions, the most pronounced differences between hypoxic and normoxic states were observed in LDH activity and apoptotic cell counts, facilitating further investigations into the effects of additional nutrients. The impact of MitoTEMPO was ambiguous; while it reduced LDH levels and apoptotic cell numbers, higher concentrations did not result in better cell viability. Cytoplasmic calcium levels increased under oxygenation conditions, but antioxidant treatment led to an even greater calcium rise in normoxia. The antioxidant's effects were primarily observed in the expression of *Canca1c* and *Ryr2*, where increasing MitoTEMPO concentrations reduced their expression under hypoxia.

In conclusion acute 0.1% hypoxia-reoxygenation induced cell membrane damage, while MitoTEMPO showed promising protective effects against such damage.

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F13

METABOLIC REPROGRAMMING USING RIBOFLAVIN TO INDUCE SYNTHETIC LETHALITY BY CAIX INHIBITORS

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Carbonic anhydrases (CAs) catalyze the reversible hydration reaction of carbon dioxide to bicarbonate ($HCO3^-$) ion and a proton (H^+). These enzymes are involved in many physiological activities in the body, such as acid-base homeostasis, calcification, carbon dioxide transport, pH control, and fluid balance control [1].

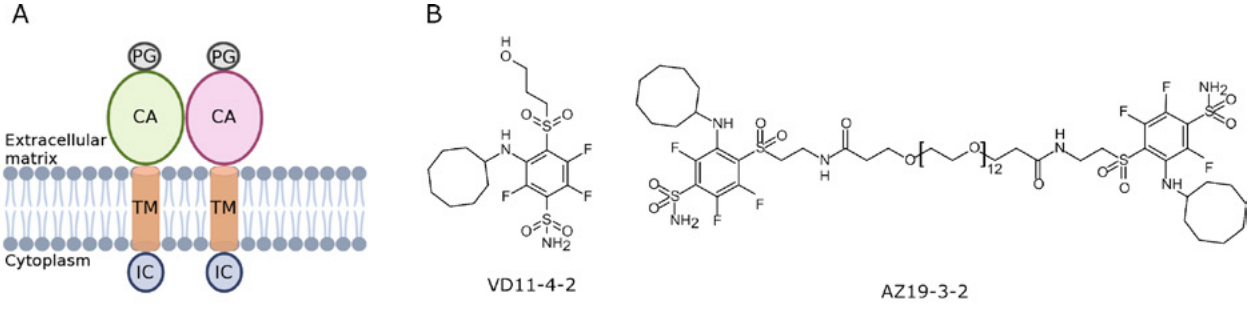
CAs are divided into eight families and are found in various organisms: protozoa, algae, plants, fungi, and animals. Humans have fifteen distinct carbonic anhydrases, all of which fall within the α-CA family. Impaired CA activity has been associated with various diseases and pathological conditions including glaucoma, obesity, epilepsy, acute mountain sickness, idiopathic intracranial hypertension, neuropathic pain, rheumatoid arthritis, oxidative stress, Alzheimer's disease and cancer [2].

Carbonic Anhydrase IX (CAIX) (Figure 1. A) is a dimeric membrane protein expressed in many types of malignancies, and in the stomach and testes of healthy people. Mostly present on the surface of cancer cells, CAIX helps tumour development and spreading under hypoxic conditions. Therapeutic targeting of CAIX is a highly relevant approach due to the critical role of this enzyme in maintaining acidic tumour microenvironment and its association with cancer progression and invasion. Developed inhibitors (Figure 1. B) of the CAIX activity must be effective and selective, i.e., they should not interact with other carbonic anhydrases, preventing undesired side effects.

In cancer cells, metabolic reprogramming is the bioenergetic shift that advances malignant transformation and tumour growth. A well-known example, a Warburg effect, is a reprogramming whereby cancer cells switch from oxidative phosphorylation to glycolysis – as manifested by the rise of glucose absorption and lactate generation. Scientists want to exploit this naturally occurring metabolic transformation of cancer cells for therapeutic purposes. For example, strategies using the vitamin riboflavin (RF) have been investigated to weaken cancer cells by disrupting their metabolism. The lack of RF alters the stability and activity of the respiratory complexes of complex I and II in the mitochondria, which makes cancer cells dependent on glycolysis as the sole source of energy. A combination of CAIX inhibition and metabolic reprogramming provides a novel strategy that, by changing cellular energy metabolism, might influence cancer cell viability and thus improve therapy outcomes.

The main aim of this work was to sensitize tumours to CAIX inhibitors using metabolic reprogramming in tumour cells. The cytotoxicity of CAIX inhibitors in osteosarcoma cancer cells (143B) was tested using the sulforhodamine B (SRB) reagent (a colourimetric cell viability assay). Concentrations that were lethal for 50% of the cell population (LC_{50}) grown with or without RF were determined by applying the Hill equation. In addition, the effect of inhibitors on 143B cells was evaluated using flow cytometry. Lastly, the effects of CAIX inhibitors on mitochondrial respiration were quantified using the Seahorse assay. The Seahorse assay confirmed that growing cells without RF indeed inactivated mitochondrial respiration. Using the SRB assay, toxic inhibitor concentrations were determined in both normal and RF-deficient mediums. No significant differences between these conditions were seen. But using different, more sensitive methods, like the Seahorse assay and flow cytometry, we saw differences in the CAIX inhibitor effect on cells grown with or without RF.

Figure 1. Structure of CAIX; PG, proteoglycan-like segment; CA, carbonic anhydrase catalytic domain; TM, transmembrane anchor; IC, intracytoplasmic tail (A). Structures of inhibitors used in this work (B).



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F14

BREAST CANCER 3D CULTURE GROWTH TENDENCIES IN DEFINED MICROENVIRONMENTS

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Breast cancer to this day is the most frequent and aggressive form of cancer among women and around 30 % of patients are likely to die in 5 years after diagnosis. Three-dimensional (3D) cell cultures provide more physiologically relevant models for understanding cancer tumour biology, offering insights into cell behaviour, drug response, and therapeutic efficacy. 3D models can be used for diagnostics and drug performance testing, as they better mimic the in vivo tumour microenvironment-driven behaviour compared to traditional 2D cultures.

This research study focuses on 3D culture-forming tendencies of diverse breast tumour and normal cell lines relevant for in vitro and in vivo antitumor drug testing under defined and controlled microenvironmental conditions.

Different breast cancer subtypes show different trends when forming 3D cultures. Luminal A subtype cells MCF-7 are expressing estrogen (ER) and progesterone (PR) receptors exhibit a less aggressive phenotype, whereas mesenchymal stem-like (MSL) cells such as MDA-MB-231 has negative ER, PR and human epidermal growth factor (HER2) expression which makes them aggressive (higher motility, proliferation rates) triple-negative breast cancer cell type [1]. Due to the more migratory nature and weaker cell-cell adhesions invasive cancer cells tend to form loose or irregular spheroids. When standard spheroid forming models fail, Matrigel the solubilized basement membrane matrix secreted by Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells can be used instead. However, due to the influence of growth factors, the true effects of drugs on the cells may not be fully reflected. As an alternative to the solubilized basement membrane matrix, methylcellulose (MC) is chosen, a natural polymer that is added to the growth media does not enrich it with external growth factors and 3D cultures forming depends on cells-autonomous secretion of proteins that support cell-cell and cell-matrix interactions. MC medium is scaffold-like material and functions as a viscous, gel-like matrix that provides cells' mechanically stable environment which likely imitates epithelial tissue conditions.

We assess that MCF-7 cells' capacity to form spheroids when the growth environment isn't enriched with growth factors is low. It might be connected to dysregulated pathways of cell-cell signalling, though loose aggregates are most likely formed because of a non-adhesive growth environment. We observe that MCF-7 cells are more likely to form 3D cultures in MC without any growth-stimulating factors than those formed in ultra-low attachment micro-wells. We also evaluate MSL subtype MDA-MB-231 cells' tendencies to form aggregates and spheroids. Negative expression of E-cadherin, N-cadherin, P-cadherin and low secretion of cadherin-11 and β-catenin might be the reason why MDA-MB-231 are less likely to form properly structured spheroids in micro-wells and observed 3D structures are loose and irregular [2]. Though cells are assembling small three-dimensional structures in MC enriched medium because of MC provided extracellular-like matrix microenvironment.

Both methods try to observe the formation of 3D cultures in the absence of exogenous growth factors which may play a role in spatial body formation. Different breast cancer subtypes provide contrast when comparing cells' tendencies to form 3D cultures in defined microenvironments.

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F15

MITOCHONDRIAL FUNCTIONS CHANGE OVER TIME IN HUMAN KERATINOCYTES AND FIBROBLASTS UNDER PSORIATIC INFLAMMATION

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Pro-inflammatory cytokines, including TNF-α, IL-17, and IL-22, are key mediators in the pathogenesis of chronic psoriasis and, in conjunction with psoriasis-affected fibroblasts, promote keratinocyte hyperproliferation and inflammation [1]. *In vivo* studies indicate that mitochondrial ROS (mtROS) is essential for activating inflammatory signaling pathways and altering cellular respiration in dendritic cells exposed to psoriatic inflammation [2]. Mitochondrial dysfunction is established as a key factor in tissue damage and the progression of disease in skin cells during psoriasis [3]; however, the time-dependent influence of psoriatic inflammation on mitochondrial function in keratinocytes and fibroblasts has not been thoroughly investigated.

This study aims to explore the effects of psoriasis-like inflammation (PLI) over 24, 48, and 72 hours on mitochondrial functions in human keratinocytes and fibroblasts, focusing on mitochondrial membrane potential, mtROS production, and cellular respiration.

To induce PLI, keratinocytes and fibroblasts were treated with a cytokine mixture comprising TNF-α, IL-17, and IL-22. At 24, 48, and 72 hours, mitochondrial membrane potential and mtROS levels were measured using TMRM and MitoSox Red staining, respectively, with fluorescence microscopy performed on an Olympus APX100 microscope. The image analysis conducted using Fiji software. Cellular respiration was evaluated at 24 and 72 hours using the Seahorse XFp Analyzer and Mito Stress Test Kit, with data normalized to total protein content through the Bradford assay and subsequently analyzed using the Infinite 200 Pro Nano Plex reader.

Results indicated that in keratinocytes subjected to PLI, mitochondrial membrane potential temporarily increased at 24 and 48 hours, alongside a rise in mtROS production that peaked at 24 hours and remained stable thereafter. These mitochondrial changes were associated with significant reductions in mitochondrial respiration, ATP synthesis, and glycolytic activity at 24 hours, all of which returned to baseline by 72 hours. In PLI-affected fibroblasts, mitochondrial membrane potential initially increased at 24 hours and normalized by 72 hours, coinciding with a progressive rise in mtROS production that peaked at the 72-hour mark. In contrast to keratinocytes, fibroblast cellular respiration and glycolysis remained stable initially but exhibited a decline in cellular respiration at 72 hours, accompanied by an increase in glycolytic efficiency.

The study demonstrates distinct cell-type-specific and time-sensitive responses to PLI, with keratinocytes displaying rapid mitochondrial dysfunction associated with decreased cellular respiration, both of which recover as membrane potential and mtROS levels normalize. In contrast, fibroblasts exhibited a delayed accumulation of mtROS, leading to subsequent impairments in mitochondrial respiration and ATP synthesis. These findings offer significant insights into the role of mitochondrial function in skin cells within the context of psoriasis pathogenesis and aid in the identification of therapeutic targets for restoring mitochondrial function.

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F16

VERIFICATION OF P53 TRANSCRIPTIONAL ACTIVITY AND UPSTREAM PATHWAY INTEGRITY IN HTERT-RPE1 CELLS USING A SPECIFIC P53 REPORTER

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HTERT-RPE1 cells are human retinal epithelial cells immortalized by the introduction of the human telomerase *(hTERT*) gene under the control of a constitutive promoter. This cell line is widely used as a model of "healthy" (non-cancerous) cells in various scientific studies, including the modeling of the cell cycle, cell division, DNA repair, and ciliogenesis [1].

However, our sequencing results confirmed that the HTERT-RPE1 cell line harbors a heterozygous gain-of-function (GOF) mutation in the *KRAS* gene. This mutation is expected to induce oncogenic stress and trigger cell cycle arrest via P53 transcriptional activation [2]. However, these cells continue to proliferate under standard culture conditions, suggesting that a critical regulatory pathway involved in DNA damage response and cell cycle control—the P53 pathway [3] —may be compromised. Since HTERT-RPE1 cells are widely used as a non-cancerous model [1], verifying the integrity of the P53 pathway is essential to ensure their validity in research involving DNA damage response and cell cycle modeling.

It is well established that under genotoxic stress, P53 stabilizes and induces the expression of its target genes, such as *CDKN1A* (which encodes the P21 protein) and *MDM2* [4]. However, in HTERT-RPE1 cells, the activation of these genes following P53 stabilization [4] does not necessarily confirm direct P53 transcriptional activity, as P53 target genes contain additional transcription factor-responsive elements in their promoters [5]. For instance, it has been demonstrated that the *CDKN1A* promoter can be transactivated in *TP53*$^{-/-}$ cells [6].

Therefore, we aimed to directly verify P53 transcriptional activity as well as assess the integrity of the upstream P53-dependent DNA damage response pathway. Since RAS signaling activates PI3K/Akt [7], which in turn can suppress P53 [8], we investigated whether this pathway remains functional in the presence of an oncogenic *KRAS* mutation.

To achieve this, we designed and constructed lentiviral vectors carrying a specific P53 reporter cassette, consisting of six P53-responsive elements, a minimal CMV promoter, and either eGFP or destabilized eGFP (deGFP). HTERT-RPE1 cells were transduced with these reporter constructs, selected using puromycin, and stable cell lines HTERT-RPE1-P53rep-eGFP and HTERT-RPE1-P53rep-deGFP were established.

Treatment of these cell lines with nutlin-3a (an MDM2 inhibitor) or etoposide (a type II topoisomerase inhibitor) resulted in a dramatic increase in green fluorescence, alongside an increase in P53 protein levels, as measured by flow cytometry and Western blotting. These findings indicate that P53 transcriptional activity and the P53-dependent branch of the DNA damage response pathway remain intact in HTERT-RPE1 cells.

To evaluate the inhibitory effect of PI3K/Akt signaling on P53 transcriptional activity, we treated cells with a chemical AKT inhibitor. However, this treatment did not enhance P53 transcriptional activity, suggesting that this upstream P53 regulatory pathway may not be fully functional.

In conclusion, our study demonstrates that P53 retains its transcriptional activity in HTERT-RPE1 cells, and the P53-dependent DNA damage response pathway remains intact, suggesting that HTERT-RPE1 cells are a suitable model for DNA repair studies. However, the lack of response to PI3K/Akt inhibition indicates dysfunction in this upstream regulatory pathway, potentially leading to a loss of cell cycle control due to oncogenic RAS signaling. These findings suggest that HTERT-RPE1 cells may have limitations as a model for studying cell cycle regulation and division, particularly in contexts where fully functional P53 signaling is essential.

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F17

Hybrid Trachea: Bioprinting with Living and Synthetic Materials for Enhanced Transplantation

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Problem Statement and Research Goal: Tracheal transplants remain a complex challenge due to issues such as limited donor availability, immune rejection, and the suboptimal functionality of synthetic grafts. Traditional methods, including autografts and allografts, are often hindered by the inability to fully restore the complex structure and function of the trachea. This research proposes the development of a hybrid trachea by combining living cells and synthetic materials using bioprinting techniques. The goal is to create a fully functional, biocompatible graft that can overcome the limitations of current transplantation methods, providing a more reliable solution for patients requiring airway reconstruction.

Significance and Potential Impact: Tracheal transplants are essential for patients with severe airway damage or congenital defects. However, the shortage of suitable donor tissues and the immune complications associated with conventional grafts pose significant barriers. Bioprinting offers a groundbreaking solution by allowing for the precise placement of living cells and synthetic polymers in a layered structure, mimicking the natural geometry of the trachea. By integrating mesenchymal stem cells and chondrocytes with bioresorbable materials like polycaprolactone (PCL), this hybrid approach has the potential to enhance both the mechanical properties and biological functionality of the trachea. This innovation could reduce dependency on donor organs, minimize immune rejection, and pave the way for scalable, long-lasting tracheal transplants, ultimately improving patient outcomes and survival rates.

Methodology: This study will utilize a multi-step bioprinting approach to fabricate the hybrid trachea, with the following key steps:

1. **Material Selection**: A combination of biocompatible synthetic polymers (PCL, PLGA) and living cells (mesenchymal stem cells, chondrocytes) will be used to form the hybrid scaffold. These materials were chosen for their mechanical strength, biodegradability, and ability to support cell growth.
2. **Bioprinting Process**: A 3D bioprinter will precisely deposit the bioinks, layer by layer, to replicate the native tracheal structure. The design will incorporate cartilage rings and a smooth muscle layer, ensuring optimal functionality and structural integrity.
3. **Scaffold Maturation**: Following the printing process, the hybrid trachea will undergo maturation in a bioreactor, which will promote cell differentiation and tissue development, mimicking the natural growth process of the trachea.
4. **In Vitro Evaluation**: The mechanical properties (tensile strength, flexibility) and cellular integrity of the printed trachea will be evaluated through in vitro tests, including cell viability, proliferation, and the formation of cartilage and epithelial layers.
5. **In Vivo Testing**: Once successful in vitro evaluation is completed, the hybrid trachea will be implanted into animal models. The in vivo testing will focus on graft integration, immune response, and the long-term functionality of the graft in an anatomical airway setting.

Expected Outcomes: The hybrid trachea is expected to demonstrate enhanced mechanical strength and biocompatibility compared to conventional synthetic grafts. It will likely support the growth of both epithelial and cartilage tissues, integrate seamlessly with surrounding tissues, and offer improved durability and functionality under physiological conditions. Additionally, the bio-printed trachea should exhibit reduced risk of immune rejection, providing a promising alternative for patients requiring tracheal transplants. This research has the potential to transform tracheal transplantation, creating a viable and sustainable solution to a critical healthcare challenge.

Conclusion: The development of hybrid tracheas through bioprinting could revolutionize the field of organ transplantation. By combining the advantages of living tissues with synthetic materials, this approach addresses key challenges in airway reconstruction, including donor organ shortages and immune rejection. The successful implementation of this research could significantly improve the quality of life for patients in need of airway reconstruction and provide a model for other bioengineered organs in the future.

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Genetics

G1

EVALUATION OF GENOTOXIC, CYTOTOXIC, AND HEART RATE CHANGES IN SEA TROUT (*SALMO TRUTTA*) EARLY DEVELOPMENTAL STAGES AFTER EXPOSURE TO COMMERCIALLY AVAILABLE UREA

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The main applications for urea, a fertilizer with 46% nitrogen, are in aquaculture, horticulture, and the cosmetics sector. Urea is widely used in both agriculture and cosmetics and dermatology due to its unique properties. In aquaculture, urea is used in combination with trimethylamine oxide (TMAO) to improve the production and growth rate of natural fish feed. Studies show that modified feed with urea and TMAO not only enhanced the osmoregulation of fish, but also improved meat color and reduced the development of skin ulcers [1][2]. However, excessive use of urea in farms can increase ammonia concentrations in water, which leads to behavioral changes, stress and even mortality in fish [3]. Therefore, it is important to study the effects of urea on fish and its potential toxicity, especially considering the importance of fish for human nutrition.

This study aimed to evaluate the effects of varying concentrations of urea (0.125 – 10 g/L) on the heart rate, genotoxicity and cytotoxicity in the sea trout (*Salmo trutta*) eyed embryos after four days of exposure. Urea (UAB AGROCHEMA) was used for the exposure experiments, choosing six concentrations and a control group, with two replicates for each group. Urea was dose-balanced according to the LC50 limits, and the exposure experiments lasted 4 days. Heart function was measured using a stereo microscope, recording heart rate on the first and fourth days. Cytogenetic analysis was carried out using erythrocytic nuclear abnormalities assay. Biomarkers of genotoxicity (micronuclei, nuclear buds, blebbed nuclei) and cytotoxicity (fragmented nuclei, apoptosis, eight–shaped and bean–shaped nuclei) were assessed according to the methodology of *Fenech et al*. [4] and *Stankevičiūtė et al*. [5]. Statistical analysis was performed using R software [6] (version 4.2.3).

At a urea concentration of 0.5 g/L, the occurrence of eight-shaped nuclear abnormalities increased 4.2 times, suggesting a cytotoxic effect without any evident genotoxic impact. The findings indicate that urea may exert cytotoxic activity and influence the heart rate of fish embryos, despite the absence of observable biomarker alterations at other concentrations. Throughout the initial day of exposure, heart rates consistently decreased. By the fourth day, heart rates increased by 1.2 to 6.8 beats per minute; however, they remained lower than those observed in the control group. It is noteworthy that the chorion of fish embryos acts as a mechanical barrier, safeguarding the embryo from environmental stressors, including pollutants. This study suggests that urea may have cytotoxic effects on fish erythrocytes and affect heart rates, yet further extensive and long-term research is necessary to confirm these findings.

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G2

DIAGNOSTIC POTENTIAL OF *C-MYC* AND *CTNNB1* EXPRESSION IN PLASMA SAMPLES FROM CLEAR CELL RENAL CELL CARCINOMA PATIENTS

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Clear cell renal cell carcinoma (ccRCC) represents approximately 75% of all renal cell carcinomas and forms in the epithelium of the nephron tubule. The standard method for diagnosing kidney cancer is tissue biopsy [1]. However, this method poses challenges in the early stages of the disease when the tumor remains small. This difficulty contributes to the high mortality rates associated with kidney cancer. Furthermore, tissue biopsy is an invasive procedure, limiting its feasibility for use in cancer prevention programs aimed at reducing kidney cancer mortality [2].

Liquid biopsies, such as plasma or urine samples, offer a promising alternative for cancer diagnosis due to their non-invasive or minimally invasive nature. These methods are safer, easier to perform, and can be repeated, making them more suitable for integration into cancer prevention programs. Liquid biopsies have the potential to detect cancer at earlier stages, facilitating timely treatment and improving patient survival rates [3].

The MYC and WNT signaling pathways are integral to tumorigenesis, as they regulate critical cellular processes that are often disrupted in cancer. Dysregulation of these pathways contributes to uncontrolled cell proliferation, survival, and tumor progression. The core gene of the MYC pathway is *C-MYC*, while the WNT pathway is centred on the *CTNNB1* gene [4][5].

Liquid biopsy-based detection of ccRCC could significantly enhance ccRCC diagnostics by providing a faster and more cost-effective method for identifying malignant renal tumors. Accordingly, the primary aim of this study was to evaluate whether *C-MYC* and *CTNNB1* could serve as novel genetic biomarkers for ccRCC.

In this investigation, RNA was extracted from plasma samples of 53 ccRCC patients and 12 individuals with benign renal tumours and analysed using quantitative reverse transcription PCR (RT-qPCR). ROC analysis was used to determine the diagnostic potential of the *C-MYC* and *CTNNB1* genes, with *GAPDH* serving as the reference gene.

The findings revealed that *CTNNB1* expression levels differ significantly between ccRCC cases and controls. *CTNNB1* was able to differentiate ccRCC from benign tumors, achieving an area under the curve (AUC) of 0.67, while *C-MYC* AUC was 0.64. Additionally, *CTNNB1* expression levels were found to correlate with tumor diameter in ccRCC patient group.

Changes in the expression of *C-MYC* and *CTNNB1* genes, detectable in plasma samples, may contribute to the early diagnosis of ccRCC. However, further research is required to confirm the potential of *CTNNB1* and *C-MYC* as non-invasive biomarkers for clear cell renal cell carcinoma.

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G3

INVESTIGATION OF THE GENETIC BASIS OF DROUGHT RESISTANCE IN LITHUANIAN BARLEY (*Hordeum vulgare* L.) CULTIVARS

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Barley (*Hordeum vulgare* L.) is a widely cultivated cereal grain and due to its rich nutritional profile, including fiber, complex carbohydrates, vitamins and minerals, it is a key component of the human diet in many developing countries, where access to more productive cereals might be limited. Additionally, barley is used as livestock fodder owing to its high fiber content, as well as for malting in alcoholic beverage brewing [1]. During the 2000–2022 period, Europe experienced an increase in drought caused by a combination of factors, including low precipitation, heatwaves and high evaporation rates [2]. This climate disbalance, negatively affecting natural ecosystems and cultivated cropland peaked in summers of 2018 and 2019, resulting in billions of euros of damages. Studies show that without action to counteract climate change and develop sustainable drought-management strategies, the agricultural sector will continue to experience worsening losses [3]. In order to protect cropland from decreasing yield, it is necessary to identify and breed potential drought-resistant cereal cultivars, as well as to expand the understanding of the genetic mechanisms of drought tolerance. Barley is an ideal candidate for this, as an important cereal crop itself and a convenient model organism for other cereals.

