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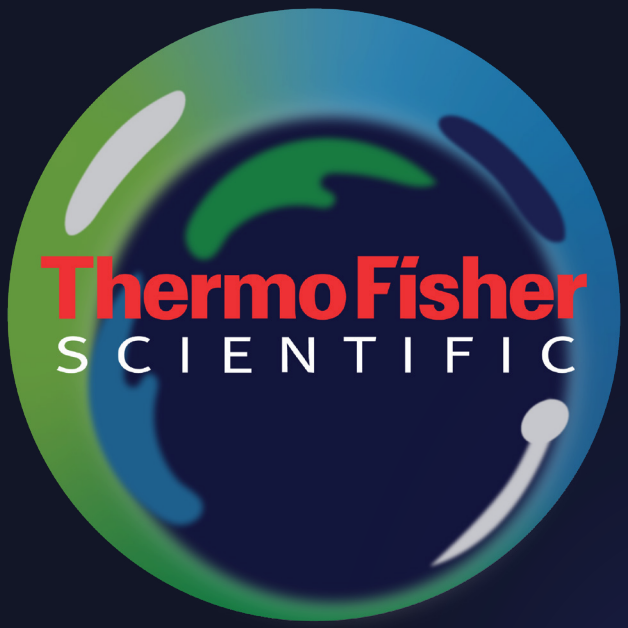

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Contents

Keynote Speakers.....4

April 15th

Microbiology and Biotechnology.....15
Biomedicine.....59
Bioinformatics.....73
Immunology.....81

April 16th

Biology and Ecology.....90
Biochemistry and Molecular Biology.....123
Oral Presentations: Medicine.....167

April 17th

Biophysics and Neurobiology.....170
Genetics.....200
Cell Biology.....224
High School Students.....238
Oral Presentations: Enviromental Sciences.....251
Sponsors 254

Keynote Speakers

Kostas Kostarelos



Everything you always wanted to know about Taking Graphene in the Clinic*

*But were afraid to ask

The use of advanced materials and, more precisely, nanoscale materials in medicine has been growing at an unprecedented rate for a variety of therapeutic, diagnostic or combinatory applications. The clinical translation of advanced materials that are being discovered or synthetically engineered is considered by many the critical factor to determine the ´success´ or ´failure´ in nanomedicine. In this talk, our decade-long experience in the clinical translation of graphene two-dimensional nanosheets will be discussed. Recent progress in the development of clinically used graphene oxide will be described, with emphasis on two very different first-in-human clinical investigation studies undertaken recently using graphene-based technologies. Emphasis will be placed on common lessons learned and whether graphene can serve as a case study on the early-stage clinical translation of advanced nanomaterials.

Nanomedicine Lab
ICREA & Catalan Institute of Nanoscience & Nanotechnology (ICN2)
UAB Campus, Barcelona, Spain

Institute of Neuroscience, Faculty of Medicine
Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain

Faculty of Biology, Medicine and Health,
National Graphene Institute
Manchester Cancer Research Centre
The University of Manchester, Manchester M13 9NT, United Kingdom

Aistė Vitkūnaitė



Precision medicine for everyone: latest technologies and global trends

Medical geneticist who worked for several years in molecular and digital pathology laboratories. Currently, Aistė is a field application specialist at UAB Linea libera, working on innovative genetic analysis solutions and their application in laboratories. Aistė is convinced that precision medicine is the future of medicine in Lithuania and worldwide. While studying biochemistry at VU, she was a member of the 2016 European Biochemistry Programme. She is very happy to be back here as a speaker and participate in a great event.

Precision medicine is an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person. Over the last years, pharmacogenomics has become an essential tool for personalized medicine to examine genetic variations that affect drug metabolism. But what are the different genomic tools for novel associations and biomarker discovery? Routine analysis and screening? How can we turn our current sick care into actual health care? Could a genetic passport be our future?

Attila Gacser



Et tu, mi fili, Brute?": Can even fungi cause cancer?

The correlation between tumorigenesis and alterations in the microbial flora composition has long been recognized. However, the tumor-microbiome relationships have primarily been characterized through the bacterial and viral components of the microbiota, leaving a gap in our understanding of the role of fungal species within the microbiome in cancer pathogenesis. Over the past decade, it has been established that, similarly to the bacteriome and virome, the fungal components of the microbiome - the mycobiome - play an important role in maintaining a healthy state. While the potential role of the mycobiome in cancer was already under investigation as early as 2015 – mainly through altered gut flora compositions - it was not until 2022 that the mycobiome's exact contribution to neoplastic tranformation was elucidated, following the detection of fungal cells in the microenvironment of tumor tissue samples. Recent research advancements have shed more light on the role of the mycobiome in various diseases, thereby elevating the study of fungal flora to a focal point of scientific interest. In our review, we discuss the known correlations between certain cancers and altered mycobiome compositions, and how the mycobiome may possess oncogenic potential, delving into the cellular and molecular mechanisms underlying this phenomenon. Finally, based on the available literature, we explore the diagnostic opportunities the fungal flora offers in cancer detection and its potential role in anti-tumor treatments.

Attila Gácser is a Professor of Microbiology and Immunology at the University of Szeged where he is the Head of the Institute of Biology. Dr. Gácser completed his PhD at the University of Szeged and during his first post-doctoral training at the University of Hamburg, Germany as a Marie Curie Fellow, he started to study the opportunistic Candida species. In 2005, he joined to Prof. Josh Nosanchuk's laboratory at Albert Einstein College of Medicine in New York where he investigated the virulence and pathogenesis of several different fungal pathogens such as Histoplasma capsulatum, Cryptococcus neoformans and Candida albicans and Candida parapsilosis. In 2008, he returned to Hungary and established his laboratory at the Department of Microbiology. He received numerous Hungarian and International grants, such as the EMBO Installation Grant, The Momentum Grant, OTKA grants, he was the coordinator of the EU-funded ERA-Net Pathogenomics consortium and participated in two Marie Curie ITNs. Since 2018 he is a visiting professor at the Federal University of Rio de Janeiro. The current research in his laboratory focuses on developing tools for the genetic manipulation of fungal pathogens and studying Candida virulence and pathogenesis, host response, and immunology of fungal infections. More recently he has been involved in human mycobiome research and the development of new innovative therapies against fungal pathogens.

Contact: Dr. Attila Gacser, gacsera@bio.u-szeged.hu, 6726 Szeged Közép fasor 52. Hungary

Laima Budėnaitė



Exploring the Engineering and Genome Editing Potential of Miniature SpaCas12f1

Laima Budėnaitė is scientist with extensive experience in the biotechnology sector, currently working at Caszyme, a company specializing in genome editing technologies. In 2022, she embarked on her Ph.D. journey at Vilnius University in collaboration with Caszyme, focusing her research on the advancements of CRISPR-based tools for precise genetic manipulation in mammalian cells. In her presentation, Laima will delve into the fundamental principles of CRISPR-Cas and its revolutionary implications for biomedical research. Additionally, she will present the results of miniature SpaCas12f nuclease engineering, noting its potential for genome editing.

It was recently shown that the miniature (497 aa) CRISPR-Cas12f1 nuclease from Syntrophomonas palmitatica (Spa) with a 5′ TTC PAM cleaves double-stranded (ds) DNA targets and prefers elevated temperatures, 44-58°C. To broaden its utility as an editing tool, we have performed protein and guide RNA (gRNA) engineering to enhance cellular activity at lower temperatures. For protein evolution and guide RNA engineering we used yeast as a model system to screen for alterations that enhanced the cleavage. Beneficial alterations were combined, tested for additive effects and selected for further experiments in human cells. Our advancements enhanced SpaCas12f activity in HE293T cells at 37°C over a 10-fold. The specificity of the most active versions was found to be sensitive to single base pair mismatches across most of the guide RNA DNA target recognition. Netherless we used SpaCas12f1 protein engineering to simplify its PAM to expanded the targeting range. Altogether, these results expands the type V CRISPR-Cas nuclease toolbox and highlights the SpaCas12f nuclease as a novel tool for DNA manipulations and human therapeutics.

Wanessa Melo



Development of photo-active hydrogel to treat chronic wounds

Wanessa Melo has completed PhD in Science with a focus on Microbiology at the University of São Paulo – Brazil (Department of Bioengineering) and part of her work was accomplished at Harvard Medical School – USA (Department of Health Science and Technology). Additionally, she has graduation in Biomedicine, Master's degree and a post-doctoral in Biotechnology. She also has experience as director of the Biomedicine course and professor of microbiology, molecular biology and cytology. Currently, Wanessa Melo occupies the position of Senior researcher at FTMC, Vilnius – Lithuania. Wanessa's research interest includes the development of new approaches to overcome biofilm infection on medical devices and prevent the adhesion of microorganisms on nosocomial environment surfaces. Additionally, she is an associate professor at Vilnius University, teaching in the Master's program of Molecular Biotechnology.

Chronic wounds have been an important therapeutic challenge for the last several decades, causing considerable patient morbidity/mortality and a burden for economics and health systems [1]. Worldwide it has been estimated that 20 million individuals have chronic wounds (predominantly patients aged older than 60 years and women), and the cost of their management and treatment exceeds €26 billion a year [2]. It is estimated that, at any given time point, around two million people in Europe have wounds that require medical care. Moreover, between 25-50% of acute hospital beds are occupied by patients with wounds and many of which were developed during hospitalization [3]. In addition, the most common forms of chronic wounds are related to diabetes mellitus, vascular diseases and pressure ulcerations.
The current medical treatments rely heavily on long-term usage of systemic antibiotics combined with topical antiseptics that usually fail [2]. To overcome this issue, alternatives have to be seriously considered. A promising candidate is microbial photodynamic therapy (aPDT), which is the photoinactivation of microorganisms by reactive oxygen species produced by a light-activated photosensitizer (PS) [6]. Much is already known about aPDT advantages over antibiotics, including no microbial resistance development, as it is a multi-target process and minimal damage to the host tissues [6]. Studies reported that superficial wound infections caused by biofilms are potentially suitable for treatment by aPDT because of the ready accessibility of these wounds for both topical delivery of the PS and light [7-9]. However, due to the complexity of chronic wound infections, PS diffusion and uptake are limited, restricting aPDT effectiveness and consequently wound healing. Chemically cross-linking the PS to a hydrogel may be an alternative to overcome this issue, once this device is recognized as an active wound dressing that can deliver drugs or other therapeutic substances to the wound site [10]
So, the presentation aims to show the progress of aPDT against biofilms and its combination with hydrogel against chronic wound infection.

Laboratory of Bioelectric, Department of Functional Materials and Electronics, State Research Institute Center for Physical Sciences and Technology, Savanorių pr. 231, 02300 Vilnius, Lithuania

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6. doi.org/10.1080/21505594.2021.1960105; 7. doi: 10.1039/b502125a; 8. doi: 10.1016/j.pdpdt.2009.10.008; 9. doi.org/10.1111/iwj.13269; 10. doi: 10.3390/biom10081169

Michael C. Jewett



Establishing cell-free synthetic biology for people and planet health

Michael Jewett is a Professor of Bioengineering at Stanford University. Dr. Jewett received his PhD in 2005 at Stanford University, completed postdoctoral studies at the Center for Microbial Biotechnology in Denmark and the Harvard Medical School, and was a guest professor at the Swiss Federal Institute of Technology (ETH Zurich). He is the recipient of the NIH Pathway to Independence Award, David and Lucile Packard Fellowship in Science and Engineering, Camille-Dreyfus Teacher-Scholar Award, and a Finalist for the Blavatnik National Awards for Young Scientists, among others. He is the co-founder of SwiftScale Biologics, Stemloop, Inc., Pearl Bio, and Synolo Therapeutics. Jewett is a Fellow of AIMBE, AAAS, and NAI.

Synthetic Biology is one of the most promising fields of research for the 21st century. It offers powerful ways to improve human health, build the global economy, manufacture sustainable materials, and address climate change. However, current access to biotechnology breakthroughs is unequal, largely due to bottlenecks in infrastructure and education. Here, I describe our efforts to re-think the way we engineer biology using cell-free systems to address these bottlenecks. We show how the ability to readily store, distribute, and activate low-cost, freeze-dried cell-free systems by simply adding water has opened new opportunities for on-demand biomanufacturing of vaccines for global health, point-of-care diagnostics for environmental safety, and education for synthetic biology literacy and citizenship.

Jorūnė Sakalauskaitė



Establishing cell-free synthetic biology for people and planet health

Jorune Sakalauskaite is a scientist working in an interdisciplinary area that intersects palaeoproteomics, molecular archaeology and marine biology. She has a background in chemistry and biological sciences, having obtained her PhD from University of Turin in Italy and University of Burgundy in France. After her postdoctoral work at the University of Copenhagen, Denmark, in 2022 she moved to Lithuania, Life Sciences Centre, Vilnius University. With the help of EMBO, MSCA and LMT funding and together in co-partnership with dr. Maria Fernanda Torres Jimenez, Jorune co-established a joint research group, EvoChrOmics.
Her work is largely focused on studying mollusc shells and ancient proteins found in their biomineral structures. Her most recent research project aims to decipher the biochemistry of shell colouration with the use of omic tools and gene editing technologies. She is interested in how molecular techniques can help to study complex living creatures and answer long-standing questions about the stability of mineralised shell pigments. For instance, what leads to their remarkable preservation deep in time?

We live in a very colourful world. Yet, rarely do we step aside to think how animals generate colours or what myriad of functions they encode. Mollusc shells are one of the most widespread examples of biomineralized structures on our planet, with an incredible diversity of colours and patterns. No wonder they were greatly appreciated and used by humans since ancient times. But despite the advancements in molecular biology techniques, the biochemistry of shell colours is still poorly understood. For example, what is the full spectrum of biogenic shell pigments, how do they get incorporated into the mineral or what function do they play?
In our current project we aim to tackle this question by using omic analyses and application of CRISPR-Cas9 gene editing tools. I will present a case story with Littorina shells, the Atlantic flat periwinkles, which have now become our model organism to study shell colouration. We use proteomics together with transcriptomics to identify intracrystalline shell chromoproteins while gene knock-out experiments will be used to verify their function.
I will share the backstage nuances about our development of gene editing approach to Littorina, a technique that has only been successfully applied to a handful of molluscan species so far. Interdisciplinary in nature, this project requires active engagement of different fields of knowledge, from biochemistry, to molecular sciences and marine biology.
We hope our research will help to uncover the mechanism behind colour biomineralization in shells and open new avenues to study pigments from ancient records. Colourful biominerals are not just pretty - they may spur new ideas on bioinspired systems: durable in time, and friendly to our planet Earth.

Linda Mezule



The hidden treasures of sewage: how to extract valuable molecules from sewage sludge

Linda Mezule is an Associate Professor and senior researcher at Riga Technical University (Latvia) Water Systems and Biotechnology Institute where she currently works on topics related to waste and biomass degradation, enzymatic hydrolysis, and resource recovery from various biological waste materials, including sewage sludge.

Sewage sludge, a by-product of wastewater treatment is a material of underestimated potential. In the EU, around 7.5 million tonnes of dry sewage sludge were produced in 2021, however, its efficient management is still limited to landfilling, incineration, or application on land. At the same time EU legislation has proposed a waste management hierarchy based on an environmentally-sound treatment option: reduce, reuse, and recover the energy. As currently seen, the reduction is still limited due to the employed wastewater treatment technologies and constant flow of wastewater.

However, the conventional methods do not take full advantage of the sludge properties, since the complex chemical composition provides the opportunity to recover proteins, carbohydrates, and lipids. To assess the potential use of sludge for high-value biomolecule production, sludge samples from 13 Latvian wastewater treatment plants were collected and their chemical analysis performed. Proteins, lipids, carbohydrates and cellulose were quantified in primary, secondary and anaerobically digested sludge. This assessment of Latvian municipal sewage sludge revealed a considerable amount of carbohydrates and proteins present in primary sludge (10.0% dw and 23.9% dw, respectively) as well as in secondary sludge (8.7% dw and 18.5% dw). Further, technologies for recovery of cellulose, proteins and lipids have been developed and tested at a pilot scale level.

In conclusion, primary sludge is a prime supply for cellulose recovery. Digested sludge has already undergone resource recovery through biogas production and, thus, offers lower yields of secondary resources. Correct selection of target molecule and sludge type can result in efficient recovery of high value molecules from this resource.

Andrius Šiaulys



Chasing an impossible dream: from the first photography of Baltic Sea reefs towards AI assisted imagery analysis of seabed habitats in front of melting Arctic glaciers

I am a marine biologist and ecologist at the KU Marine Research Institute.My main research interests and activities include benthic ecology, seafloor habitats, their mapping using in situ (e.g. diving) and remote (e.g. underwater filming systems) methods, development of underwater image analysis techniques, and working in marine ecosystems from the Baltic Sea to the Arctic regions. Less relevant to the subject of the report are modelling of species distributions, assessment of the ecological status of macrozoobenthos communities and benthic habitats, and the development of status indicators. At Klaipėda University I teach hydrobiology, blue technologies, marine landscape and habitat ecology.

Reef habitats in the Lithuanian coastal area of the Baltic Sea was Terra incognita until early 1990s. While there was knowledge of its existence, little was known of its biology, structure and spatial distribution due to the lack of scientific studies, since it was not accessible for large monitoring vessels due to shallow depths and heterogeneous seabed topography and was a restricted border area. The first underwater photography of Lithuanian coastal reefs was taken in 1993 by the team of Klaipeda University marine biologists with the assistance of German colleagues. These images revealed a complex nature of reef habitats and in turn initiated an entirely new field in Lithuanian marine biology – underwater imagery (UI).

For more than a decade UI was sparsely used for variety of studies, i.e. distribution of fish spawning grounds, however it was restricted only to descriptive surveys. A major breakthrough happened in 2006-2007 when methods for the extraction of quantitative features from UI were developed while estimating the stock of the red alga Furcellaria lumbricalis [1]. From this point UI gained the same status as a benthic sample and was used extensively, especially in reefs where traditional soft-bottom sampling gear could not be deployed due to hard substrates. With the advancement of underwater video equipment (drop-down cameras, towed cameras, hand-held cameras, remote operated vehicles), the amount of UI data recorded soon was overwhelming for manual analysis thus the search for automated methods commenced.

In this lecture the journey towards the AI supported UI analysis will be presented, starting form a semi-automatic UI analysis based on color extraction [2], detection and shape measurements of the dinoflagellate Prorocentrum cordatum [3], automated quantification of brittle stars along melting Arctic glaciers [4] to the most recent progress in coverage estimation of benthic habitat features in Lithuanian coastal reefs [5], in the same place where the first photography was taken more than 30 years ago.

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Burak Yelmen



Generative AI in functional and evolutionary genomics

I obtained my undergraduate degree in molecular biology and genetics and master's degree in biological sciences at the Middle East Technical University. I obtained my PhD at the University of Tartu focusing on human population genetics and bioinformatics. During my PhD studies, I got interested in generative AI and developed models for generating in silico artificial human genomes. I continued working on my artificial genomes project during my postdoc in Paris-Saclay University. I'm currently a research fellow at the University of Tartu. My research is centered around interpretable deep learning, generative AI and genetic privacy for human genomics and complex diseases.

In recent years, generative modelling has gained substantial momentum in genomics research thanks to increased availability of computational resources, expanding biobank datasets and development of deep generative models (DGMs) equipped with novel algorithms. DGMs can learn the complex structure of genomic data and can be utilised for a variety of tasks such as generation of realistic synthetic data, nonlinear dimensionality reduction and prediction of function or evolutionary parameters, through unsupervised, semi-supervised or supervised learning schemes. In this talk, I will provide a broad overview of generative AI in contemporary genomics research and present our work on generation of artificial human genomes using generative neural networks with applications and implications for future research.

April 15th

Microbiology and Biotechnology

A1

MOLECULAR EPIDEMIOLOGY STUDIES OF INVASIVE *LISTERIA MONOCYTOGENES* ISOLATES

Ugnė Meškytė[1], Anželika Slavinska[1], Aurelija Petrutienė[2], Svajūnė Muralytė[2], Nomeda Kuisienė[1]

[1]Department of Microbiology and Biotechnology, Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
[2]Clinical Testing Department, National Health Surveillance Laboratory, Lithuania
ugne.meskyte@gmc.stud.vu.lt

Listeria monocytogenes stands out as one of the most prevalent foodborne pathogens, presenting a significant health risk. This emerging bacterium can lead to human listeriosis, a condition with severe symptoms and a mortality rate of up to 30 percent. Cases of listeriosis have been observed in both minor and major outbreaks, particularly affecting immunocompromised patients, newborns, the elderly, and pregnant women [1]. Molecular typing of this pathogen is essential for understanding its genetic diversity and tracking outbreaks. The decreasing effectiveness of antibiotic therapy highlights the importance of this research. Such detailed information provided by molecular typing is invaluable for managing contagion and guiding potential treatments.

The aim of the present study was to investigate the molecular characteristics of *L. monocytogenes* as well as gain insights into its epidemiological aspects. Seventy-seven isolates were collected from Lithuanian hospitals during the period of 2016-2021. Isolates were serotyped and genotyped using PCR-based methods. Isolates were distributed into 1/2a (58 isolates), 1/2b (1 isolate), 1/2c (1 isolate) and 4b (17 isolates) serotypes. A genotyping dendrogram showed high diversity among isolates. One was selected due to its origin from the cerebrospinal fluid and its prevalence as one of the most frequently encountered serotypes (1/2a) associated with severe human infections. Subsequently, the genome was sequenced via the Illumina method, marking Lithuania's inaugural contribution to the BIGSdb-Pasteur database. This study represents the first analysis in Lithuania of *L. monocytogenes* characteristics using the whole genome sequencing (WGS) method. The sequenced genome resulted in a size of 2990056 bp, composing 16 contigs. The multilocus sequence typing (MLST) scheme, based on seven house-keeping genes (*abcZ*, *bglA*, *cat*, *dapE*, *dat*, *ldh*, *lhkA*), assisted in assigning this strain to sequence type 155 (ST155) and clonal complex 155 (CC155). Furthermore, a comparative analysis was performed among invasive isolates from countries worldwide presented in the database. For comparative analysis, conventional MLST, core genome MLST, ribosomal MLST, PCR-serogrouping, antimicrobial resistance, and virulence typing schemes were used. These schemes provided insights into genetic relatedness, evolutionary patterns, strain diversity, pathogenic potential, and antimicrobial susceptibility [2].

Typing schemes offer a simple and rapid way to compare *L. monocytogenes* strains. It enables the reconstruction of ancestral and evolutionary linkage among the isolates. This technique helps identify transmission routes of high-risk strains and their link to outbreaks [2]. Consequently, it helps to monitor *L. monocytogenes* infections, reducing their impact on public health.

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A2

CONTROL OF MICROBIAL CONTAMINATION OF CLUB MOSS SPORES BY ESSENTIAL OILS

Urtė Busilaitė[1,2], Modestas Petrauskas[1,2], Živilė Strazdaitė-Žielienė[1], Elena Servienė[1]

[1]Nature Research Centre, Vilnius, Lithuania
[2]Life Sciences Center of Vilnius University, Lithuania
urte.busilaite@gmc.stud.vu.lt

Plant essential oils (EOs) are hydrophobic liquid concentrates of plant secondary metabolites that can be extracted from almost all parts of plants. Although the composition of each oil is different, the main components are lipophilic, low molecular weight, volatile molecules – mostly terpenes, their oxidated derivatives – terpenoids, and phenolic compounds. Club moss (*Lycopodium clavatum L.*) spores' powder is used for a multitude of skin diseases that require a rash or sore to stay dry. However, club moss spores could be contaminated with microorganisms. It may include bacteria, fungi, and molds. Microbial contamination can occur during the harvesting, storage, and processing of the plant material. In recent decades, the investigation of the antimicrobial activity of various plant extracts, especially essential oils, is constantly growing up. Therefore, this study aimed to investigate the antibacterial and antifungal effects of coriander (*Coriandrum sativum*), cinnamon (*Cinnamomum cassia*), geranium (*Pelargonium graveolens*), ginger (*Zingiber officinale*), and oregano (*Origanum vulgare*) EO's against microorganisms isolated from club moss (*Lycopodium clavatum L.*) spores' powder.

During the research, club moss spores were washed out with MD medium and applied for direct isolation of microorganisms. The antibacterial and antifungal activities of five EOs were tested using evaporation test mode and counting of viable microbial colonies. It was demonstrated that volatiles of all tested EOs expressed antimicrobial and antifungal activity on microorganisms isolated from club moss spores. Cinnamon, oregano, and coriander EOs reduced the total viable bacterial counts by more than 50 %. Ginger and oregano EOs were the most effective against molds – they inhibited the growth of fungi by 89% and 67% respectively. By using molecular methods spores-inhabiting and EO treatment proceeded bacteria were identified as *Bacillus subtilis, Bacillus altitudinis* and *Acinetobacter oryzae/johnsonii*. It was determined that pure bacterial isolates differ in their resistance to EOs. Tested essential oils exhibited a stronger inhibition on *A. oryzae/johnsonii*, than *B. altitudinis* and *B. subtilis*. During this study, it was found that antimicrobial and antifungal action is affected not only by EO type but also by the treated object characteristics (such as rough spore surface) and widespread microbial species features. However, further studies are necessary to investigate the impact of essential oils on microorganisms and evaluate its potential in the industry.

A3

PLASMID DIVERSITY IN CLINICAL *ACINETOBACTER BAUMANNII* ISOLATES

Joris Naujalis[1], Tomas Liveikis[1], Edita Sužiedėlienė[1]

[1]Department of Biochemistry and Molecular Biology, Vilnius University, Lithuania
joris.naujalis@gmc.stud.vu.lt, tomas.liveikis@gmc.vu.lt, edita.suziedeliene@cr.vu.lt

Acinetobacter baumannii, a Gram-negative opportunistic pathogen, has become a significant global cause of nosocomial infections. *A. baumannii* infections require complex treatment because the bacterium rapidly acquires resistance to antibiotics. The spread of antibiotic resistance is significantly influenced by horizontal gene transfer via mobile genetic elements, such as plasmids. Insights into plasmid diversity may help control the epidemiological situation when treatments become ineffective. This study aimed to evaluate the diversity of plasmids from clinical *A. baumannii* isolates in terms of plasmid replicon types and plasmid profiles.

A. baumannii isolates (n = 165) were collected from Vilnius University Hospital from 2022 to 2023. Then, PCR-based replicon typing was applied to identify GR1-GR34 replicons. Subsequently, plasmid profiles were tested with pulsed-field gel electrophoresis (PFGE), focusing on the number of plasmids per isolate and plasmid size within the 10-300 kb range. Finally, the results were compared with global data. For this purpose, the NCBI BLASTn MegaBLAST algorithm was applied to search for GR1-GR34 *rep* gene sequences. The results obtained were complemented with information from the GenBank database.

Regarding the distribution of replicon types, GR2, GR6, GR8, and GR24 replicon types were detected among clinical *A. baumannii* isolates (n = 165). The majority of isolates (68%) had both GR2 and GR6 replicon types identified. A smaller proportion of all isolates (26%) had either GR2 or GR6 replicon types detected. Also, no tested replicon types were found in 6% of the isolates. Finally, only one isolate had GR8 and GR24 replicon types detected.

Subsequently, plasmid profile assessment with PFGE revealed that the majority of the isolates had a single plasmid (69%). Most isolates from this group (87%) had plasmid-like fragments of ~90 kb. However, plasmids were not found in 22% of the isolates in the collection. In addition, isolates with two fragments of different sizes were the least abundant (9%). Finally, comparison with global data showed that GR2, GR6, GR8, and GR24 are the most common replicon types globally, accounting for 48% of all GR1-GR34 *Acinetobacter spp.* replicon types (n = 1330). Also, the global size distribution of plasmids with GR2, GR6, GR8, and GR24 replicon types was found to be similar to the research findings. For instance, the median size of GR6 plasmids was found to be 73 kb. Consequently, it is likely that some of the plasmids found in the collection of ~90 kb and close in size have GR6 replicons. However, the median size of the GR2 plasmids was 9 kb; therefore, not all GR2 plasmids may have been detected under PFGE conditions.

Of the GR1-GR34 replicon types, GR2, GR6, GR8, and GR24 replicon types were found in the A. baumannii collection studied. According to GenBank data, it is likely that the isolates studied have globally dominant plasmids in regards to replicon types. Plasmid profiles were dominated by isolates with a single ~90 kb plasmid, and the plasmid sizes found in the collection of isolates are similar to those found worldwide. However, possibly not all smaller plasmids were detected under PFGE conditions.

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A4

APPLICATION OF ANTIBODIES FOR THE INVESTIGATION OF *GARDNERELLA* SPP. PUTATIVE ADHESION PROTEINS

Danas Ivanauskas[1], Aistė Bulavaitė[1], Aurelija Žvirblienė[1], Milda Plečkaitytė[1], Indrė Dalgėdienė[1]

[1]Institute of Biotechnology, Life Sciences Center, Vilnius University
danas.ivanauskas@chgf.stud.vu.lt

Bacterial vaginosis (BV) is a disease caused by changes in normal vaginal flora and is common in women of reproductive age. The disease may be asymptomatic, but about 50% of women with BV experience symptoms such as itching, bad vaginal odour, vaginal discharge, and elevated vaginal pH [1]. The normal vaginal microbiota is dominated by bacteria of the genus *Lactobacillus*. A decrease in these bacteria is associated with BV [2]. It is also thought that most BV infections start with *Gardnerella* spp. which creates a biofilm that allows other BV-associated bacteria to grow [3]. *Gardnerella* spp. can adhere to vaginal epithelial cells via adhesion proteins. The M protein repeat (MPR) protein and collagen-binding (CNA) protein are the most abundantly expressed on the bacteria's surface. Delving deeper into the molecular mechanisms of these adhesion proteins may help to elucidate their role in the pathogenesis of BV and develop immunological tools for the diagnosis and treatment of the disease [4].

In this study a collection of mouse monoclonal and polyclonal antibodies (MAbs and PAbs respectively) against recombinant *Gardnerella* spp. surface adhesion proteins, CNA and MPR, were used. Several MAbs were selected for antibody fragmentation to generate $F(ab')_2$ fragments. Removing the antibody Fc region is necessary to evade the *Gardnerella* spp. defence strategies against the host's immune system. Antibody samples were treated with the endopeptidase pepsin, which was immobilized on agarose beads. Purification of $F(ab')_2$ fragments was achieved using Protein A chromatography columns. The fragmentation process was evaluated using SDS-PAGE and enzyme-linked immunosorbent assay methods. The aforementioned antibody collection was also utilized in flow cytometry with three strains of *Gardnerella* spp. that differ in the expression of the adhesion proteins CNA and MPR on the cell surface. At first, the optimization of CFDA-SE dye, primary and secondary antibody quantities were determined, followed by the analysis of the whole antibody collection.

Electrophoresis showed that the fragmentation of the antibodies to $F(ab')_2$ fragments was complete and in higher yield in the case of the anti-CNA MAbs but not the anti-MPR MAbs. The functional activity of the generated $F(ab')_2$ fragments was compared with the unfragmented antibodies, showing a decreased affinity for the antigen. Antibodies specific for *Gardnerella* spp. CNA and MPR were tested with *Gardnerella* spp. strains by flow cytometry method and anti-MPR MAbs and PAbs showed a stronger interaction with surface MPR than anti-CNA with surface CNA.

The antibody modification by removal of the Fc region and generation of $F(ab')_2$ fragments was partially successful. Future work will aim not only at a high-quality fragmentation but also at higher yields of $F(ab')_2$ fragments. Flow cytometry results indicate that antibodies developed against recombinant MPR are more active *in vitro* against *Gardnerella* spp. compared to antibodies developed against CNA.

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A5

BIODEGRADATION OF 2-AMINOPYRIMIDINE BY *NOCARDIOIDES* SP. AM1

Aušrinė Butrimaitė[1], Renata Gasparavičiūtė[1], Justas Vaitekūnas[1], Rolandas Meškys[1], Jonita Stankevičiūtė[1]

Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University
ausrine.butrimaite@gmc.stud.vu.lt

The discovery of antibiotics is one of the most significant medical breakthroughs of the 20th century; however, their misuse has led to antibiotic resistance increasing at an alarming rate. In terms of chemical and molecular structure, the most common antibiotics are β-lactams, macrolides, tetracyclines, quinolones, aminoglycosides, sulfonamides, glycopeptides, and oxazolidinones [1].

Sulfonamides are commonly used in treating urinary tract or respiratory tract infections. Due to incomplete degradation in the human and animal intestines, sulfonamides significantly contribute to soil contamination through three primary sources: irrigation with reclaimed water, the use of sludge and manure as fertilizers, and the application of animal waste. In the biodegradation of sulfadiazines (a group of sulfonamides), 2-aminopyrimidine (2-AP) has been identified as the major intermediate. However, the further biotic degradation of this compound has been little studied, and the genes involved in its biodegradation remain unknown [2].

Given that soils harbor the greatest accumulations of sulfonamide waste, our objective was to isolate and characterize bacteria capable of degrading 2-AP from various environmental samples. To achieve this goal, environmental samples were first collected from soils and incubated in selective media with 2-AP. Subsequently, periodic HPLC-MS analyses were carried out to screen the samples. The culture that completely degraded 2-AP was isolated, purified, and identified by 16S rRNA analysis. The genome was then sequenced using Illumina technology to identify the genes responsible for 2-AP degradation (Fig. 1).

Among 46 soil samples incubated in a selective medium with 1 mM 2-AP, only one isolate was able to degrade 2-AP. The identified Gram-positive bacterium belonged to the genus *Nocardioides* and was named *Nocardioides* sp. AM1. The degradation of 2-AP was found to be an inducible rather than a constitutive process. Isocytosine was identified as the intermediate metabolite. In this study, we also investigated the capability of the AM1 strain to transform different pyrimidine compounds. The results revealed that 2-amino-4-chloropyrimidine is transformed into another accumulating product. Finding the 2-AP breakdown enzymes could be crucial in developing new targets for antibiotics, which would be a significant weapon against antibiotic resistance.

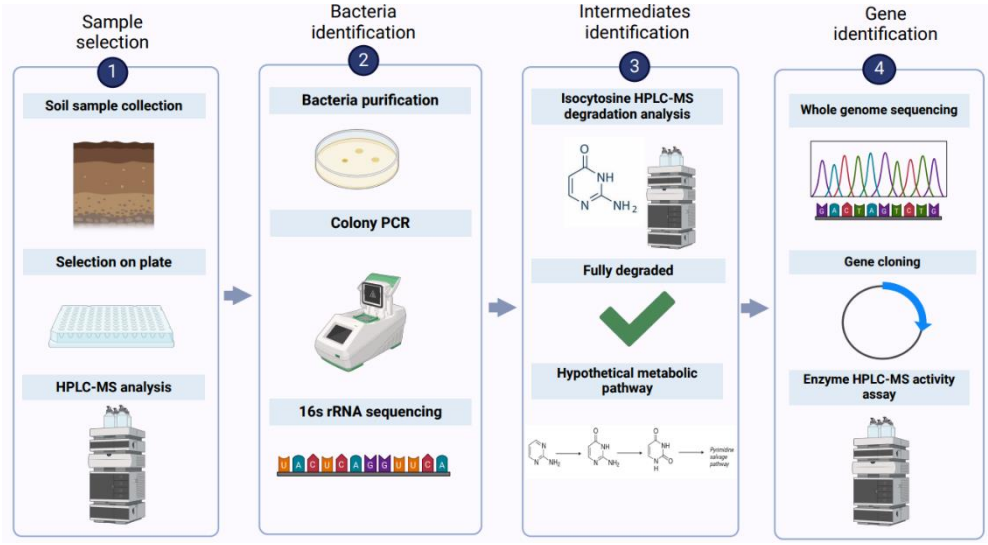

Figure 1. Isolation and characterization of microorganisms capable of degrading 2-AP. Created with BioRender.com

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A6

ISOLATION OF ENDOPHYTIC MICROORGANISMS FROM WILD CRANBERRIES (*VACCINIUM OXYCOCCOS*), LINGONBERRIES (*VACCINIUM VITIS-IDAEA*) AND THEIR ANALYSIS

Asta Bubnelytė[1], Dorotėja Vaitiekūnaitė[2], Ingrida Bružaitė[1]

[1] Department of Chemistry and Bioengineering, Faculty of Fundamental Sciences, Vilnius Gediminas Technical University, Vilnius 10223, Lithuania
[2] Lithuanian Research Centre for Agriculture and Forestry, Laboratory of Forest Plant Biotechnology, Institute of Forestry, Lithuania
asta.bubnelyte@stud.vilniustech.lt

Cranberries and lingonberries are among the most common edible berries in Lithuanian forests. They are also widely studied for their bioactive compounds [1], however, their endomicrobiota is not well researched, especially endophytes contained in the berries. Bacterial endophytes can promote plant growth in two ways. Indirect plant growth promotion is associated with endophytic bacteria's ability to outcompete pathogenic microorganisms by producing a wide range of biologically active compounds and enzymes, which can inhibit growth. Moreover, bacterial endophytes can directly affect plant growth by synthesis of phytohormones, production of siderophores and solubilization of phosphates as well as fixation of nitrogen.

In this study, five microorganisms were isolated from cranberry fruit and one microorganism from lingonberry fruit. Namely *Staphylococcus hominis*, *Staphylococcus caprae*, *Bacillus paramycoides*, *Bacillus altitudinis*, *Bacillus xiamenensis.*

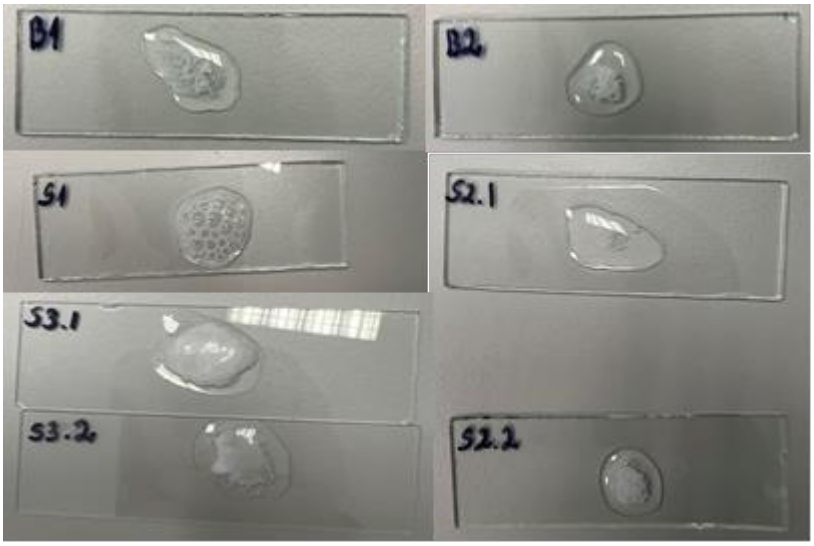

Figure 1. Determination of catalase activity. *B1*, *B2* –endophytes isolated from lingonberries; *S1*, *S2.1*, *S2.2*, *S3.1*, *S3.2* –endophytes isolated from cranberries.