In previous studies, we determined leaf relative water content (RWC) and proline content to be the most differentiating parameters. Based on these traits, four barley cultivars were selected for further analysis – 2 as the most resilient with 'RGT Planet' and 'Rusnė', and 2 as the most susceptible – with 'Laureate' and 'Ema'. The expression of key drought-responsive transcription factors CBF, CO5, DRF, EIL1, ERF4, FUS3, NAC6, WRKY and key proline synthesis player P5CS1 were analyzed using real-time quantitative PCR (qPCR) in 'Rusnė', 'RGT Planet', 'Laureate', 'Ema' barley cultivars. These hub genes were previously identified in the meta-analysis by Javadi et al. (2021) as critical regulators of drought stress in barley. In this case, the qPCR method was used to directly measure mRNA expression levels to determine how predicted hub genes are actually regulated under drought conditions and how that correlates to observed morphological and biochemical phenotypes.

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G4

SINGLE NUCLEOTIDE POLYMORPHISM ANALYSIS OF THE *CD274* GENE IN A COHORT OF CASTRATION-RESISTANT PROSTATE CANCER PATIENTS

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Prostate cancer (PCa) is the most commonly diagnosed type of cancer among men in Lithuania [1]. Approximately 10–20% of PCa cases progress to castration-resistant prostate cancer (CRPC), an advanced form of PCa with an average survival rate of less than 2 years [2]. The onset and progression of the disease are often linked to genetic alterations, including variations in the *CD274* gene, which may be a potential risk factor [3][4]. The *CD274* gene encodes the PD-L1 protein, whose increased expression may enhance the suppression of immune cells whithin the tumor microenvironment, thus promoting the development of PCa [4]. Therefore, genetic variants, such as single nucleotide polymorphisms (SNPs), may serve as potential diagnostic and therapeutic biomarkers, enabling earlier and more precise diagnosis while aiding in the management of PCa progression.

The aim of this study was to investigate the frequencies of SNPs in the *CD274* gene among CRPC patients and analyze their associations with clinical characteristics.

In this study, we used quantitative PCR (qPCR) to identify the genotypes and alleles of five *CD274* gene SNPs (rs4143815, rs2282055, rs822335, rs3780395, and rs1411262) in 134 CRPC patient samples, and evaluated their associations with clinical traits (age, PSA level, ISUP group and pathological stage T score). We detected statistically significant associations between the rs822335 C/C and C/T genotypes and PSA levels ($p = 0.0001$), the rs3780395 A/A and G/G genotypes and pathological stage T score ($p < 0.001$), as well as the rs1411262 C/T and C/C genotypes and the rs2282055 G/T and T/T genotypes and ISUP group ($p = 0.003$ and $p = 0.005$, respectively).

In conclusion, our study identified the frequencies of five *CD274* gene SNPs in CRPC patients and determined statistically significant associations between the examined SNPs and clinical characteristics. To gain a more comprehensive understanding of the *CD274* genotype prevalence, larger study involving more patients could be conducted.

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G5

ASSOCIATIONS BETWEEN GENETIC VARIATIONS OF α-AMYLASE AND TEETH LOSS IN INDIVIDUALS WITH INTELLECTUAL DISABILITY

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Dental caries, a major contributor to teeth loss, is caused by soft plaque (biofilm) and is influenced by various risk factors (biological, behavioral, genetic, socioeconomic, etc.) [1]. Some of them, like oral hygiene habits, are well understood, while the role of genetic factors remains less clear. *AMY1* gene encodes the salivary α-amylase enzyme, which hydrolyzes α-1,4 glycosidic bonds in starch, breaking it down into maltose, maltotriose, and other small carbohydrates [2]. Such molecules can be easily metabolized by cariogenic bacteria, which produce acids leading to the development of caries. [3]. On the other hand, undigested starch adheres better to the surfaces of the teeth, remains for a longer period, and is gradually metabolized by the cariogenic bacteria themselves. The aim of this study was to assess the association between *AMY1* copy number variation (CNV) and teeth loss in individuals with intellectual disabilities.

This pilot study included 48 individuals (24 males and 24 females) with intellectual disabilities, who received oral disease treatment under general anesthesia at the Zalgiris Clinic, a branch of Vilnius University Hospital Santaros Clinics, Vilnius. The mean age (±SD) was 29±7 and 31±9 for males and females, respectively. During dental examination, a single trained clinician assessed the number of teeth lost, based on this, participants were referred to three study groups (a group with no missing teeth, a group with 1-2 missing teeth, and a group with ≥3 missing teeth). The buccal swabs were collected for molecular analysis. The MagMAX Nucleic Acid Isolation Kit and KingFisher Flex automated extraction system were used for DNA purification. *AMY1* CNV values were determined using real-time PCR assays with the QuantStudio Pro 7 Real-Time PCR system (Thermo Fisher Scientific). Statistical analysis was conducted using IBP SPSS 27.0 software, analyzing possible trends in the correlation between *AMY1* CNV and number of lost teeth.

The mean *AMY1* CNV value was 14 and ranged from 4 to 33 (Fig. 1), while the mean number of lost teeth was 4 (from 0 to 14). A tendency toward a negative association between *AMY1* CNV and teeth loss was observed. Specifically, individuals with no missing teeth had a mean *AMY1* CNV of 16±7, those with 1-2 missing teeth had a mean CNV of 14±7, and individuals with ≥3 missing teeth – 13±6 gene copies. No significant correlation between *AMY1* CNV values and age (n = 46) was observed, nor were there any differences between males and females.

In conclusion, our pilot study suggests a potential link between lower *AMY1* copy numbers and increased teeth loss in individuals with intellectual disabilities, indicating a possible genetic influence on oral health outcomes. However, larger studies are needed to confirm these findings and further explore underlying mechanisms.

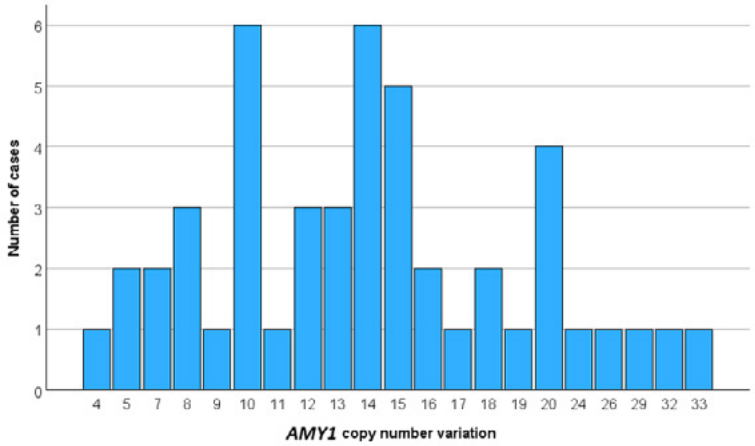

Figure 1. Distribution of copy number variation (CNV) values of *AMY1* gene locus in individuals with intellectual disabilities who underwent dental examination.

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G6

GENETIC DIVERSITY OF THE LACTASE GENE AND ITS PHENOTYPIC ASSOCIATION WITH LACTASE PERSISTENCE IN THE LITHUANIAN POPULATION

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This study analyzed the genetic diversity of lactase (*LCT*) and related genes, including *GALM, HK2* and *MCM6*, in the Lithuanian population, focusing on their association with phenotype and evolutionary significance of lactase persistence. The objectives included assessing allele frequencies, evaluating the functional relevance of genetic variants through *in silico* methods, and comparing genetic diversity across populations using principal component analysis (PCA).

Whole-genome sequencing was performed on 49 healthy individuals using the Illumina NovaSeq™ 6000 system. Genetic variants were annotated using ANNOVAR. Allele frequency distributions and Hardy-Weinberg equilibrium were analyzed using PLINK v1.9 and VCFtools v0.1.16. PCA was conducted using SmartPCA from the EIGENSOFT v7.2.1 suite to compare Lithuanian genetic structure with populations from the 1000 Genomes Project. Data visualization was performed in R using ggplot2 package.

PCA revealed that the Lithuanian genetic structure closely aligns with European populations (CEU, FIN, TSI), forming a distinct cluster separate from African (YRI) and Asian (JPT) populations. Within the European cluster, Lithuanians displayed unique allele frequency patterns, highlighting regional genetic differentiation. Sequencing data analysis revealed 126 variants in target *GALM, HK2, LCT*, and *MCM6* genes.

The study identified key genetic variants associated with lactase persistence, lipid metabolism, and disease susceptibility. For example, the rs678350 variant in the *HK2* gene is linked to glaucoma risk, while rs632632 in *MCM6* influences cholesterol and LDL-C levels. Variants in the *LCT* gene, such as rs2278544, rs1011361, and rs892715, are associated with lactase persistence and evolutionary adaptation to dairy consumption. Additionally, rs2164210 regulates lactase enzyme activity, enhances gut microbiota, and provides protective effects against autoimmune diseases, while rs2322659 impacts lactase persistence and cardiovascular health [1-5].

The alternative allele frequencies of lactase persistence variants in Lithuanians - rs2278544 (70%), rs1011361 (62.82%), rs892715 (60.25%), rs2164210 (60.25%), and rs2322659 (68.75%) - closely align with European populations. These findings reflect evolutionary adaptations to dairy consumption, highlighting distinct genetic patterns compared to African and Asian populations.

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G7

Barley TPS1 gene knock-out creation using Crispr/Cas9 system

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Barley is the fourth most important agricultural crop and is often used as a model organism for genetic research due to its relatively small diploid genome compared to polyploid species like wheat and rye [1]. As environmental challenges such as climate change intensify, developing barley varieties with enhanced stress tolerance and higher productivity is critical to ensure global food security and agricultural sustainability [1]. This study focuses on modifying the *HvTPS1* gene barley Golden Promise variety using CRISPR-Cas9 system to create a gene knockout. This gene encodes an enzyme involved in trehalose-6-phosphate (T6P) synthesis, which plays a crucial role in sugar metabolism and heat stress responses [2]. It is also thought to influence branching and spike architecture, making it a valuable target for genetic improvement [3]. Little is known about the molecular basis of cereal inflorescence architecture, so studies like this will improve knowledge about inflorescence genetic development pathways, by exporting gene – phenotype relationships.

Previously, a construct was created containing a plasmid with CRISPR-Cas9, hygromycin resistance genes (used for transformed plant selection) and two single-stranded guide RNAs containing 20-nucleotide target sequences. In this experiment, immature barley embryos were carefully excised by detaching the seed coat and embryonic axis. Each embryo was inoculated with a small drop of the prepared *Agrobacterium* suspension, containing the construct for *HvTPS1* gene knockout. Subsequently, the embryos were placed on a callus induction medium. After six weeks, once callus formation was observed, the embryos were transferred to a barley regeneration medium to stimulate shoot formation and placed in a growth chamber. The chamber was maintained at 80% humidity with a light cycle of 6 hours of the day and 8 hours of the night. The light and hormones in the medium provided the necessary signals to initiate plant regeneration. Some embryos successfully regenerated, resulting in the development of early-stage plants, which are further regenerating in rooting tubes.

In the future, once the plants reach an appropriate size, they will be transferred to soil and grown to maturity. The next step will involve extracting DNA and analysing it using Sanger sequencing to determine whether the *HvTPS1* gene has been successfully altered and knocked out. The knockouts will then be phenotyped and analysed under stress conditions to investigate the impact of the *HvTPS1* gene on stress response, spike architecture and barley development.

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G8

Evaluating *KIF2C* Expression as a Novel Biomarker for Early Detection and Prognosis of Ovarian Cancer

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Background: Ovarian cancer is the most lethal gynecologic malignancy, accounting for over two-thirds of deaths from gynecologic cancers [1]. The high mortality rate is primarily due to late-stage diagnosis, emphasizing the critical need for reliable biomarkers to facilitate early detection and improve treatment outcomes. Enhancing diagnostic methods for cancer detection has the potential to significantly improve treatment outcomes and overall survival rates. Currently, to detect ovarian cancer, the main biomarker used is serum antigen 125 (CA 125), along with the diagnostic tool: transvaginal ultrasound (TVUS). However, these methods lack sufficient sensitivity and specificity, particularly for early-stage ovarian cancer, limiting their utility for early diagnostics [2]. Mitotic centromere-associated kinesin (MCAK, also known as KIF2C), a microtubule-depolymerizing protein, plays a crucial role in chromosomal alignment and segregation during mitosis. Aberrant regulation of *KIF2C* has been linked to genomic instability, increased invasiveness, metastasis, and drug resistance across various cancer types [3]. Notably, *KIF2C* overexpression has been associated with poor prognosis in multiple cancers, including breast cancer [4]. Changes in *KIF2C* expression may offer new potential of improving the diagnosis of ovarian cancer.

This study aims to evaluate the potential of *KIF2C* as a novel diagnostic and prognostic biomarker for ovarian cancer by analyzing its expression levels and correlation to clinical features.

Methods: 28 of ovarian cancer patient samples were included in this study. *KIF2C* expression was analyzed by using reverse transcription quantative PCR (RT-qPCR). Results were compared to control gene *GAPDH*. Statistical analyses, including Student's t-test, were employed to assess the correlation between *KIF2C* expression and clinicopathological parameters. Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic performance of *KIF2C* expression levels.

Results: The analysis of *KIF2C* gene expression revealed significant differences across ovarian cancer types. Expression levels were significantly higher in high-grade serous ovarian carcinoma than in benign gynecologic tumors ($p = 0.013$). ROC curve analysis showed that the classification model effectively distinguishes high-grade serous ovarian carcinoma from benign gynecologic tumors (AUC = 0.854). These findings suggest *KIF2C* as a potential biomarker for identifying high-grade serous ovarian carcinoma.

In conclusion, *KIF2C* demonstrates potential as a novel biomarker for the diagnosis and prognosis of ovarian cancer. Its overexpression correlates with advanced disease stages and may contribute to chemoresistance mechanisms. However, further large-scale studies are needed to validate these findings, including research utilizing non-invasive sample types to determine *KIF2C*'s practicality in routine clinical diagnostics.

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G9

MITOCHONDRIAL GENETIC VARIATION IN THE LITHUANIAN CLEAN-UP WORKERS OF THE CHORNOBYL NUCLEAR DISASTER: POSSIBLE IMPLICATIONS FOR BIOLOGICAL AGEING

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Biological ageing is closely connected to mitochondrial function, which depends on the integrity of mitochondrial DNA (mtDNA) and nuclear-encoded mitochondrial genes. Numerous chronic disorders, particularly those associated with ageing, have been linked to mitochondrial dysfunction that impairs energy production, metabolism, and cellular signalling [1]. Nonetheless, mtDNA and nuclear-encoded mitochondrial gene variants have also been associated with longevity and a lower risk of developing certain age-related diseases [2,3,4]. Considering the involvement of mitochondria in biological ageing processes, the aim of this study was to determine the possible protective effect of mitochondrial genetic variants in a cohort of 40 Lithuanian clean-up workers of the Chornobyl nuclear disaster (LCWC). These individuals were exposed to high doses of ionising radiation yet exhibit relatively healthy ageing that could potentially be influenced by their mitochondrial genetic variation.

Whole genome short-read sequencing data were used to identify nuclear-encoded mitochondrial gene and mtDNA variation of the LCWC cohort. Nuclear-encoded genes that are associated with mitochondria were selected based on the data provided in MitoCarta3.0 [5]. Allele frequencies of mtDNA and nuclear variants from the LCWC cohort were compared with allele frequency data of the non-Finnish European population from the gnomAD database. Statistical analysis was conducted using the χ^2 test and Fisher's exact test. Results were considered statistically significant at $p \leq 0.05$ for mtDNA and $p \leq 7\times10^{-6}$ for nuclear variants.

In comparison to the non-Finnish European population dataset, the LCWC cohort had a higher prevalence of fifty nuclear genome variants and six mtDNA variants (m.146T>C, m.204T>C, m.477T>C, m.2623A>G, m.3244G>A, m.16086T>C). Twenty-eight out of the fifty nuclear variants are in genes (*COQ8B*, *FHIT*, *MCCC2*, *METTL4*, *MSRA*, *OXR1*, *PRKN*, *RHOT2*, *SLC25A21*, *TIMM23*, *TOP3A*, *TXNRD2*) that have been previously associated with biological ageing processes.

The nuclear and mtDNA variants identified in this study may have a protective effect in the LCWC cohort by altering mitochondrial function, but additional research is required to support this theory.

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G10

FEATURES OF CENTROMERIC FESREBA SEQUENCES ACROSS POACEAE SPECIES

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Poaceae family species are widely cultivated worldwide and are important forage grasses. Most of the plants in this family are polyploid and readily interbreed, so the origin of most polyploid species remains unclear. Polyploid species are characterized by a variety of repetitive sequences that play different roles in the nuclear genome. In a recent study, Zwyrtková *et al*. (2020) identified retrotransposon elements named Fesreba in the centromeric regions of *Festuca* and *Lolium* species. Using the FISH method (fluorescence *in situ* hybridization), it was demonstrated that Fesreba could act specifically as chromosome centromere markers in some fescue and ryegrass species [1]. In plant genetics, the development of specific centromere probes is essential for studying species evolution and genome organization. Therefore, the aim of this study was to test the Fesreba centromeric element as a potential universal marker and analyse its characteristics across various species belonging to the Poaceae family.

To achieve this, we amplified and sequenced the Fesreba RT element (~300 bp) and constructed a phylogenetic tree including 25 species/varieties from the collection maintained at the Botanical Garden of Vilnius University. From these results, we concluded that Fesreba sequences are mostly conserved across the species examined, except for some *Bromus*, *Panicum*, and *Vulpia*, showing low linkages in the phylogenetic tree (Selected parameters: Neighbour-joining tree algorithm; Bootstrap cut-off value = 50%). Further on, we developed species-specific fluorescent probes and tested them on the chromosomes in interphase and metaphase plates. We found that the Fesreba probe works well, highlighting the centromeres of the chromosomes in four *Festuca* species (*F. altissima, F. amethystina, F. arundinacea, and F. cinerea*), as well as in *Deschampsia caespitosa, Sesleria caerulea* and *Sesleria nitida*. Whereas phylogenetically distant species, such as *Panicum virgatum*, lacked a fluorescent Fesreba signal on their centromeres, which could mean that Fesreba elements evolved differently in some Poaceae species.

In summary, our findings highlight the potential of Fesreba as a specific probe for centromere studies and provide valuable insights into the evolution of centromeric regions in a broader context of plant species.

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G11

IMAPCT OF *TNF* POLYMORPHISMS ON VISUAL ACUITY BEFORE AND AFTER TREATMENT IN OPTIC NEURITIS

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Optic neuritis (ON) is a leading cause of acute optic nerve damage in adults [1]. Characterized by inflammation of optic nerve and myelin sheath, ON results in secondary damage to nerve fibers, ultimately leading to apoptosis of retinal ganglion cells. ON can be caused by a wide range of etiological factors, broadly classified as typical and atypical. Usually, typical ON is subacute, unilateral, painless, has better prognosis and associated with multiple sclerosis [2, 3]. Atypical form is thought to be bilateral, has higher recurrence rate and associated with autoimmune disorders, bacterial and viral infections [3, 4].

Tumor necrosis factor (TNF) is a potent pro-inflammatory cytokine [5]. The *TNF* gene is localized on the short arm of chromosome 6 at position 21.3 (6p21.3). It is though, that single nucleotide polymorphisms (SNP) located in the regulatory region affect the expression of *TNF-α*. Several polymorphisms have been associated with various infectious and inflammatory diseases at different locations in the *TNF-α* promoter region [6].

It is observed, that ON patients have ophthalmological changes, such as reduced visual acuity, visual field abnormalities, color vision impairment and afferent defect of the affected pupil [1]. Visual acuity (VA) is a fundamental measure of central vision, assessing the eye's ability to distinguish fine details [7]. Assessing VA changes before and after the treatment could help to determine the effectiveness of therapeutic interventions and the impact of TNF polymorphisms on visual outcomes for ON patients.

This research aims to determine the associations between *TNF-863A/C, TNF-308A/G and TNF-238A/G* SNPs with ON and visual acuity (VA).

DNA was extracted from peripheral blood leukocytes using DNA salting-out method. Genotyping of *TNF-863A/C, TNF-308A/G* and *TNF-238A/G* SNPs was performed by real-time polymerase chain reaction (RT-PCR). Visual acuity (VA) was evaluated for 75 patients before and after ON treatment. Statistical analysis was performed using the "IBM SPSS Statistics 29.0.2.0" package.

We analyzed the associations of *TNF-863A/C, TNF-308A/G* and *TNF-238A/G* SNPs with VA in ON patients. Comparison of the genotype and allele distributions did not reveal statistically significant differences in VA before and after treatment in the affected eyes.

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G12

EFFICIENT EGFP ELECTROPORATION IN LYMPHOCYTE CELLS

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Jurkat cells, a human T lymphocyte cell line, serve as a valuable model for studying T-cell signalling and gene expression. However, efficient electroporation of exogenous DNA into these suspension cells remains challenging. Electroporation is a widely used technique that facilitates transient or stable gene expression by delivering nucleic acids into cells through short, high-voltage pulses. In this study, we optimized electroporation conditions for efficient transfer of an Enhanced Green Fluorescent Protein (EGFP) plasmid. Jurkat cells were electroporated with an EGFP-expressing plasmid using a [NEPAGENE 21] with varying pulse voltages, pulse durations, and DNA concentrations. Post-electroporation, cell viability and efficiency of electroporated cells were assessed using flow cytometry and fluorescence microscopy. Various parameters, including pulse voltage, duration, and DNA concentration, were tested to achieve high electroporation efficiency while maintaining cell viability. Fluorescence microscopy and flow cytometry confirmed successful EGFP expression, with optimal conditions we achieved high transfection efficiency. These results demonstrate the effectiveness of electroporation for gene delivery in Jurkat cells which may provide a reliable approach for further genetic and immunological studies.

Keywords: Electroporation, Jurkat Cells, EGFP, Gene Transfection, Flow Cytometry

G13

THE IMPACT OF CAPSULAR POLYSACCHARIDES PRODUCED BY *ACINETOBACTER BAUMANNII* ON THE RESISTANCE TO PHOTODYNAMIC THERAPY

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The opportunistic pathogen *Acinetobacter baumannii* can produce biofilm on abiotic surfaces and is frequently found in hospitals, where it causes severe nosocomial infections [1]. One of the important virulence factors of this pathogen is the capsular polysaccharides. They help the bacteria infect patients and protect them from various external factors [2]. The increasing *A. baumannii* resistance to antibiotics is being observed around the world; therefore, there is a need to find the alternative treatment options, such as chlorophyllin-based photodynamic therapy. Photodynamic therapy causes oxidative stress to the bacteria and inactivates them [3].

This study aimed to determine how capsular polysaccharides produced by *A. baumannii* contribute to the resistance of its biofilms against chlorophyllin-based photodynamic therapy and investigate the changes of *galU* gene expression in response to the treatment. We investigated a wild-type *A. baumannii* isolate and its Δ*galU* mutant, which does not produce a polysaccharide capsule. The *galU* gene encodes UTP-glucose-1-phosphate uridylyltransferase, which is needed for UDP-glucose synthesis. The effects of chlorophyllin-based photodynamic therapy were evaluated by exposing the *A. baumannii* biofilms to 402 nm light at different irradiation dosages, followed by quantitative CFU/mL analysis. The expression of the *galU* gene was analyzed by performing qPCR and using the ΔΔCt method for data analysis.

Our research demonstrated that chlorophyllin-induced photodynamic therapy caused viability changes in both the wild-type *A. baumannii* isolate and its Δ*galU* gene deletion mutant. The non-capsulated isolate was approximately 12 times more sensitive to 402 nm light and photodynamic therapy compared to the wild-type isolate.The preliminary qPCR results showed that the expression of the *galU* gene in the biofilm formed by wild-type *A. baumannii* isolate did not exhibit statistically significant changes after exposure to 402 nm light or chlorophyllin-based photodynamic therapy. Further research is needed to explore the role of capsule-related genes expression during *A. baumannii* exposure to photodynamic therapy.

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G14

ANALYSIS OF THE COMPONENTS OF HIPPO SIGNALING PATHWAY AS A POTENTIAL BIOMARKER FOR OVARIAN CANCER DIAGNOSIS

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Ovarian cancer is one of the most common types of gynecological cancer with a relatively high mortality rate. Approximately 232 000 women are diagnosed with ovarian cancer and of these, 152 000 die. Only 30 percent of patients are estimated to survive more than 5 years after diagnosis. One of the reasons for this is that ovarian cancer is quite difficult to diagnose at an early stage. A large proportion of cases are diagnosed at a late stage, also called high-grade serous ovarian cancer (HGSOC). Also it is difficult to distinguish the symptoms of ovarian cancer as many of them are similar to those associated with other diseases. Therefore, it is significant to detect any potential biomarkers that could help to diagnose ovarian cancer at an early stage. Cancer is associated with dysregulation of cell signaling pathways and rapid cell division. One of the main pathways involved in cell growth is the Hippo signaling pathway. The Hippo signaling pathway plays an important role in organ development and regeneration. Some of the most important components of this signaling pathway are the YAP and MST proteins, encoded by the *YAP1* and *MST1* genes respectively. Downregulation of these genes and, consequently, of the signaling pathway has been associated with cancer.