The main morphological and biochemical characteristics of endophytic microorganisms were evaluated during the research. Nitrogen fixation test showed that only two bacteria were able to grow on nitrogen-free medium. Higher concentrations of produced IAA showed *Bacillus paramycoides* isolated from cranberry berries. All isolates showed positive results for siderophore production.

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A7

KNOCKOUT MUTANTS OF ESSENTIAL AND NON-ESSENTIAL GENES OF FELIXOUNAVIRUSES

Emilija Urbaitytė, Aurelija Zajančkauskaitė, Monika Šmoliūnienė, Lidija Truncaitė

Vilnius University Life Sciences Center Saulėtekio av. 7, Vilnius LT-10257, Lithuania
emilija.urbaityte@gmc.stud.vu.lt

Bacteriophages (phages) of *Felixounavirus* genus are lytic bacterial viruses that infect *Escherichia* and *Salmonella* strains including those of clinical importance [1]. Lytic phages, as natural enemies of bacteria can be used for control of these pathogens, but laboratory strains would be best suited for their research. However, only few phages of this genus infecting laboratory strains of E. coli have been characterized to date. Two of them, vB_EcoM_Alf5 (Alf5) and vB_EcoM_VpaE1 (VpaE1), have been isolated and characterized in Lithuania [2, 3]. As genetically similar representatives of *Felixounavirus* genus, these phages are attractive models for molecular studies of this little-studied phage group. To investigate molecular processes of infection by these phages, both in vitro and in vivo experiments are needed, with gene knockout mutants required for determination of gene essentiality and in vivo functions of certain genes.

In this study, we describe construction of VpaE1 and Alf5 gene knockout mutants carrying deletions of genes that were replaced by molecular markers *trxA* or *lacZa*, or amber mutations in some of their essential genes. BRIP method [4] was used to insert the in vitro-designed mutations into phage genome by homologous recombination. The deletion mutants were selected based on the presence of certain marker gene in their genomes. The amber mutants were selected using Cas9-based counter-selection against wild-type phages followed by testing them on amber-suppressing and non-suppressing laboratory strains of *E. coli*.

The knockout mutants of Alf5 and VpaE1 carrying deletions of non-essential genes were viable under laboratory conditions. Meanwhile, the essential genes could only be deleted in the presence of functional complementation in trans. For this, the essential genes were cloned into expression vectors and the strains to be infected by phage mutant were transformed with these recombinant plasmids. Different inductor concentrations were needed to support growth of certain phage mutants. Deletion analysis of phage genomes is necessary to determine gene essentiality. In addition, these phage mutants can further be used for in vivo tests of gene function or to support the results of in vitro studies.

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A8

CAROTENOID ACCUMULATION IN RHODOTORULA YEASTS

Saulė Pranckevičiūtė[1,2], Regina Losinska-Sičiūnienė[1], Živilė Strazdaitė-Žielienė[1], Elena Servienė[1]

[1]Nature Research Centre, Vilnius, Lithuania
[2]Life Sciences Center, Vilnius University, Vilnius, Lithuania
saule.pranckeviciute@gmc.stud.vu.lt

Due to the rapid expansion of the global carotenoid market, there is a growing demand for costeffective and efficient methods for carotenoid synthesis. Microorganisms such as yeast have emerged as one of the best alternative sources of natural carotenoids, presenting a sustainable and environmentally friendly way to meet rapidly increasing industry demands [1].

Carotenoids are bioactive molecules, known for their bright yellow, orange, and red coloring, and a variety of biological activities. The provitamin A, antioxidant, and colorant properties of carotenoids are widely researched for applications in phytochemical, chemical, pharmaceutical, food, and other industries. Traditionally, industrial carotenoid production methods require high production costs and generate low pigment yields and environmentally hazardous wastes. [2].

Rhodotorula is an oleaginous yeast that possesses a diverse carotenoid profile with commercially attractive β-carotene concentrations [3]. *Rhodotorula* can utilize a broad range of refined and natural carbon sources, including commercial cultivation medium and agro-industrial waste [4].

Our work aims to optimize carotenoid extraction from oleaginous yeasts using N, N-dimethylformamide as a solvent. Cultivable *Rhodotorula* species were isolated from environmental samples and identified using molecular methods. Morphological analysis of *Rhodotorula diobovata*, *R. mucilaginosa*, and *R. glutinis* was performed alongside an examination of the effect of temperature on pigment synthesis in yeast. Consequently, yeast growth was evaluated at temperatures of 20 °C, 25 °C, 30 °C, and 37 °C. For *R. diobovata* and *R. glutinis*, the optimal cultivation temperature was observed at 25 °C, with lesser growth at 20 °C and 30 °C temperatures. Samples cultivated at 37 °C showed minimal to no growth. *R. mucilaginosa* was found to have an optimal cultivation temperature range of 20 °C – 37 °C. Light absorbance was measured at a wavelength spectrum of 380 – 530 nm to determine carotenoid level in extracts. The highest carotenoid content was observed in *R. diobovata* and *R. glutinis* samples cultivated at 25 °C. *R. diobovata*, *R. glutinis*, and *R. mucilaginosa* displayed similar carotenoid content in samples grown at 30 °C.

The obtained data suggests that isolated *Rhodotorula* yeasts can be promising for carotenoid production.

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A9

DNA MODIFICATIONS AFFECT TRANSCRIPTION

Kornelija Tučinskytė[1], Miglė Tomkuvienė[1], Laura Manelytė[2]

[1]Department of Biological DNA Modification, Institute of Biotechnology, Life Sciences Centre, Vilnius University, Lithuania.
[2]Department of Biochemistry III, Institute of Biochemistry, Genetics and Microbiology, University of Regensburg, Germany.
kornelija.tucinskyte@gmc.vu.lt

Gene expression regulation is primarily dependent on transcription factors and *cis* - regulating sequences. Eukaryotic gene expression regulation adds a level of complexity to this process with epigenetic DNA modifications alongside chromatin structure [1]. The most common DNA modifications found in eukaryotic cells are 5-methylated and 5-hydroxymethylated cytosines (5mC and 5hmC, respectively). 5mC is introduced by specific enzymes – methyltransferases, which transfer the methyl group from S-Adenosyl-methionine (SAM) cofactor to the fifth carbon of the cytosine ring in CpG sequences most often. 5hmC is obtained through oxidation of 5mC by Ten-Eleven Translocation (TET) proteins [2]. Although natural DNA modifications have been studied extensively, they are poor reporter groups for further research. To expand the possibilities of DNA modification, the mTAG (methyltransferase-directed Transfer of Activated Groups) technology was developed by the group of prof. S. Klimašauskas. This method is based on transferring longer and more reactive groups from synthetic cofactor analogs onto DNA chain by engineered methyltransferases, making it possible to expand the use of DNA modification in biotechnological approach [3].

In this study, both natural and synthetic DNA modifications were studied in regard of their effects on DNA transcription. Transcription assays were performed both *in vitro* and *in cellulo*. In order to see modification's effect on transcription *in vitro*, tailed templates were made and modified throughout the DNA chain with 5mC, 5hmC or synthetic (5synC) modifications. Transcription assays were performed with yeast *Saccharomyces cerevisiae* RNA polymerase I (RNAP I) and RNAP II. Results showed no significant differences among 5mC, 5hmC as well as 5synC modifications in between the two polymerases, although a trend towards increasing transcription efficiency with RNAP I was observed in samples containing modified DNA. To study transcription *in cellulo* two different human CpG-rich promoters were cloned into CpG-free plasmids upstream of a reporter Luciferase gene. Modified with 5mC, 5hmC or 5synC, the constructs were transfected into human embryonic kidney cells HEK293 and the activity of the reporter Luciferase enzyme was assayed. Results indicated that 5mC and 5synC modified promoters were blocking transcription effectively, while 5hmC-modified promoters allowed partial transcription. As expected, natural modifications in the whole DNA chain region led to slightly increased transcription efficiency while modified promoters blocked the transcription. Surprisingly, our transcription efficiency studies showed no significant differences of the synthetic modification compared to natural modifications, both *in vitro* and *in cellulo*.

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METAGENOMIC PREDICTIONS OF BACTERIAL PHENOTYPE IN STREET GREENERY OF VILNIUS

Viktorija Kalasinskaitė[1], Nomeda Kuisienė[1]

[1]Department of Microbiology and Biotechnology, Vilnius University, Lithuania
viktorija.kalasinskaite@gmc.stud.vu.lt

Functional and biogeographical properties of soil microbial communities in urban ecosystems are poorly understood despite their role in metabolic processes underlying valuable ecosystem services [1]. It is essential to understand the microbial ecosystem in an urban environment for cities to monitor the microbial community assembly processes that shape urban microbial diversity and function.

Objective of this study was to characterize the bacterial phenotype diversity of the street greenery in the streets of different activity. The sample sites in the study were selected from the four boroughs of Vilnius - Sevcenkos, Antakalnis, Baltupiai, and Gerosios vilties. Samples were taken in the second half of October of 2023 year. Sites include a distribution of three newly installed (2-3 months apart) green areas and one that was installed 2 years before sampling. In this study we investigated whether newly installed greenery in Vilnius had distinct microbial community composition and trait-associated diversity compared to older site, and if these patterns were consistent with divergent community assembly processes associated with those sites.

We found that greenways in different streets have diverse bacterial communities, that were not associated with geographic locations. Sevcenkos sample has shown the greatest phenotypic diversity. Unlike Baltupiai sample, Sevcenkos, Antakalnio and Gerosios vilties samples did not contain microorganisms that carry out reductive acetogenesis and sulfate respiration. The lowest proportion of nitrogen fixation and highest nitrite respiration, denitrification, nitrate reduction proportion were detected in Antakalnio sample. No significant differences were observed between chemoheterotrophy proportions across all samples. The Baltupiai and Sevcenkos samples were characterized by the highest relative abundance of mobile elements and biofilm formation. Phyla *Armatimonadota*, *Pseudomonadota*, and *Desulfobacterota* had the highest motility among all samples, when phyla *Bacteroidota*, *Chloroflexota* and *Fusobacteriota* had the lowest motility. Compared to the other samples, Baltupiai sample had a relatively even distribution of gram negative and gram positive bacterial strains.

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A11

INDUSTRIAL SYMBIOSIS: POTATO PEEL WASTE AS FEEDSTOCK FOR STREPTOMYCES MOBARAENSIS CULTIVATION

Augustinas Vadeišа[1], Ieva Ožiūnaitė[2], Inga Matijošytė[3]

Sector of Applied Biocatalysis, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
E-mail: augustinas.vadeisa@gmc.stud.vu.lt

Potatoes a widely consumed vegetable globally, with popular food such as french fries, potato chips, mashed potatoes. However, the preparation of these products generates a significant amount of potato peel waste (PPW), which can make up 15% to 40% of the initial potato mass. This results in 70 – 140 thousand tons of PPW ending up in landfills annually. Despite this, PPW is rich in nutrients and can be repurposed for animal feed and composting. Additionally, PPW is used for bioethanol production [1].

One interesting approach to utilizing PPW is using it as a feedstock for microorganisms, as it is mainly composed of starch (66.8%), along with protein, cellulose, and pectin [2]. However, the starch in PPW is not readily available to microorganisms as feedstock and needs to be hydrolysed into smaller molecules. Although, acidic hydrolysis is effective, it is harmful and toxic to the environment, making enzymatic hydrolysis the preferable option. Enzymes such as amylase, glucoamylase, and cellulase can break down starch and cellulose into smaller molecules, such as monosaccharides and disaccharides, which can be consumed by microorganisms.

To achieve the project goal, we conducted experiments on wet and dry PPW samples, using different enzyme combinations, pH levels, and temperatures to determine the optimal conditions for obtaining the highest amount of reduced sugars. The samples were used for cultivating of *S. mobaraensis* to produce transglutaminase enzyme. Further details on the hydrolysis experiments will be presented during the poster session.

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A12

EXPLORING CULTIVATION CONDITIONS FOR THE PRODUCTION OF BACTERIAL SERRATIOPEPTIDASE

Stefano Giuliani[1,2], Ieva Ožiūnaitė[1], Inga Matijošytė[1], Diego Romano[2]

[1]Sector of Applied Biocatalysis Institute of Biotechnology LSC Vilnius university, Lithuania
[2]Department of Food, Environment and Nutritional Sciences, Università degli studi di Milano, Italy
stefano.giuliani@studenti.unimi.it

Income growth, urbanization, population growth and globalization have all contributed to a shift in the composition of diets with processed foods and high protein content becoming central. In the next decade, protein demand is expected to increase by 17% in middle and low-income countries [1]. Finding alternative proteins will be necessary to meet future prospective requirements for feed and food. Plant-based proteins could be a viable option to achieve the goal. In addition, the absorption and bioavailability of protein can be increased, food properties can be enhanced, and anti-nutrients can be reduced through protein pretreatment with enzymes, especially proteases. Proteases are one of largest classes of industrial enzymes, accounting for approx. 60% of the world's enzyme market [2]. These enzymes catalyze the cleavage of peptide bonds in proteins, reducing their length and producing smaller peptides by hydrolysis. Enzyme production relies mostly on microbes due to their numerous benefits such as simple handling, high yields, fast duplication time in controlled bio-reactors, and easy genetic modification [2]. *Serratia marcescens*, a gram-negative opportunistic bacterium, produces a specific protease called serratopeptidase, which has a broad substrate specificity. Since the origin host of serratopeptidase is an opportunistic pathogen, a suitable recombinant protein expression system is needed.

The aim of this project was to develop an expression system for the production of serratopeptidase. The methylotrophic yeast *P.pastoris* was chosen as the expression host. It has strictly regulated methanolinducible promoter, AOX1, and an α-factor that directs the recombinant protein to the medium. Pic9K cloning vector was used to obtain extracellularly expressed recombinant protein. The serratopeptidase gene has its own native propeptide sequence that maintains correct protein folding [3]. To assess influence of the native propeptide on recombinant protein expression, two different plasmids were designed – with and without the propeptide sequence. The gene manipulation experiments were successful. Therefore, further we optimized different expression parameters such as temperature, pH, aeration, methanol concentration, and media composition to improve both the production and activity of the recombinant protein in liquid media. The results will be presented during the poster sessions.

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A13

SURFACE DISPLAY OF LIPASE FROM *Serratia* sp. ON YEAST *Saccharomyces cerevisiae*

Arūnė Verbickaitė[1], Jokūbas Krutkevičius[2], Inga Matijošytė[2], Rasa Petraitytė-Burneikienė[1]

[1]Department of Eukaryote Gene Engineering, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
[2]Sector of Applied Biocatalysis, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
arune.verbickaite@gmc.vu.lt

Yeast surface display (or yeast display) is a protein engineering technique that involves the expression of recombinant proteins incorporated into the yeast cell wall. This versatile platform expands the applications of *Saccharomyces cerevisiae* in various scientific, biotechnological, and biomedical applications. This technique leverages yeast cell wall proteins to anchor target proteins onto the cell surface [1]. The yeast display technique was first published in 1997 [2], and until now, there is no data that it has been studied in Lithuania. The display of proteins on yeast cell surfaces is facilitated by wall protein a-agglutinin, which mediates cell-cell contacts during yeast-cell mating. A-agglutinin consists of an Aga1 subunit linked to an Aga2 subunit by two disulfide bridges. Genetic fusion of the target protein with the Aga2 subunit enables its display on the cell surface through interaction with the Aga1 subunit, which is directly anchored to the cell wall [1].

The exposure of enzymes on the yeast surface allows biocatalysis reactions to be carried out easily. This system simplifies the delivery of substrates that are difficult to penetrate the cell, and overcomes challenges of immobilization, and to ensure the necessary optimal conditions for enzyme activity. It also avoids the possibility of contamination and unnecessary purification of the enzyme. Moreover, this approach allows for continuous biocatalysis reactions. Lipases are widely used in industrial biotechnology. They are important in organic chemistry, the food industry, pharmaceuticals, the production of active substances and drugs, the transformation of oils and fats, and the production of detergents. Esters obtained with the use of lipases are used in cosmetics and perfumery. Also, these enzymes are valuable for pollutant degradation and development of pesticides, insecticides, or biosensors [3].

The aim of this study was to investigate the expression and display of lipase enzyme from *Serratia* sp. [3] on the surface of *S. cerevisiae* yeast cells using various linker peptides. The Aga2 subunit was fused with the target lipase using linkers containing different configurations: a double extension of the original glycine and serine linker, single and double α-helix-forming sequences, and an α-helix-forming sequence with serine and glycine amino acid termini. All constructed fusion proteins were successfully displayed on the surface of yeast cells. Preliminary studies on lipase activity are currently being conducted.

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A14

INFLUENCE OF PLASMID DNA MODIFICATION ON THE TRANSFORMATION EFFICIENCY OF *Parageobacillus thermoglucosidasius* DSM 2542T

Elina Guogytė[1], Arnoldas Kaunietis[1], Rūta Kananavičiūtė[1]

[1]Life Sciences Center, Vilnius University, Vilnius, Lithuania
elina.guogyte@gmc.stud.vu.lt

Parageobacillus thermoglucosidasius is a facultative anaerobic thermophilic bacterium that grows at an optimum temperature of 55-65℃. It is characterised by its ability to use plant-derived raw materials such as the lignocellulosic carbohydrates hexose and pentose and by its ability to synthesise thermostable enzymes such as lipase, protease or cellulase. *P. thermoglucosidasius* has recently gained ground in scientific and industrial research due to its ability to grow at high temperatures, synthesise heat-tolerant enzymes, and participate in bioconversion and bioremediation [1, 2]. However, their use is limited by the lack of genetic tools.

The development of a genetic transformation system for the genus *Geobacillus* is currently a major attention. One of the most efficient types of transformation is electroporation, but this method requires the appropriate preparation of electrocompetent cells and the establishment of optimal conditions, which is what determines the high efficiency of transformation because is known that each species can be influenced by many different factors [3]. Also, the use of bacteria is limited by the shortness of suitable plasmids. Previously used plasmids were characterised by exaggerated expression from natural promoters. Standardized, universal plasmids with higher transformation efficiency are being developed. One of these plasmids pG2K is used in our work, it contains a strong constitutive promoter for expression in *P.thermoglusidasius* and *Escherichia coli*, two replicons and a kanamycin resistance marker [4].

This study aims to determine the effect of plasmid DNA modifications on the transformation efficiency of *P. thermoglucosidasius* DSM 2542T. For what purpose, we used pG2K shuttle plasmid isolated from 3 *E. coli* strains characterised by different DNA methylation patterns (DH5α (dam+, dcm+), RR1 (dam-, dcm+) and GM2163 (dam-, dcm-) and from the *P. thermoglucosidasius* DSM 2542T. The study showed which of *E. coli* strains suits best for the propagations of plasmid for its further transfer to *P. thermoglucosidasius* cells. Moreover, the obtained results contribute to the development more effective transformation protocol of these biotechnologically relevant thermophilic bacteria.

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A15

ANTIMICROBIAL PHOTOINACTIVATION OF THERMOPHILIC SPOILAGE BACTERIA

Deimantė Dukalskytė[1], Irina Buchovec[2], Alisa Gricajeva[1]

[1]Department of Microbiology and Biotechnology, Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Institute of Photonics and Nanotechnology, Faculty of Physics, Vilnius University, Vilnius, Lithuania
deimante.dukalskyte@gmc.stud.vu.lt

Bacterial ability to form biofilms creates a huge problem in several fields due to the biofilms' strong resistance to chemical and physical disturbances. Biofilms formed on food or food-related equipment can cause foodborne infections, spoilage, and corrosion of equipment, leading to health risks and huge economic problems worldwide. [1] Quorum sensing (QS) is a way for bacteria to communicate and regulate vital processes, including biofilm formation. In the latter case, quorum quenching (QQ) could be an attractive method for biofilm disruption. [2] The significant increase in resistance of biofilms leads to difficult ways of disrupting biofilms and a need for novel control methods.

Thermophilic bacteria such as *Geobacillus* sp. and *Anoxybacillus* sp. are known as spoilage-causing bacteria in the dairy industry which are often overlooked. These species tend to grow on heat exchange plates and evaporators, where temperature can reach 70°C. *Geobacillus* sp. and *Anoxybacillus* sp. secrete enzymes, which deteriorate the nutritional value of dairy products and can cause changes to flavor. Under harsh conditions, thermophilic bacteria can form endospores, which are found in the final dairy products. [3] Therefore, new and safe approaches are needed to combat these microorganisms. Antimicrobial photoinactivation (API) - a known safe and convenient method [4], combined with QQ could be an efficient way to fight strongly resistant biofilms of thermophilic bacteria.

This study aimed to find out the effect of API (based on natural photosensitizers (PSs)) on *Geobacillus stearothermophilus* and *Anoxybacillus tepidamans* planktonic and biofilm forms. Chlorophyllin (Chl) - a natural and safe-to-use compound, was used as a PS. PS solution was added to the suspension of the selected optical density planktonic cells or pre-formed biofilms and irradiated with 402 nm light using LED-based light source (constructed in Institute of Photonics and Nanotechnology, Faculty of Physics). The mentioned light was used as optimal to excite the molecules of Chl, so that as a result the reactive oxygen species would form and disrupt the bacterial cells/biofilms. Results showed a clear difference between light exposure doses for planktonic and biofilm forms. As expected, biofilms were more resistant to photoinactivation than the planktonic forms. Additionally, QS pheromone molecules used for biofilm formation of selected thermophilic model strains were investigated in this work. The latter is being performed to elucidate the signal transduction system used in thermophilic bacteria which can eventually help employ QQ to combat thermophilic and other bacteria biofilms.

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A16

SYNTHESIS OF RECOMBINANT *ARTEMISIA VULGARIS* ALLERGEN ART V 3 IN YEAST

Emilija Dobrovolskytė, Laima Čepulytė, Rasa Petraitytė-Burneikienė

Department of Eukaryote Gene Engineering, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
emilija.dobrovolskyte@gmc.stud.vu.lt

Allergic diseases are one of the most prevalent chronic health conditions worldwide and their morbidity is increasing at an alarming rate. Based on the statistical research data, about 20%-40% of the world's population suffers from allergies, but these numbers are considered underestimated because many patients do not consult a doctor about their symptoms or are not correctly diagnosed [1]. People can bear different types of allergies – food, insect, inhalant, contact, and drug allergies. Allergy can cause many symptoms such as a swollen tongue, itchy or/and watery eyes, etc., and also can cause chronic conditions like allergic asthma and eczema. Allergens most often are proteins from dust, animal fur, pollen, etc., which can trigger allergic reactions. In response to allergens, the immune system of an allergic individual produces immunoglobulin E (IgE) [2].

Whole-allergen extracts are still widely used for allergy diagnostics and immunotherapy. Allergen extracts contain mixtures of many allergenic substances and are produced from natural allergen sources, therefore they are difficult to standardize. The usage of natural extracts is limited due to several reasons including variable number of different allergens, different biological activity of those allergens and contaminants, that can cause false positive/negative results and ineffective treatment [3]. Single recombinant allergen molecules could be used for allergy diagnostics and help to overcome these problems.

In this study, recombinant allergen Art v 3 from common mugwort (*Artemisia vulgaris*) was analyzed. Common mugwort has 6 different allergen components (Art v 1-6). During *Artemisia vulgaris* pollen season in late summer and fall, this plant is one of the main causes of allergic reactions. Many patients, who are sensitized to Art v 3, can also feel allergy symptoms upon exposer to other cross-reactive allergens, such as Pru p 3 from peach, Cor a 8 from hazelnut, or Par j 2 from short ragweed [4]. During this study, genes encoding two Art v 3 protein variants – single allergen component Art v 3 and Art v 3, fused with maltose binding protein (MBP) – were cloned to the yeast expression vectors pFX8 and pKDA1-Kan-GAL7. Experiments of recombinant protein synthesis were conducted in the yeast species *S. cerevisiae, K. lactis,* and *K. marxianus*.

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A17

INVESTIGATION OF POLYURETHANE HYDROLYZING MICROORGANISMS FROM VARIOUS EUROPEAN ENVIRONMENTAL SAMPLES

Dominykas Musneckis, Jokūbas Krutkevičius, Inga Matijošytė

Sector of Applied Biocatalysis, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
Email: dominykas.musneckis@gmc.stud.vu.lt

Polyurethane (PU) has become a widely used polymer due to its exceptional durability, resilience, and versatility. These properties make PU the material of choice in various industries, including automotive, construction, electronics, and medical [1]. In 2023, the global polyurethane market was valued at 83.54 billion USD, and it is projected to grow at a compound annual growth rate (CAGR) of 5.88% between 2024 and 2030 [2].

While PU products are known for their longevity, they eventually wear out and become a waste. This waste can be categorized as end-of-life (EOL) and post-consumer (PC) products or as scraps from PU manufacturing. The latter accounts for up to 10% of all PU production and results from manufacturing and processing flaws. However, EOL and PC waste pose a more significant challenge, as they are often contaminated or distorted, making reuse less likely. An alarming 50% of PU waste ends up in landfills. To mitigate this, recycling is a valuable option.

Polyurethane recycling can be accomplished through mechanical or chemical methods. Mechanical recycling involves breaking down PU into flakes, granules, or powder. However, this method is inefficient and yields low-quality products, limiting their marketability. Chemical recycling, on the other hand, requires substantial resources, including high temperatures and additional substrates, to break down PU into its raw components for various uses [3].

While chemical degradation currently dominates the landscape of PU disposal worldwide, there exists a promising approach for revolutionizing the industry – enzymatic degradation. Enzymatic degradation for PU has two main advantages – enhanced efficiency and environmental sustainability. Unlike chemical methods, enzymatic breakdown of PU would demand less energy and produce fewer harmful byproducts. This new recycling approach would promote sustainability and circular economy [4].

In the initial phase of our research, the objective was to identify microorganisms capable of polyurethane hydrolysis. The search of these microorganisms was carried out in collaboration with our partners. Samples were received from different European regions: Bosnia and Herzegovina caves, Baltic Sea shores and Lithuanian soil. 16 microorganisms from these samples were identified and screened for polyurethane hydrolyzing abilities using functional analysis. Out of these 16 microorganisms, 6 of them showed ability to hydrolyze ester-base PU, and 4 could hydrolyze ether-based PU. Based on these results, we continue our investigation by constructing genomic libraries and performing functional analysis to identify the coding sequences of these PU hydrolysis enzymes. Results in greater detail will be presented during the poster session.

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A18

INVESTIGATION OF THE BIOSYNTHESIS OF THE 2,5-DIMETHYLPYRAZINE AND ITS DERIVATIVES IN THE RECOMBINANT *PSEUDOMONAS PUTIDA* KT2440 STRAIN

Justė Juknevičūtė[1], Vytautas Petkevičius[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Vilnius University, Vilnius, Lithuania
juste.jukneviciute@gmc.stud.vu.lt

Pyrazine is a six-membered aromatic heterocycle that contains two nitrogen atoms positioned in a 1,4-orientation within a carbon framework [1]. Pyrazine compounds have significant importance in various fields, including food and fragrance, agricultural, and pharmaceutical industries [2]. For instance, pyrazine-containing drugs are used worldwide for various treatments (acipimox and glipizide) [3], while most recently four compounds (amiloride, bortezomib, paritaprevir, pyrazinamide) were included in The World Health Organization's (WHO) Model List of Essential Medicines [1].

There are several methods of obtaining pyrazine compounds. Extraction from natural sources is the "greenest" way to acquire pyrazines but is inefficient for industrial scale due to small yields (0.01% pyrazine kg−1 biomass). Currently, chemical synthesis is the method of choice; however, it also has some downsides. Harsh reaction conditions, such as higher temperatures and pressure, the use of organic solvents, and hazardous reagents do not align well with sustainable practices and are not environmentally friendly. Since the use of certain microorganisms is well-established and declared as natural and safe by food authorities (EFSA, FDA etc.), biosynthesis and biocatalysis methods using natural or recombinant microorganisms are viewed as attractive alternatives [2].

In previous works of our department, a well-known laboratory work-horse *Pseudomonas putida* KT2440 strain was engineered for 2,5-dimethylpyrazine (2,5-DMP) production. This was accomplished via metabolic engineering of the L-threonine (precursor of 2,5-DMP) biosynthesis pathway. For this reason, 6 genes from diverging pathways were identified and deletions of those genes were carried out to decrease consumption of threonine, making a recombinant *Pseudomonas putida* KT2440 strain (designated as KT2440Δ6). Also, it has been shown that the insertion of mutated bifunctional aspartokinase/ homoserine dehydrogenase (*thrA*) and L-threonine-3-dehydrogenase (*tdh*) genes was a key step in making biosynthesis of 2,5-DMP from glucose (via L-threonine metabolism) possible in recombinant KT2440Δ6 strain.

This work aims to investigates strategies to augment the production of 2,5-DMP in *Pseudomonas putida* KT2440 and expand the pathway to produce 5-methyl-2-pyrazinecarboxylic acid (MPCA) and 2,5-dimethylpyrazine-N-oxides (2,5-DMP-N-OX). Initially, we compared the production capabilities of a recombinant *Pseudomonas putida* KT2440 strain (KT2440Δ6) against the wild-type (WT) by comparing L-threonine and 2,5-DMP synthesis yields. Subsequently, we pursued 2,5-DMP biosynthesis optimization through metabolic pathway analysis, identifying additional genes, including homoserine kinase (*thrB*) and threonine synthase (*thrC*), which were overexpressed to evaluate their impact on yield and productivity. Additionally, various combinations of plasmids were explored to further enhance production efficiency. Furthermore, we explored the functionalization of 2,5-DMP through the utilization of PmlABCDEF monooxygenase (for 2,5-DMP-N-OX production) and XylMABC monooxygenase (for MPCA production). These discoveries provide valuable insights into enhancing the production of 2,5-DMP, with potential applications spanning biotechnology and industrial processes.

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A19

PHYSICOCHEMICAL PROPERTIES OF SACCHAROMYCES CEREVISIAE K2 TOXIN-LOADED FUCOIDAN PARTICLES

Aistė Galinskaitė[1], Rūta Gruškienė[1], Tatjana Kavleiskaja[2], Elena Servienė[3], Ramunė Stanevičienė[3], Jolanta Sereikaitė[1]

[1]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania
[2]Department of Polymer Chemistry, Vilnius University, Vilnius, Lithuania
[3]Nature Research Center, Vilnius, Lithuania
aiste.galinskaite@stud.vilniustech.lt

Killer K toxins are small proteins produced by yeasts. K2 mycocin is active at the cell wall where it disrupts the structural and functional integrity of the plasma membrane [1]. This toxin could be used to prevent the growth of pathogenic yeast in food products. The main problem is that environmental stresses, proteolysis, and interactions with food components could reduce the activity of natural antimicrobial agents [2]. To prevent this, K2 toxin could be encapsulated.

Fucoidan is an anionic, sulfated polysaccharide produced by brown seaweed and in this study, it was used for the encapsulation of K2 toxin. This polysaccharide also has antivirus, antitumor, antithrombotic, anticoagulant, anti-inflammatory, and antioxidant activity which could lead to positive beneficial properties when used in food products [3].

In this study, K2 toxin was purified using ion exchange chromatography. Then K2 toxin-fucoidan particles were synthesized by the complexation method at different pH values (3.5; 4.2 and 4.8). The final concentration of fucoidan was 0.1 or 0.4 mg/mL and K2 toxin concentration was 2.6 or 4.0 µg/mL. Control samples without K2 toxin are also prepared respectively. The size and zeta potential of the particles were measured at the initial moment and after 4 weeks of storage at 4 °C temperature using a Zetasizer NanoZS device and compared to control samples without K2. The smaller size of the particles provides a high surface area, which can lead to higher antimicrobial activity and better interaction with substrates [2].

The size of fresh K2 toxin-fucoidan particles was found to be similar at all pH values and was often in the range of 180-290 nm. Comparing results at the initial moment and after 4 weeks, it was found that the particles' size often decreases. When comparing the sizes of particles to the corresponding controls without K2, it was observed that the addition of K2 at pH 3.5 and pH 4.2 generally resulted in a slight increase in size. In contrast, at pH 4.8 a decrease in size was observed.

The Zeta potentials of particles were always negative and in the range between -15 and –27 mV. There were no significant changes when comparing zeta potential values at the initial moment and after 4 weeks. When comparing the zeta potential of the particles with the corresponding controls without K2, it was observed that the addition of K2 generally made the zeta values less negative.

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SYNTHESIS AND THERMAL STABILITY OF *TORULASPORA DELBRUECKII* TDV1 VIRUS-LIKE PARTICLES

Laura Plakytė[1], Enrika Celitan[1], Saulius Serva[1]

[1] Department of Biochemistry and Molecular Biology, Institute of Biosciences, Vilnius University, Life Sciences Center, Vilnius, Lithuania
laura.plakyte@gmc.stud.vu.lt

VLPs are nanostructures that self-assemble from viral capsid proteins and resemble or mimic the original virus in size, structure and symmetry. VLPs differ from viruses because they lack any genetic material and are therefore incapable of replicating independently and infecting host cells [1]. The absence of genetic material also means that VLPs are promising delivery vehicles for proteins, nucleic acids or small molecules. These particles were first used to create antiviral vaccines, and nowadays they can be used as display platforms for any kind of vaccine. Recently, VLPs have been utilized to deliver pharmaceutically active products to specific sites and into specific cells of the body [2].

TdV1 virus was first identified in *Torulaspora delbrueckii* yeast strain Td70 and belongs to the *Partitiviridae* family's genus *Cryspovirus,* which previously had been found exclusively in protozoa hosts. The TdV1 virus genome, like all *Partitiviridae* genomes, is comprised of two dsRNA segments encoding only two proteins: an RNA-dependent RNA polymerase (RdRp) and a coat protein (CP). TdV1 viral capsid is around 30 nm in diameter and is made of 120 capsid protein subunits. It has been shown that VLPs of TdV1 can be synthesized using a constitutive expression system in *Saccharomyces cerevisiae* [3]. However, at present much about TdV1 VLPs, including thermal and long-term stability, remains unexplored.

The objective of this study was to synthesize and purify TdV1 VLPs using an inducible protein expression system in *S. cerevisiae.* To achieve this, the synthesis of recombinant viral protein was induced with 2 % galactose for 24 hours in *S. cerevisiae* strain BY4741-S2 transformed with pFX7 plasmid encoding TdV1 CP protein sequence. Viral particles assembled in yeast were purified from the lysate using sucrose cushion ultracentrifugation followed by cesium chloride density gradient ultracentrifugation. After both rounds of purification TdV1 CP protein with purity of 96 % and a yield of 0.078 mg of protein per gram of biomass was obtained. Finally, the stability of purified VLPs was tested using Fluorescent Thermal Shift Assay (FTSA). TdV1 VLPs had the lowest thermal stability in citrate-phosphate buffer at pH 3 with an average melting temperature (T_m) of 31.7 °C, at a pH of 4 the T_m was 52.4 °C, while in the pH range from 5 to 8 VLPs had the highest thermal stability (T_m 63.0–67.8 °C). In phosphate buffer, thermal stability of TdV1 VLPs at pH 6 to 8 (T_m 66.6–67.8 °C) was comparable to stability in phosphate-citrate buffer. Particles had a slightly better stability in phosphate buffer with higher ionic strength (T_m 67.5 °C) in comparison to low ionic strength (T_m 65.6 °C). In general, TdV1 virus-like particles demonstrated high thermal stability in all tested conditions.

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HYDROLYSIS OF ALKYL FUCOSIDES BY ALPHA-L-FUCOSIDASES

Daniel Parvicki[1], Rūta Stanislauskienė[1], Rasa Rutkienė[1], Agnė Krupinskaitė[1], Jonita Stankevičiūtė[1], Rolandas Meškys[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania
daniel.parvicki@gmc.stud.vu.lt

Fucosylated compounds are associated with a wide range of biological processes in different forms of life. In mammalian cells, they play an essential role in various biological and pathological processes (embryogenesis, cell adhesion, signaling, regulation of the immune response, etc.). Fucosylated human milk oligosaccharides (HMOs) offer benefits to infants by acting as prebiotics, preventing the attachment of pathogens and potentially providing protection against infections. Although there is a clear demand for fucosylated compounds, their availability is limited due to the difficult and expensive chemical synthesis. Therefore, enzymatic synthesis using α-L-fucosidases is considered a better alternative. These enzymes catalyse the removal of L-fucose from glycosides by the cleavage of O-glycosyl bonds. Moreover, under certain conditions, they can perform a transfucosylation reaction (the transfer of a fucosyl group from the donor to the acceptor molecule) [1, 2]. The variety of fucosylated substrates for enzymatic transfucosylation is limited. Substrates such as p-nitrophenyl-α-L-fucopyranoside (pNPFuc) are not suitable for the synthesis of prebiotics due to the toxicity of p-nitrophenol, which is formed during the hydrolysis of pNPFuc. Therefore, alternative fucosylated substrates are being explored to perform enzymatic transfucosylation reactions with high yields and easily removable, less harmful by-products. As methyl-α-L-fucopyranoside and 1-methoxyethyl-α-L-fucopyranoside could be potential alternative fucosyl group donors, we aimed to investigate whether α-L-fucosidases could hydrolyse these alkylated compounds and carry out transfucosylation.

We screened proteins from metagenomic libraries for their ability to hydrolyse methyl-α-L-fucopyranoside and 1-methoxyethyl-α-L-fucopyranoside. Several metagenomic enzymes with hydrolytic activity towards both mentioned compounds and pNPFuc were selected for further investigation. Based on sequence bioinformatic analysis, these proteins were confirmed as α-L-fucosidases belonging to GH29 hydrolase family. Using protein molecular structure predicting tools we identified conservative motifs and crucial aminoacid residues that could be linked to the enzymatic catalysis. Homology search of the investigated fucosidases revealed that they are homologous to proteins from bacteria belonging to the Actinomycetota and Bacillota phylum. A phylogenetic tree analysis further showed that the fucosidases in question are not closely related. Recombinant proteins were successfully synthesised in *Escherichia coli* and purified. Although the yields were lower than with pNPFuc as fucosyl donor, the enzymes studied were capable of using both methyl-α-L-fucopyranoside and 1-methoxyethyl-α-L-fucopyranoside in transfucosylation reactions.

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A22

WATER SOLUBLE BETA-CAROTENE/XYLAN COMPLEXES: OPTIMIZATION OF SYNTHESIS

Elžbieta Karvovska[1], Rūta Gruškienė[1], Jolanta Sereikaitė[1]

[1]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Lithuania
Elzbieta.karvovska@stud.vilniustech.lt

Carotenoids are colored fat-soluble pigments found in nature. These pigments not only give color to certain animals, plants, and microorganisms but also perform important, sometimes even critical functions in biological systems. The most important carotenoid in human nutrition is β-carotene, which has the highest antioxidant activity among all carotenoids and is the precursor of vitamin A [1]. Despite the advantages of β-carotene, it has poor solubility in water, is sensitive to light, temperature, and oxygen, and is therefore chemically unstable. To solve this problem, various encapsulation methods are used, which are constantly improved to achieve higher encapsulation efficiency and stability of the encapsulated material. The most commonly used methods for β-carotene encapsulation are based on liposomes, emulsions, polysaccharide complexes, nanoparticles, and drying technologies. Encapsulation techniques have been found to offer possible solutions to enhance the bioavailability, water solubility, and stability of hydrophobic carotenoids [1].

One of the polysaccharides that can be used for carotene encapsulation is xylan. The hemicellulose xylan is a plant cell wall polysaccharide that accounts for a considerable mass percentage of many commercially relevant agricultural products, including food, forage, and timber [2]. Xylans have special gelling and film-forming properties for producing hydrogels that can be used as encapsulation matrices for the slow release of bioactive agents. Although there are studies demonstrating the successful application of xylan to encapsulate β-carotene [3], this area of research still needs to be developed.

This study aims to prepare a water-soluble β-carotene system by applying the encapsulation technique described in [4]. Xylan, derived from beechwood, was used as wall material. The synthesis of the β-carotene/xylan complexes is based on the addition of β-carotene in acetone to heated water dispersion of xylan and the following instant evaporation of the organic solvent. Different ratios of compounds (carotene:xylan), i.e., 1:1, 1:3, 1:5, 1:10, and 1:20 (w/w), were tested to optimize the preparation of complexes. It was evaluated how the amount of entrapped β-carotene and the yield of the complex depends on the ratio of the components. To evaluate the relative stability of encapsulated β-carotene, the complexes were kept in different conditions for four weeks. Changes in the absorbance at 450 nm were measured at certain time points. HPLC analysis was performed to ensure that no carotene degradation occurs during the synthesis.

Based on the amount of entrapped β-carotene and the relative stability of the obtained complexes, the most favorable ratio of carotene/xylan was determined. Obtained water-soluble carotene complexes could find wider applications in the food industry, and not only.

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A23

OPTIMIZATION OF RECOMBINANT LIPOXYGENASE EXPRESSION IN *PICHIA PASTORIS*

Vytautė Blekaitytė, Dovilė Daunoraitė, Inga Matijošytė

Sector of Applied Biocatalysis, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
vytaute.blekaityte@gmc.stud.vu.lt

Lipoxygenases (LOX) are enzymes that catalyse the peroxidation of unsaturated fatty acids into fatty acid hydroperoxides. These hydroperoxides are of particular interest due to their ability to be readily transformed into a variety of valuable compounds. These compounds can be used across a broad spectrum of applications, ranging from the production of oleochemicals to the creation of flavour compounds, the synthesis of signalling compounds, and their use as food additives [1]. While the most extensive research has been conducted on human enzymes, bacterial lipoxygenases were only recently discovered, and research in this area is still in its early stages. In this study we employed transgenic *Pichia pastoris*, which contains *Pseudomonas aeruginosa* lipoxygenase (PaLOX) gene integrated into its genome. The construct ensures methanol-induced protein expression and secretion. PaLOX is a resilient enzyme, that exhibits considerable activity at high temperatures and can peroxidize not only linoleic and linolenic acids, but also arachidonic acid and oleic acid, demonstrating a diverse range of substrates [2]. Thus, this enzyme can be efficiently used as an eco-friendly alternative to traditional chemical catalysts.

Initial experiments with the aforementioned construct revealed successful LOX expression, but low protein yield. It is common for recombinant protein expression systems to require optimization. Furthermore, it is important to note that each protein expression system is distinct, and the optimal conditions may vary depending on the target proteins. Therefore, this study aimed to optimise the expression of lipoxygenase in the chosen construct. The optimization process involved determining optimal cultivation temperature and duration. Additionally, experiments were carried out to optimise LOX expression induction, including testing the impact of different methanol concentrations, feeding intervals, and methanol/sorbitol cofeeding strategies. Optimal conditions have been determined by analysing protein concentration measurements, SDS-PAGE and lipoxygenase activity assay. The detailed presentation of the findings will be showcased at the poster session.

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A24

STABILITY AND CARGO ENCAPSULATION OF L-A1 VLPs PURIFIED FROM *SACCHAROMYCES CEREVISIAE*

Kamilė Vaišaitė[1], Enrika Celitan[1], Ramunė Stanevičienė[2], Elena Servienė[2], Saulius Serva[1]

[1] Vilnius University, Life Sciences Center, Vilnius, Lithuania
[2] Nature Research Centre, Vilnius, Lithuania
kamile.vaisaite@chgf.stud.vu.lt

While most chemical drugs readily diffuse into cells, their non-specific action on healthy cells can substantially reduce therapeutic efficacy and result in adverse health effects. Virus-like particles (VLPs) are envisioned as a tool to enhance the efficacy and tissue-specificity of drugs by taking advantage of viruses' innate capacity to protect and transport cargo (viral genome) to their intended destination. These are self-assembling nanoparticles made of viral capsid proteins that resemble natural viruses in all but the ability to replicate, which is impossible due to the lack of genetic material. Their empty internal space can be utilized to encapsulate small molecules, peptides, proteins, and nucleic acids, while their surface can be modified to achieve targeted delivery [1].

ScV-L-A1 virus is a member of the virus family *Totiviridae* which was found to be stably maintained in 15 out of 70 wild *Saccharomyces cerevisiae* strains by means of its' double-stranded RNA genome being shielded and delivered from cell to cell by a capsid composed of 120 units of major capsid protein Gag [2]. Given the prevalence of *S. cerevisiae* in the natural human environment [3], it may be possible to employ L-A1 VLPs for nano-delivery of biomaterials into human or animal cells. Therefore, the main goal of this study was to examine the properties of L-A1 VLPs purified from *S. cerevisiae* to evaluate their potential use as a nano-delivery system.

The VLPs were obtained by inducing synthesis of the recombinant major capsid protein of L-A1 in *S. cerevisiae* and consequently purifying them by ultracentrifugation of lysate through a sucrose cushion and cesium chloride density gradient. The formation of 41.3±1.6 nm L-A1 VLPs, which were similar in size to the original L-A1 virus (~40.0 nm) [4], was demonstrated using transmission electron microscopy (TEM) and dynamic light scattering (DLS) techniques. To gain an insight into the particle stability, the effects of buffer identity, ionic strength, pH, and Mg^{2+} ions on the size of L-A1 VLPs were evaluated by DLS method. Yet after monitoring the particle size in different conditions for 8 weeks, it was found that none of these factors had affected particle size stability. Fluorescent thermal shift assay confirmed this observation by finding that the previously listed factors had no substantial influence on the two distinctive melting temperatures of L-A1 VLPs (~58 °C and ~65 °C). Given that the particles demonstrated stability across a wide range of conditions, the development of the nano-delivery system was advanced by performing encapsulation through passive diffusion. For this purpose, the particles were incubated with the antimicrobial peptide nisin, and later exposed to *Bacillus subtilis* and *Staphylococcus aureus* to determine the efficacy of encapsulation. Encapsulation and cargo transport into the cells were successful, as evidenced by the similar diameter of the inhibition zones with both free and encapsulated nisin. Even though the refined VLPs were shown to be highly stable and the efforts to entrap cargo by passive diffusion succeeded, more research on cytotoxicity and alternative encapsulation techniques must be done before applying them for nano-delivery.

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A25

DEVELOPMENT OF BACTERIAL AMIDOHYDROLASE YQFB WITH ALTERED SUBSTRATE SPECIFICITY

Kamilė Dzisevič[1], Viktorija Preitakaitė[1], Rolandas Meškys[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Saulėtekio av. 7, Vilnius, Lithuania
kamile.dzisevic@gmc.stud.vu.lt

Amidohydrolases are a large class of enzymes catalysing 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 researches 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. Nevertheless, insufficient choice of therapeutic substrates and the complex adaptation of already existing therapeutic agents remain a common problem regarding the application of amidohydrolases in biotherapy.

Therefore, the aim of this study was to select variants of YqfB bacterial amidohydrolase capable of converting N^2-pivaloylisocytosine, thus expanding the substrate spectrum of this enzyme. Firstly, random mutagenesis of the sequence encoding YqfB amidohydrolase was performed by error-prone PCR. Next, a uracil auxotrophy-based *Escherichia coli* strain (HMS174Δ*pyrF*Δ*yqfB*Δ*codA*) was transformed with the putative mutant sequences and grown on agar plates with synthetic minimal medium supplemented with N^2-pivaloylisocytosine as the sole source of uracil. The plasmid used in the transformation also contained a gene encoding Vcz isocytosine deaminase, suggesting that if the mutant YqfB variant is capable of hydrolyzing N^2-pivaloylisocytosine, isocytosine will be generated and then converted to uracil, allowing bacteria to grow in mineral medium. After incubation at 37 °C overnight, a few of the largest bacterial colonies were selected and transferred to a new plate together with wild-type YqfB amidohydrolase-containing bacteria as a negative growth control. After a 48-hour incubation at 37 °C, the bacterial clones with the highest growth efficiency were selected and plasmids encoding potentially mutant YqfB amidohydrolases were purified and sequenced. The results showed nucleotide substitutions at several different positions altering the amino acid sequence of YqfB amidohydrolase. The efficient growth of bacteria expressing mutant YqfB variants suggests that the mutations detected may affect the substrate specificity of this enzyme, but further *in vitro* studies are needed to confirm this.

In conclusion, this study has shown that *in vitro* protein evolution is a promising approach to expand the substrate spectrum of amidohydrolases, which would make these enzymes more amenable to biotherapeutic applications.

A26

SEARCH AND ANALYSIS OF PYRETHROID-DEGRADING MICROBIAL LIPOLYTIC ENZYMES

Jūratė Udraitė[1], Ugnė Rickevičiūtė[1], Alisa Gricajeva[1]
[1]Department of Microbiology and Biotechnology, Vilnius University, Lithuania
jurate.udraite@gmc.stud.vu.lt

Pyrethroids are synthetic pesticides used worldwide to control pests in agricultural and public environments [1,2]. Different pyrethroids have high insecticidal potential, however, they do not degrade immediately after use and their persistent use can have detrimental effect on non-target organisms and contaminate natural habitats. As one of the solutions to the problem, microbial lipolytic enzymes (carboxylesterases EC 3.1.1.1, lipases EC 3.1.1.3), among some other enzymes, due to their broad substrate specificity, thermos-, pH and other attractive characteristics can be a new and effective means of removing pyrethroids from the environment [3].

In this study samples of plant growing substrate from two Lithuanian farmlands were used for the conventional search of the target, pyrethroid-degrading bacteria. For this purpose, enrichment culture using pyrethroids as a sole carbon source was employed for the isolation of the bacteria breaking-down permethrin. Isolated cultures were selected and identified employing 16S rDNA analysis: 16S rRNA gene PCR, gene sequencing and phylogenetic analysis. After identification of target bacteria, 11 cultures belonging to *Pseudomonas*, *Staphylococcus*, *Micrococcus*, *Rhodanobacter*, *Priestia* sp. were evaluated by the size of hydrolysis zones formed around their colonies in solid minimal salts media enriched with permethrin and the most active were selected or the further study. To determine that particularly lipolytic enzymes synthesized by the selected bacteria are involved in the degradation of pyrethroids, zymographic analysis using tributyrin and permethrin as substrates was employed. Two cultures belonging to *Pseudomonas* sp. (according to the phylogenetic analysis most likely belonging to *Pseudomonas knackmussii*, *Pseudomonas fluorescens*) were determined to have enzymes of ~70 kDa in the zymograms active towards both tributyrin and permethrin. Other cultures have shown to have enzymes (50-100 kDa) active towards tributyrin only.

Further, enzymes from supernatants of most active strains – *Pseudomonas*, *Rhodanobacter*, *Priestia* sp., were precipitated with ammonium sulphate, dialyzed, and concentrated. Fractions (supernatants, precipitates before and after dialysis and concentration) were tested for lipolytic activity using a synthetic substrate *p*-nitrophenyl butyrate (*p*-NPB). Hydrolysis product of *p*-NPB allowed to determine lipolytic activity colorimetrically. *p*-NPB substrate was chosen to preliminarily assess the possibility that pyrethroid-degrading lipolytic enzymes may be present in the purification fractions through the detection of overall lipolytic activity (possibly derived from both lipases and/or carboxylesterases).

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A27

MODIFIED KOMAGATAEIBACTER XYLINUS CAPABILITY TO PRODUCE BACTERIAL CELLULOSE-CHITIN COPOLYMER

Augustė Stankevičiūtė[1,2], Jonita Stankevičiūtė[2], Marija Duchovskytė[3], Vincentas Raudys[3], Augustas Cibulskis[4], Vytautas Savickas[1], Justas Vaitiekūnas[2], Rolandas Meškys[2]

[1]Faculty of Chemistry and Geosciences, Vilnius University, Vilnius, Lithuania
[2]Institute of Biochemistry, Department of Molecular Microbiology and Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[3]Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[4]LSC-EMBL partnership for genome editing technologies, Vilnius University, Lithuania
auguste.stankeviciute@chgf.stud.vu.lt

Biopolymers have gathered increasing attention over the last few years as a sustainable and convenient option for specialized uses. One such remarkable biopolymer is bacterial cellulose produced by various species of bacteria, including the genera *Acetobacter*, *Gluconobacter*, *Komagataeibacter*, *Rhizobium*, *Agrobacterium*, and *Sarcina*. The unique properties that make bacterial cellulose applicable in the medical field and food industry include biocompatibility, mechanical stability, and high water retention. However, bacterial cellulose must be modified to fully meet the needs of wound care, localized delivery systems, or in vivo degradability [1]. The readily functional material manipulation of biological systems can be applied for the most optimal production process. Introducing the chitin monomer *N*-acetylglucosamine into the bacterial cellulose structure achieves a modifiable platform through deacetylation and amino group reactivity. Additionally, incorporation of *N*-acetylglucosamine monomers into the bacterial cellulose structure offers recognizable sites for *in vivo* degradation.

The aim of this study is to assess Komagataeibacter xylinus ability to produce bacterial cellulose – chitin copolymer using feeding experiments and genetic engineering. Based on previous work with *K. xylinus*, cellulose synthase can recognize UDP-*N*-acetylglucosamine as well as natural substrate UDP-glucose and insert into bacterial cellulose structure, yet further analysis on such biopolymers and their properties is still needed [2]. To produce bacterial cellulose-chitin copolymer, varying amounts of *N*-acetylglucosamine were supplemented to cultivation media as an additional carbon source for *K. xylinus*. In addition to feeding experiments with wild type *K. xylinus*, bacteria were modified using chitin metabolism genes from *Candida albicans* to improve the intracellular concentration of UDP-*N*-acetylglucosamine. Two strategies of engineering metabolic pathways were applied: either the operons for *N*-acetylglucosamine uptake or *in vivo* synthesis were put under constitutive expression of strong Anderson promoter or inducible P_{BAD} promoter. The composition of bacterial cellulose copolymers was analyzed by thin layer chromatography and HPLC-MS.

In the future, deacetylation of bacterial cellulose-chitin would result in an enhanced chemical reactivity of bacterial cellulose-chitosan polymer. This would allow specific functionalization in the biomedicine field, such as scaffold design. In addition, incorporation of *N*-acetylglucosamine would result in a material for wound care that can be degraded by the lysozyme [2].

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A28

EXPLORING NOVEL INDUCERS: BEYOND IPTG IN GENE EXPRESSION REGULATION

Jokūbas Daunoras[1], Renata Gudiukaitė[1]

[1]Department of Microbiology and Biotechnology, Life Sciences Center, Vilnius University, Lithuania
jokubas.daunoras@gmc.stud.vu.lt

In the constantly evolving field of molecular biology and biotechnological applications, the identification and deployment of efficient gene expression inducers stand as a cornerstone for advancing research and industrial processes. Traditionally, Isopropyl β-D-1-thiogalactopyranoside (IPTG) has dominated this space, known for its effectiveness in activating the lac operon in prokaryotic models such as *Escherichia coli* [1]. However, the limitations of IPTG, including its cost and potential cellular toxicity [1], prompt the search for alternative inducers that are not only cost-effective but also environmentally benign and biologically efficient [1]. This study addresses this gap by evaluating the potential of milk permeate (MP) and Galacto-oligosaccharides (GOS) as novel inducers, benchmarking them against IPTG in terms of gene expression efficacy.

Our research objectives were twofold: first, to ascertain whether these alternative inducers could match or exceed IPTG's efficiency in gene expression regulation; and second, to evaluate the sustainability and economic viability of using MP and GOS in biotechnological applications. To achieve these objectives, we use *E. coli BL*21 (DE3) recombinant cells, that produce three different proteins – GD-95RM lipase [2], GDEstLip fused enzyme [3], and *Streptomyces scabiei* cutinase [4]. We induced them using three inductors and compared the protein expression levels and qualities between IPTG, MP and GOS.

The findings of our study reveal that MP and GOS not only present a comparable ability to induce gene expression in *E. coli* BL21 (DE3) but also highlight the added benefits of these inducers in terms of their environmental impact and cost-effectiveness. Both MP and GOS, readily available by-products of dairy processing, demonstrate significant promise as a sustainable alternative, showcasing similar, if not enhanced, protein expression levels in comparison to IPTG.

The significance of this research lies not only in its contribution to expanding the current toolkit of gene expression inducers but also in its alignment with the urgent need for more sustainable and economically feasible biotechnological practices. By presenting MP and GOS as viable alternatives to IPTG, this study paves the way for future innovations in gene regulation, promising a new era of efficient, cost-effective, and environmentally friendly biotechnology.

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A29

EXPLORING THE DEPOLYMERASE ACTIVITY OF TAILSPIKE PROTEIN gp45 FROM *Aeromonas*-INFECTING PHAGE vB_AveS_KLEA5

Vedegytė M.[1], Krylovaitė E.[1], Šimoliūnas E.[1] and Šimoliūnienė M.[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Centre, Vilnius University, Saulėtekio av. 7, Vilnius, Lithuania
migle.vedegyte@gmc.stud.vu.lt

Bacterial resistance to antibiotics is a huge problem rising constantly and spreading rapidly since the past decade. It is crucial to explore alternative approaches for combating them. One potential option is to use bacteriophages and/or their components as antibacterial agents. Therefore, we aimed to study a specific *Aeromonas* infecting phage vB_AveS_KLEA5 (shorter, common name KLEA5) by investigating the probable depolymerase-like activity of its tailspike protein gp45 *in vitro*.

Bioinformatics analyses showed that the tailspike protein gp45 had no close homologues in NCBI database and demonstrated the highest BLASTp similarity (29.55% identity) to tailspike protein (YP_010356196) from *Dompsiwa* phage TSP7_1. The gp45 contained conserved Tail_spike_N domain, which is associated with receptor-binding proteins and potentially harboring depolymerase activity crucial for recognizing bacterial cell surface elements.

To study the potential activity of gp45 *in vitro*, the *g45* of KLEA5 phage was successfully cloned into two inducible vectors, pET21a and pCDF, with His-Tag attached to the C- or N- terminus, respectively. The recombinant proteins were expressed in *E. coli* BL21(DE-3), purified and analyzed by SDS-PAGE. It was showed that proteins were soluble, with a size of approximately 70 kDa. To explore the activity of the recombinant gp45, spot tests on double-layer agar using *Aeromonas veronii* strain KR2-5 as host were performed. After 20-hours of incubation at 22°C, both gp45N-his and gp45C-his variants formed visible turbid zones - indicators of depolymerase activity of these proteins. Additionally, protein-cell interaction studies were conducted, demonstrating that gp45 of KLEA5 potentially is not a receptor-binding protein. However, we hypothesize that protein potentially hydrolyzes a certain part of LPS, as we observe reduced cell viability. Nevertheless, further investigation is required to confirm this hypothesis.

The results of this study not only expand our understanding of *Aeromonas*-infecting bacteriophages but also provide innovative insights into their potential therapeutic and biotechnological applications for addressing bacterial infections.

DESIGN AND SYNTHESIS OF MUTANT *ARTEMISIA VULGARIS* ALLERGEN COMPONENT ART V 3 VARIANTS

Eva Kupetytė, Laima Čepulytė, Rasa Petraitytė-Burneikienė

Department of Eukaryote Gene Engineering, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
eva.kupetyte@gmc.stud.vu.lt

Allergic diseases are a common health problem that affects all of humanity. There is no precise statistical data on the prevalence of allergies, but studies have shown that the percentage of people with allergies varies between 20% and 40% in different countries [1]. According to the World Health Organization, by the year 2050, as much as 50% of the world's population will suffer from allergic diseases. The high prevalence of allergic diseases in the human population is due to human diet, climate change, and air pollution [2]. Sources of allergens can vary and may include pollen, dust mites, certain food, and pharmaceuticals. The most common allergens are proteins. Allergies are an overreaction of the immune system to molecules that are harmless to most people. The presence of allergens in the human body triggers the synthesis of antigenspecific immunoglobulin E, which can cause allergy symptoms such as coughing, skin rashes, a runny nose, and sneezing. Allergic asthma, allergic rhinitis, and eczema are the most common allergic diseases caused by a compromised immune system [3]. Allergen-specific immunotherapy is so far the only therapeutic approach that allows for modulating the immune response and can prevent the development of allergic diseases. The use of recombinant hypoallergens in allergen-specific immunotherapy may help to modulate the immune response to the target allergen and induce immune tolerance to it. Molecules known as hypoallergens are less likely to induce an allergen-specific IgE response but have the ability to elicit the T cell response. Also, the use of hypoallergens in therapy reduces the risk of side effects such as allergy symptoms or even anaphylactic shock [4].

The aim of this study was to generate and analyze mutants of the *Artemisia vulgaris* allergen component Art v 3. Amino acids potentially involved in IgE-binding epitope formation were identified based on the literature review and bioinformatic analysis. Eleven variations of the Art v 3 allergen component were selected for the research. Three of them were successfully generated, and following experiments were focused on the synthesis of these mutants in *E. coli*. The results of these experiments provide important findings for the elaboration of immunotherapeutic tools for allergen-specific immunotherapy.

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A31

DEVELOPMENT OF A NOVEL SCRNA-SEQ METHOD UTILIZING SEMI-PERMEABLE CAPSULES

Denis Baronas, Simonas Norvaišis, Linas Mažutis

[1]Department of Single Cell Analytics, Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio 7, Vilnius LT-10257, Lithuania
denis.baronas@gmc.vu.lt

One of the most important techniques in modern biological research is single-cell RNA sequencing (scRNA-seq), which enables single-cell gene expression pattern analysis, cellular heterogeneity characterization, and cell type identification [1]. Its significance extends across various fields, including immunology, developmental biology, and cancer research. To this day, droplet-based scRNA-seq techniques like Drop-Seq, inDrop, and Chromium 10X remain the most widely used and accessible methods. However, one of the biggest limitations of such techniques is the low RNA capture efficiency and increased background noise when processing challenging samples.

Here, we present a newly developed scRNA-seq technique based on semi-permeable capsules (SPCs). SPCs allow passive diffusion of small DNA molecules like primers or double-stranded adapters, harsh cell lysis conditions, as well as multiple buffer exchanges [2]. Therefore, cell inhibitors, RNases, proteinases, and other components that interfere with downstream processes can be removed with ease before conducting an enzymatic reaction on single cells. Moreover, the SPCs-based scRNA-seq method boasts ultra-high throughput due to its combinatorial indexing (split-pool) approach, allowing the processing of up to millions of cells and multiple samples simultaneously with easy sample demultiplexing.

In this study, we showcase the scRNA-seq application on white blood cells using SPCs and recover cell types that usually remain undetected when using regular droplet-based scRNA-seq techniques.

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A32

CONSTRUCTION OF A *KLUYVEROMYCES MARXIANUS* YEAST STRAIN USING CRISPR-CAS9 SYSTEM FOR SECRETION OF RECOMBINANT ANTIBODIES

Justina Žičkutė[1,2], Danguolė Žiogienė[2], Alma Gedvilaitė[2]

[1]Department of Biological DNA Modification, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
[2]Department of Eukaryote Gene Engineering, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
justina.zickute@gmc.vu.lt

Recombinant antibodies (RAbs) are generated *in vitro* using synthetic genes and are crucial in diagnostics, research, biotechnology, and therapeutics due to their remarkable specificity for targets, stability, and ease of modification [1]. Yeasts are often preferred in RAbs production systems due to their ease of genetic modification and cultivation, which helps improve cost-effectiveness. *Kluyveromyces marxianus* is recognized for its efficient production and secretion of properly folded and active native and recombinant proteins, including RAbs [2]. To enhance RAbs production technologies, yeast strains can be genetically modified to improve their protein secretion properties.

Protein glycosylation, an essential protein post-translational modification, involves attaching glycans to proteins, ensuring their proper folding, activity, and stability. Dolichol kinase (DK), encoded by the *SEC59* gene, plays a crucial role in glycosylation within the endoplasmic reticulum [3]. Lower DK activity and subsequent changes in glycosylation levels, along with alterations in the activity of other proteins in the secretory pathway, may result in enhanced secretion of recombinant proteins [4]. Additionally, improved secretion of RAbs can be achieved by reducing the activity of intracellular peptidases, as RAbs are highly prone to proteolysis and degradation [5].

The aim of this study was to construct a *K. marxianus* strain capable of secreting RAbs more efficiently, using CRISPR-Cas9 technology. To achieve this, we engineered a yeast *K. marxianus* WSS-Δ*pep4* strain by introducing mutations that resulted in G418S and I432S changes in the DK amino acids, while also disrupting the gene encoding vacuolar peptidase (*PEP4*). The mutations in the DK-encoding gene resulted in noticeable changes in DK activity, as indicated by reduced glycosylation efficiency of carboxypeptidase Y in the WSS strain. Additionally, disruption of the *PEP4* gene in yeast led to reduced subsequent degradation of RAbs. Western blot analysis showed that the constructed *K. marxianus* WSS-Δ*pep4* mutant strain demonstrated enhanced secretion capability for the single-chain antibody fragment (scFv) linked to an antibody fragment crystallizable Fc (scFv-Fc) when compared to the wild-type *K. marxianus* strain. This enhanced secretion efficacy was observed for scFv-Fc against *Gardnerella vaginalis* vaginolysin. However, it is important to note that the secretion of RAbs in yeast also depends on the specific characteristics of the protein, indicating the need for further studies. This newly developed *K. marxianus* WSS-Δ*pep4* mutant strain holds promising potential for future research aimed at improving RAbs production technologies.

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A33

THE IMPACT OF GRAPHENE MODIFICATION BY FUNCTIONAL GROUPS ON THE PERFORMANCE OF AN AMPEROMETRIC UREA BIOSENSOR

Gerda Šimėnaitė[1], Vidutė Gurevičienė[1], Julija Razumienė[1], Marius Butkevičius[1]

[1]Department of Bioanalysis, Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekio Av. 7, LT-10257 Vilnius, Lithuania
gerda.simenaite@gmc.stud.vu.lt

The identification of urea in serum, urine and other biological fluids is an essential diagnostic test routinely performed in clinical laboratories. Urea is a waste product generated during protein metabolism, which is excreted by the kidneys through the urine. Elevated levels of urea in the blood and decreased in the urine, may indicate liver and kidney dysfunction and some other health issues. Therefore, measuring urea levels is crucial for diagnosing and monitoring various medical conditions, especially those related to renal function. [1]

Traditional methods for urea determination often involve complex procedures and time-consuming analyses. Electrochemical biosensors offer an innovative and effective alternative to these challenges with the potential applications in various fields. These biosensors can be an important resource for the development of modern diagnostic tools, which can be applied in personalized medicine [2]. Biosensor technologies have created the ability to make high-sensitivity and quick measurements, while providing valuable information in real time monitoring. Electrochemical biosensors are analytical instruments that combine biological elements with a transducer to detect and quantify the composition of biological samples and to determine the concentration of an analyte in a sample. [3]

A graphene-based nanomaterials are being discovered to be promising for the development of amperometric biosensors. These nanomaterials have a lot of advantages such as large surface area, which is beneficial for the immobilization of enzymes, also high electrical conductivity, conditionally low prices, and good biocompatibility. The synergistic effect of functional graphene groups influences biosensor's sensitivity, selectivity, and overall electrochemical performance. [4]

The aim of the study is to determine the influence of graphene functional groups on the performance of the amperometric urea biosensor. In this study, enzyme urease (EC 3.5.1.5) was immobilised into a synthesized layer of different carbonaceous nanomaterials by forming an adjustable membrane for amperoemtric biosenor, which can be easily replaced when the biosensor's sensitivity decreases. Thus, six different biosensor's membranes were formed: 1) with reduced graphene oxide and amino functional chemical groups, 2) with reduced graphene oxide and carboxy functional chemical groups, 3) with reduced graphene oxide and sulfo functional chemical groups, 4) with graphene, 5) with graphene oxide, 6) with thermally reduced graphene oxide. The amperometric urea biosensor was adjusted to operate in a three-electrode electrochemical cell (counter electrode – Titanium plate, reference electrode – Ag/AgCl and working electrode – constructed biosensor). The linear limits, sensitivity and stability of the biosensors were determined. Also, the optimal operating conditions (temperatures, pH, electrode potential) of the biosensors were determined. After comparison between the membranes and selection of the best one, the selected biosensor was tested with serum and artificial urine. The results were compared with data obtained using an analogous colorimetric method.

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EFFECT OF CARBONIC ANHYDRASE IX INHIBITORS ON 2D AND 3D CANCER CELL CULTURES

Agnė Kvietkauskaitė[1], Jurgita Matulienė[1]

[1]Department of Biothermodynamics and Drug Design, Life Sciences Center, Vilnius University, Lithuania
agne.kvietkauskaite@gmc.vu.lt

Carbonic anhydrases (CA) are enzymes that catalyze the reversible reaction of the hydration of carbon dioxide to the bicarbonate ion and the acid proton ($CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$). These enzymes are involved in a range of physiological processes, such as acid-base homeostasis, calcification, carbon dioxide transport, pH and fluid balance regulation in the body [1]. Total 12 active CA isozymes are found in the human body, all belonging to the α-CA family. Impaired activity of CAs has been associated with a variety of diseases such as glaucoma, obesity, epilepsy, and perhaps gaining the most attention recently, cancer [2].

Carbonic anhydrase IX (CAIX) is a dimeric membrane protein (Fig. 1A), that is overexpressed in many human cancerous tumors, but also can be found in the stomach and testes of healthy individuals. CAIX is mainly found on the surface of cancer cells and promotes cell proliferation and tumor growth under hypoxic conditions. This is the reason why CAIX is considered to be a promising target for cancer treatment and diagnosis. For this purpose, CAIX activity inhibitors are being developed. They must be not only effective, but also specific, i.e. not interacting with other CAs, thus avoiding unwanted side effects. Compounds that exhibit high affinity and selectivity for CAIX show promising therapeutic and tumor visualization potential.

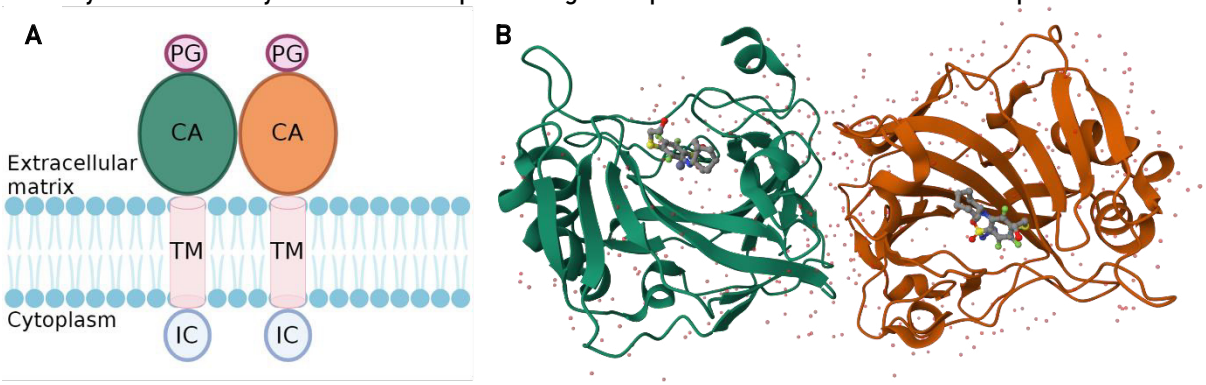

Figure 1. A. Structure of CAIX; PG, proteoglycan-like segment; CA, carbonic anhydrase catalytic domain; TM, transmembrane anchor; IC, intracytoplasmic tail. **B.** Three-dimensional structure of human CAIX catalytic domains in complex with VD11-4-2 (PDB ID: 6FE0).

When developing new drugs, compounds are first evaluated using *in vitro* systems and only later moved to preclinical and clinical studies. Both 2D and 3D cell culture models can be used in preliminary studies to determine the cellular effects. Chemical compounds, used in this work, were high affinity and selectivity CAIX inhibitors, synthesized in the Department of Biothermodynamics and Drug Design. The structure of one such inhibitor, VD11-4-2, bound to CAIX is shown in Fig. 1B [3].

The main aim of this work was to determine the effect of human CAIX inhibitors on 2D and 3D cancer cell cultures. Cytotoxicity of compounds in HeLa cells (2D) was tested using the MTT reagent. Concentrations that are lethal for 50% of the cell population (LC_{50}) under normoxic (37°C, 21% O_2) and hypoxic (37°C, 1% O_2) conditions were determined by applying the Hill equation. In addition, the effect of inhibitors on HeLa cells grown as spheroids (3D) was evaluated. The spheroids were observed and photographed with an EVOS FL Auto microscope every 2 days for 14 days after compound addition to the medium. The diameter of the spheroids was analyzed with AnaSP program. Inhibitors had similar effect on 2D and 3D cultures, however, 3D model showed higher sensitivity. In addition, the dissociation constants of CAIX inhibitors in live HeLa and A549 cells were determined by applying a competitive dosing method.

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A35

FERROCENE-MODIFIED CARBON ELECTRODES FOR FABRICATION OF REAGENTLESS BIENZYMATIC SARCOSINE BIOSENSORS

Kamilė Ratkevičiūtė[1], Skomantas Serapinas[1], Marius Butkevičius[1], Lidija Tetianec[1]

[1]Vilnius University, Life sciences center, Institute of Biochemistry Lithuania, Vilnius

kamile.ratkeviciute@gmc.vu.lt

Enzyme biosensors are beneficial tools in pharmacology, clinical practice, agriculture, food quality control, monitoring of infectious disease pathogens and the spread of environmental pollution factors [1–4].

Sarcosine is a non-proteinogenic amino acid derivative playing an important role as a metabolic intermediate in glycine methylation and creatine hydrolysis. While typically present in trace amounts in human serum or urine, elevated levels may signal various diseases including Alzheimer's, dementia, sarcosinemia, and prostate cancer, making it a promising biomarker [5]. Additionally, sarcosine is found in a variety of food sources such as egg yolks, legumes, nuts, vegetables, and meats. Therefore, the accurate quantification of sarcosine holds significant importance in clinical chemistry, as well as in the food and fermentation industries [6].

Ferrocene and its derivatives are commonly employed as mediators due to their advantageous qualities for electron transfer, including reversibility, low-potential regeneration, and stable redox states [7]. However, the use of free mediators can pose challenges such as mediator leakage or sample contamination. Therefore, reagentless devices can be developed, where all components are secured on the electrode surface.

In this study, we demonstrate the application of ferrocene modified electrodes in construction of a sarcosine biosensor by employing two enzymes – peroxidase and oxidase. Two types of bioelectrodes were fabricated. In the electrodes of Type I the ferrocene derivative was covalently attached to the graphene oxide and applied onto the surface of the glassy carbon electrode. Modification of graphene oxide with ferrocene was verified through differential pulse voltammetry measurements. In the electrodes of Type II ferrocene derivative was covalently attached to the surface of graphite rod electrode. The modification of graphite rod electrode surface was verified by cyclic voltammetry.

Both biosensors exhibited responses to sarcosine (the substrate of oxidase) and hydrogen peroxide (the substrate of peroxidase). The response was dependent on the electrode potential, with an optimal potential of 0 V vs Ag/AgCl. Incorporating peroxidase in the Type I biosensor notably enhanced its response to hydrogen peroxide, exhibiting a 14-fold increase compared to biosensors containing only ferrocene-modified graphene oxide. Moreover, the quantity of functionalized graphene oxide used in biosensor fabrication influenced the linear range of the biosensor's response to sarcosine. For Type II biosensor the modified surface generated a response to hydrogen peroxide itself, but incorporation of HRP increased the response 2-fold. Modified graphene oxide-based electrode (Type I) exhibited about 4 times greater sensitivity compared to modified graphite rod electrode (Type II) due to larger surface area. However, the modified graphite rod electrode had a much shorter response time, generating a response in a few seconds compared to a few minutes for the modified graphene oxide electrode.

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A36

CATABOLISM OF *N, O, S*-METHYLATED HETEROCYCLIC BASES IN *E. COLI* BACTERIA

Aušrinė Čekytė[1], Gabrielė Slaboševičiūtė[1], Vilija Lapinskaitė[1,2], Aistė Ramanauskaitė[1], Jaunius Urbonavičius[1], Daiva Tauraitė[1]

[1]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Lithuania
[2]Nature Research Centre, Vilnius, Lithuania
ausrine.cekyte@stud.vilniustech.lt

Ribonucleic acid is crucial to many life processes. There are many various types of RNA and they all have different modified chemical stuctures in order to fulfil their function. 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. These modifications are currently being investigated for their importance in mitochondrial diseases, neurological disorders, obesity, diabetes and cancer, and may be used to develop novel and innovative therapies [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, *N, O, S*-methylated pyrimidine and purine heterocyclic bases 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 purines and pyrimidines were used as substrates to select enzymes involved in the catabolism of the modified heterocyclic bases. For this purpose, uracil and purine auxotrophy-based selection systems were used, which are similar to previously described [2]. These systems allow the selection of enzymes that convert modified pyrimidines and purines, respectively, into unmodified derivatives. It was found that some tested methylated pyrimidines and purines, such as N^4-methylcytosine and 1-methylguanine, support the growth of uracil and purine 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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A37

ASSESSING THE IMPACT OF CAIX INHIBITOR EA2-3 ON THE MEDULLOBLASTOMA CELL LINE UW228

Sascha Arnold[1], Justina Musvicaitė[2], Audrius Zakšauskas[2], Edita Čapkauskaitė[2], Asta Lučiūnaitė[3], Agne Petrosiute[2]

[1]Faculty of Medicine, Vilnius University, Vilnius, Lithuania
[2]Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center, Vilnius, Lithuania
[3]Department of Immunology, Institute of Biotechnology, Life Sciences Center, Vilnius, Lithuania
sascha.arnold@mf.stud.vu.lt

INTRODUCTION. Medulloblastoma [MB] is defined as a highly malignant tumor derived from primitive, neuroectodermal tissue. It presents as the most prevalent type of pediatric central nervous system (CNS) tumor, comprising over 90% of all childhood embryonal tumors (15% of all pediatric CNS tumors) [1]. With an incidence of 5-11 cases per million people, they account for 40% of all tumors in the posterior fossa and tend to spread throughout the brain and spinal cord. Despite significant advances in the treatment of MB, outcomes remain poor for advanced and relapsed tumors. [1]. Hypoxia proves to be an important factor in enhancing tumor progression and creating a tumor microenvironment, which also impacts therapeutic efficiency [2]. Carbonic anhydrase IX (CAIX), hypoxia-induced enzyme, helps the cancer cells to survive in hypoxic conditions by maintaining the physiological pH in cancer cells and concurrently acidifying the tumor microenvironment [3]. It is associated with worse prognosis in MB [4]. Our laboratory has developed a CAIX inhibitor EA2-3 [5]. We aimed to study the effects of this compound *in vitro* in CNS cancer- MB cell line UW228.