The aim of our research was to analyze the change of *YAP1* and *MST1* gene expression in ovarian cancer samples to define them as a potential biomarker for ovarian cancer diagnosis. In this study 28 samples were included – 10 of HGSOC, 9 of benign and 9 of other ovarian tumor samples. Gene expression changes were analyzed by preparing an RT-qPCR. Results were normalized to the reference gene *GAPDH*.

Statistical analysis showed significant differences between benign and HGSOC, benign and other ovarian tumors groups for *YAP1* gene (both $p<0.001$), also for benign and HGSOC ($p=0.002$), HGSOC and other ovarian tumors ($p=0.001$) groups for *MST1* gene. Also, our ROC curve when comparing benign and HGSOC groups showed an AUC of 0.911 for *YAP1* gene with specificity of 90 percent and sensitivity of 80, and an AUC of 0.9 for *MST1* with a specificity of 100 percent and sensitivity of 70.

In conclusion, we suggest that the components of Hippo signaling pathway could be used as a potential biomarker in ovarian cancer diagnosis. Although this study shows promising results, further larger studies including other Hippo signaling pathway components are essential to validate these components as potential biomarkers for cancer diagnosis.

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G15

SRA GENE FUNCTION IDENTIFICATION IN BARLEY, USING TARGETED MUTAGENESIS

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Barley (*Hordeum vulgare*) ranks as the fourth most widely cultivated cereal crop in the world, making it a valuable object for genetic research [1]. Understanding unknown barley genes functions can reveal the path to developing superior varieties with higher yield [2]. Barley inflorescence architecture has been studied and altered for many years, as it is one of the main yield-relevant parameters [3]. Still, many questions about the inflorescence genetic regulatory pathways remain unanswered. *HvSRA* (*SISTER OF RAMOSA3*) gene, a paralog of maize *RA3*, is involved in the development of barley spikelets, but the exact gene function is yet to be determined [4]. The emergence of CRISPR-Cas9 has provided scientists with an easier way to create knock-out mutants, which are a great reverse genetics tool. By combining *Agrobacterium tumefaciens* T-DNA-mediated transformation with CRISPR-Cas9 vectors, it is possible to introduce targeted mutations into the barley genome [5].

This study aimed to explore *HvSRA* gene function by creating gene knockouts in the 'Golden Promise' barley. Initially, two binary vectors were constructed, each containing hygromycin resistance cassette, cas9 cassette and two guide RNA's. Before transforming *A.tumefaciens*, constructs were cloned in *Escherichia coli* bacteria and verified by agarose gel electrophoresis and sequencing. Immature embryos of 'Golden Promise' were isolated and inoculated with *Agrobacterium* containing T-DNA binary vector. After inoculation, embryos were cocultivated with *Agrobacterium* on callus induction medium without antibiotics and then transferred to a medium containing antibiotics for transgenic callus selection. After 6 weeks of callus induction and selection, callus was moved into barley regeneration medium. Regenerating plants were already observed after one week. So far, this study managed to successfully construct T-DNA binary vectors, introduce them to 'Golden Promise' via *A. tumefaciens* T-DNA-mediated transformation and obtain early development stage *HvSRA* knock-out barley plants.

Future work will be focused on growing mature barley knockouts and verifying targeted mutations using sequencing. If *HvSRA* knockouts are successfully created, phenotyping will be done to determine the potential function of *HvSRA*.

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G16

SHORTENED TELOMERES AND THEIR ASSOCIATION WITH SARCOPENIA AND FRAILTY IN OLDER ADULTS

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Background. In response to an aging society, there is a growing interest in research on the cellular and molecular mechanisms of aging. Telomere shortening is considered one of the potential molecular causes of the risk of developing age-related pathologies. Sarcopenia and frailty are overlapping geriatric syndromes that, due to reduced muscle mass, strength, and functional capacity, lead to impaired quality of life, increased morbidity and mortality in older adults. However, the pathogenesis and molecular mechanisms of these syndromes remain poorly understood. Therefore, the aim of this study was to investigate the properties of leukocyte telomere length in older adults with sarcopenia and frailty.

Methods. This study included 197 older adults (aged 82.2±7.6 years): 121 individuals with sarcopenia and/or frailty (aged 85.3±6.7 years) and 75 community-dwelling older adults (aged 80.2±7.5 years) without sarcopenia and/or frailty. Sarcopenia was confirmed according to the European Working Group on Sarcopenia in Older People (EWGSOP) guidelines, and frailty was assessed by Fried's criteria. Phenotypic data were collected via questionnaires, scales (e.g., Physical Activity Scale for Elderly (PASE)), and measurements of anthropometric and physiological characteristics. Leucocyte relative telomere length (LrTL) was determined by real-time quantitative polymerase chain reaction (qRT-PCR) on DNA extracted from blood samples and quantified as the telomere/single-copy gene (T/S) ratio. Statistical analysis was performed using R Studio (version 4.3.1).

Results. The average LrTL in older adults participating in this study was 0.408±0.262 T/S units. Individuals with sarcopenia and/or frailty had shorter LrTL (0.345, 95% CI [0.310, 0.380]) compared to community-dwelling older adults (0.469, 95% CI [0.398, 0.539], $p<0.002$). A significant negative correlation was found between LrTL and the age of elderly participants ($r = –0.29$, $p<0.001$), with no significant gender differences in LrTL. The likelihood of developing sarcopenia and/or frailty increased by 12.5% with each additional year of age (OR=1.125, 95% CI [1.033, 1.228], $p=0.007$). An interaction was also observed between LrTL and physical activity level: longer LrTL was associated with higher PASE scores ($\beta=46.10$, 95% CI [20.22, 71.99], $p<0.001$), although this relationship weakened with increasing age.

Conclusions. Our findings indicate a significant correlation between shorter telomeres and greater manifestations of frailty and sarcopenia, suggesting that telomere length may serve as a biomarker for these age-related conditions. A deeper understanding of this association could inform interventions aimed at improving healthspan and quality of life in the aging populations.

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G17

GENOTOXICITY ASSESSMENT OF BALTIC SEA CONTAMINANTS IN PERIPHERAL BLOOD LYMPHOCYTES OF JUVENILE GREY SEALS (*HALICHOERUS GRYPUS*)

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The Baltic Sea is heavily impacted by anthropogenic pollution, leading to the accumulation of hazardous chemicals that may pose a risk to marine organisms [1]. This is a persistent problem as many contaminants are not easily degradable and are prone to bioaccumulation. Grey seals (*Halichoerus grypus*), as top predators of the Baltic Sea, are particularly vulnerable to bioaccumulation of these contaminants. Moreover, pollutants are passed to younger generations through the mother: as seal pups acquire nutrition through their mother's milk, they also accumulate a significant portion of contaminants, potentially affecting their health [2].

In this study, we evaluated the genotoxicity of various contaminants (polychlorinated biphenyls, bis(4-chlorophenyl)sulfonate, dichlorodiphenyldichloroethylene, allethrins, methylbenzylidene camphor, homosalate and alpha-tocopheryl acetate) of the Baltic sea in peripheral blood lymphocytes of juvenile grey seals (*Halichoerus grypus*).

Blood samples were collected in Estonia from 20 grey seal pups during the spring of 2024 and cryopreserved for later analysis. To conduct our research, we have made several adaptations: first, we identified a suitable approach for genotoxicity evaluation in cryopreserved samples; second, we adapted the high-throughput 12 gels comet assay [3] in our laboratory; and finally, we determined suitable concentrations of contaminants for our experiment. Using these optimized conditions, which were achieved using human peripheral blood cells to minimize the use of grey seal blood, we proceeded to conduct experiments on grey seal samples, applying high-throughput comet assay and selected chemical concentrations. Mononuclear cells were incubated with varying concentrations of the selected chemicals (10-50 µM) for short-term (4 h) and long-term (24 h) exposure, cell viability was assessed using ethidium bromide–acridine orange staining, genotoxicity was assessed using alkaline and enzyme-linked comet assay.

Alkaline comet assay results showed no significant difference between cryopreserved and fresh blood samples, nor between the 2-gel and 12-gel comet assay approaches. For different chemicals we selected concentrations up to 50 µM, which were identified as non-cytotoxic (cell viability > 70%). As for results with grey seal blood, in most cases, we noticed a concentration dependent increase in oxidative DNA damage and DNA breaks suggesting genotoxic effect of contaminants found in the Baltic Sea. Further research is necessary to fully understand the long-term and population-level effects of these contaminants on grey seals.

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G18

DNMT3A METHYLTRANSFERASE CLINICALLY RELEVANT VARIANT INTERACTION WITH DNA

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In eukaryotes, DNA methylation is important for a large part of cellular processes. These include cell type and lineage determination, transcription, chromatin structure and genome stability. Disruption of DNA methylation and its control mechanisms can lead to atypical DNA methylation, misregulation of cell cycle or DNA repair genes, and chromosomal instability, leading to various diseases. This crucial epigenetic mark is installed by specific enzymes – DNA methyltransferases (MTases) DNMT3A, DNMT3B and DNMT1 by attaching a methyl group to a cytosine, mostly in CpG sequences. DNMT3A and DNMT3B MTases carry out *de novo* methylation that shapes cell fate [1].

Tatton-Brown-Rahman syndrome (TBRS) is a congenital autosomal overgrowth syndrome, which is caused by heterozygous mutations in the DNMT3A MTase gene. The syndrome is characterized by increased height and/or head circumference, obesity, intellectual disabilities and seizures. Subtle dysmorphic features are also common. The syndrome was first described in 2014, with data from 13 sequenced genomes and found to have heterozygous DNMT3A variants. The majority of these variants were located in one of the three known functional domains of the MTase: the PWWP domain, which binds the methylated H3K36 histone, the ADD domain, which binds the non-methylated H3K4 histone, and in the domain of the MTase that is responsible for the catalytic DNA methylation activity [2].

In 2024, another unique TBRS-causing mutation in DNMT3A MTase was described - Ser775Tyr. It was identified in a patient who was diagnosed with cardiovascular disorders at VU Hospital Santaros Clinics [3]. Ser775Tyr mutation is located in the catalytic domain of the DNMT3A MTase, but relatively distant from the active site and DNA binding loops. The seemingly indirect effect of the mutation to the catalysis mechanism, yet obvious phenotypic penetrance prompted us to investigate deeper into the molecular pathogenesis pathway of this curious case. Bioinformatical analysis suggested that the mutation may change the DNA binding loop flexibility through the network of amino acid interactions. To test that experimentally, recombinant human DNMT3A wt and Ser775Tyr variants were purified from yeast and their DNA binding affinity determined by electrophoretic mobility shift assay (EMSA). As DNMT3A is a large multidomain protein, special EMSA conditions had to be optimized. The results show that Ser775Tyr variant has significantly lower DNA binding affinity as compared to the wt DNMT3A.

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G19

Methylation of Specific Genes' Regulatory Sequences In Urine Sediments of Kidney Cancer Patients

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In 2022, Lithuania was number two in kidney cancer mortality in the world, accounting for males and females of all ages. Early detection of otherwise mostly asymptomatic kidney cancer leads to better treatment outcomes. The currently available diagnostic methods, such as medical imaging and biopsies, are either invasive or lacking in their prognostic qualities. All of this demonstrates the need for novel non-invasive biomarkers for kidney cancer diagnosis and prognosis.

As kidney cancer develops, natural cellular mechanisms, such as DNA methylation, are disrupted. These alterations, specifically promoter hypermethylation, can be detected, which could allow for the development of novel diagnostic and prognostic biomarkers for the disease. The goal of this research is to investigate the methylation of the specific gene regulatory sequences in urine sediments of kidney cancer patients to estimate their potential as non-invasive diagnostic and prognostic biomarkers.

DNA methylation differences in kidney cancerous and non-cancerous tissues have been explored in our lab before by using Agilent Human DNA Methylation 1 × 244K Microarrays. From this research, three specific genes were selected and primers for them were designed. Quantitative methylation-specific PCR was used to investigate the methylation of selected sequences in urine sediments from both kidney cancer patients and asymptomatic controls. Acquired results were analyzed utilizing t-test and ROC curve statistical analyses.

Our t-test analysis results show statistically significant higher methylation levels of all genes in kidney cancer patient urine sediments compared to asymptomatic control (all $P < 0.05$). Furthermore, ROC analysis showed all three genes' regulatory sequence methylation as significant diagnostically, with the combination of all three having the highest AUC value of 0,80 (sensitivity – 75,6% and specificity – 80,0% with $P < 0,001$). Moreover, the methylation level of one gene was found to have significant prognostic qualities ($P < 0,05$) when comparing early and late-stage kidney cancer. In conclusion, analyzing the methylation – of our selected genes' regulatory sequences in urine sediments of both asymptomatic controls and kidney cancer patients showed the diagnostic potential of all three and the prognostic potential of one of them. Overall, kidney cancer mortality can be improved with earlier diagnosis and our research seeks to expand the available potential biomarkers for the disease.

G20

GENOTOXIC EFFECTS OF ULTRA-HIGH DOSE RATE IONIZING RADIATION ON CELLS *IN VITRO* AND *IN VIVO*

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According to the WHO, at least one in five people develop cancer in their lifetime [1]. To combat it, radiation therapy is usually utilized, during which cancerous cells are irradiated, and the tumor is mitigated. In most cases, a conventional therapeutic dose rate (~0.03 Gy/s) of radiation is administered, although, in 2014, a new experimental approach to radiotherapy was discovered, FLASH therapy. In this method, an ultra-high dose rate (UHDR, >40 Gy/s) of radiation is delivered in a rapid burst (<1s), thus being a more time-efficient treatment method than conventional radiotherapy while hypothetically minimizing the damage to the surrounding healthy tissues. However, this method and its effects are not sufficiently investigated.

The aim of this study is to analyze the genotoxic effect of conventional and UHDR of acute ionizing radiation in different biological systems *in vitro* and *in vivo*.

In this study, to evaluate the genotoxic effect of different radiation dose rates *in vitro*, human peripheral blood lymphocytes were irradiated using TrueBeam 6MV linear accelerator at two dose rates (0.005 Gy/s, 3.1 Gy/s) and ten varying doses ranging from 1.22 Gy to 5.07 Gy of acute ionizing radiation. The genotoxic effect was evaluated by carrying out a comet assay, which determines the DNA damage in individual cells [1], and the micronucleus assay, in which the frequency of micronucleus (MN) formation in binucleated cells was analyzed. Based on the MN assay results, two dose-response curves were created using Biodose Tools software [2]. The comet assay results demonstrated that ionizing radiation displays the genotoxic effect for lymphocytes at both radiation dose rates and most of the doses. MN assay results showed that at lower doses of radiation (0-2 Gy) the genotoxic effect of different dose rates is very similar, however at higher doses (2-5 Gy), the UHDR induces a more substantial frequency of micronuclei, thus showing a higher genotoxic effect in the *in vitro* system.

The genotoxic effect of different dose rates *in vivo* was evaluated by the somatic mutation and recombination test (SMART) in *Drosophila melanogaste*r. This test is based on the loss of heterozygosity of two recessive markers, *mwh* or *flr*^{3}, in *D. melanogaster* wing cells. These cells are highly sensitive to genetic changes, which are visibly expressed phenotypically [3]. Second- and third-instar larvae of *D. melanogaster* were divided into two groups. The first group was irradiated with a UHDR (50 Gy/s) at four doses ranging from 3.76 to 35.58 Gy, while the second group was exposed to a conventional dose rate (0.1 Gy/s) at four doses ranging from 4 to 40 Gy. After eclosion, the viability of adult flies was assessed. At the highest dose, flies in the UHDR group exhibited more than twice the survival rate compared to those irradiated using the conventional dose rate (64.29% vs. 30.43%, respectively). Later, the adult flies' wings were analyzed in two groups based on different genotypes: *mwh/flr*^{3} and *mwh/TM3*. The frequency of *mwh*, *flr*^{3}, or *mwh-flr*^{3} twin spots was compared between UHDR and conventional dose rate groups. In *mwh/flr*^{3} genotype wings, a statistically significant difference between dose rate groups was observed at 16 Gy, with a greater genotoxic effect observed at the conventional radiation dose rate, while in *mwh/TM3* genotype wings, significant differences were detected at doses of 4 Gy, where UHDR dose rate was linked to higher DNA damage level, and 16 Gy, where the conventional dose rate had a greater genotoxic effect.

In conclusion, the preliminary results of our study reveal that the UHDR of ionizing radiation has a multifaceted and sometimes contradictory genotoxic effect in different biological systems, at some doses showing a higher genotoxic effect than the conventional dose rate, and at some smaller. Thus, the genotoxic effect of UHDR should be further analyzed.

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G21

NOVEL EPIGENETIC BIOMARKERS FOR RENAL CLEAR CELL CARCINOMA DIAGNOSIS AND PROGNOSIS

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Renal clear cell carcinoma (RCCC) is the main pathological type of kidney cancer. In the early stages of this disease, symptoms are unremarkable, and late diagnosis threatens patient survival and quality of life. A diagnostic test, designed for detecting DNA methylation changes, could be a potential solution to this problem. DNA methylation is considered a common, early, and stable event in tumorigenesis that can be detected even in small amounts of DNA [1]. Epigenetic alterations could also provide valuable insight into the progression of RCCC. DNA methylation biomarkers could be used for designing a liquid biopsy test and potentially enable diagnosis and prognosis in a non-invasive manner.

The aim of this study was to determine methylation levels in regulatory regions of three genes involved in the regulation of gene transcription in cancerous and healthy renal tissues as well as urine samples in order to investigate their diagnostic and prognostic potential.

Quantitative Methylation-specific PCR method was utilized to quantify DNA methylation levels in 126 RCCC and 45 non-cancerous renal tissue (NRT) samples as well as urine samples collected from 43 RCCC patients and 21 asymptomatic controls.

The obtained results revealed that methylation levels of all three genes were higher in cancerous tissues and RCCC patient urine samples when compared to healthy tissues and asymptomatic controls ($P < 0.05$). In order to determine the diagnostic potential of these genes, ROC analysis was used. In tissues, the panels of two to three genes showed high diagnostic capabilities (AUC = 0.90). Moreover, in the urine samples, the same gene panels demonstrated excellent diagnostic potential (AUC = 0.95). The prognostic capabilities of the investigated potential biomarkers were evaluated for tissue samples based on clinical data. This analysis showed that gene promoter methylation levels were higher in the advanced stage (pT3 – pT4) and lower differentiation tumors (Fuhrman degree $\geq$ 3 and WHO/ISUP degree = 3). Furthermore, methylation levels of the three genes were positively correlated with tumor size. In addition, methylation levels were higher in tumors with fat tissue invasion and in tumors with necrosis zones.

In conclusion, investigated genes have high potential as valuable diagnostic and prognostic biomarkers for RCCC.

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G22

GENETIC PROFILING OF HUMAN REMAINS FROM THE 13-15TH CENTURIES

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One of the oldest cemeteries of Vilnius, the capital of Lithuania, is located in the eastern part of the Old Town, an area known as the Ruthenian City, or *Civitas Rutenica*. The burial site is located at Bokšto St. 6, which at the time was associated with the Orthodox Christian community. According to the collected data, the burial site at Bokšto St. 6 dates from the end of the 13th century to the beginning of the 15th century. It was a complex period when Christianity was replacing the pagan worldview in the country. Vilnius officially remained pagan until 1387, but the cemetery discovered on Bokšto St. 6 shows Christian burial characteristics [1]. Although there is a lack of documentation on the origins of the inhabitants buried at this site, some assumptions can be made based on archaeological and historical data. Moreover, it is possible to analyze bioarcheological material genetically. The genetic analysis of ancient DNA (aDNA) provides a deeper understanding of the complex genetic and cultural history of the Medieval period.

The aim of this work was the genetic profiling of ancient human remains from the 13th to 15th centuries excavated in Bokšto St. 6 cemetery.

aDNA extraction from four samples obtained from four human individuals' remains (teeth) was carried out using a magnetic particle method (according to the commercial forensic kit guidelines). Extracted aDNA concentrations were measured by fluorometric and spectrophotometric methods. STR (Short Tandem Repeats) profiling (24 markers) was done using a commercially available PCR kit according to the manufacturer's recommendations. Fragment analysis was performed on *Applied Biosystems SeqStudio Genetic Analyzer* instrument polymer 5 using the LIZ600 size standard. Data analysis was done using *RStudio 2023.03.1+446* and the online software *STRAFF 2.2.2*.

Genetic STR profiling showed distinct genetic clustering among four individuals. Additionally, STR profiles with modern European population profiles were compared.

In conclusion, it is possible to establish a genetic structure using STR profiling from ancient human remains. STR profiles can reveal possible biological relatedness among individuals and their relation to modern European populations. These results could contribute to establishing the ancestral origin of individuals. Future analyses using large datasets and additional genomic techniques will complement the understanding of the genetic history of this historically significant region.

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G23

Identifying Novel Biomarkers for Early Detection and Treatment of Brain Tumors

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Introduction. Glioblastoma (GBM), the most common WHO grade IV glioma, is known for its aggressive nature and low average patient survival time (averaging <15 months) [1]. Glioblastoma accounts for 57% of all glioma cases and 48% of primary malignant central nervous system neoplasms [1]. RNA methylation is a new focus of research as a possible contributing factor, which influences the cell cycle, the structure and stability of RNA molecules [2]. With the development of personalized medicine, RNA methylation may be used in targeted gene therapy to alter gene expression and correct abnormal RNA methylation patterns. These regulators may serve as early diagnostic biomarkers to detect cancer in early stages. Transcription factor SOX2 is associated with tumor aggressiveness and poor prognosis, and is important for glioma development and growth [3]. Investigation of a modified SOX2 variant without the C-terminal domain, a region crucial for transcriptional activation, offers valuable insights into its impact on glioma cell survival and proliferation. These findings could support the development of non-invasive diagnostic and monitoring techniques, allowing for early glioma detection, assessment of disease progression, and evaluation of treatment effectiveness. This study investigates how methylation regulators *ALKBH5*, *FTO*, *METTL1*, and *BUD23*, along with a modified SOX2 variant, contribute to glioma initiation and progression, potentially revealing new therapeutic targets.

Aim. To explore the functions of RNA methylation modifications and SOX2ΔC in glioma and assess their potential as diagnostic and therapeutic biomarkers.

Methods. cDNA samples from tumor biopsies of 54 glioma patients were examined along with de-identified clinical information. Quantitative RT-PCR was performed to measure gene expression levels in both patient samples and modified glioblastoma U87 SOX2ΔC cell lines. Additionally, cell migration assay was performed. Bioinformatic analysis of RNA sequencing data was conducted to identify significant changes in gene expression and biological pathways following *ALKBH5* knockdown.

Results. We discovered that *ALKBH5, FTO*, and *BUD23* gene expression increased with glioma malignancy, suggesting them as potential targets for therapy or diagnostic tools. Patients with higher levels of these genes showed longer survival. The study revealed associations between gene expression and factors such as IDH mutation, gender, age, and survival time, suggesting potential applications in personalized treatment. Additionally, SOX2ΔC impacted *ALKBH5* and *BUD23* expression, suggesting its potential role in regulating cancer development. Furthermore, the study discovered that *ALKBH5* plays a critical role in gene expression. RNA sequencing analysis of *ALKBH5* knockdown revealed significant transcriptomic alterations, with over half of all detected transcripts showing differential expression, confirming its critical role in gene expression. Additionally, *ALKBH5* knockdown impacted important biological and oncogenic pathways, such as homologous recombination, mismatch repair, DNA replication, and the cell cycle, supporting its role in glioblastoma progression. Lastly, novel therapeutic targets were identified based on their functional relevance to gliomas and known implications in other malignancies.

Conclusions. This study identifies RNA methylation regulators *ALKBH5*, *FTO*, and *BUD23*, as well as SOX2ΔC, as promising biomarkers and potential therapeutic targets for glioblastoma, offering valuable insights for the development of personalized treatments.