MATERIALS AND METHODS. EA2-3 is a small molecule CAIX inhibitor that based on preliminary studies has the potential for blood-brain barrier penetration. To assess CAIX expression on UW228 cells we performed western blot analysis and flow cytometry in cells grown under normoxia or hypoxia. To determine compound toxicity in these cells, we performed an XTT assay. The functional activity of EA2-3 inhibitor in UW228 cells was confirmed by measuring the increase in extracellular pH in hypoxia compared to normoxia. Lastly, we performed a wound closure assay on these cells to assess compound effects on cell motility.

RESULTS. The UW228 cell line has elevated CAIX expression in hypoxia compared to normoxia based on protein and gene expression values. Therefore, it is a suitable model for testing CAIX inhibition. EA2-3 compound is nontoxic with IC50 of 100µM. We see a differential increase in ePH at a dose of 0.5 µM in hypoxia compared to normoxia. Wound closure assay shows slightly altered cell motility at a dose of 0.5 µM.

CONCLUSION. Our data shows that UW228 is a suitable CNS cancer cell model for CAIX inhibition studies. EA2-3 shows CAIX inhibition effects by altering the acidification of UW228 cell medium and cell motility in wound closure assay.

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A38

DEVELOPMENT OF MUTANT VARIANT K102R OF SACCHAROMYCES CEREVISIAE GENE SUP35

Ieva Jankauskaitė, Justina Versockienė, Eglė Lastauskienė

Institute of Biosciences, Life Sciences Centre, Vilnius University, Saulėtekio Av. 7, LT-10257, Vilnius, Lithuania
ieva.jankauskaite@gmc.stud.vu.lt

Prions are altered, infectious forms of native proteins that can acquire new functions or lose old ones, aggregate, spread, self-replicate, and lead to neurodegenerative diseases in humans and other mammals. Most of these diseases are infectious and are generally named transmissible spongiform encephalopathies (TSEs) [1]. They are associated with a prion protein or protease-resistant protein (PrP) encoded by the *PRNP* (prion protein) gene. In yeast, prions are passed down through the cytoplasm, whereas in mammals, they are transmitted extracellularly. *Saccharomyces cerevisiae* serves as an excellent model organism due to its similarity to higher eukaryotes in cellular machinery, a universal DNA transformation system, and the identification of at least 10 different prion domains within it. The prion [*PSI*$^{+}$] associated with the Sup35 protein is among the most extensively studied yeast prions [2].

The Sup35 protein plays a crucial role in protein translation termination, however, its conversion into a prion diminishes the efficiency of this process. The inherent resistance of prions to various elimination mechanisms prompts research into the underlying reasons and potential solutions. Studies have shown that the Sup35 protein is susceptible to protease activity *in vivo*, but its resistance to degradation is influenced by the arrangement of amino acids and their characteristics [3]. Typically, lysines in the Ndomain of a protein need to be ubiquitylated to target the protein for proteasomal degradation. Thus, this study aimed to substitute the sole lysine at position 102 in the N-domain of the Sup35 protein with arginine.

To generate the mutant variant K102R of the yeast *SUP35* gene, specific primers were designed, and site-directed mutagenesis was employed to alter a single nucleotide in the sequence through three separate PCR reactions. Subsequently, Sup35K102RGFP was integrated into the pJET1.2/blunt cloning vector and replicated in *Escherichia coli* DH5α cells. Later, Sup35K102RGFP was integrated into the pRSCup yeast shuttle vector and replicated in *E. coli* DH5α and *S. cerevisiae* cells.

For the first time, a mutant variant of the *SUP35* gene, K102R, has been developed. Now by utilizing fluorescent microscopy, the prionization of the native and mutated proteins can be compared. It is hypothesized that the mutant Sup35 protein variant K102R is more inclined to prionization and, consequently, more resistant to proteasomal degradation. However, to validate this hypothesis, further experiments are required.

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A39

THE FIRST STEP TOWARDS HUMANIZED RECOMBINANT TAU PROTEIN IN P. PASTORIS YEASTS

Airidas Jonušas[1,2], Lukas Krasauskas[1], Vytautas Smirnovas[1], Justina Versockienė[2], Eglė Lastauskienė[2]

[1]Amyloid Research Sector, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
[2]Laboratory of Molecular Microbiology of Eukaryotic Microorganisms,Institute of Bioscience, Life Sciences Center, Vilnius University, Lithuania
airidas.jonusas@gmc.stud.vu.lt

Neurodegenerative diseases are among the most common in the world. Despite intensive research, there are few treatment options available [1]. Many studies are done on disease-causing proteins, which are often produced in bacteria and differ from those in humans by post-translational modifications. Here, N-glycosylation is most interesting to us. By producing protein in a humanized expression system, we can glycosylate it similarly to humans [2]. Such proteins would enable more precise research and more effective drug discovery. Partially humanized *Pichia pastoris* M5 yeast, performing modified N-glycosylation, was chosen for our study. The goal of our project is to use gene engineering to change this N-glycosylation to be closer to the human system [3].

Using PCR, *TAU* gene was amplified with an additional sequence encoding 6xHis tag and restriction sites, cloned it into the integrating pPIC3.5K plasmid. Using the SalI REase, cutting the *HIS4* gene, Mut^+ phenotype was obtained, which efficiently uses methanol, grows faster, and produces more proteins. For homologous recombination, digested plasmid was concentrated in 3 ways: column purification, organic DNA extraction, and magnetic particles (the best method). Yeast transformation was performed using chemical and electroporation methods. By the same scheme, the pPIC3.5K-Tau plasmid was cut with BglII, resulting in a Mut^S phenotype with the *AOX1* knocked out, having slower methanol catabolism, but if this will reduce the yeald of protein will be checked in the future.

Pilot cultivation of *P. pastoris* M5 Mut^+ was done, expression induction with methanol and SDS-PAGE was performed to check expression over time. To ensure having the target protein, western blot was done with anti-His monoclonal antibodies. To enhance gene expression a Kozak sequence will be inserted upstream of *TAU*, which should enhance gene expression. Using Mega primers, an Alpha mating factor signaling sequence will be inserted, which will direct protein for secretion into the medium, facilitating protein purification. From the available literature, Tau protein was obtained from partially humanized yeasts with modified N-glycosylation for the first time. This and further glycosylation modifications will allow to test whether these modifications change Tau protein aggregation and it's structure.

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ISOLATION AND CHARACTERISATION OF BACTERIOPHIGES FROM THE GUT OF *MUS MUSCULUS*

Aušra Semenaitė[1], Aurelija Zajančkauskaitė[1], Rolandas Meškys[1]

[1]Department of Molecular microbiology and Biotechnology, Institute of Biochemistry, Life Sciences center, Vilnius university, Lithuania
ausra.semenaite@gmc.stud.vu.lt

In recent years research on bacteriophages (viruses that infect bacteria) the most abundant entities on Earth, has been gaining increasing attention, especially as they are an important part of ecosystems. The gut of animals is not an exception. The emerging field of viral metagenomics has allowed scientists to delve deeper into the world of the gut microbiome and to explore an often-overlooked part – bacteriophages. Although less labor intensive, metagenomic analysis is relies heavily on genome databases, and since viruses usually lack highly conservative regions, a great number of sequences remain unidentified. Prophages integrated into their host genomes can result in sequence similarity between bacteriophages and bacterial genomes. The ability to isolate a particular virus and cultivate it under laboratory conditions allows for in depth analysis of virion structure, phage-host interactions, its role in the ecosystem and helps to expand the database [1,2,3].

Hosts and bacteriophages were isolated from feces of mice provided by dr. Aurelijus Burokas. To expand the spectrum of hosts that can be cultivated under aerobic conditions, bacteria from the Department of Molecular microbiology and Biotechnology's collection of microorganisms were included.

Potential hosts were isolated by serial dilutions of fecal matter suspensions, plated on Luria-Bertani solid medium. Single colonies were isolated, purified by repeated quadrant streaking and identified by 16S rDNA phylogenetic analysis.

Bacteriophages were isolated using two methods. To isolate bacteriophages infecting native hosts, serial dilutions of fecal matter suspension, purified by low-speed centrifugation, were plated using a double agar overlay method. In the case of hosts from the collection of microorganisms, phage enrichment in Luria-Bertani broth was performed.

In this study, two bacterial viruses were isolated from the gut of mice. Genome restriction analysis has shown that these phages are genetically distinct. Transmission electron microscopy analysis revealed, that phages 50Rn and SuR, infecting *Enterococcus faecalis* MMBS 50 (isolated from fecal matter) and *Serratia ureilytica* DSM 16952 (from a microorganism collection) respectively, are members of *Caudoviricetes* class. Further investigations are underway to explore additional characteristics and behaviors of these phages.

The isolation and characterization of bacteriophages from the gut of mice, coupled with the scarcity of documented Serratia ureilytica phages in existing literature, highlights the diversity of phage populations within the gut microbiome and suggests avenues for further research of the gut virome.

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A41

CHARACTERIZATION OF CULTURABLE MICROBIOTA OF APHIDS *ADELGES* (*APHRASTASIA*) *PECTINATAE* (HEMIPTERA: ADELGIDAE)

Gustė Tamošiūnaitė[1], Jekaterina Havelka[2], Nomeda Kuisienė[1]

[1]Department of Microbiology and Biotechnology, Vilnius University, Lithuania
[2]Department of Zoology, Vilnius University, Lithuania
guste.tamosiunaite@gmc.stud.vu.lt

Aphids of the genus *Adelges* Vallot, 1836 are pests that feed on the phloem sap of host plants of the conifer family *Pinaceae*. Understanding the microbiota of insects is crucial as it profoundly influences their growth, development, and adaptation to the environment.

As part of this study, we investigated the culturable microbiome of *Adelges* (*Aphrastasia*) *pectinatae* (Cholodkovsky, 1888). The investigation began with the inoculation of aphid grinded mass onto various microbiological media, including R2A Agar, Tryptic Soy Agar, DSMZ medium 1021 (*Sodalis glossinidius* medium), and Luria-Bertani Agar. Bacterial inoculation, conducted under both aerobic and anaerobic conditions, involved the use of saline with and without dilution to 10x and 100x, followed by culturing bacterial populations at 28 °C to achieve specific densities. Remarkably, under aerobic conditions, six distinct colony morphologies emerged, demonstrating optimal growth on *Sodalis glossinidius* medium. Upcoming alkaline testing revealed the co-existence of 12 gram-positive and 10 gram-negative bacterial species among the colonies. The most abundant colonies were creamy-yellow colonies of medium size, exhibiting smooth edges, a round shape, elevated height, and a shiny surface. A few aerobic catalase positive isolates (C1, D8, D11, D15, and LB2) representing the most abundant colony morphology were subjected to further analysis.

In order to determine whether isolates C1, D8, D11, D15, and LB2 represent five different strains, genomic DNA was extracted, and BOX-PCR genotyping was performed. Our analysis clearly showed that these five isolates belong to three different strains. 16S rRNA genes of these isolates were amplified and sequenced. BLAST analysis showed that bacteria belonging to the genus *Frigoribacterium*, traditionally found in soil environments [1] were not previously detected in aphids. Analysis of the 16S rRNA gene region indicated the highest similarity with *Frigoribacterium faeni* species, suggesting a close evolutionary relationship between these bacteria. Currently, delineation of prokaryotic taxa based on 16S rRNA gene sequence similarity stands at 98.7% for species and 94.5% for genera [2]. The ultimate finding suggests that LB2 may belong to a particular species (sequence similarity 98.35%), whereas other strains (sequence similarity 95.95-96.43%) might potentially represent a novel species, however, additional genome analysis is needed.

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A42

INVESTIGATION OF RECOMBINANT URETHANASE EXPRESSION IN *PICHIA PASTORIS* AND *ESCHERICHIA COLI*

Eglė Gečaitė, Jokūbas Krutkevičius, Inga Matijošytė

Sector of Applied Biocatalysis, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
egle.gecaite@gmc.stud.vu.lt

Polyurethane (PU) is a synthetic polymer that has versatile applications and is used to manufacture foams, adhesives, coatings, and many more. The global production volume of polyurethane is expected to reach 31.27 million tonnes in 2030 [1]. This scope of production and usage requires effective and sustainable methods of treating PU waste that would otherwise pollute the environment. Nowadays, nearly 50% of PU waste ends up in landfills. Common treatment methods include mechanical processing, chemical recycling, and incineration. However, these techniques usually require harsh conditions, can produce toxic by-products, and some of the methods might not be applicable for post-consumer waste [2].

Biocatalysis-based (enzymatic) treatment of polyurethane provides an alternative way of recycling PU, addressing the issues mentioned above. Enzymes that are able to hydrolyse PU have already been discovered. However, they actually cleave the ester or ether bond; yet, there is still a need to hydrolyse the urethane bond which connects the monomers of PU. To date, several urethanases have been reported in literature and their activity was confirmed by hydrolysis of urethane (ethyl carbamate) [3]. However, cleavage of the urethane bond in polyurethane is yet to be demonstrated.

The study object of this research was a urethanase gene (UTH) from *Lysinibacillus* sp. (accession number MK757456.1). The goal was to obtain a soluble urethanase for further studies of its urethane bond hydrolysation abilities using polyurethane as the substrate. The UTH gene was cloned into *Pichia pastoris* yeast using vector pPIC3.5K. Codon optimization for *P. pastoris* was performed prior to cloning since the sequence was derived from a bacterium. Additionally, a chimera of urethanase and maltose-binding protein (MBP) was tested. Such fusion tags as MBP have the potential to enhance the solubility of recombinant proteins [4]. Several expression conditions were investigated; however, no noticeable recombinant protein nor enzymatic activity was observed. Therefore, expression in *Escherichia coli* was the next attempt at producing a soluble urethanase. The used vectors were pLATE31 and pLATE51, which contain His-tags for purification. Further details of the study will be discussed during the poster session.

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A43

IDENTIFICATION AND BIOCIDAL PROPERTIES OF YEASTS ISOLATED FROM PASTURE SOIL, WATER AND ANIMAL FEED

Paliovkinas D.[1,2], Strazdaitė-Žielienė, Ž.[1], Servienė, E.[1]

[1]Nature Research Centre, Vilnius, Lithuania
[2]Life Sciences Center of Vilnius University, Lithuania
deividas.paliovkinas@gmc.stud.vu.lt

Biocidal yeasts are capable of secreting toxic proteins into the environment, which can be lethal to other microorganisms. The susceptibility to these toxins varies greatly among yeast species and strains. While the diversity of fungal communities in pasture soil, environmental water, and farm animal feed has been studied separately before, there is still a lack of data on systematically investigating yeast populations across water, soil, and feed from the same pasture. This study aimed to identify biocidal yeasts in environmental samples from pastures and evaluate their biocidal properties.

Samples were collected from water, fodder, and soil. Fodder and soil samples were washed with sterile distilled water in a glass flask at room temperature for approximately half an hour by shaking, then concentrated thousand times and applied for microorganism isolation and identification. Water samples were concentrated ten times and isolated yeast-like morphology colonies proceeded to molecular identification. A total of eleven biocidal strains were isolated from the pastures. Biocidal yeasts were identified by amplifying the ITS region using ITS1 and ITS4 primers and sequencing the PCR fragments. They belonged to *Pichia*, *Williopsis*, *Cystofilobasidium*, *Candida*, *Rhodotorula*, *Debaryomyces*, and *Aureobasidium* genera. The killer assay was conducted on MB media at pH levels of 4.0, 4.8, and 5.6. Among the strains tested, eight exhibited biocidal activity only at pH 4.8, one strain showed biocidal activity only at pH 4.0, and another strain displayed biocidal activity only at pH 5.6. Additionally, one strain demonstrated killing activity at both pH 4.0 and 4.8. Upon evaluating the mechanism underlying the biocidal properties of these yeasts, it was determined that none of the eleven killer strains contained extrachromosomal elements such as DNA linear plasmids or dsRNA viruses. This indicates that the biocidal toxins of all tested killer yeasts may be encoded within their chromosomal genes.

This study, focusing on the diversity of biocidal yeasts in pasture ecosystems, is crucial for gaining a better understanding of their impact on animals' health. It represents the first comprehensive analysis of the microcosm, specifically focusing on biocidal microorganisms, present in water, soil, and feed within pastures as an integrated system.

April 15th

Biomedicine

B1

MANUFACTURING ARTIFICIAL URETHRAL TISSUE USING HUMAN STEM CELLS IN GELATIN-SILK FIBROIN HYDROGELS

Andrius Buivydas[1], Povilas Barasa[1], Egidijus Šimoliūnas[1], Ieva Rinkūnaitė[1], Emilija Baltrukonytė[1], Virginija Bukelskienė[1]

[1]Department of Biological Models, Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania
andrius.buivydas@gmc.stud.vu.lt

The urethra plays a vital role in the urinary system by enabling the passage of urine from the bladder to the external environment. Repairing urethral damage is crucial for restoring proper urinary function and preventing complications. Current treatments often involve multiple surgical interventions [1]. Our aim is to improve these treatments by utilizing 3D printing technology to develop artificial urethral tissue. This tissue is designed to create an optimal environment for cell growth, migration, and differentiation, ultimately forming functional tissue.

Our artificial tissue's foundation is a 3D scaffold composed of Gelatin Methacrylate (GelMA) and Silk Fibroin (SF). This scaffold forms a hydrogel with a firm structure when sonicated to denature the silk fibroin and exposed to UV to polymerize GelMA [2]. To evaluate the physical characteristics of the hydrogel scaffold atomic force microscopy was used to calculate Young's modulus. We found that the Young's modulus for the GelMA-SF scaffold is around 110 kPa and when the hydrogel is laden with cells the modulus significantly decreases and measures at around 10 kPa. Additionally we measured the water contents of the hydrogel and found that the construct has a swelling ratio of around 20.

For the cellular aspect of the hydrogels, it's necessary to mimic the urethra's natural structure, which consists of an epithelial layer surrounded by a muscular wall. Our previous work showed that rabbit adipose stem cells (RASC) are suitable for myogenic differentiation and rabbit buccal mucosa stem cells (RBMC) are suitable for epitheliogenic differentiation. To bring this technology to human application our next step was evaluating whether the equivalent human cells possess the same differentiation potential. As can be seen in figure 1 ACTA2, CALD1 and TAGLN have a higher expression in the differentiated group of HASC cells which is associated with myogenic differentiation [3] and FLG, RPTN genes are expressed more in the differentiated group of HBMC cells which is associated with epitheliogenic differentiation[4].

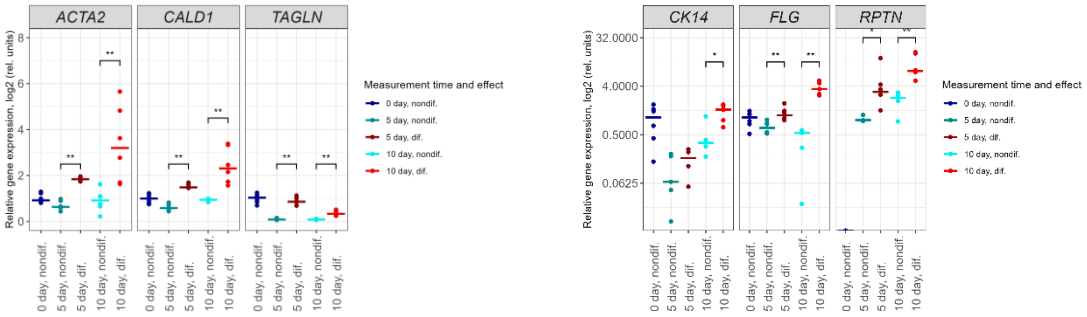

Figure 1. The results of gene expression measurements. Cells were divided into groups of undifferentiated and differentiated cells. Measurements were performed on the day of cell seeding and after 5 and 10 days. A – HASC myogenic differentiation, B – HBMC epitheliogenic differentiation. The values of measurements (points) and medians (lines) are shown. P values were calculated using the Wilcoxon rank sum test. n=6. Asterisks indicate p values: * p ≤0.05, p≤0.01.

To summarize GelMA-SF hydrogels display physical properties conducive to cell growth due to their relative softness as measured by AFM and ability to hold high levels of water. Human adipose stem cells and human buccal mucosa stem cells can be successfully differentiated into myogenic-like and epitheliogenic-like cells respectively as proven by the increase of expression of genes associated with myogenic and epitheliogenic differentiation respectively. These results are promising for applying this technology to treat urethral strictures in a clinical setting.

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B2

GRAPHENE OXIDE DRUG DELIVERY FILMS AS A TOOL FOR GLAUCOMA TREATMENT

Alexandra M. L. Oliveira[1,2], Austėja Kvedaraitė[1], Mónica Machado[1,2], Diogo B. Bitoque[1,2], Luís Abegão Pinto[3,4], Quirina Ferreira[1,2], Gabriela A. Silva[1,2]

[1]NOVA Medical School|Faculdade de Ciências Médicas, NMS|FCM, Universidade Nova de Lisboa; Lisbon, Portugal
[2]iNOVA4Health, NOVA Medical School|Faculdade de Ciências Médicas, NMS|FCM, Universidade Nova de Lisboa; Lisbon, Portugal
[3]Ophthalmology Department, Centro Hospitalar Universitário de Lisboa Norte, 1649-035 Lisbon, Portugal
epartment of Cell Engineering, University of Solar System, Country Three
[4]Visual Sciences Study Centre, Faculty of Medicine, Universidade de Lisboa, 1649-028 Lisbon, Portugal
austeja.kvedaraite@nms.unl.pt

Glaucoma, a leading cause of global blindness, is characterized by the death of retinal ganglion cells and optic nerve atrophy, often linked to increased intraocular pressure. Initial treatment typically involves eye drops, yet patient compliance is low and success rates are poor. Surgical intervention becomes necessary upon treatment failure, with valve implantation to drain the aqueous humor being a common approach. However, this procedure presents challenges, including post-surgical complications that could be mitigated by coating the valve with nanostructured thin films capable of controlled anti-mitotic drug release, specifically targeting fibroblast proliferation.

To tackle this problem, nanostructured thin films were developed, consisting in alternate layers of an antimitotic drug 5-fluorouracil (5FU), PBAE as a structural layer, and graphene-oxide (GO) as a barrier and/or capping layer. For improved stability and biocompatibility, the 5FU was previously encapsulated in β-cyclodextrin (βCD).

Different concentrations of 5FU were tested on a retinal cell line (D407) either in the free form or encapsulated, at different timepoints, to determine the effect in the cell cycle. Flow cytometry results confirm 5FU arrests the cell cycle in the S phase, being concentration dependent. No significative differences were found between the free form or the βCD encapsulated form of 5FU, and βCD alone does not seem to have an impact in the cell cycle.

Dissolution studies in physiological conditions were performed to determine if 5FU release was occurring. These release studies were carried out for two weeks and revealed no detectable amount of 5FU released as detected by HPLC. This indicates any drug being released was below the detection limit of 4 ng/mL.

These results highlighted the need to determine the total amount of 5FU present in the films and for this, the films were pulverized, and HPLC was used to ascertain the total 5FU amount. For a film with 8 alternating bilayers of PBAE and 5FU and 8 alternating quadrilayers of PBAE, βCD-FU, GO+ ang GO- we determined a total amount of drug to be 292.67 ng.

Overall, our findings suggest that to achieve higher drug loading efficiency, the amount of graphene oxide present in the films needs to be increased. This can be achieved simply by increasing the number of layers to achieve a sustained release.

B3

ANTICANCER ACTIVITY OF SOME 1-(2,4-DIFLUOROPHENYL)-5-OXOPYRROLIDINE-3-CARBOXYLIC ACID DERIVATIVES

Guoda Pranaitytė[1], Birutė Grybaitė[1], Ugnė Endriulaitytė[2]
Vytautas Mickevičius[1], Vilma Petrikaitė[2]

[1]Department of Organic Chemistry, Kaunas University of Technology, Lithuania
[2]Laboratory of Drug Targets Histopathology, Lithuanian University of Health Sciences, Lithuania
guoda.pranaityte@ktu.edu

Molecules containing fluorine atoms are gaining interest in medicinal chemistry due to increased lipophilicity and metabolic stability of a compound. In certain cases, the use of fluorine atoms in the molecule increases the bioavailability of the drug, provides information on the structure-activity relationship [1, 2].

The starting compound 1-(2,4-difluorophenyl)-5-oxopyrrolidine-3-carboxylic acid **2** was prepared by reaction of 2,4-difluoroaniline **1** with itaconic acid in water. From the esterification reaction 4-acetyl-1-(2,4-difluorophenyl)pyrrolidine-2-one **5** was obtained. Then methyl ester **5** was transformed into 1-(2,4-difluorophenyl)-5-oxopyrrolidine-3-carbohydrazide **6** by reaction of hydrazine monohydrate in refluxing isopropanol. The desired products **7b**, **9a**, **9c**, **9e** and **9f** were obtained by stirring carbohydrazide **6** with corresponding aromatic aldehyde in isopropyl alcohol at the boiling temperature of the mixture. The alkylation reaction of compound **9a** was carried by dissolving the starting material in DMF, using iodoethane (Figure 1).

The activity of the synthesized compounds was studied against three types of cancer cells – breast cancer (MDA-MB-23), prostate cancer (PPC-1) and melanoma (A375). It was found that carbohydrazides **7b** and **9f** had the lowest EC_{50} value against MDA-MB-231, PPC-1- and A375 cell lines (Figure 2).

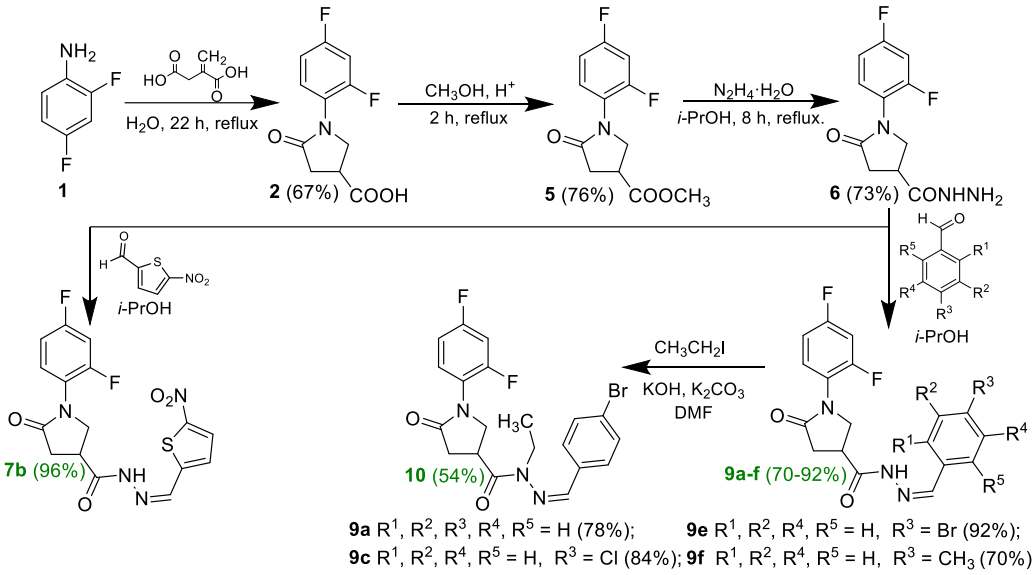

Figure 1. Synthesis of the most active 1-(2,4-difluorphenyl)-5-oxopyrrolidine-3-carboxylic acid derivatives against cancer cells

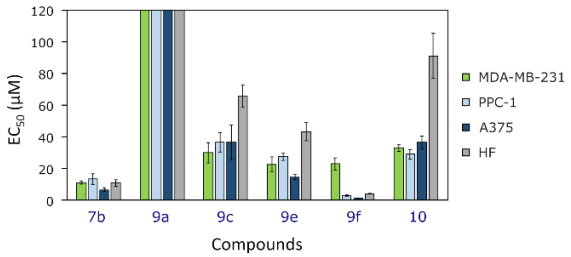

Figure 2. EC_{50} values of the most active compounds

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B4

INVESTIGATION OF ANTICANCER PROPERTIES OF 3,4'-BIPYRAZOLES

Ineta Meldaikytė[1], Aistė Drūtytė[1], Paulius Ruzgys[2], Vilija Kederienė[1]

[1]Department of Organic Chemistry, Kaunas University of Technology, Lithuania
[2]Faculty of Natural Sciences, Vytautas Magnus University, Lithuania
ineta.meldaikyte@ktu.edu

Cancer is a disease where cells divide uncontrollably. This disease is difficult to treat and one of the most common causes of death [1]. Currently, there are many ways to treat cancer, but more effective ways to do it are being sought [2].

Pyrazole derivatives are widely described in various scientific articles, and their biological activity has been determined, but more and more new pyrazole derivatives with better activity are aimed to be find [3]. Pyrazole and bipyrazole derivatives are found in many biologically active substances, as well as in drug molecules, such as celecoxib, phenylbutazone or rimonabant [4]. These compounds are widely studied by specialists in the fields of organic synthesis and medicinal chemistry. They are attractive to scientists due to their different biological properties: antibacterial, antiviral, anti-inflammatory, anticancer, analgesic, and other biological properties [4, 5]. First, the original pyrazole derivative was converted to carbaldehyde according to the already known synthesis methodology, from which the hydrazone derivative was obtained. After finding the best synthesis method, 3,4-pyrazole was synthesized. Based on the diversity of biological properties of pyrazole and bipyrazole, it was decided to perform the functionalization of new bipyrazole compounds. For this, the very widely used palladium-catalyzed Suzuki-Miyaura methodology was chosen for the functionalization of the pyrazole derivative. This synthesis was developed and first applied in 1979 by Akira Suzuki and Norio Miyaura [6]. Suzuki-Miyaura is very widely used in the formation of new C-C bonds in agriculture, pharmaceutical and other fields [7]. This reaction is characterized by mild, environmentally friendly conditions [8].

The resulting functionalized bipyrazole derivatives were investigated for their anticancer activity. Based on the obtained results, it can be stated that functionalized bipyrazole derivatives have good anticancer activity against different cancer cell lines.

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B5

EXAMINATION OF OXADIAZOLE SYNTHESIS AND PROPERTIES

Ieva Bartkevičiūtė[1], Vida Malinauskienė[1], Vilija Kederienė[1]

[1]Department of Organic Chemistry, Faculty of Chemical Technology, Kaunas University of Technology
ieva.bartkeviciute@ktu.edu

Heterocycles are a large and unique class of organic compounds with a wide variety of physical, chemical, and biological properties and constitute a large proportion of known and used organic compounds, which can be of both natural and synthetic origin. [1] This class also includes oxadiazole, distinguished by a five-membered unsaturated ring, two nitrogen atoms and one oxygen atom and having two main atomic positions: there can be 1,2,4-oxadiazoles and 1,3,4-oxadiazoles; other oxadiazoles are considered unstable. These compounds and their derivatives demonstrate anti-inflammatory, antibacterial, anti-tuberculosis, anti-diabetic, anti-cancer, anti-fungal, antioxidant, and enzyme-inhibitory effects. Commercial examples of oxadiazole-based agents would include the antihypertensive agents Tiodazosin, Nesapidil, and the antibiotic Furamizole. [2]

Since oxadiazoles have favorable physical, chemical, and pharmacokinetic properties, the synthesis and research of oxadiazole-based analogues is an important direction in the development of medicinal chemistry. In addition, the mentioned compounds are distinguished by the spectrum of interactions with macromolecules, high reactivity, and stability. These exceptional properties encourage the search and creation of an even more significant number of analogous compounds characterized by better cost and/or atom economy, greater specificity and efficiency, and new exposure possibilities. [3]

The aim of the work is to synthesize a group of new oxadiazole compounds with different aromatic structural bases by applying the principles of alkylation reactions in the hope of obtaining compounds with biological properties.

In this work, it was decided to start the synthesis of the target compounds from the preparation of oxadiazole thiol according to the known methodology. Using a two-step procedure, which first involves the use of hydrazine hydrate, the resulting product is further cyclized under basic conditions. The starting oxadiazole thiol is then treated with substituted benzyl halides and triethylamine.

Parallel synthesis of analogs characterized by other similar oxadiazole thiol structural bases was started. The methodologies are maintained as in the case of the previous synthesis routes. The starting thiols were obtained in very good yields, and three series of different target compounds were synthesized in good to very good yields.

The primary structural studies confirmed the existence and high degree of purity of the target compounds, so the compounds with all the structural bases are ready for biological studies. This is expected to further expand the library of the mentioned compounds with biological properties.

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B6

SYNTHESIS AND PROPERTIES INVESTIGATION OF BENZOTHIOPHENE

Arnas Kovševič[1], Povilas Viltrakis[1], Indrė Jaglinskaitė[1], Vilija Kederienė[1]

[1]Department of Organic Chemistry, Faculty of Chemical Technology, Kaunas University of Technology
arnas.kovsevic@ktu.edu

Benzo[*b*]thiophenes are heterocyclic compounds that have a variety of biological properties. They are widely used in pharmaceuticals due to their anti-allergic, antibacterial, anti-inflammatory, receptor modulator, and antioxidant effects. [1]

It has been established that benzo[b]thiophene derivatives may be used as drugs in cancer treatment due to their ability to inhibit protein tubulin polymerization and cell proliferation. [2] The drugs Raloxifene, intended for the treatment of osteoporosis during postmenopause, and Zileuton, an anti-asthmatic drug, are based on them. [1]

N-substituted derivatives of benzo[b]thiophene are utilized in the medicinal chemistry due to their biological properties, such as antibacterial, antifungal, and anti-inflammatory properties. [3] In addition, the drug Encenicline was discovered, which is active against schizophrenia and Alzheimer's disease. [1] In addition, these compounds are not only biologically active substances but also are used in solar cells and thin-film devices due to their fluorescent properties. [4] Benzo[*b*]thiophene compounds have luminescent properties so that they can act as efficient emitters. [5] For further synthesis, benzo[b]thiophene derivatives were chosen due to their wide application.

The abundance of synthesis and application possibilities encourages the search for more biologically active compounds, so the aim of this work is to synthesize new functionalized benzo[*b*]thiophene derivatives with different aromatic radicals by applying the principles of catalysis reactions and determining the antibacterial and antioxidant properties of the obtained compounds.

At the beginning of the study, the initial derivative of methyl 3-aminobenzo[*b*]thiophene-2-carboxylate was synthesized. Various classical reaction and catalysis methodologies were employed to investigate the reactivity of benzo[*b*]thiophene derivatives, and new benzo[*b*]thiophenes-2-carboxylate with different substituents were obtained. The obtained derivatives had good and excellent yields. A detailed analysis was conducted on the structure of the benzo[*b*]thiophene derivatives obtained using spectrometric and spectroscopic analysis methods.

The antibacterial effect of final products was determined by disk diffusion into agar; the antioxidant properties were studied according to the reduction potential and the ability to reduce the DPPH radical.

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B7

THE EFFECT OF TYROSINE KINASE INHIBITORS AND DOXORUBICIN COMBINATIONS IN 3D TRIPLE-NEGATIVE BREAST CANCER CULTURES

Ugnė Mekionytė[1,2], Vilma Petrikaitė[1,2]

[1]Laboratory of Drug Targets Histopathology, Institute of Cardiology, Lithuanian University of Health Sciences, Sukilėlių av. 13, LT-50162 Kaunas, Lithuania
[2]Faculty of Medicine, Lithuanian University of Health Sciences, A. Mickevičiaus str. 9, LT-44307 Kaunas, Lithuania
ugne.mekionyte@lsmu.lt

One of the biggest challenges of triple-negative cancer (TNBC) treatment is lack of therapeutic targets and development of drug resistance. A useful approach for this problem is to combine drugs with different mechanisms of action [1]. Doxorubicin is an anthracycline class drug widely used for the treatment of TNBC. Tyrosine kinase inhibitors, such as sunitinib (SNT), are known as targeted anticancer drugs. Our previous research revealed that sunitinib analogues and DOX may have synergistic effect in two-dimensional (2D) DOX-resistant TNBC cultures. However, there is still a need for further examination in three-dimensional (3D) cultures, that better represent the tumor microenvironment. In this study we selected SNT derivative 4001, which was synthesized in Cagliari University, Italy [2]. The aim of this study was to determine the synergistic effect of tyrosine kinase inhibitor 4001 in combination with DOX in TNBC 3D cultures.

For this research, two types of human TNBC cell lines were used. One is normal MDA-MB-231 (WT) and the other is DOX-resistant MDA-MB-231 (DR). The TNBC spheroids were formed using magnetics 3D Bioprinting method from cancer cells and fibroblasts. The concentrations of compounds in combination were calculated based on the half-maximal effective concentrations (EC50) in 2D cultures (EC50 ratio 1:1). The effect of the compound(s) on spheroid was determined by measuring change of diameter and cell viability. For cell viability, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used.

The results showed that 4001 and DOX combinations did not slow down the growth of MDA-MB-231 WT spheroids on day 10. Also, there was no difference in cell viability compared to the control on day 10. In contrast, MDA-MB-231 DR spheroids were significantly smaller when treated either with 4001 or SNT in combination with DOX (618.0 ± 47.4 µm and 502.2 ± 32.5 µm, respectively) compared with the control on day 10. The cell viability of MDA-MB-231 DR spheroids was also significantly reduced by 4001 and SNT in combination with DOX (87.8 ± 9.4 % and 62.3 ± 8.6 %, respectively) on day 10.

In summary, tyrosine kinase inhibitor 4001 in combination with DOX may be a useful tool to combat resistance in TNBC treatment.

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B8

ACINETOBACTER BAUMANNII ISOLATES PHENOTYPIC AND GENOTYPIC ANALYSIS

Salomėja Šapranauskaitė[1], Silvija Kiverytė[1,2], Asta Mačionienė[2], Edita Kazėnaitė[1,2], Tomas Liveikis[3], Julija Armalytė[3]

[1]Faculty of Medicine, University of Vilnius, Vilnius, Lithuania
[2]Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania
[3] Vilnius University Life Sciences Center, Vilnius, Lithuania
s.sapranauskaite@gmail.com

Acinetobacter baumannii is the most common nosocomial Gram-negative ESKAPE (a group of six most important and most drug-resistant) group pathogen that poses a threat to public health by causing severe and invasive infections, including bacteremia, pneumonia, and infections of the skin and urinary tract, linked with high mortality rates. During the last decades (especially during the COVID-19 pandemic) this pathogen displayed multidrug resistance (MDR) for a wide range of antibiotics. The most common risk factor for the acquisition of MDR *A.baumannii* is previous antibiotic use, followed by mechanical ventilation, hospitalization, severity of illness, and use of medical devices [1].

Different infection control strategies and procedures that are used in hospitals may lead to different selection of *A. baumannii* genes. In order to enhance infection control and reduce outbreaks of infections, great efforts are being made to analyze of *A. baumanii* antimicrobial resistance profiles. If the same bacterial strain encodes genes responsible for resistance to several classes of antibiotics, phenotyping the resistance of strains and assessing associations can be difficult. Therefore, it is extremely important to perform not only phenotypic tests, but also analyze the connections between phenotypic and genotypic profiles as best as possible [2].

The *A. baumanni* resistance capacity is generated by various mechanisms, including the modification of the target site where antimicrobial action is directed. This mechanism is mainly generated by genetic mutations and contributes to resistance against a wide variety of antimicrobials, such as beta-lactams (carbapenems, monobactams, and beta-lactamase inhibitors), aminoglycosides, tetracyclines, fluoroquinolones, polymyxins (colistin), diaminopyrimidines, sulfonamides antibiotics [3].

A total of 165 *A. baumannii* clinical isolates, isolated between December 2022 and November 2023 from 165 patients in Vilnius University Hospital Santaros Klinikos, were characterized by the phenotypic and genotypic analyses combining the antibiotic susceptibility testing and the PCR amplification methods. After a detailed comparison of age and infections, it can be seen that the highest number of infections was observed for patients between 60-79 years (51%), while the lowest number of illnesses was observed for the age group of up to 40 years (8%). During the period of observation, the biggest part of clinical samples was taken from the bronchi - 32 samples, blood - 26 samples, urine - 24 samples. The epidemiological samples were mostly collected from the feces - 20 samples. Antibiotic susceptibility testing revealed that over 90 % of isolates were resistant to meropenem, gentamicin, amikacin, tobramycin, ciprofloxacin. The results revealed that all *A. baumannii* isolates carried the $bla_{OXA\text{-}51\text{-}like}$ gene, over 80 % of isolates carried the *aph (3')-Ia*, bla_{VIM1}, $bla_{OXA\text{-}23\text{-}like}$, bla_{TEM}, *sul2* genes. However, neither the *sul3* nor *dfrA17* genes were detected in the isolates. The results are correlated with other researches that over 80 % of isolates have MDR or even XDR (extensively drug resistant) phenotypes and are resistant to beta-lactams, aminoglycosides, cephalosporins and fluoroquinolones [2] [3].

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B9

ACCUMULATION OF UPCONVERTING NANOPARTICLES FUNCTIONALIZED WITH BSA-AU NCS IN BREAST CANCER CELLS

Gabrielė Sužiedėlytė[1,2], Greta Butkienė[1], Vilius Poderys[1], Vaidas Klimkevičius[3], Vitalijus Karabanovas[1,4]

[1]Biomedical Physics Laboratory, National Cancer Institute, Vilnius, Lithuania
[2]Life Science Centre, Vilnius University, Vilnius, Lithuania
[3]Institute of Chemistry, Vilnius University, Vilnius, Lithuania
[4]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania
gabriele.suziedelyte@gmc.stud.vu.lt

Photodynamic tumor therapy (PDT) is an approach to damage and degrade cancerous tissue using the combination of photosensitizers (PS) and light irradiation. However, the typical excitation wavelength for most PS falls within the spectrum of visible or UV light and achieving sufficient light penetration in biological tissues with such wavelengths is challenging. The depth of penetration ranges between 0.1 mm and 5 mm [1] which is not optimal.

PS can be organic molecules, nanomaterials or a combination of metal atoms chelated to ligands. Here we propose gold nanoclusters (Au NCs) stabilized with bovine serum albumin (BSA). They are derivatives of 25 gold atoms in BSA, as clusters of such size exhibit photoluminescence upon excitation around 495 nm and can generate reactive oxygen species. Nevertheless, light which is used for excitation of BSA-Au NCs still is absorbed within few millimeters of tissues. A solution might be upconverting nanoparticles (UCNPs) – nanoscale compounds capable of converting lower energy photons into higher energy photons, thus turning the absorption of near-infrared (NIR) radiation into visible light and UV emission. NIR spectrum of wavelengths can penetrate tissues deeper compared to other light (up to 3.2cm) [2]. Therefore, UCNPs are beneficial when used in tandem with PS with excitation ranges in the visible light spectrum. We hypothesize that UCNPs and BSA-Au NCs should complement each other when used together to improve PDT.

In this study we investigated the accumulation of UCNPs functionalized with BSA-Au NCs using a laser scanning confocal microscope. We utilized specialized dyes such as Hoechst for the nuclei visualization, Phalloidin combined with Alexa Flour or CF594 for the cytoskeleton detection and employed transmission microscopy for the most effective imaging of breast cancer cells. We evaluated how UCNPs, BSA-Au NCs and UCNP-BSA-Au NCs complexes have internalized after a 24-hour incubation.

Composition of the coating surrounding nanoparticles determines the specific type of endocytosis mechanism involved, which includes phagocytosis, pinocytosis, clathrin-mediated endocytosis, and caveolae-mediated endocytosis [3,4]. Hence, our aim is to evaluate the accumulation of UCNPs covered with a BSA-Au NCs as surface coating. It was compared to the accumulation of UCNPs with a non-specific protein coating which formed upon exposure to components of culture media, as well as UCNPs conjugated with BSA-fluorophore. We learned that upon internalization UCNPs localize within the cytoplasm, frequently around the nucleus, but not inside it. Secondly, accumulation of nanoparticles within cells did not drastically alter the morphology of MDA-MB-231 and MCF-7 cells, which was supported by our additional cytotoxicity assay. Based on the overlay of UCNPs emission and BSA-fluorophore photoluminescence we could assume that upon internalization the nanoparticle and its coating do not separate. Our findings also suggest that the accumulation of UCNPs complexed with a BSA-Au NCs is comparable to, or more effective than, that of UCNPs alone or with BSA-fluorophore.

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B10

Changes in human ovarian tissue treated with rutin and VEGF-A after xenotransplantation

Valerija Prokofjeva[1,2], Monika Grubliauskaite[1,3]

[1]Department of Biobank, National Cancer Institute, Vilnius, Lithuania
[2]Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
[3]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
valerija.jegorova@mf.stud.vu.lt

Recent advances in early detection and treatment strategies have led to substantial improvement in cancer survival rates over the past several decades. Unfortunately, cancer treatments may compromise endocrine and reproductive functions in female patients, leading to premature ovarian failure and infertility [1, 2]. Ovarian tissue (OT) cryopreservation and autotransplantation present a promising option for fertility preservation, especially in prepubertal girls and patients requiring immediate cancer treatment with limited time for ovulation induction and oocyte collection [3]. Though hundreds of live births following cryopreservation and autotransplantation of frozen OT have been reported, this innovative technique still needs to be improved due to massive follicle loss in ovarian grafts [4]. Ischemia, hypoxia, and vascular reperfusion injury are the main factors leading to follicle loss during grafting, thus limiting the success rate of the transplantation procedure [4]. Multiple approaches have aimed to reduce damage caused by ischemia and oxidative stress via the administration of different growth factors, hormones, antioxidants, or mechanical tissue damage. This study aimed to evaluate if antioxidant (rutin) and vascular endothelial growth factor (VEGF-A) have an impact on the expression of genes involved in folliculogenesis and tissue integrity of xenotransplanted human OTs.

Cryopreserved OTs (2.5 x 2.5 mm) from 10 cancer patients were thawed, cultured *in vitro* for 36h in media supplemented with 1 µg/ml rutin and 50 ng/ml VEGF-A (n=10) or without (n=10), and transplanted to immunodeficient mice. OTs were retrieved after 4 weeks and used for gene expression analysis. Frozen-thawed OTs (n=4) were used as non-grafted controls. Vascularization was evaluated by mouse *CD31* and *ANGPT2* gene expression. Expression of genes related to the development of follicular cells (*FOXO3, FSHR, GDF9*) and tissue integrity (*PRDX1, SOD1, FN1, COL6A1, CASP3*) were analyzed. *GAPDH/Gapdh* was used as an endogenous control for gene expression data normalization. Finally, statistical analysis was performed comparing gene expression levels in non-grafted with grafted OTs and between grafted OT groups.

Four weeks post-transplantation, analysis of qPCR data revealed that genes associated with oxidative stress responses (*SOD2, PRDX1*) were significantly downregulated ($p<0.05$) in both grafted groups compared to the non-grafted control. Moreover, grafts treated with VEGF-A and rutin exhibited decreased expression of *CASP3*, a gene related to apoptotic signaling, compared to non-grafted OTs. However, after transplantation, we observed a significant decrease ($p<0.05$) in expression of the folliculogenesis-associated (*GDF9, FOXO3, FSHR*) and structural genes (*FN1, COL6A1*) in grafted OTs. The *ANGPT2* (angiogenesis regulator) gene expression in VEGF-A and rutin treated OT grafts was significantly reduced ($p<0.05$) compared to non-grafted control, although no significant difference was observed between grafted OT groups. However, increased *ANGPT2* expression can also show inflammation or hypoxia. Finally, no significant difference in the expression of mouse *CD31* gene between grafted OT groups was found.

The present study demonstrates that the administration of VEGF-A and rutin may reduce apoptosis, ischemia, and oxidative stress in xenotransplanted human OT, although follicle number diminishes significantly after grafting.

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B11

ANGIOTENSIN II MEDIATED DIVERTICULOSIS IN THE HUMAN GASTROINTESTINAL TRACT

Ignas Lapeikis[1,2], Kristina Rysevaite – Kyguoliene[1]

[1]Institute of Anatomy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Faculty of Medicine, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
ignas.lapeikis@stud.lsmu.lt

Introduction. Angiotensin II receptor type 2 (AT2) are receptors for angiotensin II, a hormone involved in regulating blood pressure and electrolyte balance. An increase in AngII receptors at myenteric plexuses in the gastrointestinal tract could lead to heightened activity in this area. The raise of AngII receptors at myenteric plexuses in the gastrointestinal tract could increase the activity of macrophages which can activate the cascade of pro-fibrotic pathway [1] to mediate fibrosis in the GI (gastrointestinal) tract.

Aim. The aim of this study is to determine whether there is an increase in the amount of AT2 receptors in the myenteric plexuses in the GI of diverticulosis patients.

Methods. Control samples were obtained from patients undergoing surgery for non-obstructing colorectal carcinoma, who did not have symptoms of complicated or uncomplicated diverticular disease (DD). This type of operation was a source of asymptomatic diverticular disease (ADD) samples if diverticula were found to be present in these patients. Tissue specimens for the symptomatic diverticular disease (SDD) group were obtained from patients who underwent sigmoid resection for diverticulitis disease. [2] Histological preparations were stained by immunohistochemistry (IHC) with primary antigens against HuC/HuD for neuronal bodies and against AT2 receptors for AngII. The area of neuronal bodies and neurons, positive to AT2 receptors in 92 myenteric plexuses were measured using the ImageJ program. The statistical data were calculated using SPSS 29.0 (using one-way ANOVA and Bonferroni post hoc tests).

Results. Data revealed a significant difference of the neuronal area proportion (AT2/neuronal bodies) between control and symptomatic DD groups ($p=0.011$). The percentage of positive AT2 receptors covering positive neuronal bodies in control at the myenteric plexuses were 42.00% compared to 56.65% in symptomatic DD. The percentage of area per unit (one myenteric plexus) showed the difference between control and symptomatic DD groups 14.48% (42.12% and 56.50% respectively). The asymptomatic group showed neuronal area (AT2/neuronal bodies) percentage of 46.00%. The percentage of area per unit marked 45.00%. There were no significant differences between asymptomatic DD and other patient groups.

Conclusions. Research results show an increase of the AT2 receptors area in the myenteric plexuses of symptomatic DD patients' group which may lead to fibrosis in the gastrointestinal tract. Fibrosis can have various consequences, depending on its location and severity. In the context of diverticular disease (DD), fibrosis could contribute to the development of diverticula (small pouches that protrude from the wall of the colon) and potentially exacerbate symptoms such as abdominal pain, bloating, and changes in bowel habits.

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B12

COMPARATIVE ANALYSIS OF MIGRATION AND GENE EXPRESSION IN BREAST CANCER CELL LINES MCF-7 AND MDA-MB-231 FOLLOWING EXPOSURE TO si-KRAS OR Anti-EGFR

Vakarė Jakimavičiūtė[1,2], Guoda Petronavičiūtė[2,3], Monika Grubliauskaitė[1,2], Daiva Dabkevičienė[1,2], Dominyka Breimelytė[1,2]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
[2]Biobank, National Cancer Institute
[3]Faculty of Medicine, Vilnius University, Lithuania
vakare.jakimaviciute@gmc.stud.vu.lt

Emerging therapies targeting EGFR and KRAS are notably advancing cancer treatment. Increased EGFR protein expression in breast cancer promotes malignant cell proliferation and survival. Therefore, targeting EGFR with antibodies can effectively inhibit or stop these processes [1]. Similarly, the KRAS gene, responsible for producing various isoforms of the KRAS protein, plays a critical role in cell growth, migration, and differentiation. Suppressing specific KRAS gene isoforms can lead to the activation of different gene signaling pathways. Evaluating EGFR and KRAS *in vitro* helps in understanding treatment responses in breast cancer cell lines. The study focused on evaluating the differential responses of breast cancer cell lines MCF-7 and MDA-MB-231 to anti-EGFR antibody treatment and KRAS isoforms A and B silencing via targeted siRNA.

First, the optimal conditions non-toxic concentrations of anti-EGFR and siRNA's treatments were determined. The 'wound healing' method was utilized for migration assay following treatments. The expression of KRAS isoforms, EGFR, and endothelial mesenchymal transition genes: *FBX7*, *MAML1*, *CDH1*, and *VIM* was measured using reverse transcription quantitative real-time PCR after 24 hours. The relative gene expression was quantified utilizing the ΔΔCT method. A gene with over a two-fold expression change compared to control was classified as significantly expressed. siRNAs were designed with Eurofins Genomics' siRNA design tools, and the primers were designed using NCBI primer BLAST software.

siRNA-KRAS-A reduced *KRAS-A* transcript by 58% in MCF-7 and 69% in MDA-MB-231 cells; siRNA-KRAS-B cut *KRAS-B* by 12% in MCF-7 and 15% in MDA-MB-231. An EGFR antibody decreased *EGFR* levels by 2% in MCF-7 and 1% in MDA-MB-231. Treatments had diverse effects on the genes' expression, influencing cell migration. *VIM* gene expression in the MCF-7 cell line was elevated after treatment with siRNA-KRAS-A and siRNA-KRAS-B. On the other hand, in the MDA-MB-231 cell line, all treatments, led to a reduction in *VIM* expression. In the MCF-7 cell line *CDH1* expression increased after anti-EGFR treatment. Meanwhile, in the MDA-MB-231 cell line, *CDH1* gene expression was reduced following treatments with siRNA-KRAS-A and siRNA-KRAS-B. Results of migration indicate significant differences in the effects of anti-EGFR ($p = 0.0002$) and both si-RNA-KRAS ($p < 0.0001$) on MCF-7 and MDA-MB-231 cell lines, showing decreased migration effect on MDA-MB-231 treatments correlating with decreased VIM and CDH1 expression.

In conclusion, the distinctive effects observed with siRNA-KRAS-A and siRNA-KRAS-B treatments on gene expression and cell migration within MCF-7 and MDA-MB-231 cells shows how the KRAS isoforms contribute to breast cancer cell behavior. In MCF-7 cells, siRNA-KRAS-A reduces migration and alters EGFR, and CDH1 expression, highlighting its influence on cell adhesion and signaling pathways [2]. siRNA-KRAS-B treatment in MCF-7 cells decreases migration more significantly and affects *CDH1* and *VIM* expression, indicating its role in modifying cell morphology [2, 3]. For MDA-MB-231 cells, both treatments lead to variations in migration and gene expression, with siRNA-KRAS-A primarily affecting *CDH1*, and siRNA-KRAS-B impacting *VIM*. These findings delineate the nuanced roles of KRAS isoforms in modulating gene expression and cancer cell migration dynamics, offering valuable insights into the response to silencing treatments.

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B13

THE ALTERATION IN EXTRINSINC GANGLION NODOSUM CAUSED BY ARTERIAL HYPERTENTION IN VERY OLD SPONTANEOUSLY HYPERTENSIVE AND WISTAR-KAYOTO RATS

Jekaterina Novikova[1,2], Kristina Rysevaite – Kyguoliene[1]

[1]Institute of Anatomy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Faculty of Medicine, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
jekaterina.novikova@stud.lsmu.lt

With a projected 22% rise in the elderly population by 2030, particularly susceptible to cardiovascular diseases (CVDs) like hypertension (HPT), understanding the underlying mechanisms becomes crucial. The aetiology of HPT is a result of imbalance in sympathetic and parasympathetic regulation of the CVS [1]. HPT manifests as endogenous neural substances such as calcitonin gene-related peptide (CGRP) - the most potent microvascular vasodilator, important in vascular-related stress, nitric oxide synthase (nNOS) - also known as endothelium-derived relaxation factor, release [2].

Aim. This study explored the link between aging and hypertension in male rats using very old (60-63 weeks) spontaneously hypertensive rats (SHR) and its age-matched Wistar-Kyoto (WKY) models in terms of area, nodal volume, and expression of CGRP and nNOS proteins.

Method. Cryosections of nodose ganglion (NG) were prepared by immunohistochemical reactions with primary antibodies against CGRP / nNOS proteins and PGP9,5 (marker for all neuronal bodies). Secondary antibodies were conjugated with fluorochrome Cy3 and AF488. Sections were examined using fluorescence microscope. The ganglia area was measured and neurons, positive to PGP9,5, CGRP and nNOS were counted using AxioImage and ImageJ programs. Statistical data was analysed using T-test and one-way ANOVA tests.

Results. Surprisingly, no significant differences ($p>0.05$) were found in nodal ganglion area, volume, or CGRP/nNOS expression between SHR and age-matched control rats. These findings suggest the greater the age of the mouse the less difference they exhibit in terms of CGRP and NOS expression, area, and volume of ganglion. The tendency between the groups shows higher expression of nNOS in SHR rats – 42.9%, whereas in WKY nNOS - 39.3%. Expression of CGRP in SHR group also showed lower results than WKY - 29.5% compared to 35.2%. The SHR rats also showed higher area of the ganglia by 3%, however smaller volume of neural bodies by 29.8%, compared to the WKY rats. Interestingly, the significant difference ($p<0.05$) was found in measurements of an area of NG in WKY rats between right and left ganglia. Right ganglia were bigger than left by 32,1% but no difference between node sides was found in SHR rats.

Conclusion. Even though, the results did not show a significant difference in SHR and WKY rats, they still exhibit different morphology and levels of CGRP and nNOS protein expressions. These distinctions are not significant due to the age of the rats. However, the tendency of SHR rats to higher nNOS and lower CGRP expression, smaller volume of the ganglion, is due to long term pathological action of HPT on blood vessels, compared to WKY rats who underwent natural ageing process [1]. Difference in WKY NG area might indicate slight difference in parasympathetic innervation and type of nerve fibres [3]. Similarly in humans, the older the human the greater the risk of naturally occurring hypertension because of morphological changes of the cardiac plexuses, this explains the huge rise of CVDs in elderly population.

Acknowledgements. This study was supported by the Grant S-MIP-19-23/PRM19-113 from the Research Council of Lithuania.

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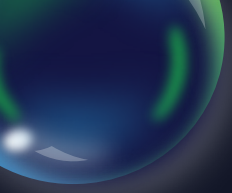
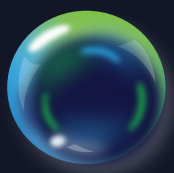
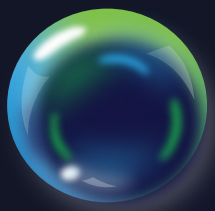
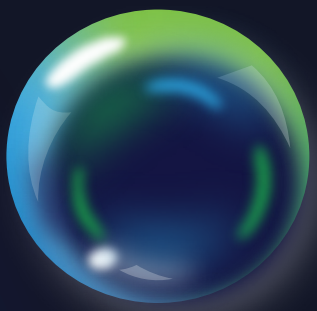
April 15th

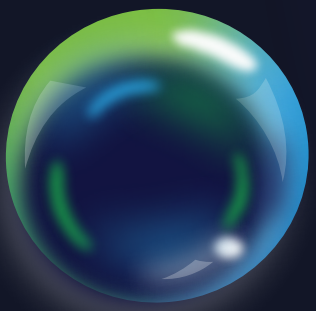








Bioinformatics









C1

THREE METAGENOMIC α-L-FUCOSIDASES BELONGING TO THE NOVEL GH151 FAMILY

Pijus Serapinas[1], Rūta Stanislauskienė[1], Agnė Krupinskaitė[1], Rolandas Meškys[1], Jonita Stankevičiūtė[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania
pijus.serapinas@gmc.stud.vu.lt

α-L-Fucose (Fuc) is a unique carbohydrate having a L-configuration and lacking a hydroxyl group on the C-6 carbon. It is found in the structure of oligosaccharides, and Fuc is also the most common terminal sugar of glycoconjugates [1]. Fucosylated glycoconjugates, present in both prokaryotic and eukaryotic (including humans) organisms, are essential for many biological processes, such as host-microorganism interactions in bacterial pathogenesis, cell-to-cell communication in plants, neurological and immunological processes in humans [1, 2, 3]. Additionally, L-fucose plays a significant role in the human body as a component of secretor blood group antigens and human milk oligosaccharides [4].

α-L-Fucosidases (EC 3.2.1.51) are exo-acting glycoside hydrolases that catalyze the removal of α-L-fucose from oligosaccharides and glycoconjugates. These enzymes can be found in a wide variety of organisms and tissue types. The diversity of α-L-fucosidases produced by gut microorganisms may provide these species with an advantage in colonizing and adapting to the gut environment [1]. In humans, α-L-fucosidase deficiency may lead to two pathological events: fucosidosis and cancer. These enzymes are applied in medicine, research, and biotechnology, as they show potential for the enzymatic synthesis of valuable oligosaccharides through transfucosylation [2].

This study introduces three novel α-L-fucosidases – FucKUR, FucLINKA, and FucMSL2, obtained from metagenomic libraries. They all belong to the glycoside hydrolase family GH151. This novel family was first recognized in 2012. To date, it contains over 260 members, of which only three are characterized and only one structure (α-L-f2wt) has been determined experimentally. The amino acid sequences of the fucosidases in question were analyzed using bioinformatic analysis tools. A phylogenetic tree constructed using GH151 family proteins revealed that the three fucosidases are quite distinct from each other. Analyzing structures modeled via AlphaFold2, the main structural differences were found in the C-terminal domain of the investigated proteins. Comparative analysis with α-L-f2wt showed that the active site residues are highly conserved among the GH151 family proteins, with only minor differences.

α-L-Fucosidases belonging to the GH151 family are novel, thus limited research has been conducted, resulting in a relatively sparse understanding. Further research is needed to gain a better understanding of the mechanisms and properties of these enzymes, as well as their potential for biotechnological applications.

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FTDMP: A FRAMEWORK FOR PROTEIN-PROTEIN, PROTEIN-DNA AND PROTEIN-RNA DOCKING AND SCORING

Rita Banciul[1], Kliment Olechnovič[1,2], Justas Dapkūnas[1], Česlovas Venclovas[1]

[1] Institute of Biotechnology, Life Sciences Center, Vilnius University
[2] CNRS Laboratoire Jean Kuntzmann, Grenoble
rita.banciul@gmc.vu.lt

Knowledge of the 3D structure of protein-protein and protein-nucleic acid complexes is crucial for understanding the molecular mechanisms that govern essential cellular processes. Experimental methods such as X-ray crystallography, NMR, and cryoEM provide high-quality structures, but are expensive and time consuming. Thus, there is a need for computational structure prediction. While the AI-based method AlphaFold has revolutionized single chain protein structure prediction, challenges remain, particularly in modeling antibody-antigen interactions, protein-nucleic acid complexes, and proteins lacking close homologs. In these cases, docking can be employed to generate structure models. As a result, effective methods for selection of the most accurate models are necessary.

Here we present FTDMP, a newly developed framework for protein-protein and protein-nucleic acid docking and scoring. The framework can be used in two ways: to perform docking and subsequent scoring, or to evaluate and rank user provided models coming from various sources (AlphaFold, RoseTTAFold, docking, etc.). The ranking in FTDMP is done by the VoroIF-jury method, that is based on the consensus of several scoring functions [1]. The protocol based on this method obtained top results in the community-wide CASP15-CAPRI scoring experiment, that evaluates computational methods for predicting protein structures and interactions [2].

The full FTDMP docking and scoring framework was tested on protein-protein, protein-DNA, and protein-RNA docking benchmarks [3-5]. Compared to currently available docking systems, FTDMP demonstrated improved results of the free unbound-unbound docking when the top-ranked model was considered. Moreover, the success rates were very high for bound-bound docking (up to 83% for the top prediction), which opens new application possibilities when the conformational changes upon binding are negligible. For example, rigid-body docking using FTDMP assisted the identification of high-quality models when AlphaFold and docking predictions were similar for hard CAPRI targets. In addition to that, the framework can be employed for fast and straightforward evaluation of new scoring functions, since it can be used not only with the built-in, but also with external scoring methods.

FTDMP, docking benchmarks and docking results are available at https://github.com/kliment-olechnovic/ftdmp.

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PINPOINTING UNCERTAINTIES IN X-RAY STRUCTURES OF PROTEIN HOMODIMERS

Simonas Ašmontas[1], Justas Dapkūnas[1], Lukas Valančauskas[1], Česlovas Venclovas[1]

[1]Institute of Biotechnology, Life Sciences Center, Vilnius University
simonas.asmontas@gmc.vu.lt

X-ray crystallography is one of the most popular methods for accurate protein structure determination, which usually provides enough data to construct macromolecular models with atomic precision. However, in protein crystals with numerous inter-chain contacts it may be difficult to distinguish interactions originating from the underlying biological assemblies and artifacts produced by crystal packing alone. To alleviate this problem, the community of computational structural biologists has created a plethora of automated methods for detection of biologically relevant protein-protein interfaces (PPIs) in X-ray crystallography data. These methods are quite diverse, ranging from purely physicochemical single structure-based models to the ones based on evolutionary distant protein comparisons. Nevertheless, none of the available methods are capable of perfect PPI annotation. While specialized methods still struggle with the said problem, they are getting complemented with other computational techniques [1]. One such case is repurposing of AlphaFold-Multimer [2], a method, initially designed to model proteins with no experimental structures available. This new application of AlphaFold-Multimer is reported to be quite successful, however in its initial study only around ~1500 protein dimers were examined, leaving a vast protein pool yet to be explored [1].

In this study we investigated a wider non-redundant dataset of crystal structures for protein complexes from PPI3D database [3], comprising of 19002 protein-only homodimers with unique PPIs, corresponding to 16667 protein sequence clusters at 95% sequence identity threshold. We discovered that only around 2/3 of these sequence clusters contain proteins which are found in the Protein Data Bank (PDB) only as homodimers, indicating some uncertainty for the rest of the dataset. Moreover, we revealed that a small fraction of the dataset sequence clusters (approximately 2%) contain homodimers, whose structures resemble cases of incomplete C3 symmetry, which is unexpected for dimeric structures. Additionally, we remodeled the entire homodimer dataset with AlphaFold-Multimer implemented in ColabFold [4]. We analyzed top ranked models and determined that most of them reproduce PDB biological assemblies. However, we identified two additional groups of models that differ from corresponding homodimer biological assemblies in the PDB. One group, making up almost 1/3 of all top ranked models, has unreliable interfaces, according to AlphaFold-Multimer self-confidence estimates. This suggests that the corresponding homodimers, annotated in the PDB as biological assemblies, may be in fact homodimers resulting from crystal packing. Another group, which comprises nearly 4% of all top models, has relatively high AlphaFold-Multimer self-confidence estimates. In this case it is most likely that structure remodeling discovered new biologically relevant assemblies that were missed in the PDB experimental data.

Based on our results, we conclude that a significant minority of PDB X-ray homodimer structures have dubiously assigned oligomeric states and/or PPIs. Although our analysis does not definitively disprove the existence of these homodimers, we suggest using these experimental models with caution.

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PREDICTING THE PROTEIN-LIGAND BINDING AFFINITY USING DESCRIPTORS DERIVED FROM VORONOI TESSELLATION

Anastasija Kudrevceva[1], Justas Dapkūnas[1]

[1]Department of Bioinformatics, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
anastasija.kudrevceva@gmc.stud.vu.lt

The protein-ligand binding affinity prediction based on 3D structural data is one of the important tasks in modern drug development, driving rational drug design, structure-based lead optimization, and drug repurposing. At the moment, a large number of computational models aiming to predict the protein-ligand binding affinity have been developed. However, designing a model able to precisely predict the binding affinity for diverse protein-ligand complexes remains an unsolved challenge [1]. Moreover, recent studies revealed the extreme impact of the presence of similar structures in the training set on the model performance, questioning the actual predictive power of existing methods [2].

Here we present the development of predictive models for protein-ligand binding affinity using descriptors based on Voronoi tessellation of biomolecular structures: inter-atom contact areas and atom volumes [3]. To account for different interaction types, the contact areas were grouped according to atom properties computed by the RDKit software. The developed models are based on different machine learning methods (PLS, Random Forest, and Gradient Boosting), and the correlation coefficient between predicted and experimentally determined binding affinity values ranges between 0.58 and 0.67.

We investigated the performance of our models using independent datasets of experimentally determined structures of protein-ligand complexes with known binding affinity. To compare the models with widely used methods, we tested their performance on CASF scoring, ranking, docking, and screening tasks designed to benchmark scoring functions [1]. Our models demonstrated better binding affinity prediction accuracy compared to the classical scoring functions but performed poorly for docking-related tasks. The models were additionally tested using a large dataset of diverse structures published after 2019 and single protein target datasets [4]. In the latter case, mimicking a realistic drug discovery scenario, we observed different prediction accuracies for different target proteins. Moreover, we demonstrated that the accuracy of prediction depends on the similarity of both protein and ligand to the training set even for the developed simplistic binding affinity predictors.

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DYNAMICS OF WATER MICROORGANISMS COMMUNITIES DURING THE SEASONAL BLOOM OF CYANOBACTERIA

Rugilė Blauzdžiūnaitė[1,2], Sigitas Šulčius[1], Mikołaj Kokociński [3]

[1]Nature Research Centre, Vilnius, Lithuania;
[2]Life Sciences Center of Vilnius University, Lithuania
[3]Jagiellonian University, Krakow, Poland
rugile.blauzdziunaite@gmc.stud.vu.lt

Seasonal fluctuations in the microbial communities of marine and freshwater bodies play a pivotal role in ecosystem dynamics [1]. Cyanobacteria, while vital for oxygen production, can also trigger harmful algal blooms due to eutrophication, posing significant challenges to water ecosystems [2]. To comprehend cyanobacteria's influence, it's crucial to discern the broader microbial population within these bodies of water. Next Generation Sequencing technologies offers a powerful tool for this purpose.

In order to understand the influence of cyanobacteria on the water ecosystem, it is first necessary to find out which microorganisms make up the population of the studied water bodies. This can be achieved by using Oxford Nanopore Technologies long-read sequencing on water samples. The main goal of this work is to find out the abundance of dominant microorganisms populations and investigate the mutual exchange of functional groups of the microbial nutritional network during the seasonal blooming and non-blooming of cyanobacteria. During this work, bacterial community samples and colony-forming phytoplankton of Curonian Lagoon water samples were collected and their DNA was purified, concentrated, and finally sequenced.

Bioinformatic metagenomic analysis was employed to visualize the results. In the waters of the Curonian Lagoon, the constancy of bacterial communities over time was observed, where the majority of the population consisted of *Actinobacteria, Betaproteobacteria* and *Flavobacteria. Acidobacteria, Actinobacteria* and *Betaproteobacteria* had the highest mutual similarity. While diatom blooms, including colony-forming *Cyclotella* sp. and *Desmodesmus communis*, were observed on September 9, 2022, no direct connection with the bacterial community was identified. This highlights the complexity of microbial interactions within aquatic ecosystems, where multiple factors influence community dynamics.

The findings show the need for comprehensive understanding and monitoring of microbial communities in water bodies, particularly in the context of cyanobacterial blooms and their ecological repercussions. Such insights can inform management strategies aimed at preserving water quality and mitigating the adverse effects of eutrophication and algal blooms on aquatic ecosystems and human health.

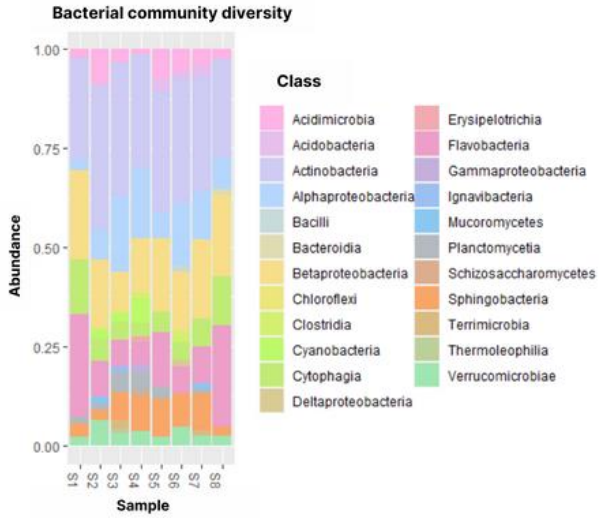

Figure 1. The diversity of the water bacterial community of the Curonian Lagoon in different weeks, taxonomic level: class. The diagram was made using RStudio.

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mK-ras INITIATED CARCINOGENESIS-MALIGNIZATION MODEL

Kęstutis K.Urba

NCI The Ras initiative international project
urbascience@gmail.com

A comprehensive molecular level model of CARCINOGENESIS-MALIGNIZATION is essential for precise drug therapy. Unfortunately, oncology articles tend to be very fragmented and clinical trials are based on too weak logic. Therefore, carcinogenesis-malignization scenarios creation also require artificial intelligence algorithms that mimic the author's deductive reasoning presented below, integrating the causal relationships of the individual facts by means of a "mosaic" method, obtained by "mining" the texts using the keywords "upregulated" and "downregulated", while also taking into account the stochasticity [1].

Let the description of a malignant tumour be $\{TUMOROEx\}_t$=UkUij fk,ij(Exξ_1, G(K)ξ_2, C(K)ξ_3, Tr(K)ξ_4, P(K)ξ_5,L(K)ξ_6,Mtl(K)ξ_7,M(K)ξ_8, F(K)ξ_9,Ep(K)ξ_{10},I(K)ξ_{11}), where U is the union of sets, k=1... n, and n is the number of clones in the tumour, ij belongs to the set of t - random, stochastic time points of the start of biological processes - their alignment yields the biological level scenarios of cancer, K denotes the fatal karyotypic phenotype, (Ex) denotes the exposome, (G) - genomic factor , (C) - cystromic, (TR) - transcriptomic, (P) - proteomic, (L) – lipidomic, (Mtl) – metallomic, (M) - metabolomic, (F) - fliucosomic, (Ep) - epigenetic, (Im) - immunomic factors, t - time, ξ - the stochastic moment of the factor's expression in carcinogenesis. Let Bi, i=1...41 be the biological hallmarks of malignancy [2]. The qualitative full sequential model of molecular level carcinogenesis-malignization is then the result of the identification of the sequences ST,i i=1...m generated by $\{TUMOROEx\}_t$, mapping FC to the set {UBi, i=1...n, n=41}.

The main result: The oncogenes K-ras and c-myc, the mTOR complex together with p53 are not only effector genes ("drivers"), but also stochastic triggers of carcinogenesis with a long time course and a long sequence of reactions and biological changes, which by the activation of RTKs - mK-ras-mTor1 - c-myc (-p53), polyamines, and the changes in the methionine cycle... pathways and scenarios determine the generation of the necessary and sufficient biological properties and signs of cancer:

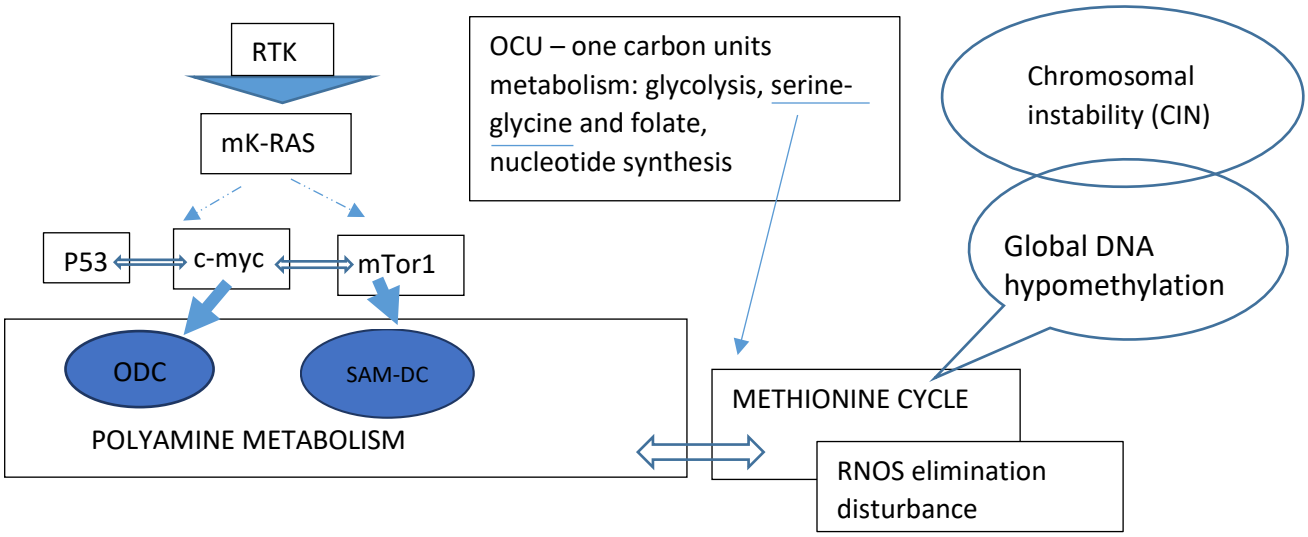

Figure 1. The overall pattern of mK-ras induced carcinogenesis-malignancy was obtained by "mosaicking" the individual fragmented results using the PubMed's articles text „mining"

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C7

SPLICING ANALYSIS OF ARTHROPOD-INFECTING ORTHOMYXOVIRUSES

Aistė Židonytė[1], Gytis Dudas[1]

[1]Institute of Biotechnology, Life Science Center, Vilnius University, Lithuania
aiste.zidonyte@gmc.stud.vu.lt

Some viruses of *Orthomyxoviridae* family are unusual amongst RNA viruses due to their distinctive transcription process, which occurs within the nucleus of the infected cell [1]. Viruses of this family have a segmented, single-stranded, negative-sense RNA genome and the number of segments typically ranges between 6 to 8 segments within different virus genera [2]. Well-researched Orthomyxoviruses, such as Influenza viruses, are known to use the host splicing machinery to modify their mRNAs from some of their genome segments, therefore expanding their proteome diversity and ensuring replication [3].