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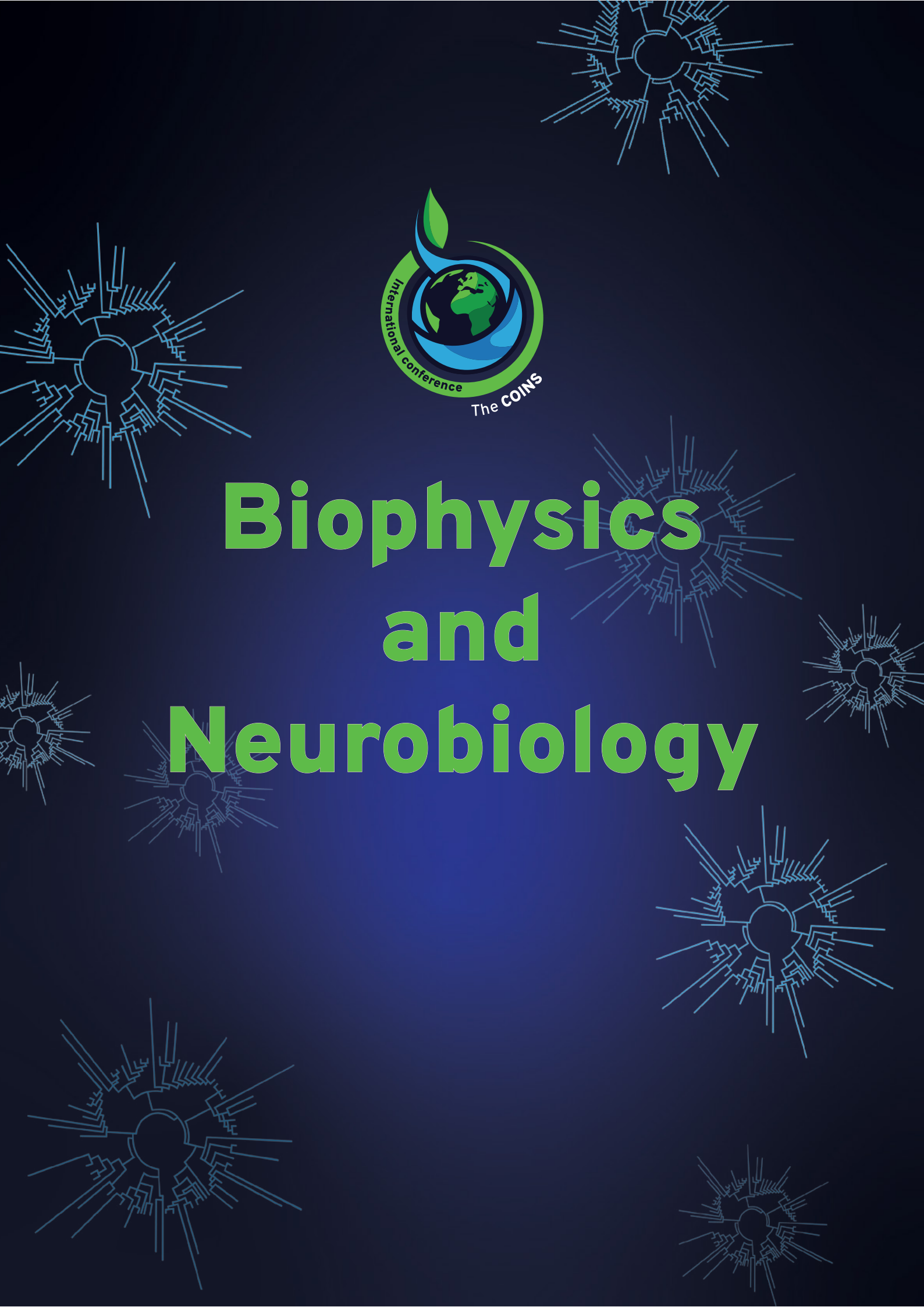

Biophysics and Neurobiology

H1

IMPACT OF CdSe/ZnS AND CuInZnS/ZnS QUANTUM DOTS ON MICROALGAE AUTOFLUORESCENCE

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Quantum dots (QDs) are nanometer-sized semiconductor particles, which have gained popularity for their unique optical and chemical properties, enabling applications in imaging, real-time sensing and monitoring [1]. However, the growing production and use of nanoparticles raise concerns about their potential release into aquatic ecosystems. Microalgae, as bioindicators, play a crucial role in assessing water quality and detecting pollutants due to their sensitivity to environmental changes and rapid physiological responses [2]. The interaction of some types of nanoparticles with microalgae can disrupt vital cellular processes, such as photosynthesis [3]. However, it is still unknown how effects of different types of QDs depends on algae species and how environmental conditions influence this.

The aim of this study is to evaluate the interaction of CdSe/ZnS-COOH (Cd-based) and CuInZnS/ZnS-COOH (Cu-based) QDs with *Desmodesmus sp.* and *Scenedesmus sp.*, comparing species-specific responses and QD-induced impact on photosynthetic efficiency and overall physiology. Microalgae were exposed to quantum dots and the images captured with fluorescence microscope as well as the parameters of photosynthetic activity of algae were registered during 96 hours. To evaluate these effects, Pulse-Amplitude modulated (PAM) fluorometry offers a reliable, non-invasive method for monitoring changes in their autofluorescence and photosynthetic parameters.

The PAM fluorometry results show a decline in F_V/F_M (maximum photochemical quantum yield of PS II) in microalgae in both species upon exposure to QDs (Fig. 1A) compared with control algae samples without QDs. However, Cu-based QDs caused a more pronounced reduction of F_V/F_M as well as the nonphotochemical quenching of fluorescence compared with Cd-based. PAM results also showed that the two microalgae species respond similar to QDs exposure, however Cu-based QDs having a stronger negative effect on cell physiology specially on *Scenedesmus sp.* microalgae. The Cd-based QDs had a weaker impact, possibly due to their tendency to form visible aggregates observed in fluorescence images (Fig. 1B), which may have reduced their bioavailability and interaction with the cells.

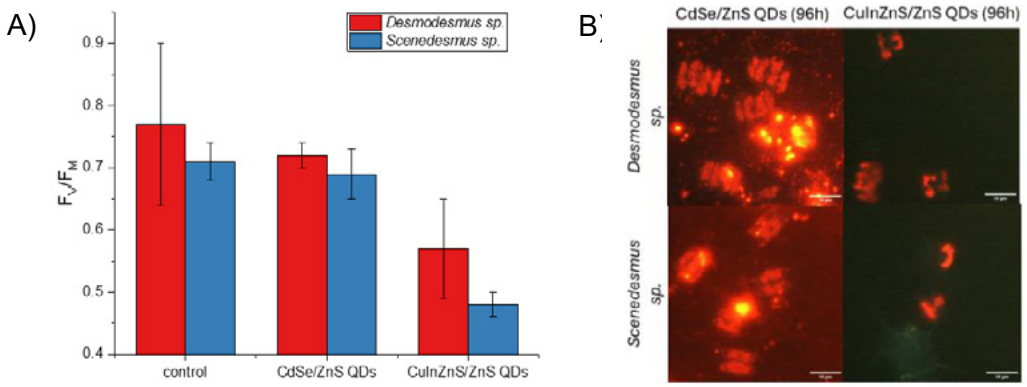

Figure 1. The F_V/F_M values in algae after 72 hours of exposure to CdSe/ZnS and CuInZnS/ZnS quantum dots (mean ± 95% CI value) (A). Fluorescence microscopy images of algae ($\Delta\lambda_{ex}$ = 380–420 nm, dichroic mirror: 430 nm, bandpass emission filter: > 450 nm) after 96 hours of exposure to QDs (B), scale bar is 10 µm.

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H2

Mg-CHLOROPHYLLIN ANTIOXIDATIVE ACTIVITY ON MICROALGAE: SPECTROSCOPIC STUDY

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Microalgae are sensitive to changes in environmental conditions and are among the first organisms to respond to these changes. Nevertheless, different nutritional conditions can lead to physiological state variation reflected in chlorophylls autofluorescence (AF). Spectroscopic AF measurements are relatively fast and non-invasive, making them suitable for studying organisms under natural conditions.

Environmental stressors, such as reactive oxygen species or nanoparticles (NPs) could lead to varying physiological responses of algae under different growth conditions. It is already known that quantum dots (QDs) as NPs could cause oxidative stress in algae [1], but research to prevent or reduce the negative effects of NPs is limited. Magnesium chlorophyllin (Mg-Chl), photoreactive molecule, derived from naturally occurring chlorophyll, is studied as a potential protective agent for mitigating oxidative stress in human and animal cells [2, 3]. However, the antioxidative effects of chlorophyllin on plants and algae have not yet been thoroughly studied.

This study aims to determine whether Mg-Chl reduces hydrogen peroxide-induced oxidative stress and negative effects of CuInZnS/ZnS-COOH quantum dots (562 nm, Nanooptical materials, USA) on *Scenedesmus sp.* microalgae in two different growth media at controlled light conditions. Prior and during experiments, algae were grown in mineral fertilizer dissolved in distilled water (FDW) or in modified Wilkins-Chalgren growth medium (MWC) under 12/12 day/night white light illumination conditions (6W, 8000K LED). Algae were incubated with 4 µM of QDs for 24 h before introducing Mg-Chl and then were divided in two sub-groups – those that were further kept under previous light conditions and those that were further kept in the dark. The steady state fluorescence spectra of the samples were measured using an AvaSpec-3648 spectrometer (Avantes, The Netherlands), and Pulsed-Amplitude-Modulation Junior-PAM fluorometer (Heinz Walz GmbH, Germany) was used to get autofluorescence induction curves and to calculate photosynthetic parameters of algae.

The QDs in MWC medium gradually reduced the electron transport rate (ETR) and quantum efficiency (Y(II)) of the second photosystem (PSII) throughout experiment. Meanwhile in samples where Mg-Chl was introduced, ETR and Y(II) decline stopped after 48 hours only under illumination and no Mg-Chl effect was registered in dark-kept samples. In addition to previously published findings on QDs effect dependency on algae cultivation media [4], these results raise further questions whether Mg-Chl effect could vary as well. Mg-Chl effect against H_2O_2-induced oxidative stress is also analysed in this study.

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H3

Full Field Optical Coherence Tomography In Vivo Retinal Imaging with Optimized Beam Splitter

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Full Field Optical Coherence Tomography (FF-OCT) is one of the most upcoming methods for *in* vivo imaging, but there is always a need to improve imaging speed, depth and quality. Fourier Domain (FD-) FF-OCT is most used for retinal imaging due to its speed and innate possibility to digitally correct aberrations [1]. Usually, interferometers for this kind of imaging are based on symmetrical, 50/50 beam splitters, thus by default losing over a half of the overall light and most importantly – more than half of the signal, since intensity of light that can be sent to an eye safely is limited.

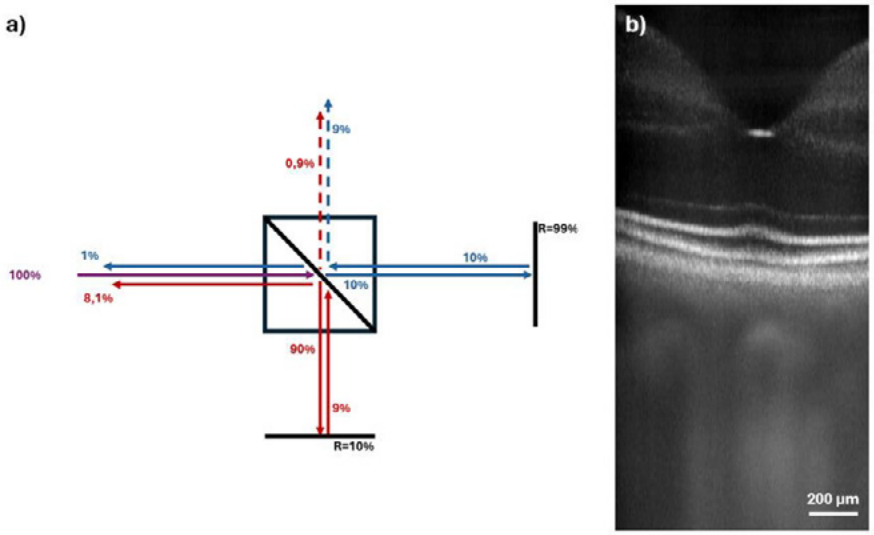

Figure 1. a) – 90/10 cube beam splitter. b) - FF-FD-OCT image of in vivo human retina registered using 90/10 cube beam splitters.

Due to these reasons, the goal of this work is to optimize beam splitting ratios for FF-OCT in both Fourier and Time Domain (TD-) FF-OCT and to improve imaging Signal to Noise Ratio (SNR). For the first time, 90/10 cube beam splitter was applied for FD-OCT imaging and a unique, low-loss ablated mirror beam splitter design was tested (Figure 1 a). As a result, it was shown that asymmetric, high throughput beam splitter configurations increase TD- and FD-FF-OCT systems' SNR by 2,5 times and that digital dispersion correction algorithm can be successfully implemented (Figure 1. b).

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H4

OPTIMIZATION OF FULL-FIELD OPTICAL COHERENCE MICROSCOPY FOR BIOMEDICAL TISSUE IMAGING

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Optical coherence tomography (OCT) is an interferometric imaging technique commonly used for visualizing various biomedical samples. Standard OCT systems typically achieve axial resolutions between 1-10 µm and lateral resolutions between 3-10 µm. The axial resolution of an OCT system is mainly influenced by the spectral width (and consequently the coherence length) of the light source. In contrast, the lateral resolution depends on the numerical aperture of the objectives. Full-field (FF-) OCT can surpass these resolutions, achieving results better than 1 µm, thanks to a combination of factors [1,2].

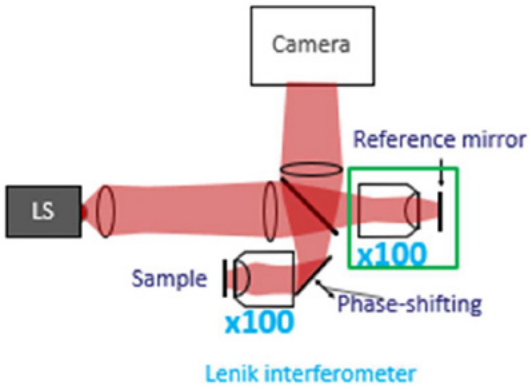

Figure 1. Principle schematics of extremely high-resolution FF-OCT system.

FF-OCT system is shown in Fig. 1, featuring Linnik interferometer with two identical high NA immersion objectives, a spatially incoherent white light source and a camera. Reference mirror is mounted on a piezo actuator to enable phase shifting.

Here we compare two incoherent white light sources: the new LS-WL1 and the Thorlabs MCWHL7, which differ in output power and etendue. By incorporating the LS-WL1, a light source with lower power but also lower etendue, into our system, we achieved a 6-fold increase in light intensity on our specimen. This resulted in significantly greater imaging depth (see Fig. 2), a 9-fold improvement in the signal-to-noise ratio.

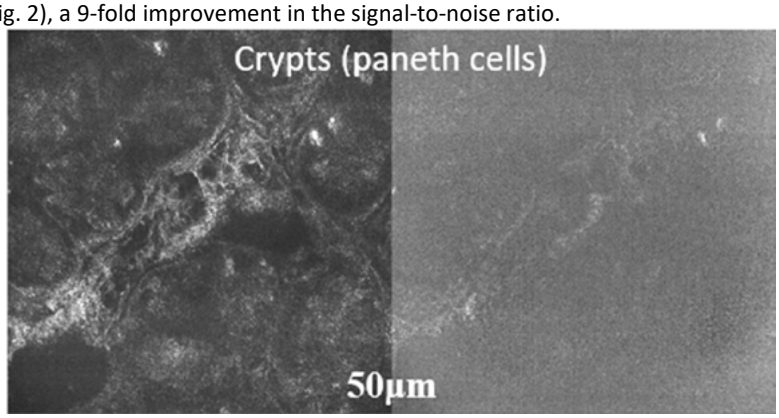

Figure 2. Nano FF-OCT images of mouse intestinal tissue at depth of 50µm beneath the sample surface. Images clearly showcase difference between the new light source (left image) and the old cold white LED light source (right image).

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H5

The activity of the rodent's primary auditory cortex during the circadian rhythm

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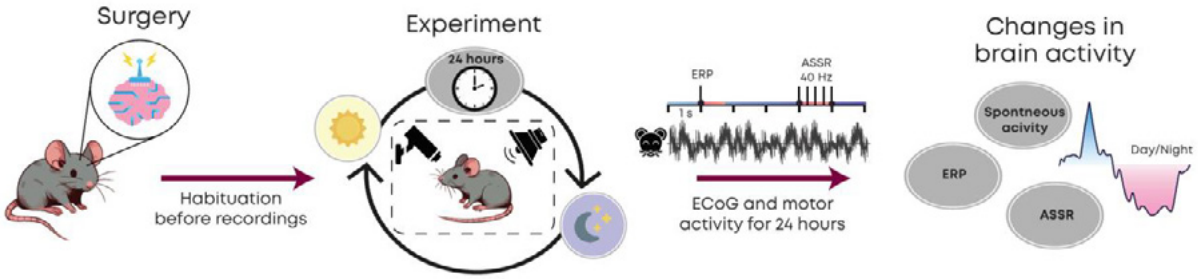
It is widely accepted that bodily processes are regulated by circadian rhythms over a 24-hour period (Logan & McClung, 2019). The circadian rhythm sets not only the sleep-wake cycle but also influences arousal, vigilance, and attention throughout the day (Schmidt et al., 2007). These internal states have been shown to have a direct impact on the brain, where low-frequency oscillations are prevalent during rest, while high-frequency oscillations occur during motor activity or increased arousal (McCormick et al., 2015). Although the diurnal changes in general brain activity are well-established, it is unclear how they affect the auditory system. There is evidence that the hearing system expresses core clock genes, which oscillate in synchrony with the suprachiasmatic nucleus (SCN), the master pacemaker entraining circadian rhythms (Meltser et al., 2014). Also, hearing sensitivity changes over the day, as demonstrated by acoustic startle response and recovery to noise exposure, but further research involving the functional changes in the auditory system remains sparse (Ngo et al., 2023). Experiments are often conducted without considering the time of day, which can have a significant impact on the results obtained and, in turn, reduce their translatability. Therefore, it is necessary to investigate how brain activity changes under the same recording conditions but at different times of the day.

Figure 1. A simplistic overview of the registration methods and obtained results.



The aim of this study was to investigate the changes in the auditory system activity over a 24-hour period. To this end, we performed chronic electrocorticogram (ECoG) recordings from freely behaving mice while simultaneously monitoring their motor activity (Fig. 1). The motor activity of the recorded mice exhibited a well-characterised circadian rhythm, with mice being active at night and resting throughout the day. In line with the circadian rhythm of motor activity, the brain oscillations also demonstrated circadian changes, with high-frequency oscillations being more pronounced at night and low-frequency oscillations being more prominent during the day. Next, we assessed the responsiveness of the auditory system through measuring event-related potentials (ERPs) and auditory steady-state responses (ASSRs). A notable change in auditory ERPs was observed over the 24-hour period, with larger ERPs present during the day. Similarly to the preceding results, the ASSRs increased in the daytime. This study demonstrates that the auditory system undergoes substantial changes during the day, highlighting the importance of accounting for the time of experiments.

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H6

MATERNAL HIGH-FAT DIET EFFECT ON THE INFLAMMATORY RESPONSE IN THE OFFSPRING'S RETINA

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Aim: The increased high-fat content in today's society's diet is one of the leading factors contributing to the rising obesity rates. Research studies indicate that maternal high-fat diet (mHFD) can cause neurodevelopmental disorders in the offspring due to induced systemic inflammation [1, 2, 3]. Furthermore, inflammatory response changes throughout different stages of female mice estrous cycle [4, 5, 6]. Proteins such as CD68 and TSPO can be used as reliable biomarkers for detecting inflammatory response and microglia phagocytic and metabolic activation in the central nervous system, including the retina [7,8]. Although some studies show HFD effect on the retina, little research has been done to investigate its impact on the retina of the offspring [9]. This study aims to evaluate maternal diet induced changes of microglial CD68 and TSPO area and Müller cell activity in the peripheral retina of the offspring and assess how they depend on the stages of the female estrous cycle.

Methods: Female C57Bl/6J mice, from weaning and during pairing, pregnancy and lactation, were fed with either control diet (CD, 10% fat) or high-fat diet (HFD, 60% fat). The offspring were weaned to normal rodient diet. Collected eyeballs of the offspring were fixed with 4% PFA, cryoprotected, and sliced using cryotome. Microglia, Müller cells, CD68 and TSPO proteins in microglia were labeled immunohistochemically using anti-RFP, anti-GFAP, anti-CD68, and anti-TSPO antibodies, respectively. Before tissue collection, the estrous cycle stages were determined by vaginal cytology in female offspring (22 weeks old).

Results: We evaluated mHFD effect on offspring Müller cells, microglia cells, CD68 and TSPO area in microglia cells in peripheral retina. Our results showed that mHFD significantly increased the area of microglia, Müller cells, CD68 and TSPO in both male and female offspring's peripheral retina. However, we observed a more subtle mHFD effect on female offspring retina. Further research showed that these different mHFD alterations could be due to female hormone changes during female estrous cycle.

Conclusion: Our findings demonstrated that mHFD had an effect on both male and female offspring peripheral retina, and that the retinal inflammatory response could be dependent on reproductive hormones.

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H7

DETECTION AND QUANTIFICATION OF SYNAPTIC EVENTS IN MOUSE HIPPOCAMPAL CA1 NEURONS DURING POSTNATAL DEVELOPMENT

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The early stages of postnatal development in hippocampal pyramidal neurons are characterized by morphological and electrophysiological changes, which support the formation of neural networks and contribute to the functional maturation of neurons [1][2]. During this period, synaptic pruning plays a critical role in optimizing signal processing and transmission within the neuronal network [3]. However, quantification of synaptic activity in hippocampal pyramidal neurons proves challenging due to noisy signal, variability, and high number (>1000) of synaptic events.

The aim of this study was to improve the synaptic event recognition and quantification in the mouse hippocampal CA1 neurons electrophysiological recordings by refining detection algorithms. To achieve our goal, we analyzed recordings previously collected by our colleagues. Firstly, we tested and selected appropriate filters, such as low-pass filtering (500 Hz and 1000 Hz) and data reduction methods. Secondly, a synaptic event template search algorithm was employed with a focus on how the template's construction and the template matching criteria parameters affect precision and accuracy of synaptic event detection. Respectively, we manually constructed synaptic event template from multiple (5, 15, or 30) synaptic events in recordings. Finally, different template matching criteria (from 1 to 10, where increasing the matching criteria, the algorithm detects and selects synaptic events that are as similar as possible to the used template) was tested in the algorithm that allow fine-tuning for optimal synaptic event recognition.

Preliminary results indicate that a 500 Hz low-pass filter is highly effective at removing noise from recordings while preserving synaptic events across all amplitude levels. Including higher (>5) number of synaptic events to the search template does not improve the accuracy of event detection. Low template matching criteria leads to detection of false events, while high matching criteria results in detection just of fraction of synaptic events.

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H8

GAZE STRATEGY OF HUMBOLDT PENGUINS (*SPHENISCUS HUMBOLDTI*) DURING U-SHAPED UNDERWATER TURNS

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All animals experience optic flow in their visual system while moving. Translational optic flow is experienced when an animal moves along a straight trajectory, and it is used to estimate relative distances to the objects and direction of movement. Whereas rotational optic flow does not provide this information, rather, it disturbs efficient use of translational optic flow. However, animals usually move in various trajectories and translational and rotational optic flow always occur together. Therefore, gaze stabilization strategies have evolved. It has been shown that flying birds use rapid head saccades to stabilize translational optic flow, but there is currently no information about saccadic head movements in penguins or any other diving birds, leaving a significant gap in our understanding of how these species stabilize gaze underwater. This research aims to investigate gaze strategy in Humboldt penguins (*Spheniscus humboldti*) during U-shaped diving turns and determine the parameters of saccadic head movements, including duration, angular velocity, and amplitude. These findings will provide new insights into the visual-motor strategies of diving birds and contribute to the broader understanding of sensory and motor adaptations in aquatic environments.

The study was conducted on ten Humboldt penguins housed in the Lithuanian Sea Museum, Klaipėda. We used seven synchronized and calibrated cameras, which captured penguins during U-shaped turns from different angles at 240 frames per second, for underwater 3D videography. Initial data processing was performed using Argus software to determine beak coordinates, followed by further analysis in Python.

For the first time, it has been shown that rapid head saccades are used in underwater gaze stabilization, as observed in flying birds. Preliminary results show that penguin saccades' duration is longer, as well as saccade angular velocity is lower, than in flying birds. The number of saccadic head movements varies between analyzed dives. These findings suggest that Humboldt penguins adapt their visual-motor strategies to ensure stable vision underwater. Further research is needed to compare penguins with other diving birds that could offer broader insights into sensory adaptations in aquatic habitats.