Several Orthomyxoviruses have been found and studied in invertebrates because they have also been recognized to cause human or other vertebrate infections (e.g. thogotoviruses). Even though many others have been identified, they are rarely researched any further [4]. Hence, there have not been cases where splicing would be described in the *Quaranjavirus* genus. Therefore, in this study, we conducted bioinformatics analysis on metagenomic data to identify if splicing is found in a virus of the *Quaranjavirus* genus – with Wuhan Mosquito Virus 6 specifically and its nearest relatives with known complete genomes.

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April 15th

Immunology

D1

CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST HOUSE DUST MITE ALLERGEN COMPONENT DER P 2

Diana Ulčic[1], Dainius Gudas[1], Martynas Simanavičius[1], Indrė Kučinskaitė-Kodzė[1]

[1] Department of Immunology, Vilnius University, Lithuania
diana.ulcic@gmc.stud.vu.lt

Allergy is a hypersensitivity reaction to non-hazardous substances such as pollen, food and animal proteins. House dust mite (HDM) allergy affecting approximately 65-130 million people worldwide ranks HDM one of the main indoor allergens [1]. *Dermatophagoides pteronyssinus* commonly found in Europe, is a prominent HDM species. One of its major allergenic molecule (allergen component) is Der p 2. Serological assays measuring allergen-specific IgE levels are one of the diagnostic measures for determining sensitization to allergens. To develop accurate quantitative serological diagnostic systems, calibrators of human IgE antibodies are needed. However, the utilization of human serum or plasma-derived calibrators presents several limitations, including low quantities, variability in antibody specificity and affinity, characterization constraints, and high costs. Recombinant chimeric antibodies offer a promising approach for standardizing such systems.

This study aimed to characterize mouse monoclonal antibodies (mAbs) against Der p 2 for chimeric antibody development. Four mAbs were generated previously in our laboratory using hybridoma technology. Folowing purification by affinity chromatography, the mAbs were analyzed using SDS-PAGE, Western blot and indirect enzyme linked immunosorbent assay (ELISA).

During this investigation isotypes of the mAbs by commercial ELISA kit were determined. MAbs 10C12, 4G7, 5E12 were of IgG1, kappa (κ) and mAb 2B4 – IgG2a, κ isotype. All mAbs were tested to determine the ability to recognize natural allergens by Western blot assay using 6 natural allergen extracts from different manufacturers. Subsequently, all mAbs were found to interact with Der p 2 in natural allergen extracts. Additionally, mAbs 10C12, 4G7, 5E12 were cross-reactive with natural allergen component Der f 2 from the HDM *Dermatophagoides farinae.* The apparent dissociation constants (Kd, nM) of the mAbs were determined using indirect ELISA, where the reactivity of the mAbs with recombinant Der p 2 was analyzed. MAbs 10C12, 4G7, 5E12 demonstrated high affinity (0.09, 0.12, and 0.13 nM, respectively), whereas mAb 2B4 – lower affinity (8.19 nM) to the recombinant Der p 2.

Hybridoma producing mAb 4G7 was chosen for chimeric antibody creation. Polymerase chain reactions were used to amplify the nucleotide sequences of the mAb heavy and light chain variable regions. Their sequence was determined by Sanger sequencing.

This study revealed that all four mAbs recognized both recombinant and natural Der p 2. MAbs 10C12, 4G7, 5E12 also recognized Der f 2. Apparent dissociation constants reveal that 10C12, 4G7, 5E12 can interact with antigen with high affinity. Variable regions of 4G7 mAb were determined and chimeric recombinant antibodies are being created.

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DRUG INDUCED PHOTOSENSITIVITY REACTIONS

Neringa Janseviciūtė[1], Violeta Kvedarienė[2,3,4]

[1]Vilnius University, Faculty of Medicine, 03101, Vilnius, Lithuania
[2]Vilnius University, Faculty of Medicine, Institute of Biomedical Sciences, Department of Pathology, 30101, Vilnius, Lithuania
[3]Vilnius University, Faculty of Medicine, Institute of Clinical Medicine, Clinic of Chest Diseases, Allergology and Immunology, 03101, Vilnius, Lithuania
4Innovative Allergology Center, 06256, Vilnius, Lithuania
neringa.janseviciute@mf.stud.vu.lt

Drug-induced photosensitivity refers to the development of skin disorders resulting from the interaction between a chemical substance and sunlight exposure. Photosensitivity is one of the most common types of adverse drug reactions related to the skin. These reactions can occur from medications taken internally or applied directly to the skin [1]. Drug-induced photosensitivity can pose significant challenges in diagnosis, often leading to underrecognition of this condition [2].

The research aimed to describe photosensitivity reactions and their mechanisms. The articles were searched in the Medline (PubMed) and Embase (ScienceDirect) electronic databases using the keywords "photosensitivity," "drug-induced," "photoallergy," and "phototoxicity." Inclusion criteria included relevance to the described topic, availability of full text, English language texts, and publication between 2013 and 2023. A total of 11 articles were selected for review.

Over 300 medications have been associated with drug-induced photosensitivity with many commonly used in everyday medical practice. Drug-induced photosensitivity can be classified into two main types: phototoxicity and photoallergy. Additionally, it can manifest as lichenoid reactions, subacute cutaneous lupus erythematosus, or pseudoporphyria, which includes bullous reactions and photo-onycholysis [2]. Phototoxic reactions occur when a light-activated compound directly damages tissue including cell membranes, and sometimes DNA. Typically, phototoxic reactions manifest as an exaggerated sunburn-like response and usually occur within minutes or hours of light exposure [3]. Photoallergy is a type IV (delayed) hypersensitivity reaction mediated by the immune system. In this reaction, the drug, activated by light, serves as the antigen. When activated by light, the drug forms a metabolite, which can bind to protein carriers in the skin, forming a complete antigen [3]. Photosensitizing drugs are increasingly linked to more cases of skin cancer. Most drug-induced photosensitivity reactions are linked to UV-A wavelengths. UV-A radiation, although less potent than UV-B, can penetrate deeper tissue layers, interacting with medications deposited there. In contrast, UVB radiation is notorious for causing sunburns, primarily penetrating the epidermal layer and playing a more significant role in carcinogenesis [4].

Numerous widely used medications have the potential to induce photosensitivity. Educating both clinicians and patients about the potential danger of sun light and photosensitivity is crucial to prevent many dangerous skin diseases.

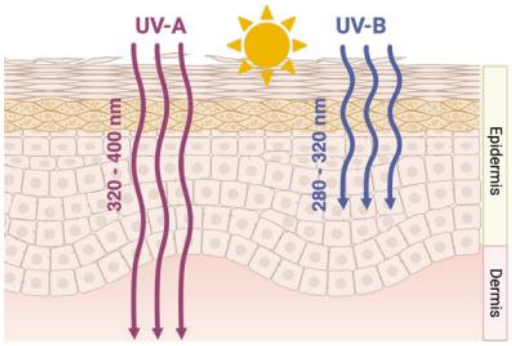

Figure 1. UV-A and UV-B wave penetration to the skin

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DEVELOPMENT OF β-LACTAMASE SPECIFIC MULTIMERIC RECOMBINANT ANTIBODIES

Adrija Buturlakina, Karolina Bielskė, Dovilė Stravinskienė

Department of Immunology, Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania

adrija.buturlakina@gmc.stud.vu.lt

β-lactamases are the enzymes produced by bacteria that provide multidrug-resistance (MDR) to β-lactam antibiotics such as penicillins, cephalosporins, cephamycins, and others by breaking the structure of the antibiotic. In the health settings β-lactams are typically used for the treatment of infectious diseases caused by the broad spectrum of gram-positive and gram-negative bacteria [1]. Therefore, rapid and reliable detection methods of MDR bacteria is clinically and epidemiologically relevant. Antibody-based assays, such as immunochromatographic tests using monoclonal antibodies (MAbs) specific to β-lactamases, are promising diagnostic tools. Immunoassays are fast, simple, easy to perform, and affordable for the clinical laboratories [2]. The sensitivity of these assays can be improved by utilizing the multimeric recombinant antibodies. Displaying Fc-fused single-chain variable fragments (scFv) on the virus-like particles (VLPs) increases avidity of the recombinant antibodies, thus improving the sensitivity of the assay [3, 4].

In this study we describe the determination of the variable region sequences of hybridoma-derived MAb 7B6 against ACT-14 β-lactamase and their application for the development of multimeric recombinant β-lactamase specific antibodies. During the study, the variable fragments to heavy (VH) and light (VL) chains of MAb 7B6 were determined. For this purpose, total RNA was isolated from the stable hybridoma and the VH and VL coding sequences were amplified by reverse transcription and PCR using a set of previously described primers [5] specific for the framework of mouse IgG heavy and light chains. PCR products were cloned, VH and VL coding DNA sequences were verified by suquencing and analysed using IMGT/V-QUEST, IgBLAST, Geneious Biologic tools. Moreover, the sequences of the complementarity determining regions (CDRs) of VL and VH were defined. The obtained VL and VH sequences were engineered as two scFv variants (VL-VH and VH-VL) displayed on the surface of pseudotype virus-like particle (VLPs) as VP2-foreign sequence (Figure 1). The particles were produced in yeast *Saccharomyces cerevisiae* expression system and purified. VLP forming VP1, VP2 proteins and scFv in the particles were verified by SDS-PAGE and immunoblotting. We believe that our study provides a new data on the Mab against ACT-14 β-lactamase, its antigen-binding region, and the development of multimeric recombinant antibodies.

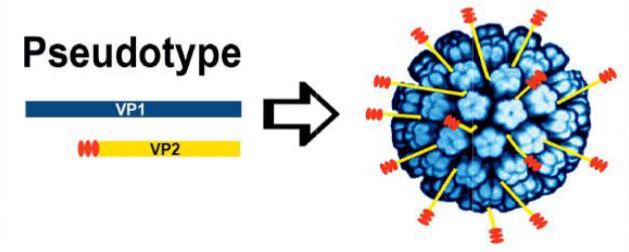

Figure 1. Pseudotype virus-like particle. VP1 – capsid protein, VP2 – foreign sequence fusion protein (scFv) [4].

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D4

CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST GRP78

Gabija Klimavičiūtė[1], Evaldas Čiplys[2], Rimantas Slibinskas[2], Aurelija Žvirblienė[1], Martynas Simanavičius[1]

[1]Department of Immunology, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
[2]Department of Eukaryote Gene Engineering, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
gabija.klimaviciute@gmc.stud.vu.lt

Glucose-regulated protein of 78 kDa (GRP78) is one of the most important endoplasmic reticulum (ER) chaperone proteins. This protein, also known as human binding immunoglobulin heavy chain protein (hBiP), facilitates protein folding, assembly and quality control, export into the ER, signal transduction, calcium ion and ER homeostasis [1]. The accumulation of unfolded proteins in the ER leads to ER stress and hBiP initiates signalling cascade of unfolded protein response (UPR) to remove/correct misfolded proteins. It was discovered that ER stress plays a role in disease progression, including heart disease, neurodegenerative disorders, diabetes, hypoxia, autoimmune inflammatory diseases and cancer [2]. There is evidence, that during ER stress GRP78 can exist outside the ER – in the cytoplasm and cell membrane. In the case of cancer, tumor cells face acidity, low glucose levels and nutrient deprivation. When hBiP is localised in the cell membrane, it acts as a multifunctional protein and plays an important role in tumor cell survival, metastasis, angiogenesis and resistance to chemotherapy. Increased levels of GRP78 expression have been noted in primary tumors in contrast to benign tissues, suggesting its role in tumor advancement and heightened aggressiveness [3]. If the diseases caused by ER stress are thoroughly monitored, patients have a better chance of survival or significant symptom relief.

Monoclonal antibodies (MAbs) are great biotechnological tools for protein detection and their function investigation [4]. Because of GRP78's ability to be localised on the cell surface during ER stress, it allows this protein to become a biomarker for the detection and monitoring of ER stress-related diseases. In this study, our goal is to characterise monoclonal antibodies against hBiP, so that research studies of mentioned diseases could be improved. Four mouse MAbs were generated using hybridoma technology. Cross-reactivity of monoclonal antibodies with other recombinant ER proteins and natural GRP78 expressed in various cell lines lysates was tested by Western blot. MAbs affinity to GRP78 was evaluated by measurement of the apparent dissociation constant by indirect ELISA. MAbs reactivity to natural GRP78 in cell lines was assessed by immunofluorescence microscopy.

In conclusion, one MAb generated against recombinant hBiP has shown the best characteristics as it was specific to natural hBiP in Western blot and immunofluorescence microscopy, and demonstrated the strongest affinity to the antigen. This antibody has a high potential for becoming a valuable reagent in GRP78 studies.

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D5

APPLICATION OF MONOCLONAL ANTIBODIES FOR THE DETECTION OF AmpC β-LACTAMASES

Karolina Bielskė[1], Martynas Simanavičius[1], Julie Nuttens[2], Julija Armalytė[3], Aurelija Žvirblienė[1]

[1]Department of Immunology, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
[2]ArcDia International Oy Ltd, Turku, Finland
[3]Department of Biochemistry and Molecular Biology, Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
karolina.bielske@gmc.vu.lt

According to the World Health Organization, antimicrobial resistance (AMR) is one of the greatest threats to human health in this century. Currently about 700 000 deaths are linked to AMR each year globally. It is estimated that 10 million lives a year may be lost due to AMR by 2050 [1]. The emergence of AmpC β-lactamases, which degrade β-lactam antibiotics, in *Enterobacteriaceae* is widely identified in health settings, and AmpC is increasingly being detected in livestock, wild and companion animals. B-lactamases of the CMY-2-type subfamily are identified as the most prevalent AmpC enzymes [2]. Currently, there are no criteria approved by the Clinical and Laboratory Standards Institute for the detection of these β-lactamases in pathogenic bacterial isolates [3]. Therefore, accurate and easy-to-perform diagnostic approaches for AmpC-producing bacteria are needed in the clinical laboratories.

The aim of this study was to apply previously characterized monoclonal antibodies (MAbs) raised against CMY-34 β-lactamase for the detection of AmpC enzymes. CMY-34 is one of the barely investigated members of the CMY family, making this β-lactamase a noticeable target for the development of detection assays. In this study the most promising and broadly reactive pair of characterized MAbs against CMY-34 was selected and diagnostic potential of these MAbs was evaluated by their ability to recognize natural β-lactamases in the CMY-positive bacterial isolates. For this purpose, MAbs were applied for the development of rapid diagnostic systems, such as quantitative lateral flow immunoassay, sandwich ELISA and two-photon excitation assay. These MAb-based immunoassays were able to detect all analyzed CMY-positive isolates, producing CMY-2, CMY-4, CMY-6, CMY-16, CMY-34 and one unidentified CMY variant. We believe that in this study described antibodies can be applied for the CMY immunodetection in bacterial isolates and are highly promising for the rapid diagnostics.

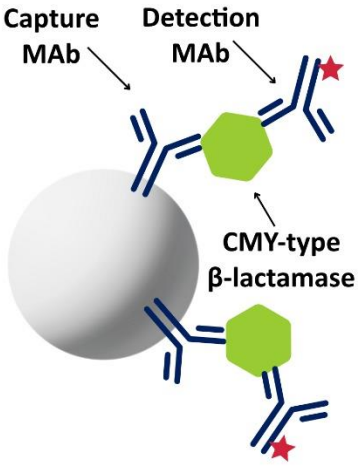

Figure 1. Schematic representation of CMY-type β-lactamase detection using two-photon excitation assay. B-lactamase is captured by MAb coated microparticles and detected by fluorescently labelled MAb

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D6

INVESTIGATION OF NLRP3 INFLAMMASOME ACTIVATION BY IMMUNE COMPLEXES OF VIRUS-LIKE PARTICLES IN PRIMARY MOUSE MICROGLIA

Kotryna Vilkaitė[1], Kristina Mašalaitė[1], Asta Lučiūnaitė[1], Aurelija Žvirblienė[1]

[1]Department of Immunology, Institute of Biotechnology, Life Sciences Center, Vilnius University
kotryna.vilkaite@chgf.stud.vu.lt

Inflammation is a protective immune response triggered by innate and adaptive immune systems in response to harmful stimuli such as pathogens or dead cells. An important part of the innate immune system is the inflammasome. It is a multiprotein complex that is activated through a group of innate immunity receptors called pattern recognition receptors that respond to structures that only pathogens possess and structures that occur in damaged cells. Inflammasomes are found in cells of the innate immune system such as monocytes, macrophages, and dendritic cells. Microglia, macrophages in the brain, are immune cells of the central nervous system and an important part of innate immunity. These cells are among the main cells in which the inflammasome is strongly activated [1].

NLRP3 inflammasome is the best-characterized inflammasome. NLRP3 inflammasome consists of three major components – nucleotide binding and oligomerization domain-like receptor NLRP3, adapter protein apoptosis-associated speck-like protein ASC, and proteolytic enzyme pro-caspase 1. Activation of NLRP3 inflammasome leads to the release of IL-1β and inflammatory cell death, i.e. pyroptosis. Importantly, IL-1β stimulates the production of other cytokines and chemokines to attract other immune cells to the site of infection [2].

Given the strength of inflammasome-dependent immune responses, it is not surprising that impaired NLRP3 inflammasome activity is implicated in several disorders, including Parkinson's and Alzheimer's disease [3]. Recent studies have reported the role of viral antigens in inflammasome activation. For instance, inflammasomes are activated in response to SARS-CoV-2 infection. SARS-CoV-2 N protein activates the NLRP3 inflammasome, which is associated with hyperinflammation in patients with SARS-CoV-2 infection [4]. Therefore, mechanisms of NLRP3 inflammasome activation and therapeutic interventions targeting structures involved in NLRP3 inflammasome signaling are promising areas of basic research. This study aims to investigate the effect of viral antigens and their immune complexes on the activation of the NLRP3 inflammasome and the release of cytokines and chemokines in primary mouse microglia.

Primary microglia cell culture was prepared from newborn C57BL/6 mice and treated with virus-like particles (VLP) and their immune complexes (IC). IC were composed of VLP of WU polyomavirus and murine immunoglobulins specific to VLP. Different subtypes of IgG class immunoglobulins were used: IgG1, IgG2a, and IgG2b to form IC. Microglia and NLRP3 inflammasome activation was studied by analysis of released inflammatory cytokines, such as TNF-α and IL-1β, and chemokines, such as CXCL1 and CXCL2 with the enzyme-linked immunosorbent assay method. Cell viability was measured by LDH release detection assay. To confirm NLRP3 inflammasome activation, the specific inflammasome inhibitor MCC950 was used.

Our study shows that VLP and their IC causes cell death and release of chemokines and cytokines, like IL-1β, indicating NLRP3 inflammasome activation. Moreover, antibodies alone did not induce any cellular response and NLRP3 inflammasome inhibitor MCC950 caused a decrease in IL-1β secretion in microglia. In conclusion, our results show that IC of VLP induces inflammatory response and NLRP3 inflammasome activation in microglia.

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D7

CHARACTERIZATION OF MODULAR DETECTION SYSTEMS TO IDENTIFY CAR RECEPTORS OF ANTI-CD19 CAR-T CELLS

Ramojus Balevičius[1], Grigorij Šutov[2,3], Jan Aleksander Krasko[4]

[1]Department of Life Sciences Center, Vilnius University, Lithuania
[2]UAB Genie Biotech EU, Lithuania
[3]Department of Immunology, State Research Institute Centre for Innovative Medicine, Lithuania
[4]Department of Immunology, National Cancer Institute, Lithuania
ramojusbalevicius@gmail.com

CAR-T cell therapy is an innovative, rapidly advancing field of immunotherapy. CAR-T cell therapy can be used to treat patients with tumours - lymphoma, leukemia, myeloma; autoimmune diseases - myasthenia gravis, systemic lupus erythematosus; and viruses - HIV, hepatitis B virus [1]. CAR-T cell therapy offers a promising alternative for patients who show resistance to conventional treatments such as surgery, radiotherapy or chemotherapy. CAR-T cell infusions are given to oncology patients to stimulate the immune system to achieve anticancer, antiviral or immunomodulatory effects. These cells are derived from genetically modified T cells ex vivo by expressing a CAR (chimeric antigen receptor) on the cell surface. CAR receptor consists of 4 parts: antigen recognition domain, hinge region, transmembrane domain and intracellular signalling domain [2]. CAR-T cells CAR receptor can recognize the specific antigens of cancer cells and initiate their death. Advantages of CAR-T cell therapy: stimulates a specific immune response, does not require antigen-presenting cells (APCs) for antigen presentation and antigen recognition stimulates the proliferation of CAR-T and T cells.

From 2017 FDA approved four anti-CD19 CAR-T cell therapies: "Kymriah", "Yescarta", "Tecartus", "Breyanzi", which are used to treat B-cell malignancies and anti-BCMA therapies "Abecma" and "Carvykti" for the treatment of myeloma [3]. In addition to anti-CD19 and anti-BCMA therapies, dozens of antigen targets are being tested under preclinical and clinical settings for solid and haematological tumours. To take CAR-T cell research from bench to bedside, first, genetically modified T cells have to be tested in vitro for CAR expression on the cell surface.

In 2022, B. Sharpless won a Nobel Prize for developing a more efficient way of conjugating molecules: he used water instead of organic solvents and catalyzed the reaction of azide and alkyne with copper [4]. The "click chemistry" reaction can take place in bioorthogonal conditions (in living cells), so the functioning of the body is not disturbed. Here, azide reacts only with compounds containing an alkyne group (BCN, DBCO, OCT).

GenieTAG technology was developed by Genie Biotech company [5] which allows the site-specific introduction of azide into the protein for subsequent "click chemistry"-based conjugation of various payloads for the vaccine, diagnostics, protein therapeutics and slow delivery applications. The linker is conjugated to proteins at a neutral pH of the solution (~7), the duration of the process is 1-3 hours, at 37 ℃.

In this project, we are developing and characterizing a novel single-step modular detection systems to determine the expression of CARs. For this purpose, we are conjugating recombinant protein L to fluorescent dyes using GenieTAG site-specific conjugation technology. We used Western blotting and flow cytometry to determine the specificity of the modular detection systems. These single-step modular detection systems will allow for faster and more precise identification of CAR receptors on engineered T cells.

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D8

QUANTIFICATION OF BLOOD CIRCULATING IMMUNE CHECKPOINTS AND THEIR LIGANDS IN BLADDER CANCER

Nerija Remeikaitė[1], Agnė Šeštokaitė[1,2], Agata Mlynska[2], Vincas Urbonas[1,2], Rasa Sabaliauskaitė[1,2]

[1]Vilnius University, Lithuania
[2] National Cancer Institute, Vilnius, Lithuania
nerija.remeikaite@gmc.stud.vu.lt

Bladder cancer (BCa) is the 10th most common malignancy with high recurrence rates compared to other genitourinary cancers with most of the primary cases (~70%) diagnosed with non-muscle invasive cancer. Nowadays one of the most promising (especially in BCa) treatments is immunotherapy. BCa is known for developing a plethora of mechanisms to evade antitumor immunity, including the overexpression of inhibitory receptors and their ligands, known as immune checkpoints (IC). ICs are responsible for keeping healthy cells from immune response, while cancerous cells are known to hijack this response to their own advantage. Therapeutic targeting of ICs recently demonstrated the great potential of such an approach, especially in BCa. Circulating forms of IC and their ligands are actively involved in immune regulation and could provide a non-invasive tool in gaining a broader understanding of ICs and their ligands.

This study is aimed at quantifying most common ICs and their ligands in BCa patients' blood and compare the levels of each protein to those found in healthy controls.

In this research we used 34 NMIBC patients' and 16 healthy controls' plasma to measure a panel of 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, using flow cytometry approach. 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.

Based on our findings, bigger concentrations of sCD25, B7.2, CTLA-4 and possibly PD-1, LAG3, Tim-3 suggest that these ICs are the most common ones in BCa plasma. Deeper understanding of these immune checkpoints and their changes in cancer could change the approach of immunotherapy and be used for non-invasive response to treatment prediction.

April 16th

Biology and Ecology



E1

DETERMINATION OF THE OOMYCETES EFFECT ON Salmo salar L. BY ASSESSING CHANGES IN OXIDATIVE STRESS BIOMARKERS

Eglė Gadeikytė[1,2], Gintarė Sauliutė[1], Arvydas Markuckas[2], Milda Stankevičiūtė[1]

[1]Nature Research Centre, Akademijos St. 2, LT-08412 Vilnius, Lithuania
[2]Vilnius University, Life Sciences Centre, Saulėtekio av. 7, 10223 Vilnius, Lithuania
egle.gadeikyte@gmc.stud.vu.lt

Oomycetes are among the most damaging of disease-causing organisms in forestry, agriculture, and aquaculture, presenting global threats to natural and anthropogenic environments, as well as to food production [1]. Infections caused by the oomycetes of the genus *Saprolegnia* are among the main parasitic diseases affecting farmed salmonids [2]. The total annual production losses in freshwater aquaculture due to saprolegniasis remain consistently high, with losses of 50% having been reported for over two decades, and with *Saprolegnia* responsible for at least 10% of all annual salmonid economic loss worldwide [3]. All fish in fresh water can possibly be infected by disease-causing oomycetes, which is termed saprolegniasis. This infection could be caused by several species within the genus *Saprolegnia*, in particular, the species *S. australis*, *S. diclina*, and *S. parasitica* [3]. Infected fish are easily recognized by the cotton-like white to greyish patches on the skin and gills [4]. However, oomycetes could infect not only adult salmonids, but also fish embryos and larvae [5]. The destructive economic impact of saprolegniasis on freshwater aquaculture necessitates further study on the range of *Saprolegnia* species within salmonids farms [3]. Because it is the only way to discover how to prevent the spread of saprolegniasis.

The study was carried out to assess the toxic effects of different Saprolegnia species oomycetes on *Salmo salar* larvae. Changes in activity of oxidative stress biomarkers, such as superoxide dismutase (SOD) and catalase (CAT), were detected in this study.. CAT was one of the first enzymes proposed to be an effective biomarker of oxidative stress, which protects tissues against damage by hydrogen peroxide [6]. SOD is a group of metalloenzymes that plays a crucial antioxidant role and constitutes the primary defence against the toxic effects of superoxide radical in aerobic organisms [7].

In this study, results showed changes in catalase activity in *S. salar* larvae, but no significant differences were observed after exposure to oomycetes. Significant changes in SOD activity in *S. salar* larvae were detected after exposure to *S. australis* oomycetes. The obtained results show changes in biomarkers of oxidative stress following exposure to oomycetes. However, further extensive investigations of antioxidant enzymes and other indicators are required to better understand the impact of oomycetes on salmonid fish.

Acknowledgments

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VIRUS INFECTION CYCLE OF CYANOBACTERIA *APHANIZOMENON FLOS-AQUAE* AND CYANOPHAGE vB_AphaS-CL131 SYSTEM RESEARCH

Augustė Matiukaitė[1], Sigitas Šulčius[2]

[1]Nature Research Center, Lithuania
[2]Life Science Center, Vilnius University, Lithuania
auguste.matiukaite@gmc.stud.vu.lt

Aphanizomenon flos-aquae is a filamentous, bloom-forming, cyanobacteria commonly found in freshwater environment, Baltic Sea, and Curonian lagoon. Intensive growth of this cyanobacteria results in a process called algal-bloom and even could be responsible in some toxin production [1]. In this study, *Aphanizomenon flos-aquae* 2012/KM1/D3 strain and cyanophage vB_*AphaS-CL131* system collected in Curonian lagoon is used, to understand the impact of viral infection not only in host dynamics but also in all aquatic environment. This cyanophage has an isometric head, 97 nm in diameter and a long, flexible non- contractile tail, 361 nm long. With a genome size of ~120 kb, it is the second largest cyanosiphovirus isolated to date [2].

This study aim is to understand the dynamics of host and cyanophage to be a step further finding a solution for algal blooms in aquatic environment. The infection cycle of *vB_AphaS-CL131* has been studied by applying adsorption test method (Fig. 1). By using different multiplicity of infection (MOI) values we tried to figure out when all the infection cycle stages (phage adsorption, replication, and cell lysis) occur.

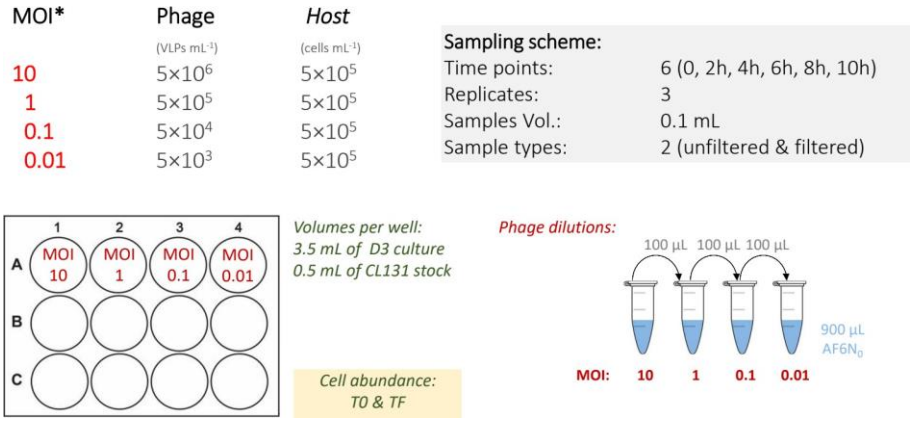

Figure 1. Adsorption test sampling scheme

Samples were examined using real time PCR method and these are following results: adsorption phase was dependent on MOI: in samples with MOI1.0 phage adsorbed in approximately 30 minutes, MOI0.1 – 60 minutes and MOI0.01 – 90 minutes. Cell lysis phase was similar in all the samples – 900 minutes (15 hours).

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SALMO TRUTTA POPULATION RESPONSE TO A DAM REMOVAL IN BRAZUOLE STREAM, LITHUANIA

SIMONAS RAČKAUSKAS[1], VYTAUTAS RAKAUSKAS[1], TOMAS VIRBICKAS[1]

[1] Nature Research Centre, Akademijos st.-2, LT-08412 Vilnius, Lithuania
simonas.rackauskas@gamtc.lt

For decades, dams on the small rivers served diverse purposes from resource management to energy production and flood control. However, these structures have negative consequences for native ecosystems, particularly on the populations of native fish species, such like brown trout (*Salmo trutta*) [1]. The first man-made riverine barrier in Lithuania was demolished in the Brazuole stream in 2020. The main aim of the present study was to investigate Brazuole stream *Salmo trutta* population dynamics before and after dam removal.

The study was conducted in the Lithuania's lowland Brazuole river, 23 km long third order tributary (Nemunas, Neris, and Brazuole river) of the Nemunas river (Baltic Sea). To analyze fish communities abundance and composition a continuous single run electrofishing survey using the same backpack unit with the same electric current settings was undertaken few days before dam removal in 2020 and repeated in the end of summer in 2021 and 2023. Fishes sampling was performed under typical flow condition in each of the studied years when total length (TL thereafter) to the nearest mm and weight (W thereafter) to the nearest 0,1 g were recorded. All fish individuals after analysis were released at the same point of capture. Fish density N (ind.) and biomass B (kg) were calculated and extrapolated for 100 m^2 (ind./100 m^2 and kg/100 m^2. Fish community diversity (H') was calculated using Shannon-Weiner diversity index.

The results showed that *Salmo trutta* density ratio between the monitoring points comparing 2020 and 2023 years went closer to each other more than 4 times (Fig. 1 A). After an increase of *Salmo trutta* density comparing 2020 and 2021 years above and below dam site, it dropped twice comparing 2021 and 2023 years (Fig. 1 A). *Salmo trutta* biomass ratio between the same monitoring points through years didn't change noticeably, but drooped twice comparing 2020 and 2021 years with 2023 (Fig. 1 B). Shannon-Wiener Diversity index between 2020 and 2023 years showed that there were considerable ratio differences and even a decline in index value (Fig. 1 C).

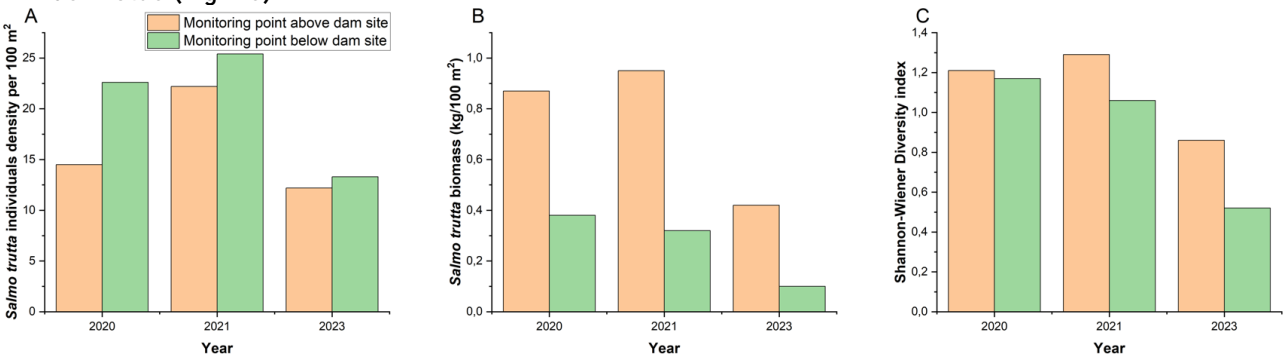

Figure 1. *Salmo trutta* individuals N (A), B (B) and fish community H' (C) above and below dam monitoring sites, before and after Brazuole stream artificial dam removal.

Despite an increase in *Salmo trutta* density, biomass, and the Shannon-Wiener Diversity index in 2021, these metrics declined in 2023. Usually in such salmonid streams like the Brazuole, the main individuals are typically 0+ or 1+ years old [2]. Therefore, this decrease could be related to the absence of artificial *Salmo trutta* stocking in 2022. Brazuole is a lowland stream, and it is very possible, that after artificial dam removal in 2020 it needs at least a decade or more time to recover [3] and further monitoring needs to be performed.

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MONKEY GOBY (*NEOGOBIUS FLUVIATILIS* PALLAS, 1814) DIET IN THE NEWLY INVADED NEMUNAS BASIN

Ieva Savickaitė[1], Vytautas Rakauskas[1]

[1] Nature Research Centre, Akademijos st.-2, LT-08412 Vilnius, Lithuania
ieva.savickaite@gamtc.lt

The monkey goby (*Neogobius fluviatilis* Pallas, 1814) is an invasive fish from the Ponto-Caspian region that has rapidly spread in European waters only in recent decades [1]. In Lithuania *N. fluviatilis* was first discovered in 2015 in the river Neris on the border with Belarus, where it entered through an artificial canal system, connecting the catchment of Neris and Berezina rivers (the right tributary of the Dnieper) [2]. Although almost a decade has passed since *N. fluviatilis* entered the Nemunas basin, there is still very little knowledge about the impact of this invasive species on native ecosystems.

The purpose of this study was to determine the diet of *N. fluviatilis* and to compare the dietary spectrum with native fishes found in similar habitats. Three sites with different biotopes were selected for the samples of monkey gobies gut content analysis – one site standing water in Kaunas water reservoir, slow-flowing river Nemunas and faster-flowing river Neris. Fish were caught in 2021 and 2022 using electrofishing in rivers and beach seine in Kaunas water reservoir. The diet of *N. fluviatilis* and the native fish – gudgeon (*Gobio gobio*) – were compared, since they occupy similar habitats. Relative importance index (IRI) was used to determine the importance of food items, Schoener's index was used to determine nutritional overlap.

A total of 272 *N. fluviatilis* and 60 *G. gobio* stomachs were analysed. Analysis of the stomach contents showed that the diet of *N. fluviatilis* is dominated by the larvae of Chironomidae in standing water, in rivers *N. fluviatilis* also feed on Chironomidae larvae, but a large part of the diet consists of bivalve molluscs (Sphaeriidae), mayflies (Ephemeridae, Baetidae) and net-spinning caddisflies (Hydropsychidae) larvae. It was found that the feeding niches of *N. fluviatilis* and *G. gobio* overlap by 52.4%. It is relevant to mention that smaller fish has been detected in the stomachs of several gobies, suggesting that monkey gobies may have a direct impact on native fish communities.

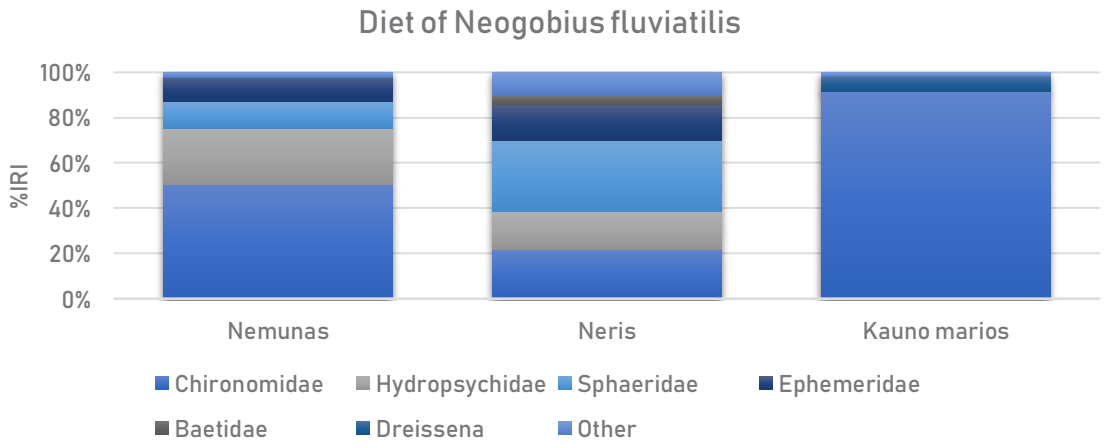

Figure 1. Percentage of relative importance index (%IRI) of *Neogobius fluviatilis* diet in different habitats.

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VARIATION IN PLANT BIOMASS BETWEEN TWO AQUATIC PLANT SPECIES OF GENUS NAJAS

Kamaitytė-Bukelskienė L.[1], Sinkevičienė Z.[1], Butkuvienė J.[2]

[1] Nature Research Centre, Laboratory of flora and geobotany, Lithuania
[2]Institute of Biosciences, Life Sciences Centre, Vilnius University, Lithuania
liucija.bukelskiene@gamtc.lt

Different species exhibit a wide range of growth rates and strategies, influenced by a complex of biotic and abiotic factors within their respective environments. Understanding these dynamics is a valuable in the assessment of species ecology, providing invaluable insights essential for the management of both aggressively spreading invasive species as well as rare and vulnerable ones [1]. Differences in growth patterns can often we quite visual, even between species that are closely related or occupy similar ecological niches. In aquatic environments, plant growth is subject to considerable variation, influenced by factors such as light and nutrient availability, as well as interspecific and intraspecific competition [2]. Therefore, understanding the dynamics of plant growth in aquatic habitats is important for elucidating ecological processes and improving plant management strategies. In this context, our research aims to investigate differences in the accumulated plant biomass of two annual aquatic plant species, *Najas marina* L. and *Najas major* All. in different depth zones, to gain knowledge about species dynamics and expansion within aquatic habitats.

During the fieldwork carried out between August and September 2022-2023, we collected plant biomass samples measuring 0.16 m² from six lakes containing communities of *Najas marina* and *Najas major*, using a Bernatowicz sampler. Samples were collected at each metre depth in three transects extending from the shoreline to the maximum depth of plant growth. The biomass samples were then carefully sorted by species and air dried in the laboratory. Each sample was evaluated to determine both mass and species composition.

Primary data analysis revealed distinct associations between *Najas marina* and *Najas major* in different species assemblages within the sampled lakes. Specifically, *Najas marina* was found predominantly in associations with stoneworts, whereas *Najas major* tended to grow solitary or in association with floating-leaved plants. Despite these ecological differences, conducted Mann-Whitney U test did not reveal any significant differences in collected plant biomass samples of *Najas marina* and *Najas major* across different depth zones.

Future studies using different designs are recommended to help reveal clearer ecological patterns.

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E6

INVESTIGATION OF POSSIBLE VECTOR-BORNE TRANSMISSION OF *SARCOCYSTIS* PARASITES

Aistė Stundžėnaitė[1,2], Agnė Baranauskaitė[2], Petras Prakas[2], Elena Servienė[2], Pavel Starkevič[2], Živilė Strazdaitė-Žielienė[2]

[1]Vilnius University, Vilnius, Lithuania
[2]Nature Research Centre, Vilnius, Lithuania
aiste.stun@gmail.com

All over the world, various insects are known as vectors that can transmit a wide variety of infections. One of the best-known infections is the mosquito-borne disease malaria, which is caused by the unicellular parasite *Plasmodium* [1]. Moreover, studies conducted in recent years show rapid changes in the epidemiology and distribution of zoonotic vector-borne pathogens. Ever growing occurrence of outbursts of parasitic diseases is affecting not only animals but also humans, since with the help of insects they can overcome long distances and various environmental conditions [2]. However, there is still a lack of research on the ability of many parasitic species to use insects as vectors. Therefore, the aim of this work was to investigate whether parasites of the genus *Sarcocystis* can be transmitted by insects using molecular-based methods.

During the work, a sample of insect washings and a sample of crushed insects were prepared. The collected insects were washed with sterile distilled water in a glass flask at room temperature for about 1 hour by shaking. After that, the outwashes were concentrated using the filtration method [3] and applied for genomic DNA (gDNA) extraction. The remaining insects were crushed using a mortar and pestle and stirred for 30 minutes with sterile distilled water. The crushed insects were removed from the sample, the remaining liquid was centrifuged, and collected sediment was used for gDNA extraction. Universal primers were used to detect the DNA of *Sarcocystis* parasites. PCR-amplified DNA fragments were cloned into pJET cloning vector with the following selection of positive transformants, plasmid DNA isolation, and sequencing.

Based on the sequencing data, the DNA of two *Sarcocystis* species infecting cattle - *Sarcocystis cruzi* and *Sarcocystis bovifelis* - was found on the investigated insects. These results suggest that insects may be vectors of *Sarcocystis* parasites. However, further research is needed to investigate the transmission of these parasites and to determine whether the transmission takes place on the body of the insect or through the intestines.

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THE DISTRIBUTION OF ANTIBIOTIC-RESISTANT BACTERIA IN LITHUANIAN FARMLAND ECOSYSTEMS

Alper Melih Ucar[1,2], Juliana Lukša[1], Elena Servienė[1]

[1]Nature Research Centre, Vilnius, Lithuania
[2]Department of Chemistry and Bioengineering, Vilniustech, Lithuania
alper-melih.ucar@stud.vilniustech.lt

Bacteria are ubiquitous in diverse environments, exerting significant influence as one of the most abundant microorganisms in nature. In contemporary agricultural landscapes, the microbial dynamics within pastoral ecosystems are pivotal for understanding the overall ecological conditions and potential threats to animal and human health. Approximately 243 of the 616 pathogens (39%) currently known to infect livestock are also infectious to humans [1].

This study aims to investigate the distribution of antibiotic-resistant bacteria (ARBs) in Lithuanian farmland ecosystems, focusing on soil, feed, and freshwater. Samples were collected from four different pastoral dairy farmland locations around Lithuania. The identification of ARBs was based on the combination of cultural and molecular methods. Collected microorganisms were cultivated on selective LB media with four different antibiotics (Ampicillin, Streptomycin, Tetracycline, and Chloramphenicol). A total of 58 bacterial strains showing resistance against antibiotics were morphologically analyzed, followed by PCR amplification of the V1 to V3 regions of 16S rRNA genes using universal primers. Restriction fragment profiling of the amplified regions using multiple restriction enzymes was conducted. Eventually, bacteria strains were grouped by morphological features and their restriction pattern. Each group's representative's PCR products were purified with a commercial purification kit, and sequenced using the Sanger sequencing method, and identified using NCBI BLAST.

The investigation revealed a higher prevalence of ARBs in soil samples with 26 strains, comprising almost half of all observed bacteria. High resistance was revealed to both Ampicillin and Streptomycin compared to other antibiotics. Where 48 % of strains were showing resistance against Ampicillin and 43 % of strains against Streptomycin. This number is significantly lower with four strains in the case of Tetracycline and only one stain in Chloramphenicol.

After identification, several bacteria genera that are considered non-pathogenic or opportunistic to humans and animals were found in soil, water, and feed, including *Solibacillus*, *Enterobacter*, *Acinetobacter* in soil, *Pantoea* in feed, and *Chryseobacterium* in water. However, some potentially pathogenic genera to both humans and animals, for instance, *Bacillus* was also identified in soil samples.

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E8

IDENTIFICATION OF ENDOPHYTIC FUNGI IN *FESTUCA GIGANTEA* AND *LOLIUM PERENNE* SPECIES AND THEIR HYBRIDS

Saulė Matijošiūtė[1,2], Violeta Stakelienė[1,2], Justas Martūnas[1], Izolda Pašakinskienė[1,2]

[1] Botanical Garden of Vilnius University, Kairėnų 43, 10239 Vilnius, Lithuania
[2]Life Sciences Centre, Vilnius University, Saulėtekio 7, 10221 Vilnius, Lithuania
saule.matijosiute@bs.vu.lt

Endophytic fungi inhabit the inner tissues of foliar parts and roots of plants. These endophytes protect the plants from both biotic and abiotic stress and enhance the uptake of nutrients, including phosphorus. Their capacity to stimulate plant growth positions them as a promising substitute for chemical fertilizers. Many studies show that most grasses are symbiotic with endophytic fungi, but roots, the hidden parts of plants, are less studied compared to above-ground organs. This work was aimed to identify endophyte fungi in the roots of herbaceous plants, *Festuca gigantea*, *Lolium perenne* and *L. perenne* × *F. gigantea* hybrids. Endophytic fungi were isolated from ~1 cm root tip cuttings on PDA medium by incubation at 27℃ in the dark. For the isolation of the genomic DNA of endophytic fungi, 10-day-old fungal colonies were used. The DNA assessment was based on ITS, *RPB2*, *TEF1-α*, *BTUB*, *ACT* sequences aligned to fungi reference DNA data by BLAST. The fungal isolates were characterized by their morphological characters, including structure, color, and colony edge. A mixture of glycerol and lactic acid (1:4) was used for fungal mycelium microphotography. Three species of Ascomycota, *Cadophora fastigiata*, *Plectosphaerella cucumerina* and *Paraphoma fimeti*, were obtained in culture from the roots of *F. gigantea* and *L. perenne* × *F. gigantea* hybrids. Microscopically, the highest frequency of endophytic fungi, up to 90%, was found in *L. perenne* × *F. gigantea* hybrids, compared to the frequency in parental *L. perenne* (74%) and *F. gigantea* (46%) species. Standardized ITS and *RPB2* sequences were more reliable for the identification of endophytic fungal species, compared to *TEF1-α*, *BTUB,* and *ACT* sequences. In this study, we discovered the occurrence of endophytic fungi in the roots of *F. gigantea* and hybrids for the first time and confirmed their taxonomy at the species level, which to our knowledge is not available in the literature. In addition, in the experiment of seed inoculation by fungal spore suspension, we demonstrated that inocula of *P. cucumerina* and *C. fastigiata* have a positive effect on the root growth of spring barley plants.

E9

Endophytic fungi in the roots of *Festuca* and *Lolium* species and their hybrids

Violeta Stakelienė[1,2], Justas Martūnas[1], Saulė Matijošiūtė[1,2], Izolda Pašakinskienė[1,2]
[1] Botanical Garden of Vilnius University, Kairėnų 43, 10239 Vilnius, Lithuania
[2]Life Sciences Centre, Vilnius University, Saulėtekio 7, 10221 Vilnius, Lithuania
violeta.stakeliene@bs.vu.lt

Endophytic fungi are associated with almost all plants found in natural ecosystems. Their necessity in the formation of plant communities became evident when their impact on plant growth was discovered. They can affect overall plant growth by regulating stress resistance mechanisms, nutrient uptake, photosynthesis and phytohormone levels. Many endophytic fungi that inhabit plant tissues have been documented so far but data on root endophytic fungi of *Festuca* and *Lolium* (Poaceae) plants are still lacking. Plants of the genera *Festuca* and *Lolium* are the dominant species of grassland communities. This study aimed to determine the prevalence of endophytic fungi in the roots of *Festuca gigantea*, *Festuca arundinacea*, *Festuca pratensis*, *Lolium perenne*, *Lolium multiflorum*, *Lolium temulentum* and *Lolium perenne* × *Festuca gigantea* hybrids. We estimated the frequency of endophytic fungi in cytological root samples by staining them with Trypan Blue (0.025%). We found that endophytic fungi colonize 44-56 percent of the roots in perennial *Festuca* and *Lolium* species and 84-94 percent of the roots of annual *L. multiflorum* and *L. temulentum*. 200-250 root fragments (1-2 cm long) were used to isolate endophytic fungi on PDA medium by incubating at 27℃ in the dark. 10-day-old fungal colonies were used for genomic DNA isolation. The sequence data of rDNA ITS, TEF, SSU, and RPB2 were obtained and aligned to fungi reference DNA by BLAST. The morphological assessment of the mycelium of fungi was carried out by describing the color and structure. Microscopically, mycelium specimens were analyzed in glycerol : lactic acid (1:4) under the phase contrast microscope. We found that endophytic fungi were more common in the roots of annual *L. temulentum* and *L. multiflorum*; and the highest abundance was recorded in *L. multiflorum*. Ten different fungi species were isolated from *L. multiflorum* roots. In total, in culture, we obtained and identified 22 species of endophytic fungi, members of Ascomycota (18), Basidiomycota (2) and Zygomycota (2). The occurrence of *Microdochium bolleyi* fungus in culture was the most prevalent from the roots of all studied plants, except for *L. perenne* and *F. pratensis*.

E10

ISOLATION AND IDENTIFICATION OF MESOPHILIC BACTERIA FROM UNDERINVESTIGATED ENVIRONMENTS

Galkutė E.[1], Šimoliūnas E.[1], Šimoliūnienė M.[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Centre, Vilnius University, Saulėtekio al. 7, Vilnius, Lithuania
emilija.galkute@gmc.stud.vu.lt

Bacteria are widely spread in every possible place in the world. They have conquered deep and dark places like the bottom of the seas, can survive in various soils and in other living organisms. Bacteria can be divided into groups by the temperature at which they exist. Mesophilic bacteria are adapted to grow in temperature range from 20°C to 40°C, and this feature makes them attractive tools for food industry, agriculture and even in biodegradation processes. On the other hand, most human pathogens are also mesophilic bacteria. Thus, it is important to search for new bacteria species, identify them and look for useful properties or ways to combat them.

In this study, environmental samples were taken from the mud of the gypsum karst lake Kirkilai (Biržai, Lithuania), from the excrement of the lynx (lot. Lynx lynx), as well as from the compost heaps. In total, eight bacterial strains were isolated and identified based on the results of 16S rRNA gene fragment sequences. It was demonstrated that isolates belonged to phylogenetically distinct groups of the bacteria from the genera *Bacillus*, *Microbacterium*, *Aeromonas*, *Exiguobacterium*, *Pseudomonas*, *Enterococcus*, and *Micrococcus*.

The results of this study not only expand our knowledge about microorganisms present in underinvestigated environments but also provide an opportunity for further research of biotechnologically attractive bacterial strains (*Bacillus* spp., *Exiguobacterium* spp.) or potential pathogens (*Aeromonas* spp., *Pseudomonas* spp., *Enterococcus spp.*).

E11

DECOMPOSITION OF FOOD WASTE USING ORGANIC MATERIAL ADDITIVES AND ITS USE IN THE PRODUCTION OF HIGH-QUALITY BIOGAS: A REVIEW

Austėja Roberta Bojarinaitė[1], Alvydas Zagorskis[2]

[1]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University
[2]Associate Professor of the Department of Environmental Protection and Water Engineering, Vilnius Gediminas University of Technology
austeja-roberta.bojarinaite@stud.vilniustech.lt, alvydas.zagorskis@vilniustech.lt

In recent decades, many climate problems such as air pollution and climate warming have arisen due to human existence. With the rise in oil prices and depletion of natural energy sources, humanity must find alternative solutions and nurture them. As the number of people in the world increases, so does the consumption of plant- and animal-based foods, resulting in a large amount of food waste. Renewable energy resources are one of the most promising areas that can reduce pollution and the use and import of fossil fuels. Biogas production is one of the renewable energy use alternatives. Large amounts of food waste can become a potential energy value. Using biodegradable waste in biogas production creates an opportunity to reduce climate change and air pollution. In order to improve the decomposition of organic waste and the production of biogas, it is recommended to add organic materials as additives. One such material is zeolite, an ecologically clean, inert and non-toxic material. Zeolite is resistant to high temperature, aggressive environment, the effects of ionizing radiation, selectivity for large cations of alkaline, alkaline earth and some heavy metals.

Two-staged bioreactor can be used to transform food waste into biogas, it is breakthrough in the field of renewable energy technology. This process can provide increased energy efficiency and process stability.

The literary analysis will aim to clarify how it is possible to improve the decomposition of food waste using organic material additives and their use to produce high-quality biogas.

SPECIES DISTRIBUTION MODELING OF UNDERSAMPLED PREDATORY ARTHROPODS: HOW LITTLE IS TOO LITTLE?

Milda Riepšaitė[1], Maria Fernanda Torres Jimenez[2]

[1,2]Department of Zoology, Vilnius University Life Sciences Center, Lithuania
milda.riep@gmc.stud.vu.lt

Species distribution models (SDMs) can be used to estimate current and future patterns of biodiversity and to help discover new localities of known species. One of the key requirements for robust SDMs is wellsampled and abundant species occurrence data. This is not always possible for understudied, rare, or endangered species that often have little distribution data available. Consequently, SDMs that are trained on sample sizes that are too small, tend to perform poorly, lead to incorrect assumptions, and have the potential to misdirect conservation efforts [1]. This problem is particularly prevalent when it comes to small arthropods, such as spiders (order Araneae) [2]. Despite being abundant and highly diverse generalist predators, spiders are rarely included in research and conservation efforts across Europe [3], making it difficult to protect rare or vulnerable species and estimate their distribution.

In this study, we investigate tree based SDM performances across a gradient of presence data availability for peat bog generalist and specialist spiders in Europe. We aim to: estimate the minimum range of presence records required for accurate distribution models; evaluate whether model performance depends on spider habitat specialization; identify which modeling method is most suitable for spiders with small sample sizes.

Using publicly accessible presence-only occurrence spider data from the Global Biodiversity Information Facility (GBIF) and recently collected data from Lithuania, we compiled a spider occurrence dataset comprising five specialist and five generalist European peat bog spider species, integrating it with bioclimatic variables and habitat classification from satellite data. Tuned random forest (RF) and generalized boosted regression models (GBM) were used to fit 1200 models along decreasing sample sizes from 300 to 10 observations. Model accuracy was evaluated using the area under the receiving operator curve (AUCROC) and the precision-recall area under the curve (AUC-PRC).

We found that the minimum number of presences needed for robust SDMs ranged between 50-75 observations for RF and 50-125 for GBM, with models for specialist spiders leading in predictive performance and RF models performing better than GBM. Our results show that, with consideration to their ecology and model selection, both habitat specialist and generalist spider distribution can be predicted using SDMs even at relatively small sample sizes. This study provides new insights into tree based SDMs for under-sampled invertebrate groups and helps inform conservation and research planning.

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E13

THE EVALUATION OF ENVIRONMENTAL NEURODEGENERATIVE EFFECTS IN MALE RAT PUPS

Laurynas Orla[1], Edita Paulikaitė[1], Rasa Aukštikalnienė[1], Urtė Neniškytė[1], Violeta Žalgevičienė[2], Grita Skujienė[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
[2]Department of Anatomy, Histology and Anthropology, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Lithuania
laurynas.orla@gmc.stud.vu.lt

Phthalates are diesters of 1,2-benzenedicarboxylic acid (phthalic acid). Di(2-ethylhexyl)phthalate (DEHP) and dibutyl phthalate (DBP) are the most commonly used phthalates in industry. Phthalates have been shown to affect the endocrine system [1]. They are found only in micrograms in nature, but if released into water, they can pose a serious health risk to humans and ecosystems. **The aim of this project** was to assess the effects of two types of phthalates (which are consistently above the maximum permissible concentration in Lithuanian waters) on hippocampal CA1 and CA3 region (Fig. 1) thickness and neuronal density in rat male pups. Rat male pups for the study comes from the study carried out on 2022-08/09, which was reviewed and approved by the Ethics Committee for Animal Experiments of the State Food and Veterinary Service (No. G2-221 of 08/09/22).

36 female rats of the Wistar strain, 5-8 weeks old, were divided into control and 5 experimental groups, which received phthalates for 3,5 months: 2 months before pregnancy, and a further 1,5 months throughout pregnancy and lactation. They received standard food with additional piece of an ecological biscuit infused with different doses of phthalates dissolved in olive oil: 1) DEHP 200 µg/kg; 2) DEHP 1000 µg/kg; 3) DBP 100 µg/kg; 4) DBP 500 µg/kg; 5) mixture of phthalates (DEHP 200 µg/kg, DBP 100 µg/kg). Control animals received only a piece of the same biscuit with oil and without phthalates. After 2 months, females were mated with unaffected males. At the postnatal day 21 young male pups were selected and euthanised in a CO2 chamber. The brain was collected and placed in formaldehyde. The brains were cut in half, one half was embedded in paraffin, cut into 5 µm-thick sections, and stained with Nissl dye, where nuclei and Nissl bodies (rough endoplasmic reticulum) are stained blue purple with cresyl violet.

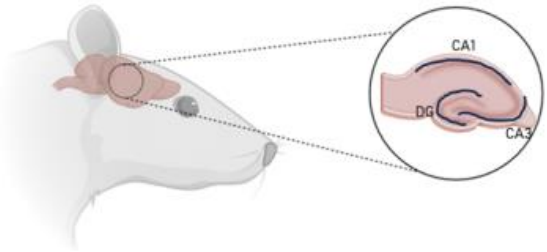

Figure 1. Schematic illustration of the hippocampus region in the rat brain, drawn with Biorender.com.

Neuronal density was counted in three sections per animal in the CA1 region in 100 x 200 µm2 and in the CA3 region in 200 ´ 200 µm2 squares [2]. For the measurement of the thickness of the regions, 10 lines per hippocampus were drawn in three sections per animal [3].

It was found that DEHP 1000 µg/kg, DBP 100 µg/kg, DBP 500 µg/kg and a mixture of phthalates have toxic effects on the hippocampus of rat pups. Statistically significant decreases in neuronal density and in the thickness of the CA1 and CA3 hippocampal regions were observed.

Acknowledgments: We would like to thank dr. Virginija Bukelskienė for help in organizing the research, medical students Justina Alčauskaitė and Evita Sėrikovaitė for assisting in the experiment. The project was financed by the Science Promotion Fund for Scientific projects of Vilnius University, agreement No MSF-JM-18/2022.

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ECOLOGY OF IMMATURITY: AGE DEPENDENT MORTALITY PATTERN IN TWO SPECIES OF ACCIPITRIFORMES

James Firkins[1], Deivis Dementavičius[2], Gintarė Grašytė[3], Saulius Rumbutis[1,2], Ričardas Patapavičius[2], Rimgaudas Treinys[1]

[1]Nature Research Centre
[2]Kaunas Tadas Ivanauskas Zoological Museum
[3]State Service for Protected Areas under the Ministry of Environment
james.firkins@gamtc.lt

Populations of raptors in Europe recovered at the end of the 20th century following heavy decline due to contaminants and persecution in the past [1], however, some species or populations in certain regions continue to face pressure from human induced mortality today. Causes of mortality may vary between, and within, raptor species and populations, therefore, it is important to understand reasons and patterns for mortality in a particular region because knowledge derived from elsewhere may not be applicable for the target population. Typically anthropogenic factors result in greater mortality of young individuals compared to adults within the same population [2] and different acting factors may be age dependent. Therefore understanding factors and age dependent effects on mortality is crucial for effective population conservation. We studied mortality patterns in White-tailed Eagle (n = 94) and Osprey (n = 45) in the eastern region of the Baltic Sea, Lithuania. Most deaths were human related with a majority caused by electrocution, and more frequently for Ospreys than White-tailed Eagles. While patterns of mortality did not significantly differ between sexes and seasons, the causes of mortality significantly differed between adults and younger birds in both species. However, the pattern of mortality was unique for each species contrary to our expectations (see **Figure 1**). Namely, human related mortality was more frequent in young individuals than adult individuals of White-tailed Eagle, however the opposite was true for Osprey. Additionally, White-tailed Eagles death recoveries were spatially aggregated with several hotspots, but deaths of Ospreys were randomly distributed throughout the region. Most likely, species-specific mortality patterns emerged because of behavioural and life history differences despite association of both raptors with aquatic habitats. We estimate that age-dependent mortality patterns formed as a result of changes in movement behaviour during maturation and, for White-tailed Eagle especially, an increase in territoriality post-maturation. This indicates that species-specific behavioural traits in adult raptors may outweigh individual experience at early life stages in susceptibility to specific mortality factors. Our results suggest that conservation efforts should be focused on dealing with the main threats for each species, but also could benefit from prioritisation of efforts in most problematic areas for raptor species if the clustered mortality pattern is evident.

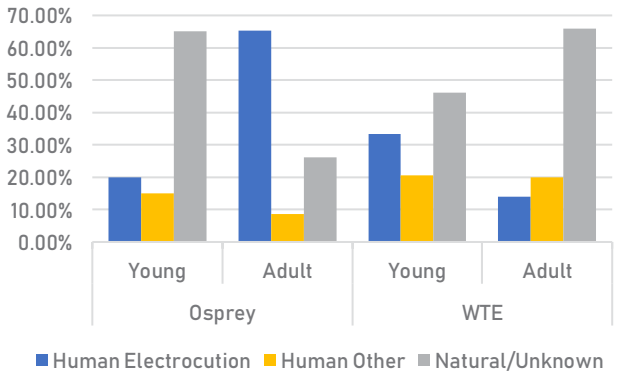

Figure 1. Causes of mortality among young and adult individuals of Osprey *Pandion haliaetus* and White-tailed Eagle *Haliaeetus albicilla* in the eastern region of the Baltic Sea.

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EFFECTS OF FLOWERING STRIPS AND LANDSCAPE STRUCTURES ON BIRDS IN INTENSIVELY FARMED AGRICULTURAL LANDSCAPE

Tedas Laurinaitis[1], Saulis Skuja[1]

[1]Institute of Biosciences, Life Sciences Centre, Vilnius University, Vilnius, Lithuania
tedas.laurinaitis@gmc.stud.vu.lt

Agriculture has been practiced in Lithuania for a long time and changes in land areas are confirmed by the statistical data of the 70-year period (1946-2016): arable land areas increased by 4.5 percent, while the decrease in the areas of grassland and natural pastures since 1946 reaches as much as 74.9 percent [1]. Although the European Union and Lithuania have set goals to stop the loss of birds in agricultural landscapes, the data collected during the long-term research of the Lithuanian Ornithological Society (LOD) Common Bird Population Monitoring Project shows that the abundance of farmland bird is decreasing [2].

The aim of this research – to examine the presence of farmland bird species in Kupiškis District Municipality, in order to derive the extent of flowering strips and other measures for the breeding birds are useful and beneficial. The positive example should later serve as a model that could be adopted on a larger scale by other farms.

We conducted the research of birds in the selected farm (owner Zigmantas Aleksandravičius) of the BASF Farm Network in Kupiškis District Municipality, Lithuania. A total of 2-areas were studied: the main area – Juodpėnai-Šileikiai (130 ha) included flowering strips and continued through several farmers' fields, and control field – Puponys II (140 ha) had no flowering strips. Six bird counts were carried at regular intervals every year between April and June 2021-2023. The breeding bird counts were made in the period from 1st to 15th and from the 16th to the end of every month at least 5 days apart in the same study site. A modified mapping-point count methodology was used for bird counts [3].

There were 1419 individuals and 68 bird species recorded in 2023. The Shannon index was calculated as a measure of diversity and Evenness as a measure of distribution (Fig. 1). The highest diversity in 2023 was reached in the Juodpėnai-Šileikiai (H = 3.16), the lowest Shannon (H = 2.09-2.47) and Evenness (E = 0.67-0.76) indices were found in Puponys II field.

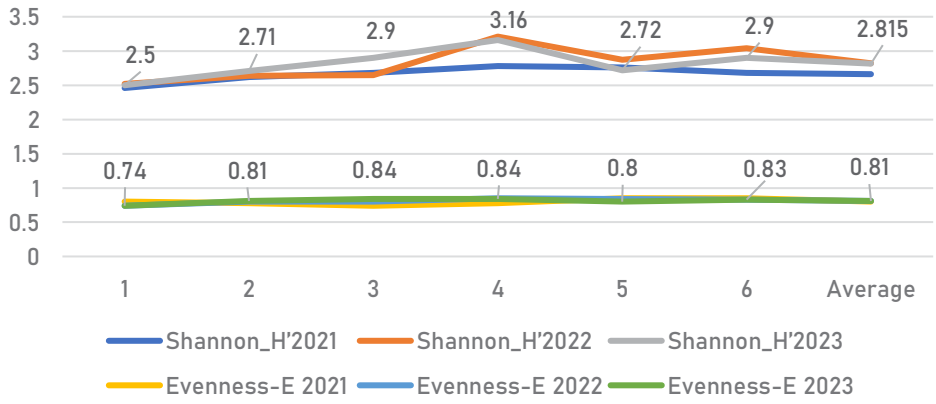

Figure 1. Shannon diversity and Evenness indices for comparing of bird species per studied Juodpėnai-Šileikiai site during different bird counts.

The results of three years research confirm that flowering strips affects the maintenance of species diversity and abundance: 1) farmland bird diversity and abundance is significantly lower in the control area; 2) a total of 9 (of 14) Lithuania farmland bird indicator species moderately increased in Juodpėnai-Šileikiai. The population of 3 breeding bird species (*A. pratensis, M. flava, S. rubetra)* slightly decreased, most possibly due to loss of grassland area in the Juodpėnai-Šileikiai research site.

Acknowledgments: We would like to thank dr. Grita Skujienė for consultations and direct help conducting research. Financial support was by the VU-BASF R&D-SERVICE AGREEMENT No. (1.57) 15600-INS-43.

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E16

FUNGUS – INSECT INTERACTION: ASPERGILLUS FLAVUS VOLATILES INFLUENCE BEHAVIOUR OF YEALLOW MEALWORM (TENEBRIO MOLITOR L.)

Ieva Lučinskaitė[1], Gabrielė Bumbulytė[2]

[1]Life Sciences Center, Vilnius University, Lithuania
[2]Laboratory of Chemical and Behavioural Ecology, Nature Research Centre, Lithuania
ieva.lucinsk@gmail.com

The escalating global human population necessitates multifaceted strategies to mitigate environmental pollution. Insects, long regarded as an alternative to conventional meat (such as pork, beef, or chicken) in Asian cultures, offer several advantages [1]. Their rapid growth, minimal environmental impact, and rich protein content position them as an excellent protein source.

Among the most widely recognized edible insects worldwide, the mealworm (*Tenebrio molitor*) stands out. Its ease of cultivation and substantial biomass gain contribute to its favorable nutritional profile. To maintain optimal production hygiene, efforts are underway to distinguish between live and deceased insects. One promising approach involves leveraging natural repellents found in the insects' native habitat—cereals. For instance, the microscopic fungus *Aspergillus flavus* colonizes mealworm food substrates, suggesting that volatile compounds produced by this fungus could serve as natural repellents.

This study aimed to assess the impact of volatile compounds generated by *Aspergillus flavus* on adult mealworm beetles. The investigation employed a two-choice pit-fall test, utilizing vials containing control grains (no stimulus) alongside vials containing *Aspergillus flavus*–infected grains at different ages (0, 5, 10, 20 days). A Petri dish served as the experimental arena.

Additionally, the study evaluated the effects of *Aspergillus flavus* compounds reported in the literature. Behavioral experiments were conducted in Petri dishes using a two-choice assay. Beetle behavior was meticulously tracked using the computer program EthoVision XT 12 (developed by Noldus, the Netherlands). Observations spanned 5 minutes, with 10 replications performed for each tested compound. Chemical compounds were applied to five spots on the side of the Petri dish (2 µl each, totaling 10 µl).

The findings indicate that female mealworm beetles detected the presence of *Aspergillus flavus* from the very onset of infection (day 0), whereas male beetles began distinguishing the infected grain only from day 5 onward. Notably, the study identified the most potent compounds, which will be unveiled in the forthcoming presentation.

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E17

OVIPOSITION-MODULATING COMPOUNDS FOR TENEBRIO MOLITOR FEMALE

Arijus Auškalnis[1], Gabrielė Bumbulytė[2]

[1]Life Sciences Center, Vilnius University, Lithuania
[2]Laboratory of Chemical and Behavioural Ecology, Nature Research Centre, Lithuania
arijusaus@gmail.com

As global population projections surge toward 10 billion by 2050, the demand for food—particularly animal-derived protein—intensifies [1]. However, this growth must align with environmental and economic sustainability. Yellow mealworms (*Tenebrio molitor* L.), notorious for causing substantial losses in agriculture, also serve as a prolific source of larvae produced on an industrial scale for both human and animal consumption. These larvae boast high protein content, rich nutrient profiles, and low-fat levels, positioning them as a sustainable alternative to traditional meat sources. Importantly, mealworm production and consumption exert a lesser environmental footprint compared to conventional meat.

For insect farmers aiming to maximize biomass yield, understanding the nutritional and chemical composition of feed is paramount. However, numerous unanswered questions persist regarding large-scale insect farming. Among these, egg quantity and collection play a pivotal role. To address this, we investigate the impact of chemicals derived from oats (*Avena sativa* L.) and fatty acids on the oviposition behavior of female yellow mealworms. Identifying oviposition stimulants could enhance egg yield within shorter timeframes, while inhibitors would guide substrate selection.

Our behavioral study utilized a two-choice test: oat-derived chemical substances (extracted) and fatty acids (administered at doses of 1 g and 0.1 g) were deposited on sand (simulating a nutritional substrate), while solvent-treated sand served as the control. In the test with a single stimulus, we employed 10 males and 10 paired females. We conducted 5 repetitions. After 24 hours of egg-laying exposure, we meticulously quantified egg numbers in each stimulus and control plate.

Notably, female mealworms exhibited significantly higher egg deposition in sand than in sand supplemented with oat extract. Furthermore, we will present data on specific fatty acids implicated in egg-laying regulation. Understanding the chemical triggers behind yellow mealworm oviposition holds promise for optimizing egg yield and advancing environmentally conscious insect farming. Future investigations should explore additional fatty acids as attractants, paving the way for efficient and sustainable mealworm production.

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E18

STUDY OF ALLELOPATHIC PROPERTIES OF SOSNOVSKY HOGWEED (*HERACLEUM SOSNOWSKYI*)

Aistė Kundrotaitė[1], Irena Nedveckytė[1]

[1]Life Sciences Center, Vilnius University, Vilnius, Lithuania
aiste.kundrotaite@gmc.stud.vu.lt

Organisms (plants, animals, fungi) that have been accidentally or purposefully introduced from other countries have a significant impact on the biocenoses that have historically been established in a particular country, and can disturb completely change the balance of entire ecosystems. This poses a threat to the region's biological diversity [1]. Since 2001, Sosnovskiy hogweed (*Heracleum Sosnowskyi)* has been the only plant species in Lithuania included in the list of harmful and endangered species of wild plants and mushrooms due to its ability to release physiologically active substances that can harm flora, fauna and humans. Phototoxic organic compounds, such as furanocoumarins, can react with DNA bases to form adducts. Under the influence of further UV-A radiation, they can react with other bases in the DNA strands and form cross-links between the strands. This can eventually lead to cell death [2].

The study of allelopathic properties of Sosnovsky hogweeds is presented in this research. The aim of this study was to investigate the allelopathic effect of the Sosnovskiy hogweed on model species: Garden cress (*Lepidium sativum*), Lettuce (*Lactuca sativa*), and native plant species: White clover (*Trifolium repens*), Perennial ryegrass (*Lolium perenne*), Timothy grass (*Phleum pratense*) using different concentrations of aqueous solutions of leaves, roots and flowers (0.1; 0.5; 1.0). One of the main tasks was to identify the most allelopathically active morphological part of the invasive plant. Seed germination tests were used for this purpose.

The study results indicate that the aqueous extracts of Sosnovsky hogweed significantly inhibited the germination and growth of the selected model and native meadow plants (One-way ANOVA test, Tukey's test, $p<0.05$). The highest concentration of leaf aqueous extract (1.0) had the greatest impact on the germination of model plant species. Garden cress and lettuce had germination rates of 27.22 ± 0.08% and 25.56 ± 0.08%, respectively. The most allelopathically sensitive native species was identified as White clover. The germination rate of seeds exposed to the strongest concentration of leaf solution was 0%. Among the native species tested, Perennial ryegrass was the least affected by aqueous extracts of Sosnovsky hogweed.

Results of this study may contribute to the better understanding of the phenomenon of allelopathy, and may help in the control of the spread of invasive plants.

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KAZOKIŠKĖS LANDFILL SOIL: CONTAMINATION AND TOXICITY

Miglė Krasauskaitė[1], Irena Nedveckytė[1], Virginija Kalcienė[1]

Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
migle.krasauskaite@gmc.stud.vu.lt

Approximately 95% of all municipal solid waste collected worldwide are deposited in landfills. The increasing generation of municipal solid waste (MSW) has become a major burden on our society, causing serious environmental and economic problems [1]. Physical, chemical and biological interactions in landfills result in the formation of toxic landfill soils. Landfill soil is particularly toxic and hazardous, accumulating hazardous substances, and posing a serious threat to living organisms, surface water, and groundwater due to its complex components, such as ammonia, metals, and organic compounds [2]. Eventually, the toxicity to organisms depends on the waste disposed of in the landfill, the type of landfill in operation, and the stage of decomposition of the waste [3].

The aim of this study was to evaluate metal pollution and ecotoxicity in soil from the Kazokiškės landfill. Five samples of landfill soil were collected from different sites (from the oldest operating site (KZ3-KZ5) to the newest (KZ1-KZ2)) in November 2023 (Figure 1). The concentrations of heavy metals in the soil samples were determined using X-ray fluorescence spectrometry. The following model organisms were used for ecotoxicity testing: lettuce (*Lactuca sativa* L.), garden cress (*Lepidium sativum* L.) and the bioluminescent bacterium *Aliivibrio fischeri* (Beijerinck).

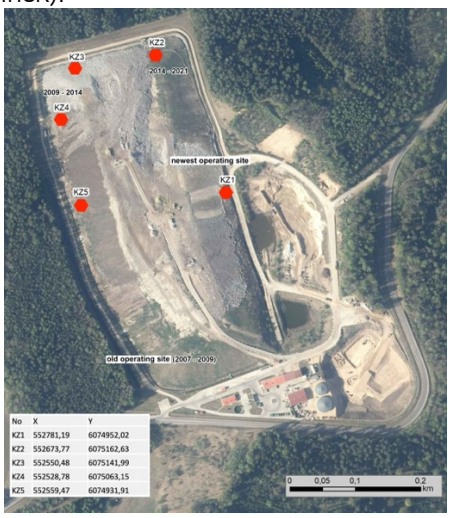

Figure 1. Kazokiškes landfill soil sampling plan.

Comparison of the determined metal concentrations with the limit value (LV) of the Lithuanian Hygiene Standard (60:2015) showed that the concentrations of Zn and Cu in samples KZ3 and KZ4 are above the LV: 2 and 2.3 times more zinc and 1.4 and 1.6 times more copper, respectively. Data from ecotoxicological bioassays showed that the sample of KZ1 has the strongest negative effects, significantly inhibiting the bioluminescence of *A. fischeri* by 78.17% and 37.9% at concentrations ranging from 72% to 36%. The model plants germination test showed that the KZ1 soil sample was the only one (at 100% concentration) that inhibited plant growth. Based on the results, it was concluded that the toxicity of the KZ1 sample may be related to contaminants other than heavy metals. Further research is needed to determine the cause of the observed adverse effects.