H9

KETAMINE CHANGES THE RESPONSIVENESS AND SYNCHRONISATION OF THE AUDITORY SYSTEM BY ACTING ON SUB-CORTICAL BRAIN REGION

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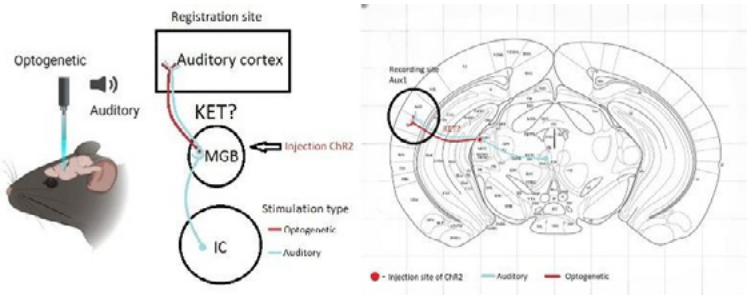
To model schizophrenia in rodents, N-methyl-D-aspartate (NMDA) antagonists are often employed [1]. The administration of these antagonists has been observed to induce behavioural and brain activity changes that are analogous to the symptoms of schizophrenia in humans [2,3]. A prominent characteristic of NMDA hypofunction is the alteration in auditory steady-state responses (ASSRs), which has been proposed as a potential biomarker for schizophrenia [4]. The administration of ketamine (KET) has been demonstrated to reduce ASSRs recorded in the auditory cortex [5,6]. However, the specific region of the auditory pathway affected by KET remains to be elucidated. The aim of this study was to identify the target of KET action, specifically whether changes in ASSRs occur in the auditory cortex or already in subcortical regions.

Methods. Wild-type C57BL/6 mice were used in this study. To record brain activity, Teflon-coated stainless steel electrodes were implanted into the primary auditory cortex (A1). Direct activation of A1 was achieved by means of a viral vector (pAAV-CaMKIIa-hChR2(H134R)-mCherry (AAV1), Addgene) encoding excitatory opsin ChR2, which was injected into the medial geniculate body (MGB). To activate projections of MGB, an optic fibre (FT400EMT, 400 µm 0.39 NA, Thorlabs) was implanted above A1. The excitability of the entire auditory pathway and A1 itself was tested by presenting 2 ms white noise stimuli (clicks, 70 dB) and 2 ms light pulses (470 nm, 1-5 mW) through the implanted optic fibre at 1 Hz, respectively. ASSRs and direct steady-state responses (dSSRs) were evoked by presenting the same auditory clicks or light pulses respectively at 40 Hz for a duration of 1 second, with 2-second quiescence intervals. Brain activity was recorded before and 5 minutes after the administration of a sub-anesthetic dose of KET (i.p., 20 mg/kg). Power spectral density was calculated to evaluate spontaneous activity. The excitability of the auditory system was evaluated as the amplitude of the response to 1 Hz stimulation, and the brain synchronization was determined by conducting time-frequency analysis using the Morlet wavelet transformation, with the power (induced & evoked) and phase-locking index (PLI) calculated.

Results. The administration of KET resulted in a reduction of low-frequency oscillations and an increase of high-frequency oscillations. Furthermore, KET reduced responses to auditory stimulation, while responses to direct activation of MGB projections, remained unchanged. Finally, KET reduced the synchronisation of ASSRs but did not change the synchronisation of dSSRs.

In summary, the results demonstrate that KET acts primarily on sub-cortical brain areas of the auditory pathway, while the functions of the auditory cortex remain unchanged.

Figure 1. Scheme of research methodology.



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H10

APPLICATION OF FLUOROGENIC DYES FOR EVALUATION OF PROTEIN STABILITY BY THERMAL SHIFT ASSAY

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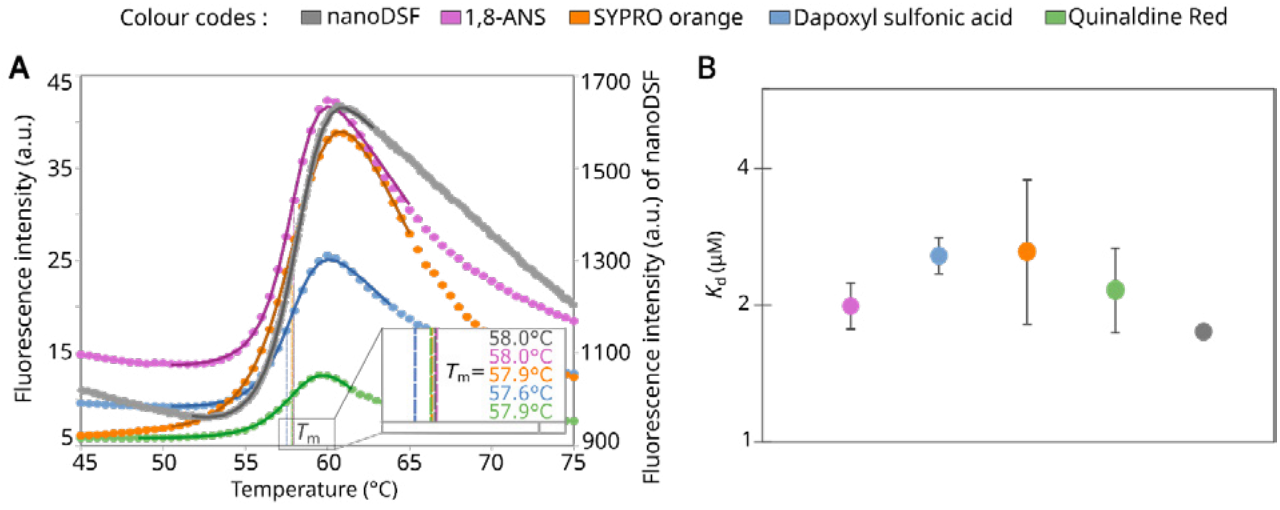
Fluorescent Thermal Shift Assay (FTSA) is a method used to assess protein stability by monitoring fluorescence intensity as proteins unfold with increasing temperature. Extrinsic solvatochromic fluorophores (dyes) are necessary for generating fluorescence signals. The fluorescence of such dyes is quenched in water but increases in a less hydrophilic environment, i.e., when bound to exposed hydrophobic sites of unfolding proteins. The protein unfolding curve generated during the assay helps to determine the melting temperature T_m of a protein. FTSA is a widely used technique in drug discovery research due to its efficiency, high throughput, ease of implementation and versatility. FTSA can be applied to both single-protein stability evaluation and protein-ligand binding affinity studies [1].

Although several fluorophores, such as 1-anilino-8-naphtalenesulfonate (1,8-ANS), are commonly used in FTSA experiments, they come with certain limitations. Some dyes tend to interact with proteins by destabilizing them and altering T_m value. Dyes can also exhibit quite high affinity to proteins and thus may interfere with the binding of ligands. Moreover, the fluorescence of a probe can sometimes overlap with the autofluorescence spectrum of a ligand, leading to increased background noise. Lastly, some fluorophores can be sensitive to additives, such as reducing agents commonly used to preserve proteins. These dyes may interact preferentially with additives than proteins themselves, resulting in decreased fluorescence or inaccurate reporting of protein unfolding [2]. Thus, new fluorophores must be found to overcome these limitations.

This study aims to identify fluorescent dyes suitable for examining protein stability using FTSA. Eight dyes, including Quinaldine Red, were tested. Dye efficacy was evaluated by the fluorescence signal generated by dye binding with unfolded proteins, which should be high with an acceptable signal-to-noise ratio enabling precise determination of the T_m of the protein. Ten recombinant human carbonic anhydrase (CA) isozymes were used to measure their thermal stability. Acetazolamide (AZM), a sulfonamide-based inhibitor, binding to CA isozymes was measured and dissociation constants K_d were determined. Control experiments were performed using a fluorophore-free assay nanoDSF. The comparison of binding affinities of AZM to recombinant CA isozymes, obtained through nanoDSF and FTSA assays, revealed no significant differences in K_d values between the dye-free and dye-based thermal shift assays. Results from the study of one of the CA isozymes, CAI, are shown in **Figure 1**.

Figure 1. Unfolding curves of CAI obtained from monitoring intrinsic fluorescence (nanoDSF) or fluorescence of different dyes with increasing temperature providing T_m values (**A**). The K_d values for AZM binding to CAI determined by FTSA and nanoDSF (**B**).



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H11

STRUCTURE-AFFINITY RELATIONSHIPS OF IMIDAZOLE-BEARING CARBONIC ANHYDRASE INHIBITORS

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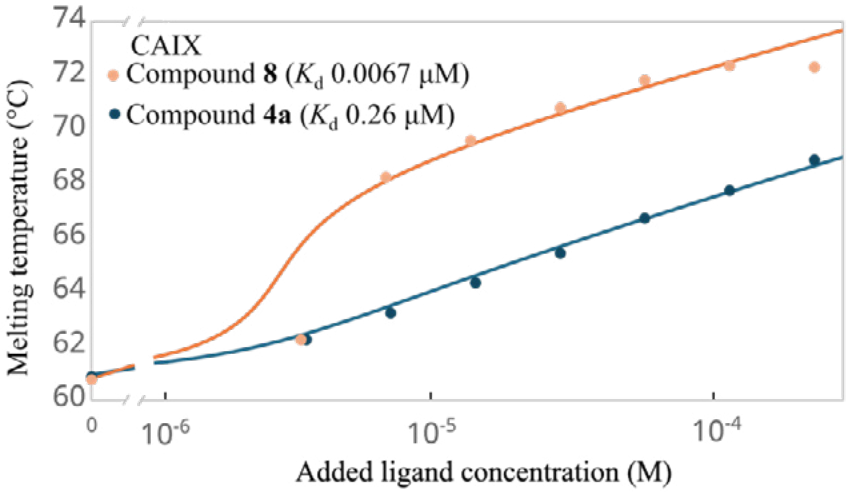
In the early phases of drug development, a lead compound is chemically modified to enhance its affinity and selectivity for target protein. Achieving improved selectivity involves modifying the lead compound to increase its binding affinity for the disease-related protein while reducing its affinity for off-target proteins.

Carbonic anhydrases (CAs) are zinc metalloenzymes that catalyze the reversible conversion of carbon dioxide and water into protons and bicarbonate ions. Fifteen carbonic anhydrase isozymes are found in humans, which play a crucial role in various physiological and pathological processes, including pH homeostasis, respiration, glucogenesis, bone resorption, glaucoma, epilepsy, oxidative stress, obesity and cancer. CAs became drug targets several decades ago. Compounds inhibiting the activity of carbonic anhydrases, thereby affecting various physiological processes such as fluid balance, pH regulation, and ion transport, have therapeutic applications in several medical conditions. Nowadays, significant research attention is being directed toward CAIX and CAXII isozymes due to their overexpression in various tumors and contribution to tumor progression.

The catalytic efficiency of CAs is driven by their active site, where a zinc ion is coordinated by three histidine residues and a water molecule. Benzenesulfonamides, the most investigated class of CA inhibitors, directly interact with zinc ion through the sulfonamide nitrogen atom. Benzenesulfonamide scaffold modifications enable the development of inhibitors with different binding affinity and selectivity for a particular CA isozyme [1].

Here we present the binding affinities of ten newly synthesized benzenesulfonamide derivatives containing a 2-substituted imidazole fragment at the para position. The fluorescence thermal shift assay (FTSA) [2] was used to determine the binding constants (K_d) of these benzenesulfonamides with CAI, CAII, CAIX, CAXII and CAXIII isozymes. Structure-affinity relationship analysis of the compounds was performed to show how small changes in the substituents at the 2-position of the imidazole ring affect binding affinities to CA isozymes.

Figure 1. The dosing curve illustrates the interaction of newly synthesized compounds **4a** and **8** with CAIX. Compound **4a** is the initial acyclic compound, while compound **8** contains a cyclic imidazole ring with an attached ethyl sulfide substituent. Such modifications increase the binding affinity to CAIX by 40-fold.



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H12

COGNITIVE REAPPRAISAL IN MALES: CUES FROM EEG, PUPILS AND PERCEIVED NEGATIVITY

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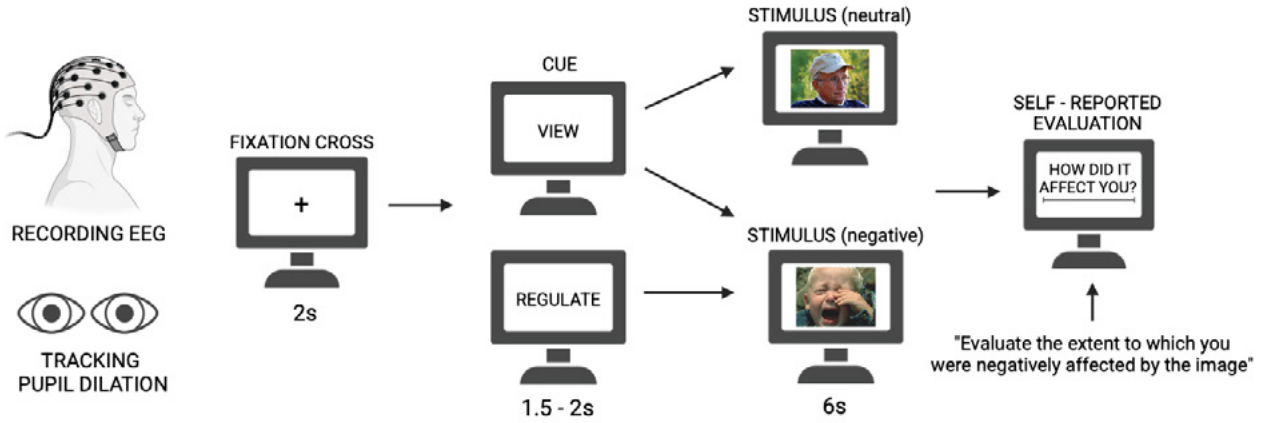
Cognitive reappraisal is an emotion regulation strategy involving a cognitive effort to alter the interpretation of an emotional stimulus, thus changing the emotions it evokes. This strategy is usually examined using psychological (e.g., questionnaires), physiological (e.g., pupillometry), or neuroimaging (e.g., electroencephalography (EEG)) measures. Although commonly used individually in emotion regulation studies, in this study they were integrated in this study to provide a multimodal perspective. We combined self-reported perceived negativity measures with EEG and pupillometry parameters to provide profound insights into emotion regulation in males.

Event-related potentials (ERPs) were used to investigate brain activity. ERP amplitudes were analysed in specific time intervals characterised by the late positive potentials (LPPs) that are linked to both emotional and cognitive processes. Larger LPP amplitudes indicate greater emotional processing, arousal, and/or cognitive effort to process the stimulus [1]. Similarly, arousal and cognitive effort can be inferred by evaluating pupil dilation in response to emotional stimuli [2]. The cognitive effort to regulate emotions was assessed by investigating the slope of pupil dilation, defined as the rate of change in pupil size. The self-reported evaluation of stimuli was used to investigate participants' perceived negativity.

In this study, 35 males performed an emotion regulation task in which they either watched or reappraised visual stimuli depending on the cue shown before the image. If the word "view" was present, participants watched neutral or negatively valenced images. If the word "regulate" was shown, participants had to regulate negative emotions by reinterpreting the stimulus positively or imagining a better outcome of the shown situation. We analysed three different conditions: "view neutral", "view negative", and "regulate" (see Figure 1). After each stimulus, participants evaluated how negatively it affected them. Throughout the task, participants' brain activity was recorded using EEG, and pupil size was recorded using an eye tracker.

Figure 1. Study design



The analysis revealed that LPP amplitude and the velocity of pupil dilation did not differ significantly between the "view negative" and "regulate" conditions. However, both were higher in these conditions compared to the "neutral" condition, indicating that participants were least emotionally and cognitively engaged while watching neutral stimuli. Perceived negativity measures were the highest when viewing negative stimuli and lower during "regulate" condition, suggesting that reappraisal effectively reduced the subjective emotional impact of negative stimuli.

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H13

FULL-FIELD OPTICAL COHERENCE TOMOGRAPHY WITH LOW COHERENCE NOISE

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Optical coherence tomography (OCT) is a non-invasive technique widely used for three-dimensional retinal imaging, which is applied in the detection, diagnosis and monitoring of various retinal diseases. However, OCT technology still faces challenges related to resolution, imaging speed and imaging depth. In order to increase the imaging speed of OCT, Fourier space full-field OCT (FF-OCT) technology has been recently developed, which uses an ultrafast camera and a tunable wavelength laser source to obtain three-dimensional images of an object. FF-OCT can capture interferometric images from deep layers of biological tissue. However, the efficiency of FF-OCT can be limited by crosstalk noise caused by the spatial coherence of the laser. To reduce this noise, a rapidly deformable membrane was used, which reduces the spatial coherence of the laser within a few microseconds [1]. Later, it was shown that multimode fiber can be used for the same purpose [2]. Unfortunately, multimode fibers are characterized by modal noise, as the fiber itself produces coherent artifacts that manifest as speckles at the end of the fiber. To avoid these fluctuations, in this work we combined a multimode fiber (50 μm core diameter and 200 meters long) with a rapidly deformable membrane (Dyoptyka, "Anti-Speckle Technology Evaluation System"), as shown in Fig. 1. The deformable membrane creates various phase distributions that change the laser wavefront. Such light directed into the fiber excites different sets of modes in the fiber. Due to this light randomization, the light exiting the fiber no longer forms bright speckles, as the various realizations of the membrane shape average the speckles into a more homogeneous distribution. Such light is directed into the FF-OCT system described earlier [2, 3].

This improved method demonstrates a significant improvement in imaging quality [4], underscoring the effectiveness of the applied methods. These findings highlight the potential of advanced optical techniques to enhance diagnostic capabilities in ophthalmology, paving the way for more accurate and reliable retinal imaging in clinical settings.

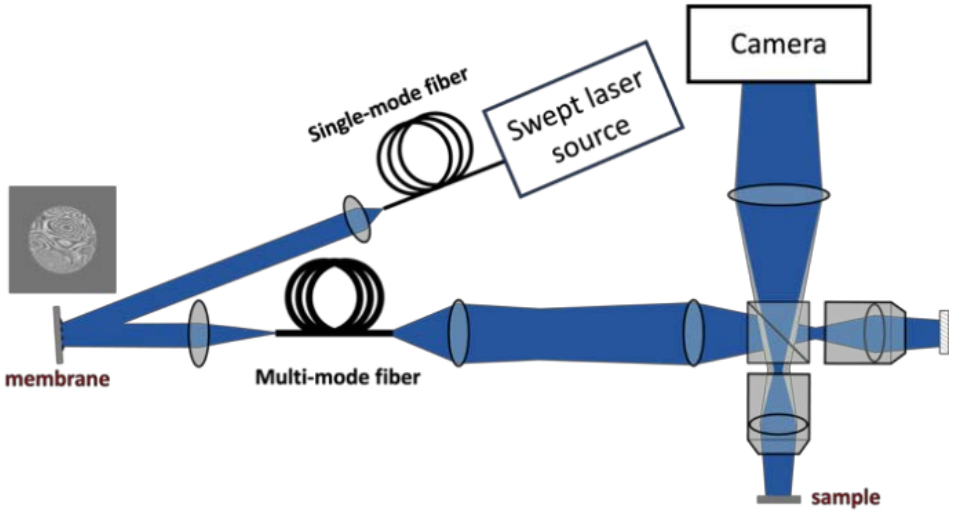

Figure 1. The STOC-T system consisting of swept source laser, a combination of multimode fiber and deformable membrane to reduce coherent noise, interferometer and ultrafast camera.

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H14

Neural Synchronization in Music Interaction: Insights from EEG Hyper-scanning and Granger Causality Analysis

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Introduction: Hyper-scanning records brain activity from multiple individuals simultaneously, offering insights into coordination, emotions, and neural synchronization. In music, synchronized brain oscillations shape interactions between musicians and audiences, fostering cohesion. EEG-based hyper-scanning enables real-time analysis of these connections, revealing how music strengthens social bonds. However, in order to transfer the knowledge to support Brain-Computer Music Interfaces (BCMI), , a detailed analysis grounded on simple well-controlled conditions is first necessary

Methodology: EEG data from 32 channels were recorded simultaneously from 15 pairs of subjects (designated as leader and follower) as they played a musical score on drum pads for 40 seconds under different conditions. The experiment included the following tasks: Familiarization (leader played while the follower listened), Recall (follower played while the leader listened), Rehearsal (both played to practice synchrony), Rhythmic (both played while being told this was the main performance), Improvisation (both improvised rather than following the score), Mechanical (both hit the drum pads at a constant frequency), and Baseline (both remained inactive).

For this study, two conditions—Rehearsal and Rhythmic—were selected for analysis. EEG data were synchronized using event markers and concatenated into a 64-channel dataset. Preprocessing included artifact removal, Independent Component Analysis (ICA), band-pass filtering (0.5–80 Hz), and event-based segmentation.

To extract information flow, Granger causality was computed across frequency bands using a 3-second sliding window, yielding 64×64 matrices representing intra- and inter-brain connectivity. Significant connections ($p < 0.01$, permutation test with 1000 repetitions) were retained, and only connections with $GC > 0.75$ were included in the final analysis. Connection maps were normalized against the resting-state and mechanical tasks. Electrodes were grouped into 10 Regions of Interest (ROIs), with connections averaged across ROIs for simplified representation.

Results: Our results demonstrate increased inter-brain connectivity during rehearsal compared to rhythmic tasks, likely due to the learning process, especially in the beta and gamma bands [1]. Leaders exhibit occipital gamma activity for synchronization, while followers show frontal activation, likely reflecting control mechanisms [2]. Research supports that gamma-band synchronization facilitates coordinated musical performance [1], that, according to our outcomes, is evident with well-controlled simple tasks. Additionally, studies indicate that beta-band activity predominantly flows from the follower to the leader, suggesting anticipation and adaptation [3].

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H15

THE LINKS BETWEEN WOMEN'S SUBJECTIVE VIEW TOWARDS THEIR BODIES AND CHANGES IN BODY COMPOSITION DURING THE MENSTRUAL CYCLE

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Women's self-perception and attitude towards their bodies may change throughout the menstrual cycle. These fluctuations often coincide with mood alterations such as irritability, anxiety, and shifts in overall well-being. Additionally, women report variations in body image, social motivation, and clothing choices. However, objective data on these changes remain limited. This study examined the relationship between women's subjective self-perception and body image across the menstrual cycle and compared these experiences with objective measurements.

Naturally cycling women (n=34) filled out questionnaire forms every 2 days in 35day period. Participants completed 18 diary-type forms, each containing 11 questions. The diary-type questionnaire assessed eight aspects of subjective views: body dissatisfaction, body satisfaction, willingness to dress up, choice of figure-empowering clothes, willingness to participate in various activities, perceived desirability, general self-esteem, and anxiety. The results of the questionnaires were categorised and analysed according to the six phases of the menstrual cycle: early follicular (eF); follicular (F); peri-ovulatory (pO); early luteal (eL); mid-luteal (mL); late luteal (lL). To investigate the relationship between changes in body composition and subjectively perceived body satisfaction, body parameters such as weight and body mass index were measured during the eF, pO, and mL phases.

The estimates of overall self-perception were substantially higher during the pO phase compared to the start and the end of the menstrual cycle ($0.001 < p < 0.035$). Similarly, the desire to dress up and choices of figure-empowering clothes were higher during the pO phase compared to the beginning of the cycle. Also, the desire to dress up peaked in the eL ($0.004 < p < 0.020$), while figure-enhancing clothing choices peaked in both eL and lL ($0.005 < p < 0.049$) phases. Self-perceived desirability increased significantly during the pO phase and remained elevated during F and until eL phase ($0.001 < p < 0.089$). Willingness to participate in activities was higher in the F, pO, and mL ($0.001 < p < 0.031$) compared to the eF phase. Body satisfaction increased from the F phase, peaked in pO, and remained elevated until the mL phase ($0.001 < p < 0.045$). Despite these fluctuations, objectively measured body parameters did not change significantly (all $p < 0.05$). Meanwhile, anxiety decreased significantly from the F to the eL phase ($0.003 < p < 0.009$) and was related to other parameters. Participants reported higher self-perceived desirability, self-perception, body satisfaction scores, and willingness to participate when anxiety was low ($0.001 < p < 0.38$). Interestingly, women were willing to participate in various activities more when their anxiety was high.

Findings suggest that women's self-perception, desirability, and motivation for self-enhancement behaviours peak during the peri-ovulatory phase, aligning with the increased likelihood of conception.

H16

RESEARCH ON CELL PLASMA MEMBRANE REPAIR MECHANISMS AFTER ELECTROPORATION BASED ON ANEXIN A4 PROTEINE ACTIVITY

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When mammalian cells are exposed to induced electric fields (IEFs), the potential of the outer cell plasma membrane (PM) increases, eventually reaching a critical value of 0.2–1 V [1]. This leads to an increase in PM permeability to various molecules and electrical current [2], a phenomenon referred to as electroporation (EP) or, more broadly, electropermeabilization. Research on EP, particularly in the context of medical applications, has expanded into areas such as electrochemotherapy (ECT), gene electrotransfer (GET), and tumor or tissue ablation. Typically, reversible electroporation (RE) techniques are used in ECT and GET studies, while irreversible electroporation (IRE) is employed in tumor and tissue ablation research. To enhance the safety and efficacy of EP-based methods, it is crucial to better understand not only how cells respond to electric fields and introduced molecules but also how affected cells restore their homeostasis and membrane integrity after EP, as well as the underlying mechanisms involved.

Electropermeabilization can activate various membrane repair mechanisms in cells, including exocytosis, pore sealing, and membrane shedding [3]. However, current electroporation research often overlooks one of these mechanisms, specifically the ability of annexin A4 to seal electropermeabilized PM. Although it is known that annexins, a multifunctional protein superfamily, are involved in cellular apoptosis (where they regulate phosphatidylserine (PS) externalization and Ca^{2+} signaling [4]) and necrosis (where they regulate membrane repair and inflammatory responses [5]), studies evaluating the role of annexin A4 in cell recovery after electroporation are scarce. For this reason, we conducted a study to investigate the distribution of annexin A4 in cells following exposure to electric fields and its importance for cell viability.

During this study, we used wild-type MCF7-WT (WT) and MCF7-ANXA4-KO (KO) cells, in which the expression of an annexin A4 protein was knocked out using CRISPR/Cas9. To evaluate the distribution of annexin A4 in cells, KO cells were transformed with the annexin A4-GFP gene, labeled with a GFP tag, thereby creating MCF7-ANXA4-KO ANXA4-GFP^+ cells. The distribution of the GFP-labeled protein in KO-ANXA4-GFP^+ cells was assessed using fluorescence microscopy, and cell viability was evaluated using the MTS assay.

In this study, we determined that a $CaCl_2$ enriched medium significantly accelerates the redistribution of the ANX A4 protein from the cytosol toward the plasma membrane. The rate and intensity of ANX A4-GFP^+ protein redistribution from the cytosol to the plasma membrane strongly correlated with the strength of the electric field (EF) pulses, with increasing strength leading to faster and more intense ANX A4-GFP^+ activity. We observed that in a $CaCl_2$-enriched EP medium, ANX A4-GFP^+ also moves toward the nuclear envelope, whereas this phenomenon was not observed after electroporation in calcium deficient EP medium, even when using extremely strong electric field pulses. However, the $CaCl_2$ enriched medium negatively affected the viability of both WT and KO cells. It is also worth noting that WT cells exhibited higher viability compared to KO cells at the same electric field strength. In summary, this study demonstrated the importance of the annexin A4 protein in maintaining cell viability by revealing that annexin A4 deficient cells are significantly more sensitive to the effects of an electric field. Together, fluorescence microscopy results revealed that ANX A4 protein response after EP is tightly related to the redistribution of ANX A4 protein from cytosol towards plasma membrane or nuclear envelope emphasizing that active plasma membrane repair mechanisms are activated in cells after EP.

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Immunology

I1

ANALYSIS OF IMMUNOEPIGENETIC MARKERS AND THEIR ASSOCIATION WITH CLINICAL PARAMETERS IN BLADDER CANCER

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Bladder cancer (BCa) ranks as the 10th most prevalent malignancy and has a notably high recurrence rate compared to other genitourinary cancers. Approximately 70% of primary cases are diagnosed as non-muscle invasive cancer and currently immunotherapy is emerging as one of the most promising treatment approaches. This type of cancer is recognized for its ability to develop various mechanisms to escape antitumor immune responses, such as changes in immune checkpoints' (IC) concentration, aberrant DNA methylation in the promoter regions of immune response related genes, which can impact treatment responsiveness. Aberrant DNA methylation could be a significant marker in determining patients that are at risk for poor response to treatment and disease progression.

This study is aimed at identifying significant changes in 10 most common ICs and their ligands, including sCD25 (IL-2Ra), 4-1BB, B7.2 (CD86), Free Active TGF-β1,CTLA-4, PD-L1, PD-1, Tim-3, LAG-3, and Galectin-9 in plasma samples using flow cytometry approach and DNA methylation changes in *CD223*, *LGALS9*, *PDCD1*, *FOXP3*, *FOXP1* regulatory regions in 50 patients' paired tumor and urine samples by means of qMSP in hopes to assess if the changes have significant associations with clinical outcomes.

After analysing the results, we determined three ICs (sCD25 (IL-2Ra), B7.2 (CD86) and CTLA-4) were at least 4 times higher ($p<0.05$) vs controls. Also, a comparison of clinicopathological characteristics such as stage and grade revealed that there are some considerable distinctions between some of the groups: there is a variation of PD-1 and LAG-3 concentrations in pTa, pT1 and pT2a stages while sCD25 (IL-2Ra), B7.2 and Tim-3 vary in G1, G2 and G3 grades. As of DNA methylation results, we determined that *PDCD1* methylation levels significantly differ between urine and tumor tissue, with urine samples showing at least 8-fold higher methylation ($p<0.05$). Additionally, clinicopathological analysis revealed a notable distinction in methylation levels based on tumor invasiveness — invasive bladder cancer (BCa) exhibited 1.5 times higher methylation compared to non-invasive cases ($p<0.05$).

These results suggest that sCD25, B7.2, CTLA-4, PD-1, LAG-3, and Tim-3 are the most common immune checkpoints in BCa plasma and could serve as potential biomarkers. Additionally, *PDCD1* methylation in urine may also act as a biomarker for BCa detection and stratification based on tumor invasiveness, with both immune checkpoints and methylation levels offering valuable insights into disease progression.

I2

ROLE OF *ACINETOBACTER BAUMANNII* VIRULENCE FACTORS IN THE INTERACTION WITH HUMAN NEUTROPHILS

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Acinetobacter baumannii is an opportunistic pathogen that belongs to the "ESKAPE" group of microorganisms known for their high antibiotic resistance and prevalence in hospitals [1]. In 2017, the World Health Organization published a list of 'priority' pathogens, and carbapenem-resistant *A. baumannii* was given priority for antibiotic research and development [2]. Despite some identified virulence factors, little is known about its pathogenicity compared to other Gram-negative bacteria. Its antibiotic resistance, ability to survive on medical equipment, and lack of a clear toxin or molecular determinant suggest a “persist and resist” strategy [3]. Understanding host-pathogen interaction is crucial for the treatment of multidrug-resistant *A. baumannii*. During infection, bacteria first encounter the key effectors of the innate immune system – neutrophils. They are essential for controlling *A. baumannii* infections using three main mechanisms: phagocytosis, degranulation, and NETosis [4]. During NETosis, neutrophils release neutrophil extracellular traps (NETs), which are large, web-like extracellular structures that help trap, inactivate, and destroy bacteria [5]. However, it is still not fully understood how *A. baumannii* virulence factors regulate NET production during infection. The aim of this study is to evaluate the role of surface-associated virulence factors of *A. baumannii* during interaction with neutrophils.

In this study, genes of interest were those associated with surface virulence factors: *galU* encodes an enzyme involved in the biosynthesis of capsular polysaccharide, *pmrC* – an inner membrane protein that adds phosphoethanolamine to lipid A and *ompA* – an outer membrane porin. These genes were each individually knocked out of *A. baumannii* clinical isolate by generating markerless gene-deletion mutants. The *galU* mutants were then used to infect human neutrophils. Wild type (WT) strains and complemented mutant strains were also used for infection. Neutrophils were isolated from human peripheral blood by centrifugation using density gradient. Confocal fluorescence microscopy was used for imaging, using DAPI and Phalloidin-Fluor647 dyes. The images were analyzed and the level of NETosis induction was evaluated and compared between different strains.

The genes *pmrC* and *ompA* were successfully knocked out from *A. baumannii* clinical isolates and confirmed by DNA sequencing. The *A. baumannii galU* mutant strain, which does not produce a polysaccharide capsule, was used in the neutrophil experiments. Comparing the WT strain with uninfected neutrophils, the production of NETs was around 3 times higher. The *galU*-deletion mutant strain caused higher NETosis levels than the WT strain. However, this tendency was not observed with the complemented strains. Therefore, *A. baumannii* induces NET production in neutrophils, but preliminary data do not show a significant difference between WT and the capsule-non-producing mutant. Further studies are needed to investigate the role of other surface-associated virulence factors on the induction of NETosis in neutrophils during *A. baumannii* infection.

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I3

PROFILING OF IMMUNE CHECKPOINTS AND THEIR LIGANDS IN NON-SMALL CELL LUNG CANCER PLASMA SAMPLES

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Non-small cell lung cancer (NSCLC) is one of the main subtypes of lung cancer, resulting in approximately 80–85% of all lung cancer cases and remaining the leading cause of cancer-related deaths [1]. Currently, breakthrough in oncology - immunotherapy with immune checkpoint inhibitors (ICI) alone or in combination with chemotherapy is the new standard of treatment in metastatic (stage IV) NSCLC. The main immunotherapy approach consists of the inhibition of programmed death-ligand receptor 1 (PD-L1) and its ligand, PD- L1, by using monoclonal antibodies. Preclinical and clinical data indicated that monoclonal antibodies could significantly enhance patient antitumor immune responses and lead to higher survival rates [2]. However, according to the third phase of clinical trials, an objective response to treatment is observed in approximately 45% of cases [3,4]. Additionally, the identification and characterization of new ICIs could help to improve prognosis and treatment possibilities in advanced stages of NSCLC patients.

The purpose of our study is based on the search for new predictive and prognostic markers that complement PD-L1 expression.

A flow cytometry method was used to evaluate the 12 most common ICs and their ligand concentration in plasma samples. In this research two main histopathological subgroups of NSCLC i.e., adenocarcinoma (AC) and squamous cell carcinoma (SCC) were analysed. In this study 98 serial samples were taken from different treatment periods: before, one and two months after pembrolizumab consumption. 7 from 12 ICs and their ligands showed significant differences in NSCLC vs control samples ($p<0.05$). CTLA-4 concentration in all serial samples was approximately 2-fold lower compared to the control. Serial samples were also compared by histological (AC vs SCC) profile, however, neither group has shown significant differences.

This analysis demonstrates the potential ICIs application in immunotherapy as a non-invasive treatment possibility. Further studies should be done to better understand inhibitors' working mechanisms and new ICIs complementation in PD-L1 expression.

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I4

SYNTHESIS OF A RECOMBINANT *APIS MELLIFERA* ALLERGEN API M 7 IN *ESCHERICHIA COLI*

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Between 30% and 40% of the world's population suffers from allergies [1]. One of the most severe allergic reactions is anaphylactic shock [2]. It is dangerous because it can be life-threatening if the allergic person cannot receive timely treatment, such as an epinephrine injection [3]. One of the most common allergens that can trigger anaphylactic shock is the venom from hymenopteran insects, such as bees and wasps [2, 3].

Allergy diagnostic methods are rapidly improving. This is evident by the shift towards using specific allergens in *in vitro* tests, which are replacing the traditional use of allergen extracts in *in vivo* tests as well as expanding the diagnostic capabilities of *in vitro* testing. This change is advantageous due to three main reasons. Firstly, allergen extracts can lead to inaccurate results due to their varying compositions. Secondly, since allergen extracts are derived from natural sources, a possibility of cross-reactions arises. Lastly, conducting allergy tests *in vitro* instead of *in vivo* eliminates the risk of causing allergic reactions in patients, which is highly beneficial when working with potentially dangerous allergens, such as the aforementioned hymenopteran insect venoms that can cause anaphylactic shock [4].

A recombinant Api m 7 allergen, naturally found in European honey bees (*Apis mellifera*), was synthesized during this study. European honey bees are the most common and the most studied species of bees [5]. Up to 80% of people allergic to honey bee venom are sensitive specifically to the 7th of the 12 known Api m allergens [6]. In this study, the synthesis of a recombinant Api m 7 allergen was induced in different *E. coli* strains and under different temperatures, to optimize the synthesis.

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I5

Macrophage response to extracellular inflammasome components – ASC specks

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Innate immune system activation can be described by various signaling pathways. One of them is the inflammation-related mechanism – inflammasome activation. It is characterized by the production of inflammatory molecules and inflammatory cell death – pyroptosis. Inflammasomes can be activated by numerous factors, including microbes and sterile inflammation-related factors, such as cholesterol crystals. Inflammasomes are responsible for the maturation of pro-inflammatory cytokines interleukin 18 (IL-18) and IL-1β [1]. Inflammasomes are multimeric protein complexes. There are a few inflammasome types, but they all have a common adapter protein – apoptosis-associated speck-like protein containing a CARD, ASC [2]. ASC is necessary for the inflammasome complex assembly and activation. During inflammasome activation, ASCs oligomerise to about 1 µM size particles called ASC specks [3]. During pyroptosis, these specks are released into extracellular space. They can accumulate in inflamed tissues and continue to mature cytokines or be phagocytized by surrounding cells to activate inflammasomes further. Prolonged inflammasome activation can be a reason for various diseases, such as Alzheimer's disease, atherosclerosis, and various autoimmune diseases [4]. Thus, ASC proteins can be used as targets to inhibit the inflammatory response. For this purpose, further research must be conducted to determine the exact effect that ASC specks have on cytokine production in macrophages. This research aimed to investigate how ASC specks affect macrophage activation by viral antigens.

We prepared ASC speck fractions using human THP1-ASC-GFP cell line. We settled on an optimal technique to gather ASC specks using various cell lysis methods and centrifugation steps. THP-1 macrophage-like cells, prepared from THP-1 monocytic cell line, were treated with ASC specks and viral antigens – human KI polyomavirus virus-like particles (KIPyV VLPs). We assessed cell viability and inflammatory response via cytokine secretion to analyze the effect of ASC specks and KIPyV VLPs on macrophages. Cell viability was evaluated by lactate dehydrogenase (LDH) assay. Enzyme-linked immunosorbent assay (ELISA) was applied to measure the release of cytokines, including IL-8, IL-1β and CCL2.

Our results demonstrated that ASC specks and a mixture of ASC specks with KIPyV VLPs induced a significant increase in LDH release in macrophages. We also observed the tendency - the higher the concentration of ASC specks used for macrophage treatment, the higher the LDH concentration was detected in the cell medium. Macrophages treated with KIPyV VLPs released a higher LDH concentration than the control group, but it was insignificant. We also found that viral antigens KIPyV VLPS induced a higher IL-8 and CCL2 secretion in macrophages than ASC specks. However, macrophages treated with ASC specks released more IL-1β than viral antigen-activated cells. Interestingly, ASC specks significantly reduced the KIPyV VLPs-induced effect on macrophages regarding CCL2 secretion. CCL2 is a significant chemokine that recruits other immune cells to the infection site. It could be that ASC specks cause the ongoing inflammation by modulating chemotaxis. A mixture of ASC specks and KIPyV VLPs induced different secretion of CCL2 and IL-1β than KIPyV VLPs alone. However, the opposite effect was observed in the case of IL-8 secretion – ASC specks alone had less of an impact on IL-8 secretion than the mixture of viral antigens and ASC specks.

Our results suggest that ASC specks induce inflammasome assembly and promote pyroptosis in macrophages. The viral antigen KIPyV VLP stimulates the release of pro-inflammatory cytokines, and ASC specks modulate their secretion. In conclusion, ASC specks affect the KIPyV VLP-induced inflammatory response in macrophages.

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MODELING OF DENDRITIC CELL VACCINES FOR OVARIAN CANCER BASED ON TUMOR IMMUNE SUBTYPES

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Ovarian cancer remains one of the deadliest gynecologic malignancies, particularly in advanced stages, where innovative treatment options are scarce. The tumor immune microenvironment plays a crucial role in therapy response, yet ovarian cancer exhibits high heterogeneity at the immune level. Based on immune infiltration patterns, tumors are categorized into three major subtypes: inflamed (characterized by abundant immune cell infiltration), immune-excluded (immune cells present but restricted to the stromal compartment), and immune-desert (lacking significant immune infiltration) [1]. Given the limited efficacy of immune checkpoint inhibitors in ovarian cancer, dendritic cell-based immunotherapy emerges as a promising alternative [2].

Dendritic cells (DCs) are key antigen-presenting cells responsible for initiating and regulating adaptive immune responses. In this study, we aimed to model monocyte-derived DC-based vaccines *in vitro* and investigate how tumor-derived factors from different immune subtypes of ovarian tumors affect DC maturation and functionality. Peripheral blood mononuclear cells from 10 healthy donors were isolated, and monocytes were differentiated into immature DCs using a standard GM-CSF and IL-4 protocol over three days. These immature DCs were then matured for six hours using lysates derived from previously characterized ovarian tumors representing immune-desert, immune-excluded, and inflamed subtypes (3).

To evaluate the immunogenic properties of these DCs, we analyzed the expression of key maturation and functional markers via flow cytometry. Interestingly, DCs matured with lysates from immune-desert tumors displayed the highest expression ($p<0.05$) of CD80 (co-stimulatory molecule critical for T-cell activation), CD83 (maturation marker), and CD11c (marker of conventional DCs), suggesting that tumor-related factors within this subtype may induce a more potent antigen-presenting phenotype in DCs.

These results highlight the strong influence of the TME on DC maturation and suggest that tumor subtype-specific factors should be considered when designing DC-based immunotherapies. Future studies will focus on identifying the molecular signals responsible for this differential DC activation and evaluating their effects on T-cell priming and anti-tumor immune responses. Ultimately, our findings support the development of personalized DC-based immunotherapy approaches tailored to the immune subtype of the tumor, potentially improving treatment efficacy in patients with ovarian cancer.

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I7

OPTIMIZATION OF SEMI-SOLID MEDIA FOR HYBRIDOMA CLONING AND SELECTION

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In hybridoma technology, the most labor-intensive and time-consuming stage is cell selection and isolation of the monoclonal population. One of the traditional methods for cloning hybridomas is limiting dilution cloning, which requires a lot of work and repetitions and does not guarantee that the isolated cell population will be monoclonal. Cell cloning in a semi-solid medium and isolation of grown clones manually or by robotic systems is another way to select monoclonal populations. Cells immobilized in a semi-solid medium are separated, thus preventing cell mixing and promoting clonal growth from a single cell.

This work aims to develop and optimize a cost-effective semi-solid medium production protocol for hybridoma selection and cloning. Different hybridoma lines were cloned in a semi-solid medium made based on methylcellulose (MC). By seeding hybridomas into semi-solid medium with different MC concentrations, the optimal MC concentration was sought at which the highest number of viable round-shaped clones would form. The optimal cell seeding concentration was determined by seeding hybridomas at different densities into the semi-solid medium.

Based on the seeding results of three stabilized hybridoma lines (5H6, 12E12, and 25E6), the optimal 1 % MC concentration for semi-solid medium production and the recommended cell seeding into 6-well plates 2×10^4 cells/well concentration were determined. Seeding fused cells into a semi-solid medium with components of the HAT selection system allowed hybridoma selection and cloning to be carried out simultaneously. Combining the processes of hybridoma selection and cloning into one streamlined process allowed for the savings of time and financial resources for reagents. Based on the ELISA results, it can be stated that hybridomas grown in a semi-solid medium produce antibodies against target antigens. The potential of the semi-solid medium for selecting monoclonal populations is already being utilized for hybridoma cloning in the laboratory where this work has been conducted.

High School Posters

M1

IŠ MOLIUSKŲ IŠSKIRTŲ PARAZITINIŲ TREMATODA CERKARIJŲ RŪŠIES IDENTIFIKAVIMAS

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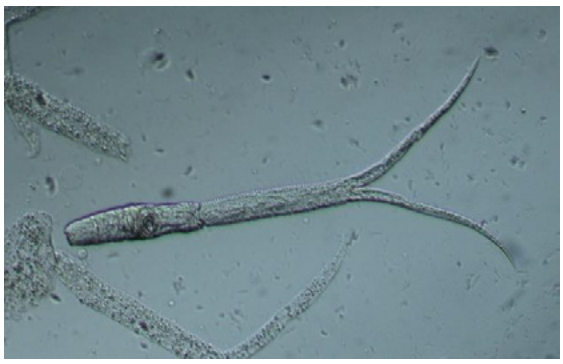
Trematodai (Trematoda) yra parazitinės plokščiosios kirmėlės, turinčios sudėtingą gyvenimo ciklą, kuriame dalyvauja keli šeimininkai [1]. Pirmasis tarpinis šeimininkas – vandens moliuskai, kuriuose vystosi aktyvios lervos – cerkarijos. Cerkarijos, palikusios moliuską, virsta metacerkarijomis ir migruoja į antrąjį tarpinį šeimininką (dažniausiai žuvis arba kitus vandens bestuburius), o pasiekus galutinį šeimininką (dažniausiai vandens paukščius), jos išsivysto į suaugusias trematodų formas, kurios subręsta ir dauginasi [2].

Trematodai gali daryti reikšmingą poveikį vandens ekosistemoms. Pavyzdžiui, jų cerkarijos įsiskverbia į žuvų akies audinius, sukeldamos uždegimus, skatindamos cistų susidarymą ir pažeisdamos akių struktūras, kas gali lemti regėjimo sutrikimus. Dėl šių sutrikimų žuvys tampa labiau pažeidžiamos plėšrūnams, nes jų gebėjimas atpažinti grėsmes sumažėja. Be to, pastebėta, kad trematodai gali manipuliuoti žuvų elgesiu, skatindami jas atlikti neįprastus judesius arba laikytis pavojingose vietose. Tokiu būdu parazitai siekia patekti į galutinį šeimininką (vandens paukštį). Tai gali turėti neigiamų pasekmių žuvų populiacijoms, o ekologiniai pokyčiai taip pat gali sukelti ekonominių nuostolių, ypač žuvininkystės ūkiams [3].

Šio tyrimo tikslai buvo išskirti parazitines Trematoda cerkarijas iš vandens moliuskų ir nustatyti jų rūšį, bei palyginti morfologinio ir molekulinio identifikavimo metodų tikslumą ir patikimumą.

Moliuskai buvo surinkti iš upės Šalčia pakrančių, daugiausia Lymnaeidae ir Planorbidae šeimų atstovai. Iš viso surinkta 25 moliuskai, iš kurių 7 buvo infekuoti trematodų cerkarijomis. Cerkarijų morfologija buvo tiriama naudojant fazinio kontrasto mikroskopiją. Pagal morfologinius požymius (kūno ilgis, siurbtukų ir liaukų struktūra, uodegos forma) buvo preliminariai nustatyta parazitų taksonominė grupė. Molekulinė identifikacija buvo atlikta iš cerkarijų gryninant DNR, amplifikuojant ITS regioną ir vykdant Sangerio sekoskaitą. Gautos sekos buvo lyginamos su NCBI BLAST duomenų baze, kas leido nustatyti cerkarijų rūšį. Sekvenuoti buvo 4 mėginiai, tačiau tik 2 iš jų gautos sekos buvo tinkamos identifikavimui dėl nepakankamos kitų mėginių DNR kokybės ar prastos sekoskaitos rezultatų.

Gauti rezultatai rodo, kad nagrinėtoje vandens ekosistemoje egzistuoja bent dvi skirtingos trematodų rūšys - *Tylodelphys clavata* bei *Cotylurus gallinulae*. Palyginus abiejų metodų rezultatus, nustatyta, kad morfologiniai duomenys suteikia pradinę informaciją apie cerkarijų grupę, tačiau jie yra nepakankami tiksliam identifikavimui iki rūšies lygio. Molekuliniai metodai pasižymi aukštesniu tikslumu ir patikimumu, todėl jie yra rekomenduojami kaip pagrindinis identifikavimo metodas.



Pav. 1 Parazitinės Trematoda cerkarijos vaizdas, fazinės kontrastinės mikroskopijos metodas, objektyvo didinimas x40.

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M2

ANTHOCYANINS-ENRICHED INDICATOR BIOPLASTIC FOR ASSESSING THE QUALITY OF DAIRY PRODUCTS

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Aim: to develop bioplastic that alerts the consumer when dairy products begin to spoil.

Food waste is a specific loss of food that USDA ERS describes as "food products that are thrown away due to unpleasant color or look"[1]. During the warm season, meat and dairy products can spoil more quickly thus making the expiration date inaccurate. In this case, usage of an anthocyanins-enriched bioplastic as a food package would indicate the level of product freshness – it could change its color according to the pH level of the product. Anthocyanins are suspended into a biopolymer matrix made out of glycerol and starch. Bioplastic naturally degrades in the environment without forming microplastics or other particles.

Anthocyanins are extracted from 25 grams of red cabbage and butterfly pea using Soxhlet extractor (solvent – water). 4 grams of starch, 2 grams of glycerol, 5 milliliters of anthocyanin extract, and 15 milliliters of distilled water are mixed and heated. 3 milliliters of 0.1M HCl solution is added. The mixture is heated until thick paste formation and is neutralized (pH=7) using 0.1M NaOH. The mixture is spread into a mold and left to dry. Anthocyanins are immobilized by adding sodium alginate and calcium chloride in the primary mixture.

To determine the indicator properties of determine the indicator properties of the extracts, acetic acid – sodium acetate buffer solutions with pH 5.0-5.8 and phosphate buffer solutions with pH 6.0-7.2 were prepared with 0.5 milliliter of buffer and 0.5 milliliter of extract were pipetted into cuvettes, and color changes were observed (Figure 1) and (Figure 2).

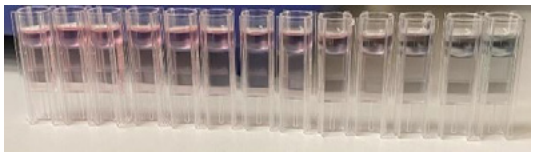

Figure 1. Indicator properties of red cabbage extract using sodium acetate and phosphate buffer.

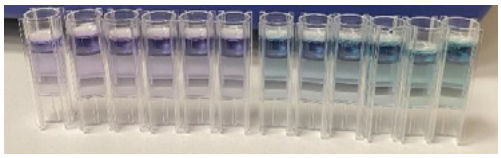

Figure 2. Indicator properties of butterfly pea extract using sodium acetate and phosphate buffer.

Butterfly pea extract showed more promising results by visualy observing various color changes in different pH environments. A colorimetric test of indicator properties was performed. Buffer solutions are prepared in the range of pH at 5.0-7.2. In a cuvette, 1 milliliter of the extract is mixed with 1 milliliter of buffer and the absorption spectrum (190-800 nm) is recorded using a spectrophotometer. The obtained data is normalized in range 300-800 nm and the spectra is ploted (Figure 3). Butterfly pea extract has better indicator properties than red cabbage, as a clear shift towards longer and shorter wavelengths is visible.

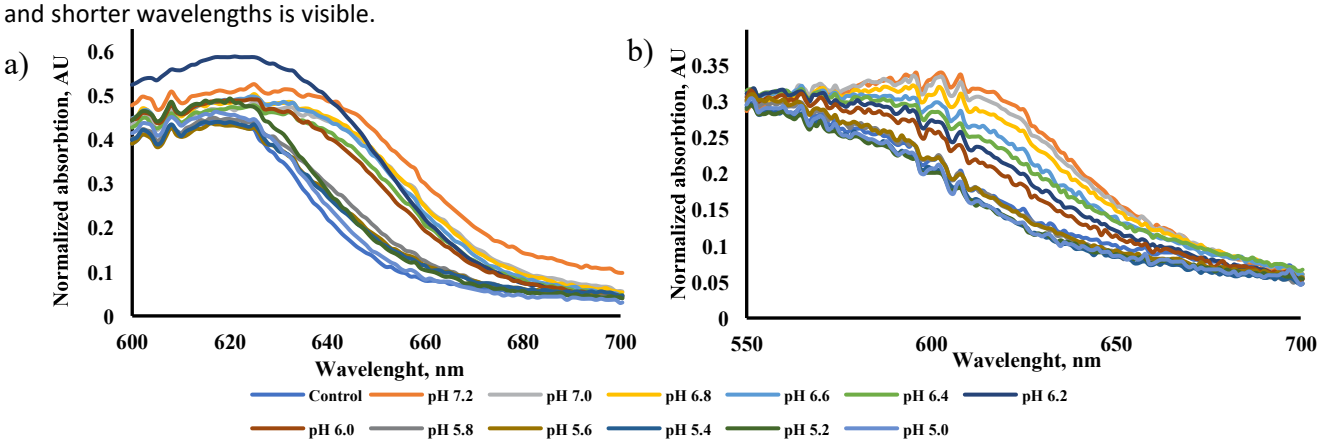

Figure 3. a) Corrected absorption spectrum of butterfly pea extract treated with buffer solutions (pH 5.0-7.2); b) Corrected absorption spectrum of red cabbage extract treated with buffer solutions (pH 5.0-7.2).

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M3

Violacein biosynthesis optimization using L-tryptophan

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Introduction: L-tryptophan (L-Trp) is an essential amino acid that is most commonly found in oats, bananas, dried prunes, milk, tuna fish, cheese, bread, chicken, turkey, peanuts, and chocolate and is a precursor for biologically significant molecules, including violacein.[1] Violacein is a purple, water-insoluble pigment produced by several Gram-negative bacteria with antibacterial, antiviral, and antiparasitic properties.[2] However, its complex purification and limited production make it highly expensive, with prices exceeding $553.70 per 1 mg.[3] One of the most studied violacein-producing bacteria is Chromobacterium violaceum, a Gram-negative facultative anaerobe found in tropical and subtropical environments.[4] Optimizing bacterial growth using L-tryptophan and refining violacein extraction techniques from Chromobacteriumviolaceum may increase violacein availability for pharmaceutical use.

Purpose: Optimize violacein biosynthesis using L-Tryptophan.

Methods: *Chromobacterium violaceum* was cultivated on nutrient agar (NA) with varying L-Trp concentrations at 30°C for seven days with constant shaking at 140 rpm. Samples were centrifuged for 10 minutes at 6000 rpm. The biomass was weighed and extracted with methanol over multiple cycles until violacein was fully dissolved. The final solutions were stored in a refrigerator. From each concentration, 0.5 ml of the violacein-methanol solution was analyzed using an optimized HPLC-DAD method to assess the influence of L-Trp on violacein production. A spectrophotometer was also used to measure extracted violacein concentration in solvent.

Results: Biomass measurements showed samples with **0,4 mg/ml** and **0,8 mg/ml** of L-Trp had higher biomass than those with 1.6 mg/ml, 3.2 mg/ml or no supplementation. Spectrophotometry and HPLC-DAD analysis confirmed maximum dissolved violacein concentration in methanol.

Conclusion: L-Tryptophan supplementation influences violacein production in *C. violaceum*. Optimizing biosynthesis by adjusting L-Trp concentrations enhances bacterial growth and violacein yield. Further research should refine extraction methods for cost-effective production in multigram amounts.

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M4

PLASTIKŲ DALELIŲ PAPLITIMO TYRIMAS KAUNO VANDENS TELKINIUOSE

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Plastikai plačiai naudojami kasdienėje veikloje – technologijose, medicinoje, buityje ir kitose srityse. 2019 m. plastiko gamyba siekė 368 mln. tonų [1]. Tačiau didelė dalis iš plastikų pagamintų gaminių tampa atliekomis, neretai tai įvyksta po vienkartinio jų panaudojimo. Netinkamas plastikų šalinimas sukelia rimtą aplinkos taršą. Kasdien ne tik į sąvartynus, bet ir į vandenynus ir kitus vandens telkinius patenka didžiuliai plastiko kiekiai, kurie laikui bėgant skyla į mikroplastikus ir nanoplastikus. Dėl savo cheminės struktūros mikro ir nanoplastikai ilgai išlieka aplinkoje, o dalelėse esantys priedai, tokie kaip pigmentai, plastifikatoriai ir stabilizatoriai, gali dar labiau didinti aplinkos taršą. Vandenynų ekosistemoms plastiko tarša ypač žalinga – mikroplastikas kenkia jūros gyvūnams, plinta per maisto grandinę ir galiausiai pasiekia žmones. Nanoplastikai yra pavojingi todėl kad mažas dalelių dydis leidžia jiems prasiskverbti pro biologines membranas, o jų poveikis organizmams vis dar nėra pakankamai ištirtas. [2] Mikroplastiko pašalinti mechaniškai beveik neįmanoma todėl mokslininkai ieško mikroorganizmų, galinčių jį skaidyti. Nors tokių bakterijų ir jų fermentų jau yra atrasta, jų efektyvumas vis dar nėra pakankamas. Siekiama sukurti palankias sąlygas tokių mikroorganizmų veiklai arba sukurti genetiškai modifikuotus mikroorganizmus, tačiau šie metodai dar tobulinami. Kadangi plastiko gamyba vis dar auga, būtina imtis veiksmų siekiant mažinti taršą ir ieškoti veiksmingų sprendimų kontroliuoti plastikų dalelių paplitimą aplinkoje. [3]

Lietuvoje atliekų rūšiavimas bei utilizavimas yra visuotinai aktuali problema, nemaža visuomenės dalis šiukšlina viešose vietose bei nesilaiko atliekų rūšiavimo taisyklių, todėl verta tikėtis mikro ir nanoplastikų paplitimo vandens telkiniuose, visų pirma - didmiesčių. Kontroliuoti mikro ir nanoplastikų kiekį vandenyje yra būtina, tačiau tam reikalingas greitas ir patikimas mikro ir nanoplastikų detekcijos metodas.

Mūsų tikslas atlikti mikroplastikų ir nanoplastikų aptikimą Kauno vandens telkiniuose greitos detekcijos metodu bei įvertinti šių dalelių paplitimą.

Darbo uždaviniai būtų atidirbti optimalias nano ir mikroplastiko dalelių detekcijos sąlygas taikant Nile Red dažiklį ir nustatyti nano ir mikroplastikų paplitimą Kauno vandens telkiniuose.

Šiame darbe bus naudojamas nano ir mikroplastikų aptikimo metodas, pagrįstas hidrofobinio fluorescencinio Nile Red dažiklio absorbcija ant nano ir mikroplastiko dalelių paviršiaus, dalelių surinkimu celiuliozės filtro paviršiuje ir detekcija fluorescenciniu mikroskopu. Mikroskopinės kameros vaizdo analizė leidžia identifikuoti, išmatuoti ir suskaičiuoti daleles [4]

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M5

BAKTERIJOS *PAENARTHROBACTER NICOTINOVORANS* GENŲ KLASTERIŲ TAIKYMAS NIKOTINO BIODEGRADACIJAI

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Nikotinas – toksiška medžiaga, plačiai randama tabako produktuose. Rūkymo metu nikotinas lengvai patenka į plaučius. Jis skatina oksidacinį stresą, prisideda prie uždegiminių procesų plaučiuose ir yra glaudžiai susijęs su lėtinėmis kvėpavimo takų ir kitomis mirtinomis ligomis[1].

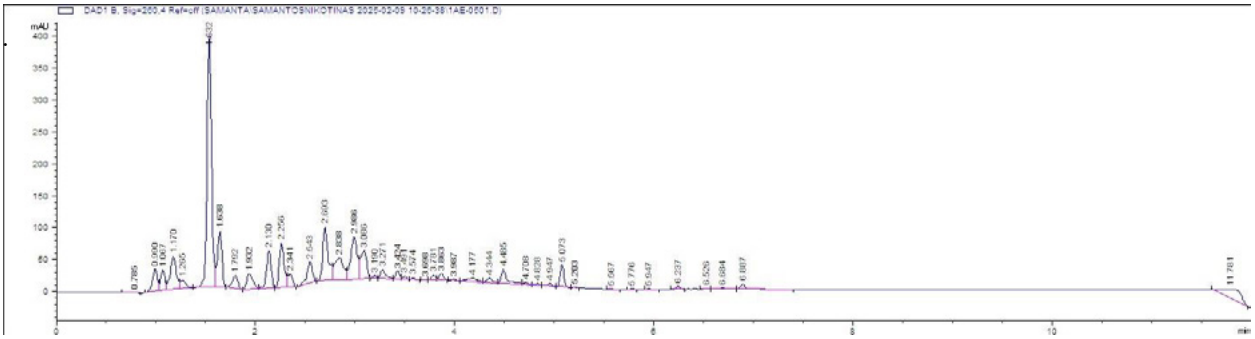
Paenarthrobacter nicotinovorans yra gramo teigiama dirvožemio bakterija, priklausanti Actinobacteria klasei[2]. Ši bakterija išskirtinai pasižymi savo gebėjimu skaidyti nikotiną. Nikotino skaidymo procesas vyksta per sudėtingą biologinį kelią, kurio svarbiausius fermentus koduoja nik-genų klasteris, esantis pAO1 megaplazmidės struktūroje[3]. Šių genų koduojami fermentai, tokie kaip nikotino dehidrogenazė, leidžia bakterijai suskaidyti nikotiną į netoksiškas medžiagas, viena jų – Nikotine Blue.[4]

Paenarthrobacter nicotinovorans pAO1 nik genų klasterio biotechnologinis taikymas gali padėti spręsti globalią žalingo nikotino poveikio žmogaus organizmui problemą. Jei pavyktų įkelti šią genetinę kasetę į žmogaus plaučiams būdingos „gerosios“ bakterijos genomą arba sukurti inžinerinę plazmidę būtų įmanoma rūkymo žalos problemą, įskaitant ir pasyvų rūkymą bei taip vadinamą „third-hand smoke“ spręsti skaidant nikotiną rūkančio žmogaus plaučiuose į netoksiškus junginius. Tai ypač aktualu atsiradus elektroninėmis cigaretėmis, kuriose, skirtingai negu paprastose cigaretėse nikotinas nesudega, bet visas patenka į plačius, o iš jų - į aplinką.

Šio tyrimo tikslas yra pritaikyti *Paenarthrobacter nicotinovorans* biologinį kelią nikotino skaidymui transformuojant tam reikalingą genų klasterį į žmogaus plačių mikrobiotą.

Metodai. Bakterijos *Paenarthrobacter nicotinovorans* bei iš aplinkos (nuo tabako lapų paviršių) izoliuotos bakterijos, užaugintos ant minimalios terpės, kuriose vieninteliu anglies šaltiniu buvo nikotinas kultivuojamos Nutrinet Agar terpėje su 0.1% nikotino koncentracija. Nikotino kiekis prieš ir po kultivavimo nustatomas HPLC-DAD metodu. Atrenkami tie mikroorganizmai kurie geba efektyviai skaidyti nikotiną.

Rezultatai. Yra atidirbtas HPLC-DAD detekcijos metodas, kuris yra efektyvus analizuoti nikotino koncentraciją mitybinėje terpėje. Taikant metodą yra nustatyta, kad *Paenarthrobacter nicotinovorans* per 7 dienas suskaidė 82,13% nikotino buvusio terpėje, kitos bakterijos nors rodė nikotino skaidymo požymius ant agarizuotų terpių, skystoje terpėje tokio rezultato neparodė. Šiuo metu tyrimas tęsiamas.



Pav.1. Nikotino (Rt: 1.532 min) aptikimas NB mitybinėje terpėje

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M6

Protease extraction and purification

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Proteases, also known as proteolytic enzymes, are enzymes that break down proteins by cleaving peptide bonds between amino acids. They can be found in various sources, such as plants, animals, and microorganisms, including bacteria. However, scientists have found that certain fruits contain a particularly high concentration of proteases, such as kiwis, figs, papayas and pineapples. It is believed that such a high concentration may be beneficial for protection against pests and pathogens, as well as for developmental processes. Genetic studies, including the analysis of mutant alleles and gene silencing, have shown that increased expression of proteases may be associated with resistance mechanisms. [1]

Proteases are widely applied in both medicine and biotechnology. In medicine, they are used in the treatment of various diseases, including AIDS therapy, where viral protease inhibitors block viral replication. [2] Furthermore, studies have shown that the protease actinidin found in kiwi juice can be used in cheese production as an alternative to traditional enzymes. [3]

To effectively use proteases in the industry, it is important to have a reliable and cost-effective method for protease purification and extraction, as well as to investigate the factors determining protease efficiency. The aim of this study is to develop an optimal method for the extraction and purification of the protease actinidin from kiwi (Actinidia chinensis) fruit to obtain an enzyme suitable for biotechnological applications.

To do so, these methods were used: cryogenic extraction, protein precipitation with ammonium sulfate, a three-phase extraction method and protein purification by ion exchange chromatography. Actinidin activity was confirmed using fast milk-clotting agar method.

As a result, a way to extract and purify the protease actinidin was found which could be used to produce chip active actinidin in multigram amounts, suitable for biotechnology applications, also for academical needs.

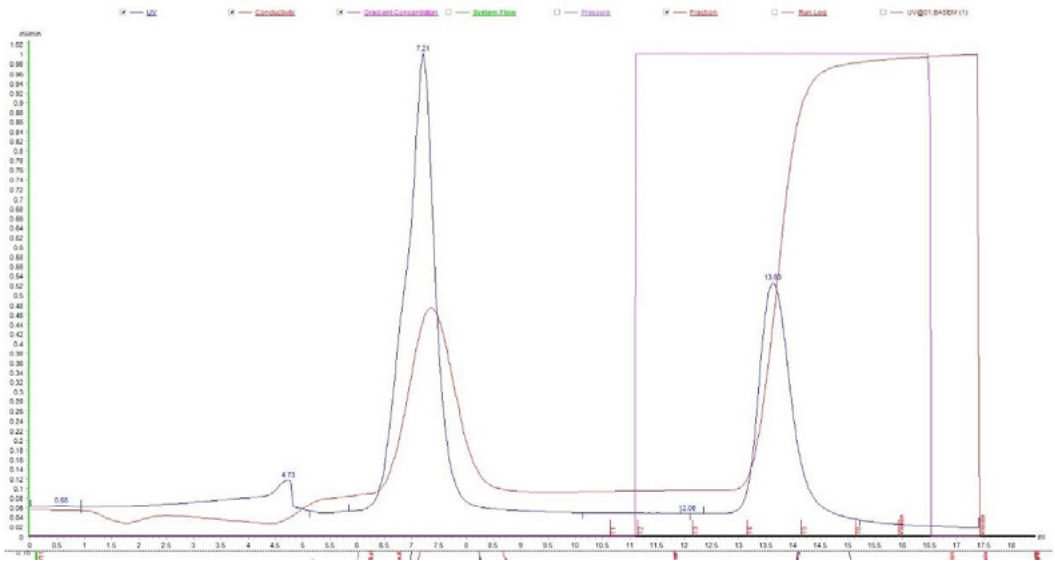

Figure 1. Ion exchange actinidin purification chromatogram

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M7

APPLICATION OF MOLECULAR BIOLOGY METHODS FOR FISH SPECIES IDENTIFICATION

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Fish is a valuable food source, however, declining resources and population growth have led to frequent mislabeling, food fraud and consumer rights violations. Many fish products lack distinct morphological differences, making species substitution common. Studies worldwide highlight this issue, such as a 2019 study in Turkey, where only 4 out of 15 fish species were correctly labeled [1]. Mislabeling causes economic loss as consumers overpay for cheaper fish sold as premium species, misrepresents nutritional value and poses health risks, especially for the 7% of people with fish allergies. Additionally, it undermines regulatory compliance, as EU laws require accurate labeling of genetically modified organisms' products and endangered species, which becomes impossible when species names are altered [2]. There is no available data on similar studies conducted in Lithuania. Usage of DNA sequencing methods could help fill this gap.

This study aims to analyze the DNA of fish sold in stores, identify their species, and evaluate species accuracy in labeling. To achieve this goal, the following objectives were set: 1. Review the literature on fish DNA barcoding to determine suitable sample preparation, analysis methods, and primer selection; 2. Extract DNA, amplify the target gene using PCR (Polymer Chain Reaction), and prepare amplicons for sequencing; 3. Analyze sequencing data and identify fish species using bioinformatics databases.

The key aspects of a successful outcome are selecting the appropriate sample, choosing a suitable DNA extraction method based on the sample, and selecting the right primers for fish DNA amplification. Three types of samples were considered: fish roe, frozen fish fillets, and never-frozen fish fillets. However, preserved fish roe (2 samples) and frozen fish fillets (4 different samples, each tested twice) employing two different DNA extraction procedures from the literature did not yield satisfactory results due to low DNA concentration obtained. Therefore, the study continued using never-frozen fish samples, specifically fresh fillets of common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*). For DNA extraction, the commercial Zymo #D4011 Genomic DNA Clean & Concentrator™ Kit [3] was used and the procedures provided in the kit description were followed. In this case, sufficient amounts of DNA were extracted, ranging from 21 to 113 ng/µL, for three replicates of two fish species: carp and tilapia. Successful DNA extractions were also confirmed by gel electrophoresis.

In the next step, four primers were selected from the literature [4], which had already been successfully applied for the amplification of different fish species DNA by PCR. After PCR amplification, the DNA was purified, with final concentrations ranging from 33.5 to 70.7 ng/µL. The results were again verified using gel electrophoresis.

The final step was to perform Sanger sequencing to identify the fish species. For this, the purified and amplified DNA was sent to the Netherlands. Samples obtained using the MiFish-U and L2513/H2714 primers showed a 99% confidence match to *Oreochromis niloticus* and *Cyprinus carpio* according to the NCBI BLAST database.

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Oral Presentations

O1

ASSESSING *TAS2R16* POLYMORPHISMS AND THEIR INFLUENCE ON VISUAL ACUITY IN OPTIC NEURITIS

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Optic neuritis (ON) is a neuro-ophthalmological disorder characterized by inflammatory optic neuropathy and is a leading cause of vision loss worldwide [1]. The pathophysiology of ON involves inflammatory and demyelinating mechanisms that disrupt visual function. This condition typically affects individuals between the ages of 18 and 45, with young women being twice as likely to develop it [2].

ON is categorized into two types - typical and atypical. Typical ON, which is often idiopathic or associated with genetic and environmental factors such as multiple sclerosis, constitutes the majority of cases. Atypical ON is associated with infections or autoimmune conditions [3].

Bitter taste receptors – TAS2R family, have been implicated in immune and inflammatory processes. Among them, the TAS2R16 receptor, expressed in various tissues. Preliminary findings suggest that *TAS2R16* SNPs may modulate immune-inflammatory pathways relevant to ON pathogenesis [4, 5].

Ophthalmological indicators, especially visual acuity, are essential for assessing the severity and progression of ON. These indicators vary significantly at different stages of the diseases. To evaluate treatment efficacy, we analyzed visual acuity in affected patients before and after treatment, focusing exclusively on the diseased eye.

This research aims to identify the associations between *TAS2R16* rs860170, rs978739, rs1357949 gene polymorphisms with visual acuity in optic neuritis.

A total of 82 patients diagnosed with ON and 160 healthy individuals participated in the study. DNA extraction from venous blood samples from both ON patients and healthy individuals was performed using the DNA salting-out method. Genotyping of *TAS2R16* rs860170, rs978739, rs1357949 was performed using real-time polymerase chain reaction (RT-PCR). Statistical data analysis was carried out using IBM SPSS Statistics 29.0.2.0.

We analyzed the associations of *TAS2R16* rs860170, rs978739, rs1357949 SNPs with visual acuity in patients with ON. Genotype and allele analysis did not reveal statistically significant differences in visual acuity before and after treatment in the affected eyes.

Comparing the visual acuity of the affected eye before and after treatment and it is correlation with *TAS2R16* rs860170, rs978739 and rs1357949 SNPs, no statistically significant differences were found.

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O2

NOT SO FANTASTIC PLASTIC: HOW MICROPLASTICS AFFECT FISH AT DIFFERENT DEVELOPMENTAL STAGES

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The rapidly growing production of plastic materials has exceeded the capacity to manage them effectively once they reach the end of their life cycle. Which leads to plastic waste becoming a major issue on the global scale. Microplastic (MP) pollution impacts all global sectors – from environmental protection to the economy – and is becoming an inevitable part of humanity's and the planet's future. The long-term ecological impacts of microplastics on aquatic life are significant and multifaceted, affecting various aspects of aquatic ecosystems [1].

Toxicological studies using fish are useful in assessing the environmental impact of anthropogenic pollution. Salmonids are particularly sensitive to pollutants, which is why they are often used as bioindicators to reflect the state of aquatic ecosystems. Fish embryos and larvae are vulnerable to stress caused by MP pollution due to intensive developmental processes, meaning the damage can have long-term consequences for their growth, reproduction and survival [2]. In the meantime, juvenile and adult fish are at a higher risk of ingesting MP during feeding, which may lead to particle accumulation in the gastrointestinal tract [3].

This work examined the genotoxic effects of common microplastic polymers, such as high-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP), polystyrene (PS) and tire particles (TP), on rainbow trout (*Oncorhynchus mykiss*) at different developmental stages. The aim of the study was to assess the response of larvae and juveniles to MP pollution in order to reveal possible differences in toxicity between these developmental stages. To achieve this goal, an erythrocytic nuclear abnormalities assay was performed according to the methodology described by Stankevičiūtė et al. [4].

The results of the study demonstrated that PP and PS caused a significant increase in the frequency of micronuclei in larval erythrocytes. The genotoxicity of MP particles in larvae increased in the following order: LDPE < HDPE < PS < TP < PP. Meanwhile, HDPE, LDPE, PS and TP, due to exposure through the diet, significantly increased the frequency of micronuclei in juvenile fish erythrocytes. The genotoxicity of MP particles in juvenile fish increased in the following order: PP < TP < PS < LDPE < HDPE.

This study emphasizes that the effects of MP may vary not only depending on the type of particle, but also that the same type of particle may have different effects depending on the developmental stage of the fish and the route of exposure. This highlights the importance of fully assessing the risks posed by different types of MP to aquatic ecosystems, ultimately leading to the creation of strategies for environmental protection and sustainable resource management.

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O3

ROS AND SHINE: ILLUMINATING PLANT RESPONSE TO OXIDATIVE STRESS

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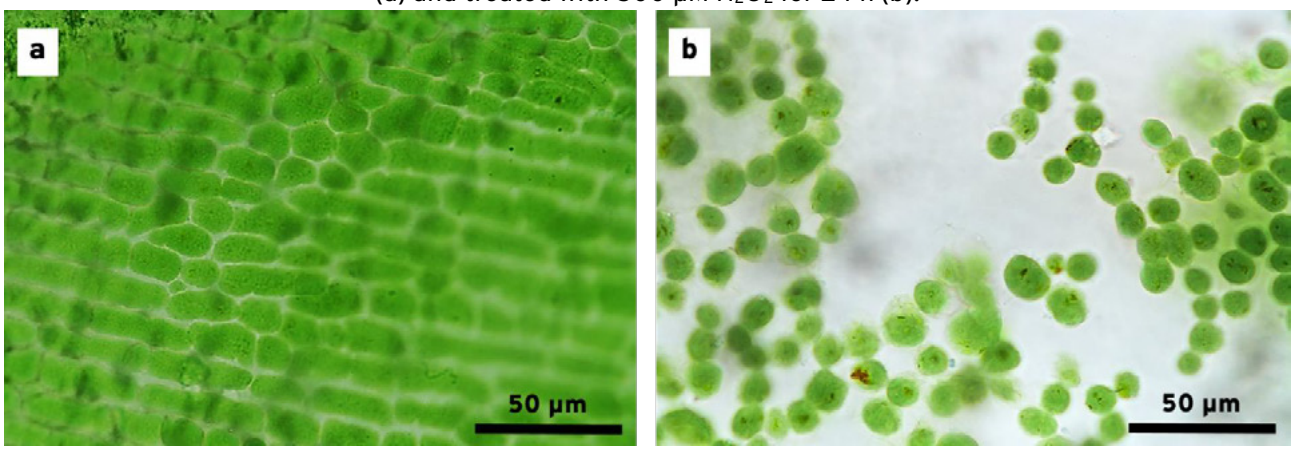
Due to the changing environment, plants are constantly exposed to a multitude of adverse environmental conditions that can reduce photosynthetic activity and lead to the overproduction and accumulation of reactive oxygen species (ROS), a process known as oxidative stress. This complex phenomenon can result in significant damage to various cellular structures, such as chloroplasts, thereby affecting plant growth and development [1]. The implementation of non-invasive early detection strategies, coupled with a better understanding of oxidative stress, could assist in the development of new approaches to mitigate the effects of adverse environmental conditions and improve plant resilience.

To evaluate the effect of ROS at the single cell level, experiments were performed on internodal cells of green macroalga *Nitellopsis obtusa*. The toxicity of externally applied H_2O_2 (500 µM) was studied over time (24 h) via cell viability assays, where cells that lost turgor were considered dead. Noticing changes in the chloroplast layers within the algal cells after prolonged exposure, microscopic images were taken to visualize the damaging effect of H_2O_2, and the concentrations of chlorophylls and carotenoids from cell extracts were evaluated via absorption spectroscopy. Photosynthetic processes of intact algal cells after 6 h incubation in H_2O_2 were monitored via steady-state and kinetic (pulse-amplitude-modulated (PAM)) fluorometry, using blue LED light (405 nm, 445 nm) for excitation.

The results showed that H_2O_2 is toxic to algal cells, with cell viability reaching 0 % after 24 h. In addition, damaged chloroplast layers were observed after prolonged exposure (Fig. 1). Spectroscopic analysis revealed that the reduction in chlorophyll content over 24 h was not significant, but the decrease in carotenoid concentration was ($p < 0.05$, Kruskal–Wallis test). Fluorometry showed that peak emission intensity at 683 nm decreased ($p < 0.05$, Mann-Whitney test) after exposure to H_2O_2 and the quantum yield of PSII (Y(II)), a measure of the efficiency of photosynthesis, was lower ($p < 0.05$, unpaired t-test), compared to the control group.

Figure 1. Microscopic images (40x) of the chloroplast layers of different *Nitellopsis obtusa* cells: untreated (a) and treated with 500 µM H_2O_2 for 24 h (b).



The study conducted shows that externally applied H_2O_2 (500 µM) is toxic to algal cells. The compound causes oxidative stress, as indicated by a decrease in carotenoid concentrations, which have antioxidant properties [2]. The effects studied were detected using non-invasive techniques before cell death, suggesting that the applied methods may be applicable for early detection of physiological stress or detection of lower, environmentally relevant concentrations of ROS.

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O4

AN INVESTIGATION OF VIBRIO INFECTIONS IN HUMANS IN LITHUANIA

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The *Vibrio* genus is a gram-negative curved rods that are facultative anaerobes and oxidase positive. They are abundant in warm estuarine and brackish coastal waters. The *Vibrio* genus comprises up to 130 bacterial species, approximately 12 of which are known to cause human infections. *Vibrio cholerae [non O1/O139]*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, and *Vibrio vulnificus* are the "big four" or the most common *Vibrio* species in the Baltic Sea region. They cause vibriosis, seafood-associated gastroenteritis, ear infections, and wound infections that may eventually lead to septicemia. Studies in Lithuania have observed the presence of *Vibrio* spp. in the Baltic Sea waters associated with rising global sea surface temperatures [1].

This study aimed to investigate the prevalence of *Vibrio* species in surface wound samples collected from humans. This study was conducted from July 1, 2024, to October 1, 2024. Vilnius University Hospital Santaros Klinikos took part in the study. Specimens from superficial wounds and infection sites from all patients over 18 years of age were included in this study. Collected specimens were assigned into four groups: skin and its derivatives, wound/ulcer, ear, and others (pus, eye). All specimens were inoculated onto Thiosulfate citrate bile salts sucrose agar (TCBS) (*VWR Chemicals BDH*, Belgium). Following growth on TCBS agar, bacterial colonies were subcultured onto Chromogenic agar (*Condalab*, Spain) and 3% salt Brain-Heart agar (*Liofilchem*, Italy). An oxidase test was performed for identification. All plates were aerobically incubated for 24–48 h at 35 ± 2°C. Conventional PCR was used to identify the four most common pathogenic *Vibrio* species in the Baltic Sea region. It was conducted on bacterial strains that showed a positive oxidase reaction and colony pigmentation consistent with the manufacturer's guidelines on TCBS and Chromogenic agar following the methodology described in Gyraite et al. [2].

The study included 194 patients from whose specimens were taken. The mean age of the participants was 49.0 years (median – 48.5, SD – 20.4). The youngest patient and study participant was 18 years old, and the oldest was 90 years old. 56.2% (n = 109) of patients were female, and 43.8% (n = 85) were male. The most significant number of specimens was taken from patients' ears (48.5%, n = 94), with additional from wound/ulcer (26.3%, n = 51), skin and its derivatives (21.6%, n = 42), and 3.6% (n = 7) from other specimens.

Bacterial growth was observed on 51.5% (n = 100) of TCBS agar plates. 45% of the plates exhibited characteristic yellow colonies, while 35% showed green colonies. Eighty bacterial isolates were subcultured onto Chromogenic agar, and 3% Brain-Heart agar and an oxidase test was performed. A total of 18 bacterial strains were subjected to a PCR analysis. The results of the PCR analysis revealed no amplification of species-specific genes of *V. cholerae [non O1/O139], V. parahaemolyticus, V. alginolyticus,* and *V. vulnificus*.

In our study, no *Vibrio* spp. were cultured from surface wound and infection specimens collected during the warm season of 2024. Nevertheless, considering the warming climate and the increased prevalence of *Vibrio* spp. in the Baltic Sea waters, we propose the routine use of TCBS agar for culturing samples from superficial wounds.

Acknowledgments. This work was supported by funding from the Research Council of Lithuania [S-PD-22-80 to GG].

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05

THE ANTIBACTERIAL ACTIVITY OF DIFFERENT FORMS OF ESSENTIAL OILS

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The rapid growth in antimicrobial resistance (AMR) has become a global concern. To stop this process of developing resistance, it is essential to explore natural alternative approaches. Medicinal and aromatic plants are an alternative and promising pathway for drug development that has historically improved. Essential oils (EOs) derived from plants that include bioactive components, and antibacterial activities could be a potential solution to arrest this problem. However, the application of EOs could be restricted due to poor solubility in water and high volatility, which leads to a reduction of their effect. Encapsulation using biopolymers such as emulsions is a promising alternative to overcome the disadvantages of using EO formulations.

This study aims to compare and evaluate the antimicrobial activity of different forms of EOs: Basil, Cinnamon, Cinnamaldehyde, Eucalyptus, Lavender, Pine, Rosemary and Tea tree. This study focused on three types of essential oils: pure, Octenyl Succinic anhydride-based emulsions containing EO (EOE), and EO freeze-dried powder (EOFD).

For this study, we selected gram-positive *Staphylococcus aureus* (ATCC 9144), methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300), *Staphylococcus epidermidis* (ATCC 14990), *Bacillus subtilis* (ATCC 6051), gram-negative *Salmonella enteritidis* (ATCC 8739), and *Pseudomonas aeruginosa* (ATCC 10145) bacterial species. The agar diffusion method was used to determine the inhibition zone and the microdilution method - to evaluate the Minimal Inhibitory Concentration (MIC), Minimal Bactericidal Concentration (MBC), and the MBC/MIC ratio [1, 3]. In addition, the anti-biofilm formation activity against *S. aureus*, MRSA, and *P. aeruginosa* was evaluated with cinnamaldehyde using the tube method [4].

As a result, cinnamaldehyde showed the highest antibacterial activity against all reference strains, with the diameter of the inhibition zone ranging from 24.37 to 50.22 mm. Cinnamon, eucalyptus, and pine EO inhibited all bacteria except *P. aeruginosa*, while basil EO did not affect any bacteria. For the antibacterial effect of EOEs, cinnamaldehyde emulsion showed an effect against all bacteria except *P. aeruginosa*, where the diameter of the inhibition zone ranged from 12.07 to 23.00 mm. As for EOFD, they had no effect.

The results of the microdilution method correlated with the agar diffusion test. Cinnamaldehyde EO showed a low MIC value of 1.31 mg/mL against all bacteria and 2.62 mg/mL against *P. aeruginosa*. The MICs of the EOEs are higher than the MICs of the EOs. The MIC of cinnamaldehyde EOE was 6.87 mg/mL against MRSA, *S. epidermidis*, *B. subtilis*, and *S. enteritidis*, and 27 mg/mL against *S. aureus*. Regarding the MBC/MIC ratio, MBC values were generally equal to MIC values or two-fold MIC for all the antimicrobial agents. Therefore, the EOs and their emulsions had a bactericidal effect on all the tested bacteria.

Regarding the biofilm assay, MRSA and *P. aeruginosa* showed moderate biofilm formation. After treatment of the bacterial biofilm with cinnamaldehyde for 1 hour, no biofilm was detected, as indicated by the disappearance of biofilm layers on the tube wall for all the tested bacteria.

In conclusion, this study showed that EOs and their emulsions could be an effective and alternative source of antimicrobials to combat bacterial strains resistant to conventional antibiotics.

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06

IMPACT OF ARTIFICIAL PANCREAS DO-IT-YOURSELF (DIY) CLOSED-LOOP SYSTEMS ON TYPE 1 DIABETES MANAGEMENT

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Type 1 diabetes mellitus (T1DM) is a lifelong autoimmune condition that requires continuous insulin therapy. Over the years, advancements in diabetes management have led to the development of continuous glucose monitoring (CGM) and insulin pumps, significantly improving glycemic control. However, traditional insulin therapy often fails to maintain optimal glucose levels, leading to the introduction of closed-loop insulin delivery systems, also known as artificial pancreas systems (APS). While commercial APS options are available, many individuals with T1DM have opted for do-it-yourself (DIY) closed-loop systems, which offer greater flexibility, improved glucose regulation, and lower costs compared to proprietary commercial solutions [1], [2]. Research suggests that DIY APS users experience improved time-in-range (TIR), reduced glycemic fluctuations, and a lower burden of diabetes management compared to those using traditional insulin delivery methods [3]. Additionally, DIY APS has been found to be a cost-effective alternative, as commercial hybrid closed-loop systems often require expensive hardware and software subscriptions [4].

This study, conducted in March 2024, surveyed Lithuanian individuals with T1DM using an online questionnaire. Among the 92 valid responses, 65.22% (n=60) reported using a closed-loop system, with 93.40% of them relying on an open-source DIY version. No significant gender-based differences were observed in system adoption ($p = 0.8623$); however, users were, on average, younger than non-users (30.43 vs. 37.00 years; $p = 0.03$). A significant decrease in glycated hemoglobin (HbA1c) levels was observed following system adoption, with the average HbA1c dropping from 7.19% (SD = 1.35) to 6.02% (SD = 0.57) ($p < 0.05$), reinforcing previous findings on the benefits of DIY APS in glycemic management [5], [6]. Additionally, the average number of hypoglycemic episodes per month declined significantly, from 24.08 to 9.67 ($p < 0.05$), consistent with prior studies on DIY APS [7].

The most commonly cited reasons for adopting DIY APS included improved glucose stability (93.4%), better sleep quality (72.1%), and greater convenience (67.2%). Meanwhile, barriers to adoption included technical challenges related to software setup (55.7%), infusion set blockages (31.3%), and limited support from healthcare providers (39.3%). Among non-users, the most frequently reported barriers were a lack of knowledge about the system (40.6%), financial constraints (25%), and concerns regarding safety and reliability (28.1%). These findings align with existing literature, highlighting the need for increased awareness and education regarding DIY APS options [8]. Despite these challenges, 90.9% of insulin pump users expressed interest in trying a DIY closed-loop system in the future.

DIY APS presents a viable, patient-driven alternative to commercial artificial pancreas solutions, offering significant improvements in glycemic control, quality of life, and financial accessibility. However, challenges such as technical complexity and limited physician involvement remain key obstacles to widespread adoption. Addressing these issues through improved education, accessibility, and healthcare provider engagement will be essential to ensuring that more individuals with T1DM can benefit from these advanced diabetes management systems [9], [10].

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O7

AN EXPANDED DIVERSITY OF PEDINOPHYTES PROVIDES A WINDOW INTO THE EVOLUTION OF THE GENETIC CODE IN ORGANELLES

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Mitochondria and plastids of various lineages exhibit genetic code alterations. However, the knowledge of the diversity and occurrence, mechanistic underpinnings, and evolutionary origins of codon reassignments in organelles remains incomplete. We focused on organelles of the neglected green algal class Pedinophyceae, as well as pedinophyte-derived secondary plastids of peDinoflagellates.

We isolated a novel pedinophyte dubbed “Okinawa strain” and sequenced its organellar genomes. Additionally, we assembled organellar genome sequences from previously unexplored pedinophyte lineages utilizing exiting raw (meta)genomic data. Bioinformatic analyses of the expanded set of pedinophyte organellar genomes paint a surprisingly complex picture of their genetic code landscape. Concerning mitochondria, the stop-to-Trp reassignment of the UGA codon appears to have evolved multiple times in pedinophytes, and the Arg-to-Ala reassignment of AGA/AGG codons was shown to be apomorphic for the whole order Marsupiomonadales. Strikingly, this order has additionally converted UUA and UUG into termination codons, relying on specific mutations in the mtRF1a protein. Finally, all pedinophytes seem to decode AUA as methionine rather than the standard isoleucine. An analogous reassignment is on the way also in plastids of two separate pedinophyte lineages. Most unexpectedly, apart from the previously reported Ile-to-Met AUA reassignment, peDinoflagellate plastids have switched the meaning of the AGA/AGG codons from arginine to another amino acid, and have modified their pRF2 protein to mediate translation termination at UUA/UCA codons.

In conclusion, pedinophyte(-derived) organelles present a broad spectrum of codon reassignments and provide important insights into the emergence and mechanisms of non-standard codon translation.

O8

ENHANCING AI-DRIVEN BIOMOLECULAR STRUCTURE PREDICTION WITH VOROMQA-AA: A NEW APPROACH TO ACCURACY ESTIMATION

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All cellular processes from energy production to cell division and signal transduction are based on interactions between proteins, nucleic acids, and other molecules. The structures of the resulting biomolecular complexes directly affect how they function. Therefore, knowing the three-dimensional arrangement is crucial for understanding fundamental biological processes, as well as designing biomolecules for therapeutic and industrial applications.

Recent advances in deep learning-based modeling have significantly improved the accuracy and accessibility of protein structure prediction. However, predictions for nucleic acids, ligands, and their complexes with proteins remain less reliable, having lower accuracy and less certain confidence metrics. While methods like AlphaFold and RoseTTAFold represent major progress, they function as black-box models, offering little transparency into their decision-making [1]. This lack of interpretability makes it difficult to assess reliability, identify errors, and fully trust their predictions, posing challenges for applications requiring high structural accuracy. Furthermore, multiple biomolecular modeling methods are available, each generating several structure models of varying accuracy, thus selecting the most reliable prediction becomes a major challenge.

To address both issues, structure quality assessment tools are essential. Previously, we developed VoroMQA [2], a quality assessment method for protein structures. However, with the release of methods capable of predicting biomolecular complexes including proteins, nucleic acids, ligands, and ions [3], there is an increasing need for accuracy estimation methods of broader scope.

Here, we present the development of VoroMQA-aa (VoroMQA all-atom), an advanced quality assessment tool capable of evaluating a broad range of macromolecular structures, including proteins, nucleic acids, and their complexes with ligands and ions. VoroMQA-aa is a knowledge-based statistical potential that utilizes contact areas derived from Voronoi tessellation to assess structure quality. To train this potential, we utilized a large dataset of experimentally determined structures. Due to the limited availability of structures containing nucleic acids, ligands, and ions, we adopted a novel training approach. Instead of relying on a clustered subset of reference structures, we incorporated all available structures, using clustering data to account for similarity of sequences and interaction interfaces. This strategy enhances the robustness and generalizability of VoroMQA-aa across diverse biomolecular systems.

With its broad applicability, VoroMQA-aa bridges the gap between AI-driven structure prediction and reliable model selection as well as interpretation, providing deeper insights into biomolecular interactions and functions, ultimately advancing our understanding of complex biological processes.

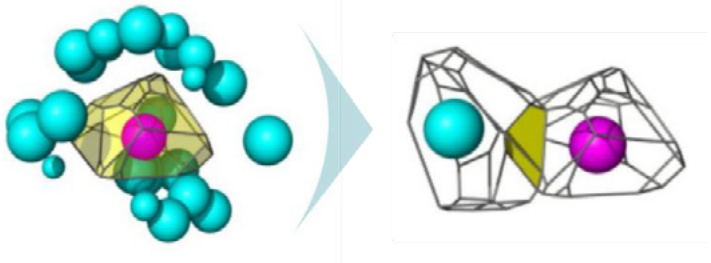

Figure 1. Constructing contact area of two atoms by Voronoi tessellation.

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09

INNATE PROGRAMMABLE DNA BINDING BY CRISPR-CAS12M EFFECTORS ENABLES EFFICIENT BASE EDITING

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The development of CRISPR technologies, particularly Cas9 and Cas12a, has transformed the field of genome editing. However, as the limitations associated with current methods reliant on double-stranded breaks (DSBs) grow, attention is shifting towards alternative approaches, like base editing. This technique enables precise and predictable modifications without introducing DSBs. Adenine base editors (ABE), which convert adenine (A) to guanine (G) within the DNA sequence, offer targeted and predictable single nucleotide alterations. Although adenine base editing activity has been demonstrated with the nuclease-impaired dCas9 and dCas12a effector proteins [1], there is a demand for more compact protein variants that can retain the precision of base editing while being compatible with efficient packaging into adeno-associated virus (AAV) vectors, that are commonly used in clinical applications [2]. Cas12 effector proteins encoded in CRISPR-Cas type V systems are characterized by a wide range of sizes. Among them are relatively small Cas12m effector proteins (~600 aa), the size of which would not limit the use of AAV vectors to deliver protein-encoding genes.

In this study, we characterize a set of compact CRISPR-Cas12m (subtype V-M) effector proteins and show that they protect against bacteriophages and plasmids through targeted DNA binding rather than cleavage. Biochemical assays suggest that Cas12m effectors can act as roadblocks inhibiting DNA transcription and/or replication, thereby triggering interference against invaders. Harnessing the innate ability of Cas12m to bind DNA targets we fused them with adenine deaminase TadA-8e and showed that GoCas12m-ABE efficiently edited A-to-G in *Escherichia coli* and human cells [3]. Overall, this study expands our understanding of the functionally diverse Cas12 protein family, revealing the DNA-binding dependent interference mechanism of Cas12m effectors that could be harnessed for the engineering of compact base-editing tools.

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O10

DNMT1-SPECIFIC METHYLOME TRACKING IN LIVE LUNG CANCER CELLS

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In mammalian development, 5-methylcytosine plays a crucial epigenetic role in the coordinated regulation of cellular functions. Nevertheless, alterations in DNA methylation may transpire, disrupting numerous cellular processes and resulting in various diseases, including cancer. The AdoMet-dependent DNA methyltransferase DNMT1 has oncogenic functions in cancer cell proliferation, invasion, and the enhancement of cancer stem cell self-renewal. Nonetheless, the full implications of DNMT1 molecular functions in carcinogenesis are yet unclear. Consequently, the advancement of a molecular method to accurately identify DNMT1-specific DNA methylation during oncogenesis is essential. To improve the comprehension of DNA methylation profiles in mammalian cells, we engineered mouse DNMT1 to facilitate the transfer of extended moieties onto DNA in vitro utilizing a synthetic cofactor analog, Ado-6-azide. Subsequently, we successfully employed modified DNMT1 and pulse internalization of the Ado-6-azide cofactor via electroporation to selectively covalently tag and accurately map catalytic DNMT1 sites in mammalian cells. By employing this genome tagging technique [1, 2] we successfully observed DNMT1-specific activity in mouse lung cancer cells. As a result, further development of this novel approach will help to establish distinctive preclinical platform to elucidate DNMT1-mediated DNA methylation alterations during cancer progression and identify prospective epidrug targets.

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O11

Mechanism of N4BP1 RNase action as a component of decapping complex

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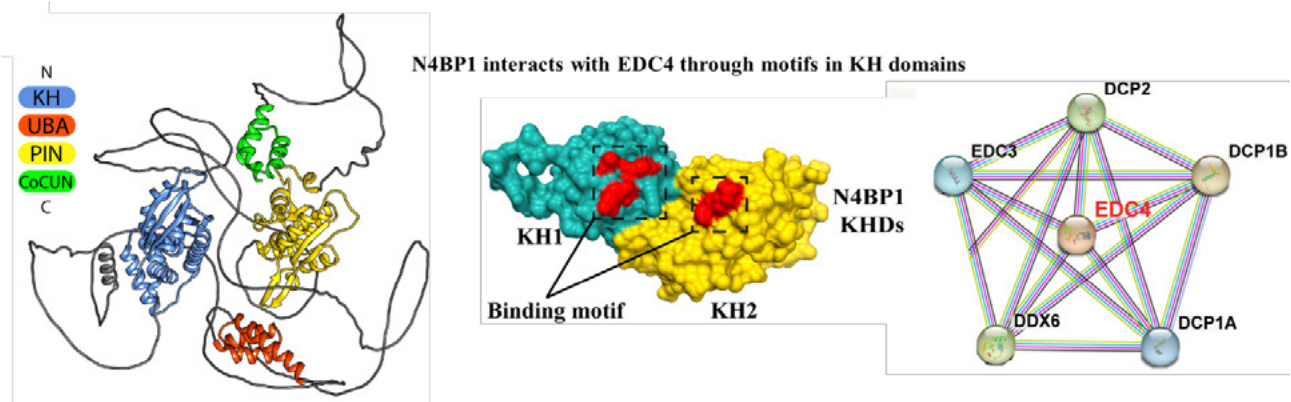
Exonuclease-mediated degradation of invasive un-capped RNA represents a key mechanism of primary immune response. In eukaryotic cells, mRNA decapping occurs within specialized cytoplasmic granules known as processing bodies (P-bodies), where the decapped transcripts are subsequently degraded through exonuclease cleavage [1]. Large molecular complexes within the cell facilitate multistep biochemical reactions between proteins and nucleic acids. Here we aimed to uncover function of human RNase N4BP1 in a macromolecular complex. Surprisingly, through immunoprecipitation coupled with mass spectrometry, we identified N4BP1 as a novel nuclease associated with the mRNA decapping complex, scaffolded around EDC4. This decapping complex comprises many proteins including a core of EDC4, EDC3, decapping proteins DCP1A, DCP1B, and the mRNA cap hydrolase DCP2.

To investigate the function of N4BP1 RNase, we analyzed the significance of its tandem KH domains by examining its crystal structure focusing on ability to interact with EDC4 and RNA motifs including stem-loop RNA. Given the numerous N4BP1 potential interaction partners and regions within the studied N4BP1 complex, we employed high-throughput cloning based on MCSG structural genomic projects to generate over 200 expression vectors so far. These proteins are intended for biochemical and structural analyses to elucidate the function of N4BP1 both as an independent entity and within the context of the partner for decapping complex. The N4BP1 comprises two KH domains that are crucial for interaction with EDC4 and PIN nuclease domain that has similarity to Regnase1 RNase. Whereas EDC4 posses WD40 domain assembled by 7 fold propeller made by β-sheets, providing stable core of complex. We plan to reconstruct whole N4BP1-EDC4 complex in vitro for structural and biochemical analysis. Therefore, we utilize mammalian expression systems, including non-adherent Expi239 and ExpiCHO cells that grow in a high density suitable for protein production. We produce N4BP1 and its macromolecular complexes that are subsequently analyzed using EM negative staining and cryo-EM.

Figure 1. N4BP1 is an RNase involved in mRNA turnover by decapping complex



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