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SCREENING OF PESTICIDE RESIDUES IN SURFACE WATER AND SEDIMENTS FROM STRAIGHTENED RIVERS GUBESĖLĖ, VADAKTIS AND NEMĖŽA

Kristina Strielė[1], Virginija Kalcienė[1], Alius Ulevičius[1], Algirdas Leonardas Suchockis[1], Vaidotas Valskys[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
kristina.striele@gmc.stud.vu.lt

Pesticides can contaminate groundwater or surface water if they are used excessively or if recommendations for their use are not followed. This accounts for between 20 and 80 per cent of the total pollution by pesticides of surface waters [1]. Pesticides can be washed out of agricultural fields by rainwater leaching or by adsorption to soil particles which then enter shallow groundwater through erosion [2]. Surface water runoff can carry pesticides to lakes, rivers and drainage canals, where they can have negative effects on ecosystems depending on their amount and duration of exposure [3][4].

The objective of this study was the assessment of the presence of pesticide residues in straightened rivers of Lithuania. The rivers selected for sampling are located in areas with different levels of urbanisation and different types, sizes and intensities of urbanisation. Water and sediment samples for pesticide pollution studies were taken from the different sites in the rivers Gubesėlė, Nemėža and Vadaktis.

A gas chromatograph coupled to a mass spectrometer was used for the determination of pesticides in the water and sediments. The sample preparation was carried out according to a modified QuEChER method. The analysis of pesticide residues included 28 different analytes. A total of 27 water and 30 sediment samples taken from the Gubesėlė, Vadaktis and Nemėža rivers were analyzed.

The water and bottom sediment samples of the rivers Gubesėlė and Vadaktis did not show any signs of pesticide contamination. Although no pesticide pollution was detected in the water samples of the Nemėža river, biphenyl was found in all sediment samples. However, additional investigation is required to determine the extent and implications of this contamination.

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ETHOLOGICAL TESTS HELP TO EVALUATE ECOTOXICITY OF PHTHALATES

Rokas Zalūba[1], Edita Paulikaitė[1], Justina Alčauskaitė[2], Violeta Žalgevičienė[2], Rokas Buišas[1], Grita Skujienė[1]

[1]Institute of Biosciences, Life Sciences Centre, Vilnius University, Vilnius, Lithuania
[2]Department of Anatomy, Histology and Anthropology, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
rokas.zaluba@gmc.stud.vu.lt

As the production of novel synthetic compounds escalates, the levels of these substances and their byproducts in the environment rise. Phthalates are ubiquitous chemical compounds produced in high volumes and extensively used as plasticizers, which have endocrine disrupting properties and impair cardiovascular health across all ages [1]. Although found in micrograms, they have a tendency to accumulate in the ecosystem and human body [1]. Therefore, analytical methods of phthalate induced behaviour play a crucial role in assessing their presence and impact. This study aim was using modern rodent's behaviour analysis program to determine whether phthalates can change rats behaviour. All experimental procedures were reviewed and approved by the Ethical Committee of State Food and Veterinary Service of the Republic of Lithuania (2022-08/09, No G2-221) and were conducted following the European Communities Council Directive (2010/63/EU) and the local Animal Welfare Act. To determine anxiety-related rats behaviour, an behaviour test and its data analysis were carried out.

During this research, 36 *Wistar* female rats, aged 1 month, were used. Standard laboratory food and tap water were provided *ad libitum* throughout the experimental period. Rats were divided into control (without phthalates) and 5 experimental groups that received different doses of phthalates: 1) DEHP 200 µg/kg; 2) DEHP 1000 µg/kg; 3) DBP 100 µg/kg; 4) DBP 500 µg/kg; 5) DEHP 200 µg/kg and DBP 100 µg/kg.

Two Elevated Plus Maze (EPM) behaviour tests were performed – after one week and a month of phthalate consumption. The ANY-maze (Ireland, version 7.20) computer software was utilized for the analysis of the video recordings (Fig.1, 2): traveled distance and entries into open zone were measured. Statistical analysis was performed using RStudio 4.3.1, one-way ANOVA and Kruskal–Wallis tests were used.

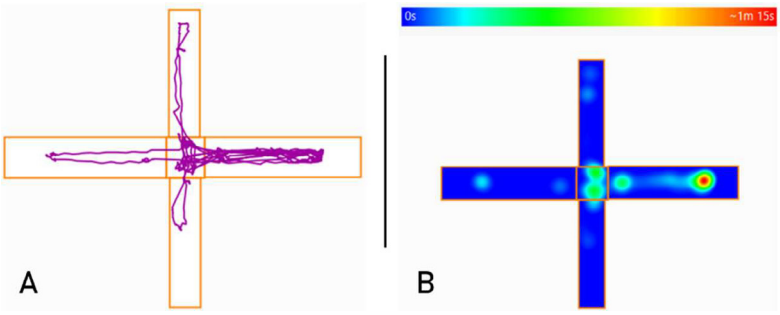

Figure 1. ANY-maze rodent's analysis results examples: track (A), heat map (B) – the longer rat stay in a specific place, the redder it becomes.

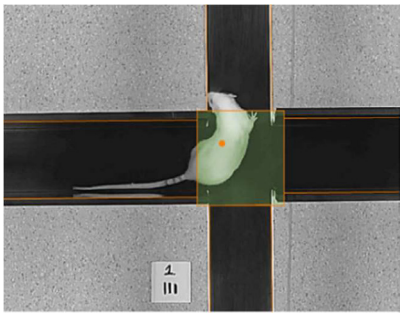

Figure 2. ANY-maze analysis process. Orange dot shows rodent's center, green color refers presence in zone.

It was found that daily, uninterrupted long-term (2 month) consumption of small doses of phthalates does not show any trends of rat behavioural changes ($p < 0.05$), but it was found significant decrease in entries into open zone of DEHP_DBP group. However, it has been observed that daily, uninterrupted short-term (1 week) consumption of 100 µg/kg DBP showed a reliable increase in traveled distance and entries into open zone.

We can conclude, that animal behavior tracking program ANY-maze, used in the current study, reveals that it is useful to evaluate ecotoxicity of environmental chemical compounds on the behavior of rats. Yet, to enhance confidence in the data it is necessary to perform more tests to state that phthalates lead to adverse outcomes.

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ALLELOPATHIC PROPERTIES OF COMMON TANSY (*TANACETUM VULGARE* L.)

Donata Stancelytė[1], Asta Judžentienė[1,2], Irena Nedveckytė[1], Jurga Būdienė[2]

[1]Institute of Biosciences, Life Sciences Centre, Vilnius University, Lithuania
[2]Department of Organic Chemistry, Center for Physical Sciences and Technology, Vilnius, Lithuania
donata.stancelyte@gmc.stud.vu.lt

Tanacetum vulgare L. – is a perennial herb of the *Asteraceae* family, cultivated in gardens and growing wild on the well-drained or sandy soils in open disturbed areas such as roadsides, pastures and hedges. Native to Europe and Asia, it was introduced to North America as a medicinal and horticultural plant. The members of the genus *Tanacetum* are widely used for a variety of purposes, including both traditional and modern medicine, food preservation, cosmetics also as insecticides, spices, dyes [1]. Thus, biological properties such as antioxidant, antibacterial, antifungal and repellent activity have been demonstrated in various extracts obtained from *T. vulgare* [2].

It is important to point out that tansy is a widespread weed that competes with agricultural crops for space, light, nutrients and as a result has a negative impact on biodiversity, ecosystems and economy. *T. vulgare* can also hinder reforestation and landscape restoration efforts. The spread of the plant is determined by several factors: great propagation via seeds, underground rhizomes and root fragments, broad climatic tolerance, phenotypic plasticity [3].

The success of the tansy's spread is also determined by its allelopathic properties. Allelopathy is defined as any direct/indirect harmful or beneficial effect of one plant on another through the production and release of chemical compounds. *T. vulgare* is rich in the phenolic acids, flavonoids and their derivatives, tannins, carotenoids. It is known that plant secondary metabolites can be obtained from different parts of the plant. They may affect other plants seed germination and root formation in the early stages, as well as the growth of the whole plant: chlorophyll production, membrane conductance, photosynthetic activity [4]. The allelopathic effect of aqueous extracts of tansy on the other plants has not been widely studied. This opens up a wide range of research possibilities.

The aim of the experiment was to determine the allelopathic activity of aqueous extracts from *T. vulgare* roots, leaves and inflorescences. Under laboratory conditions, allelopathic properties of acidic, neutral and alkaline fractions and different concentrations (0.1; 0.5; 1.0 M) were tested on the model objects Lettuce (*Lactuca sativa* L.) and Garden pepper cress (*Lepidium sativum* L.) seeds. The observed results showed that tansy extracts significantly affected the germination and growth of the seeds tested. On the other hand, the allelopathic effect of *T. vulgare* differed depending on the morphological part and the pH of the extracts. Leaf and inflorescence aqueous extracts showed the strongest inhibitory activity. The effect was most active in the acidic and neutral fractions. In the case of the leaves, at the relative concentrations of 0.5 and 1.0 M the germination and growth of *L. sativa* seeds was suppressed from 89.93 % to 98.37 % in the acidic fraction and from 66.55 % to 80.10 % in the neutral pH. *L. sativum* was reduced by 100 %. In case of inflorescences, *L. sativum* was also more sensitive: seed germination was inhibited up to 100 %. This study revealed that it is important to investigate the allelopathic mechanism and its role in the widespread weed species.

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IDENTIFICATION OF HAEMOSPORIDIAN PARASITE (HAEMOPROTEUS) VECTORS USING MICROSCOPY AND DNA DATA

Paulina Minkevičiūtė[1,2], Rasa Bernotienė[2], Rita Žiegytė[2]

[1]Vilnius University, Lithuania
[2]Nature Research Centre, Lithuania
paulina.minkeviciute@gmc.stud.vu.lt

Avian haemosporidian parasites (Apicomplexa: Haemosporida) are widespread in the wild and are transmitted by blood-sucking insects (Insecta: Diptera). They are virulent both to blood-sucking insects and birds, can cause diseases or even mortality in birds. Haemosporidian parasites (genus *Plasmodium*) can also infect humans causing malaria, a common disease in warm countries that affects and kills millions of people each year. Much research has been devoted to the investigation of genetic diversity and phylogenetic relationships of avian blood parasites belonging the genus *Haemoproteus* and *Plasmodium* but there is still insufficient information on their epidemiology, development in blood-sucking insects, and their transmission patterns [1].

The aim of this work was to get new knowledge about vectors of *Haemoproteus* parasites using microscopy and PCR-based methods. The use of both methods provides more precise information on the interactions between blood-sucking insects, their vertebrate hosts and parasites. Bloodsucking biting midges (Diptera: *Culicoides*) were collected in study sites in Curonian spit, they were sexed and females were identified, dissected for the preparations of salivary glands, which were later investigated using microscopy. The remains of *Culicoides* females were individually analysed using PCR [2] in the Nature Research Centre. The detection of sporozoites (invasive parasite stage) and the identification of the parasite genetic lineages by PCR in the same biting midge allowed us to identify vector species of avian haemosporidian parasites in the wild. In total 751 specimens were examined using both microscopy [1] and PCR-based methods[2]. So far, seven PCR positive results were obtained.

The obtained results showed that *Culicoides kibunensis* biting midge was found to be a vector of the *Haemoproteus pallidulus* (genetic line hSYAT03), and biting midge of *C. obsoletus* group was found to be the vector of the *Haemoproteus sp.* (hSYAT01) as both sporozoites and parasites DNA were detected in the same *Culicoides* female. DNA of *Haemoproteus* parasites of genetic lineage hSYAT02 (*H. parabelopolskyi*) and genetic lineage hCULKIB01 (*H. syrnii*) were also detected in *Culicoides* biting midges using PCR. The vectors of H*. syrnii* are known to be *C. impunctatus* and *C. nubeculosus* [3]. In order to better understand the *Haemoproteus* vectors in Europe, it is necessary to pay attention to other, less studied and described in the literature, *Culicoides* species of biting midges.

Acknowledgment
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THE ROLE OF RED FOX (*VULPES VULPES*) IN THE TRANSMISSION OF SARCOCYSTIS SPECIES FROM FARM ANIMALS

Naglis Gudiškis[1], Evelina Juozaitytė-Ngugu[1], Petras Prakas[1], Dalius Butkauskas[1]

[1]Nature Research Centre, Vilnius, Lithuania
naglis.gudiskis@gmail.com

Parasites of the genus *Sarcocystis* are widespread globally following a two-host life cycle. While infections in the definitive hosts may not be symptomatic, acute infections in intermediate hosts can reduce production and render meat unfit for consumption [1]. This poses ecological challenges and significant economic losses in the livestock sector. Predatory mammals of the Canidae and Felidae families play an important role in the distribution of *Sarcocystis*. One of the most widespread wild canids and terrestrial predators is the red fox (*Vulpes vulpes*), which is best adapted to survive in a wide range of environmental conditions [2]. This mammal can be found not only in the wild but also in urbanised areas, posing a significant risk to both the livestock sector and humans due to the transmission of *Sarcocystis* species [3]. Studies in Lithuania have shown common *Sarcocystis* species in farm and cervid animals with canids as definitive hosts, including *S. tenella*, *S. cruzi*, *S. hominis*, *S. miescheriana*, *S. capracanis*, *S. bertrami*, *S. iberica*, *S. morae*, and *S. venatoria* [4-6]. Infection experiments indicate some of these species can infect the red fox. To date, there is a lack of studies on the *Sarcocystis* species diversity in the faeces of Canidae. This research aimed to investigate how the red fox contributes to the transmission of *Sarcocystis* parasites in Croatia, Lithuania, and the Czech Republic, specifically by analysing the faecal matter of this predator.

In collaboration with researchers from Croatia, Lithuania, and the Czech Republic, 90 samples of red fox faeces were collected in different regions of each country. The sporocysts in the samples were isolated by flotation in sucrose solution. Subsequently, DNA isolated by a commercial kit was amplified using the *cox1* gene as a molecular marker. *Sarcocystis* species were identified using a nested PCR approach with *Sarcocystis* genus (step I) and species (step II) specific primers.

Of the 90 animals tested across the three countries, 48 (53.3%) were positive for *Sarcocystis spp.* Notably, *S. tenella*, *S. miescheriana*, and *S. cruzi* were the most common species of the parasite in all three countries. Only in the Czech Republic, a single specimen was discovered to be infected with *S. arieticanis* and *S. capracanis*. Additionally, in Croatia and Lithuania, it seems that red foxes can transmit *S. hjorti*. The primary results show that the red fox can harbour more than one species of *Sarcocystis* parasites thus posing a major threat to both the economic and health sectors.

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E25

IDENTIFICATION OF *SARCOCYSTIS* SPP. IN THE BLOOD OF THE BANK VOLE AND YELLOW-NECKED MOUSE

Dovilė Laisvūnė Bagdonaitė[1], Naglis Gudiškis[1], Neringa Kitrytė,[2] Laima Baltrūnaitė[2]

[1]Laboratory of Molecular Ecology, Nature Research Centre, Lithuania
[2]Laboratory of Mammalian Ecology, Nature Research Centre, Lithuania
dovile.bagdonaite@gamtc.lt

The genus *Sarcocystis* is an abundant group of Apicomplexa parasites found in mammals, birds, and reptiles. These parasites are characterised by the formation of sarcocysts in the muscles of intermediate hosts and the development of sporocysts in the intestines of definitive hosts.

To date, over 40 different *Sarcocystis* species have been identified in rodents. Majority of the investigations have focused on synanthropic rodent species, such as the house mouse (*Mus musculus*) and brown rat (*Rattus norvegicus*), thus data concerning *Sarcocystis* species prevalence and diversity in wild mice and voles remain sparse [1]. Yellow-necked mouse (*Apodemus flavicollis*) and the bank vole (*Clethrionomys glareolus*) are two most commonly found wild rodent species in Lithuania, that are mostly prevalent in the forests, but frequent other habitats as well [2].

Traditional research approaches prove challenging for studying *Sarcocystis* spp. in small mammals, prompting exploration of alternative methods such as screening blood samples from intermediate hosts [3, 4]. In this study the molecular identification of *Sarcocystis* spp. in the blood samples of yellow-necked mice and the bank voles was carried out using nested PCR and sequencing. *Sarcocystis* spp. were statistically ($p < 0.01$) more frequently detected in the bank vole (6.3%) than in yellow-necked mice (0.9%). Samples were collected from seven different habitats and *Sarcocystis* spp. were identified in specimens from four different habitats, such as mature deciduous forest, bog, natural meadow, and arable land.

In this study, *Sarcocystis myodes* was the only species identified in yellow-necked mice. Meanwhile three *Sarcocystis* species were confirmed in the blood of the bank voles - *Sarcocystis funereus*, *Sarcocystis myodes*, and *Sarcocystis* cf. *glareoli*. The obtained results are important in the development of molecular identification of *Sarcocystis* parasites in live animals and understanding of *Sarcocystis* species richness in wild rodents of Lithuania.

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E26

Detection of Lingonberry Stunted Yellows Disease associated with *'Candidatus* Phytoplasma trifolii' in the natural habitat of Lithuania

M. Dėlkus[1]; A. Mikalauskas[1,2]; M. Žižytė[1]; A. Ivanauskas[1]; D. Valiūnas[1]

[1]Nature Research Centre, Laboratory of Plant Pathology; Vilnius, Lithuania
[2]Vilnius university; Vilnius, Lithuania
martynas.delkus@gamtc.lt

Lingonberries (*Vaccinium vitis-idaea L.*) are low-growing, evergreen shrubs of cooler, northern regions of North America and Europe. These plants produce berries with unique flavour and high economic value, which play a vital role in maintaining the diversity of the northern ecosystems [3]. Plant yellows diseases can result in significant harm to beneficial plants and berry crops. Some plant yellows diseases, once thought to be viral, are actually caused by prokaryotic organisms known as phytoplasmas. These pathogens lack a cell wall and are limited to the phloem. Phytoplasmas exhibit pleomorphism and have not yet been successfully cultured without cells. They infect over 700 plant species worldwide, leading to symptoms such as yellowing and stunting like those caused by certain plant viruses, resulting in substantial economic impact [2]. The spread of these pathogens between plants is facilitated by insect vectors, while grafting and vegetative propagation play key roles in their dissemination in agriculture. In Eastern Europe, the prevalence of these diseases is less pronounced compared to southern regions of Europe, making early detection and control more challenging. '*Candidatus* Phytoplasma trifolii' was found in lingonberries (*Vaccinium vitis-idaea L.*) in naturally occurring forests of Labanoras Regional Park in Lithuania. Diseased plants usually showed symptoms that indicate a disturbance in the normal balance of plant hormones. *'Candidatus* Phytoplasma trifolii' is characterized by pronounced plants stunting, yellowing, small leaves, shortened internodes, and stem distortions [Fig.1]. Samples of plants with possible phytoplasma infection were collected and genomic DNA was extracted using CTAB protocol. '*Candidatus* phytoplasma trifolii' was identified using routine method by amplifying 16S rRNA region using two-step PCR protocol with P1/P7 and R16F2n/R16R2 primer pairs [4; 5]. Restriction fragment length polymorphism (RFLP) analysis of nested PCR products of 16S rRNR revealed that detected phytoplasmas belonged to 16SrVI-A subgroup. In order to better molecularly describe and sub-group the phytoplasma identified in the mentioned berry plants, another genetic marker - SecA - was analysed. Strains of 16SrVI phytoplasma group were reported in *Vaccinium myrtillus* in Austria [2] and in *Vaccinium vitis-idaea* in Canada [1]. This is the first report of '*Candidatus*. phytoplasma. trifolii' strain belonging to 16SrVI phytoplasma group infecting lingonberry worldwide. Also, this is the first report of 16SrVI-A phytoplasma group found in Lithuania. The presence of this phytoplasma poses a threat to the natural ecosystem and could eventually spread into agricultural settings in our country. Therefore, it's crucial to conduct surveillance for insect vectors, and assess effective control methods. Without proactive intervention, the long-term sustainability of lingonberries and their associated ecosystems may be jeopardized.



Fig 1. Lingonberry plants: A – plants infected with '*Candidatus* Phytoplasma trifolii' exhibiting symptoms of stunting, yellowing, small leaves, shortened internodes, and stem distortions; B - infected plants in natural habitat; C - healthy plants; D - healthy plants in natural habitat.

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TOXICITY STUDIES OF AEROSOL NANOPARTICLES USING A NOVEL *IN VITRO* "CELLS-ON-PARTICLES" EXPOSURE MODEL; SYSTEM TESTING AND VALIDATION

Elina Ridikaitė[1], Edvardas Bagdonas[1]

[1]Department of Regenerative Medicine, Centre for Innovative Medicine, Vilnius, Lithuania
eridikaite@gmail.com

Smoking has become very popular nowadays, especially among young people. Smoking of conventional cigarettes (CCs) or heated tobacco products (HTPs) results in high levels of particulate matter (PM) [1]. People very often tend to think that CCs are a safer alternative to smoking, but there is currently insufficient independent research to support this claim [2]. The current lack of research on the health harms caused by second-hand smoke underlines the importance of such studies. Current *in vitro* systems for PM or aerosol particles can be divided into monolayer cultures, cocultures, systems to simulate the air-liquid interface and lung-on-a-chip models. However, nowadays, new integrated indicators are being sought that allow a simpler and faster assessment of the health impact of aerosol particles around us.

The aim of this study was to characterise the cytotoxicity of aerosol PM from conventional cigarettes (CCs) and heated tobacco products (HTPs) using a novel *in vitro* "cells-on-particles" exposure model. Viability of BEAS-2B cells, the levels of IL-6 and IL-8 cytokines in the medium and changes in the expression of genes involved in xenobiotic metabolism were evaluated by culturing the cells on 3D poly(ε-caprolactone) (PCL) matrix. These matrixes were constructed using an innovative electrospinning technology. The relatively low cost of air toxicity analysis, the ease of use, minimal sample preparation and the possibility of real-time monitoring of the system under investigation are some of the main advantages of such systems over current cytotoxicity methods [3]. After we performed the cell viability test, we found that CCs aerosol caused a significant and dose-dependent decrease in cell proliferation, while the highest doses tested led to complete cell death. In contrast, HTPs aerosol increased cell proliferation and metabolic activity by up to 92% after 24 hours. In addition, we found by RT-qPCR that aerosol exposure to HTPs aerosol resulted in a significant up-regulation of *CYP1A1* and *NQO1* genes, while CCs aerosol particles strongly and significantly increased the expression of *CYP1A1*, particularly at higher doses. After we performed ELISA assay, we showed, that exhaled HTPs aerosol did not significantly alter the secretion of proinflammatory interleukins, but a significant decrease in IL-6 and IL-8 levels was observed in the case of the CCs in relation to the NPs concentration. Particle concentration of CCs low as 2.8 μg/cm^2 resulted in a ~1.8-fold increase in IL-6 secretion after 48 hours.

Thus, using an innovative *in vitro* "cells-on-particles" system, which is technically much simpler, faster and well suited for initial NPs cytotoxicity testing, we found that aerosol particles exhaled from HTPs products are significantly less harmful to bystanders.

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E28

GENETIC DIVERSITY OF FUNGI ORIGINATING FROM THE NATIVE AND INTRODUCED *PINUS* SPP.

Kamilė Bukėnaitė[1], Dovilė Čepukoit[2], Goda Mizerienė[2], Daiva Burokienė[2]

[1]Life Science Center, Vilnius University, Lithuania
[2]Laboratory of Plant Pathology, Nature Research Centre, Lithuania
kamile.bukenaite@gmc.stud.vu.lt

Genetic variation is an essential aspect for all organisms to survive and adapt to a rapidly changing environment. Ongoing climate change, leading to shifts in temperature and precipitation trends, and the expansion of global trade and transport, is driving the introduction of non-native species into new habitats. These events have the potential to influence genetic changes in both plants and microorganisms. It is known that endophytic fungi can enhance the host's ability to tolerate a range of abiotic and biotic stress factors, potentially reducing the demand for pesticides and fertilizers in agriculture. However, it is known that depending on the host species or environmental conditions, endophytes can switch to pathogenic behaviour, overcome the plant's immunity and cause the disease.

The plant material for the study was collected in the Kairėnai Botanical Garden of Vilnius University. The samples were collected from a total of 7 plants of the genus *Pinus* (*Pinus sylvestris* 'Beuvronensis', *Pinus mugo* 'Frisia', *Pinus strobus*, *Pinus nigra*, *Pinus Banksiana* xp *contorta*, *Pinus ponderosa* var. *scopulorum*, *Pinus parviflora* 'Glauca'). A total of 135 isolates were obtained from needles, buds and twig samples.

The fungal isolates were classified into 13 morphological groups based on the most important microscopic and macroscopic phenotypic characteristics, such as colour, shape and size of colonies, hyphae and spores. The cetyltrimethylammonium bromide (CTAB) method was used to extract genomic DNA. Molecular identification of the isolates was performed by the amplification of the internal transcribed spacer (ITS) region [1]. The genetic diversity of the isolated fungi was analysed using ISSR (Inter Simple Sequence Repeat) primers [2].

The results of sequencing revealed a diverse fungal community on pine trees. Out of 13 morphological groups, 11 fungal genera were identified. About 61 % of the isolates were related to *Sydowia polyspora*, which was identified as the causal agent of current season needle necrosis. The genera *Alternaria*, *Diaporthe* and *Fusarium* were other common potentially pathogenic fungi. Between 3 and 5% of less common species were observed, which were mainly endophytic fungi. *S. polyspora* was found to be dominant on *Pinus sylvestris* 'Beuvronensis', while fungi of the genus *Alternaria* were frequently found on *Pinus parviflora* 'Glauca' pines. In addition to *S. polyspora*, *Diplodia sapinea* and *Fusarium* sp. were frequently found on *Pinus mugo* 'Frisia'.

The ISSR analysis was carried out with 10 ISSR primers. Among the 10 tested ISSR primers, 4 primers were selected for the species *Sydowia polyspora*, *Epicoccum nigrum* and *Diaporthe eres* and the following genera: *Alternaria*, *Diplodia*, *Fusarium* and *Plagiostoma*. Five primers were used for *Pestalotiopsis* sp. and *Microsphaeropsis olivaceae*. Finally, 3 primers were used for *Mollisia albogrisea* and 2 primers for *Pezicula eucrita*.

The minimum spanning network and cladograms were generated using GenAlEx 6.51b2 [3] and Treeview X [4] and showed the association between isolates and their hosts in a genotype-dependent manner.

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E29

PREVALENCE OF FACULTATIVE SYMBIONTS AMONG SOME TREE-DWELLING SPECIES OF THE GENUS *DYSAPHIS* BÖRNER (HEMIPTERA: APHIDIDAE)

Barbora Ieva Masoit[1], Jekaterina Havelka[1]

[1]Department of Zoology, Vilnius University, Lithuania
barbora.masoit@gmc.stud.vu.lt

Like other insects, aphids can have a wide range of symbiotic bacteria, which can enhance their survival abilities. The obligate symbiont *Buchnera aphidicola* provides aphids with nutritional components, while facultative symbionts are important for the protection against parasitoid wasps and other parasites, the tolerance of high temperatures, the modification of body colour, or the use of host plant [1]. Previous study of microbiome of twelve species of *Dysaphis* Börner, 1931 aphids collected in Poland showed the presence of facultative symbionts from genera *Serratia*, *Regiella*, *Fukatsuia*, *Wolbachia*, *Gilliamella*, *Hamiltonella*, *Spiroplasma*, *Sphingomonas*, *Pelomonas*, and *Acinetobacter* [2].

The aim of the present study was to check the prevalence of *Serratia symbiotica*, *Regiella insecticola*, *Hamiltonella defensa* and *Spiroplasma* sp. among four tree-dwelling species and two species groups of the genus *Dysaphis*. Fourty nine samples of total genomic DNA extracted from individual aphids collected in Lithuania (n=40), France (n=1), Latvia (n=1), Czech Republic (n=1), Italy (n=1), Turkey(n=4), Poland (n=1) were screened for the presence of facultative symbionts using diagnostic PCR with species-specific primers as described by [3] and [4], and yielding fragments of different length, namely, 1600 bp for *H. defensa*, 1000 bp for *Spiroplasma* sp., 500 bp for *S. symbiotica* and 200 bp for *R. insecticola* [3-4].

The most abundant facultative symbiont was *R. insecticola* detected in 31 samples, followed by *S. symbiotica*, which was found in 9 samples. There were also 5 samples with *Spiroplasma* sp. and 3 samples with *H. defensa*. During this study facultative symbionts of *Dysaphis pyri* (Boyer de Fonscolombe, 1841) were detected for the first time as the single sample analyzed by [2] contained on *B. aphidicola*. The presence of *S. symbiotica* in *Dysaphis devecta* group (Walker, 1849), *H. defensa* in *Dysaphis sorbi* (Kaltenbach, 1843) and *Dysaphis crataegi* group (Kaltenbach, 1843), *R. insecticola* and *Spiroplasma* sp. in *D. sorbi* and *Dysaphis plantaginea* (Passerini, 1860) was recorded for the first time. The majority of analyzed aphid samples harboured only one species of facultative symbiont. Coinfection with two species of facultative symbiotic bacteria was observed in 11 samples, and there was a single sample with three species of facultative symbionts detected. Further studies are needed to evaluate the presence and prevalence of other facultative symbiotic bacteria species and their proportion in microbiome of different *Dysaphis* species comparing to *B. aphidicola*.

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WOOD-INHABITING FUNGI ISOLATED FROM FOUR TREE SPECIES GROWING IN PROTECTED OLD-GROWTH FOREST STANDS

Ieva Sokė[1, 2], Vaidotas Lygis[1], Simona Jaseliūnaitė[1, 2], Dovilė Čepukoit[1], Daiva Burokienė[1]

[1] Laboratory of Plant Pathology, Nature Research Centre, Lithuania
[2] Life Sciences Center, Vilnius University, Lithuania
ieva.soke@gamtc.lt

The protected old-growth forests are ecologically, economically, socially important and may serve as a key point of reference for ecological research. Compared to commercial forests, the protected old-growth forest stands are not always more species-rich, but the most obvious advantage of them in terms of biodiversity is their uniqueness. They are a refuge for rare, red-listed and even endemic species, as active forest management is usually restricted there. The flora and fauna in Lithuania's old-growth forests have been well studied. However, there is a lack of information on microorganisms in these areas, especially on the diversity of microscopic wood-inhabiting fungi. Therefore, the main aim of the present study is to assess the diversity of wood-inhabiting fungi in four economically and ecologically important tree species – Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), pedunculate oak (*Quercus robur*) and silver birch (*Betula pendula*), growing in two protected old-growth forests in Lithuania.

In the summer of 2023, a total of 115 middle-aged *P. sylvestris* (15 healthy and 15 dead trees), *P. abies* (20 healthy and 20 dead trees), *Q. robur* (20 healthy trees) and *B. pendula* (15 healthy and 10 dead trees) were sampled with an increment borer in two mixed old-growth forest stands located in the Punios Šilas Strict Nature Reserve (southern Lithuania) and in the Plokštinė Strict Nature Reserve (north-western Lithuania). A total of 311 fungal cultures were isolated from wood cores of 7 healthy and 35 dead trees. Most isolates (104) were obtained from dead *P. abies*. All fungal cultures were assigned to 66 groups according to their characteristics and micromorphological features of colonies. Twenty-six cultures of the largest morphological group were isolated from dead *P. sylvestris* (7 isolates) and *P. abies* (19 isolates). The other two main groups consisted of 25 isolates. All cultures from one of these groups were isolated exclusively from *B. pendula*. Molecular identification of the fungal cultures representing these morphological groups will be performed in the near future by sequencing the ITS region of the fungal DNA. It is expected that rare and protected fungi or even new species for Lithuania could be found in the studied old-growth forests.

E31

REVEALING THE HIDDEN DIVERSITY OF NEPTICULIDAE (INSECTA: LEPIDOPTERA) OF THE CAUCASUS

Viktorija Dobrynina[1], Svetlana Orlovskytė[1], Jonas R. Stonis[1]

[1]State Research Institute Nature Research Centre, Lithuania
viktorija.dobrynina@gamtc.lt

Nepticulidae is a highly specialized yet phylogenetically primitive lepidopteran family, distinguished by peculiar morphological and biological characteristics [1]. Up to now, the taxonomic diversity of Nepticulidae in the Caucasus, especially in Armenia, has been poorly understood. The goal of this study was to investigate the world's smallest lepidopterans of the family Nepticulidae in the Caucasus for the first time and to discuss possible cryptic taxa.

During our recent fieldwork in the Caucasus, conducted within the framework of the Agreement of Scientific Cooperation between the Scientific Center of Zoology and Hydroecology of the National Academy of Sciences of Armenia and the State Research Institute Nature Research Centre, we collected a significant amount of Nepticulidae specimens. The part of this material was evaluated not only by morphological and ecological differences but also by molecular characters [2-4].

In total, the research revealed thirty-two Nepticulidae species belonging to eight genera. Surprisingly, quite a lot of these species appeared to be previously unknown to science: *Stigmella colchica* Stonis & Diškus [3], *S. ararati* Stonis, Dobrynina & Remeikis, *S. armi* Stonis, Dobrynina & Remeikis, S*. garnica* Stonis, Dobrynina & Remeikis, *S. inopinoides* Dobrynina, *S. magicis* Stonis & Dobrynina, *Etainia caucasi* Remeikis, *Trifurcula vardenisi* Stonis, Dobrynina & Remeikis [2]. One of them is *Stigmella colchica*, well supported by both morphological and molecular data, is a previously unknown potential Nepticulidae pest infesting plum trees in the Caucasus [3]. Besides the mentioned species, we detected some specimens, which did not exhibit any obvious morphological or ecological differences from the European *Simplimorpha promissa* (Staudinger) and *Ectoedemia* (*Zimmermannia*) *longicaudella* Klimesch, but distinguished by significant molecular characters, what may actually represent possible cryptic taxa, allopatric subspecies or species [2, 4].

We assume that the currently identified species in the Caucasus may account for only about 40–45% of the species actually occurring in this region, i.e., in total, at least 70–80 species of Nepticulidae can be discovered in the future. Among those still undescribed taxa, we expect a significant portion of species similar to European but cryptic. Consequently, we believe that further research is necessary in this fascinating and biogeographically very important region.

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E32

BIOCHEMICAL EFFECTS OF MICROPLASTIC PELLETS ON *ONCORHYNCHUS MYKISS*

Vita Žvynakytė[1,2], Janina Pažusienė[2], Gintarė Sauliutė[2], Milda Stankevičiūtė[2]

[1]Vilnius University, Lithuania
[2]Laboratory of Ecotoxicology, Nature Research Centre, Lithuania
vita.zvynakyte@gmc.stud.vu.lt

The presence of microplastics (MPs) in the environment has become a critical issue in recent decades. The alarming increase in the concentration of MPs in aquatic ecosystems is a matter of utmost concern. Different MPs have various physicochemical properties, which can cause environmental stress to living organisms [1]. Accurately measuring the amount of MPs in the environment is a major challenge, given the diverse sources of MPs. In addition, the long-term effects of MPs on organisms remain largely unexplored and require further investigation. Ecotoxicological studies are widely used to evaluate the status of fish after exposure to MPs and cover a wide range of functional areas. Exposure to MPs can induce oxidative stress leading to apoptosis, inflammation reactions, and metabolic disorders [2]. Therefore, the evaluation of changes in enzymes activity is an effective tool for assessing the ecotoxicological effects of MPs in fish [3]. It is important to note that antioxidant enzymes such as glutathione S-transferase (GST) and superoxide dismutase (SOD) are important biomarkers of oxidative stress that need to be evaluated.

The aim of this study was to determine the changes in the activity of antioxidant enzymes in the liver of rainbow trout (*Oncorhynchus mykiss*) after long-term exposure to different MPs polymers. Glutathione S-transferase activity in the liver of *O. mykiss* was determined by the method of Habig et al. (1974) [4]. Total superoxide dismutase activity was measured according to the pyrogallol autoxidation method of Marklund and Marklund (1974). [5].

The obtained results showed that GST activity significantly increased in low-density polyethylene and polystyrene exposure groups compared to the control group [6]. Low-density and high-density polyethylene have been shown to significantly increase in SOD activity. In this study, the analysis of the results showed that the different types of MPs induced significant changes in SOD and GST enzyme activity, suggesting that MPs cause oxidative stress in fish. Significant changes in enzyme activity were also found between the different groups of MPs. However, more detailed studies on antioxidant enzymes and other biochemical biomarkers are needed to characterize adverse effects of MPs in fish.

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Biochemistry and Molecular Biology

STUDY OF NEW ANTI-PHAGE DEFENSE SYSTEMS

Viktorija Rainytė[1], Paulius Toliušis[1], Mindaugas Zaremba[1]

[1]Department of Protein DNA Interactions, Vilnius University
viktorija.rainyte@gmc.vu.lt

The constant arms race between bacteria and their viral predators, bacteriophages, has fueled the development of intricate bacterial immune systems. Traditionally, research focused on well-known defense mechanisms like restriction-modification (RM) systems, 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].

This study delves deeper into this newly discovered landscape, focusing on novel bacterial defense systems characterized by the presence of nuclease and ATPase domains. By analyzing how these catalytic domains interact, the research aims to shed light on the molecular mechanisms underlying the hydrolysis, or breaking down, of DNA bonds during phage defense. This work not only provides fundamental insights into the intricate machinery of bacterial immunity, but also holds significant potential for future biotechnological applications.

Exploring this newly discovered realm of bacterial defense systems promises to not only broaden our understanding of the intricate dance between bacteria and their predators, but also holds immense potential to revolutionize various fields through innovative biotechnological applications.

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PRODUCTION OF RECOMBINANT MURINE CARBONIC ANHYDRASE IX PROTEIN

Tautvydas Kojis[1], Aurelija Mickevičiūtė[1], Lina Baranauskienė[1]

[1] Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Centre, Vilnius University, Vilnius, Lithuania
tautvydas.kojis@gmc.stud.vu.lt

Carbonic anhydrase (CA) is an enzyme that catalyzes the reversible reaction of carbon dioxide to bicarbonate ($CO_2 + H_2O \rightleftarrows H_2CO_3$); because of this function, the enzyme plays a crucial role in various physiological processes, including respiration, photosynthesis, bicarbonate and CO_2 transport, pH regulation, ion and water transport, bone development, bile and gastric production, among others. Therefore, some CA isozymes are drug targets for managing fluid secretion and pH change-linked diseases, such as glaucoma and edema; specifically, CA IX has become a target for cancer [1].

CA IX is a transmembrane glycoprotein whose involvement in various cancers has been extensively studied. This isozyme has received more attention because of its limited expression in normal cells and upregulation in many aggressive cancers compared to other CA isozymes. It is now known that CA IX is important for the growth and survival of tumor cells under normoxia and hypoxia [2].

While studying carbonic anhydrase inhibitors and related diseases, it is difficult to say whether a compound will be as effective on mouse carbonic anhydrase as it is on human carbonic anhydrase, even though these organisms' CAs are homologously similar [3]. Therefore, additional screening studies with recombinant murine carbonic anhydrase proteins (Car) would help assess whether the effects of the compounds under investigation in mice can be related to the established effects on human CA.

This research presents CarIX protein purification and recombinant protein binding to several small-molecule compounds (Figure 1).

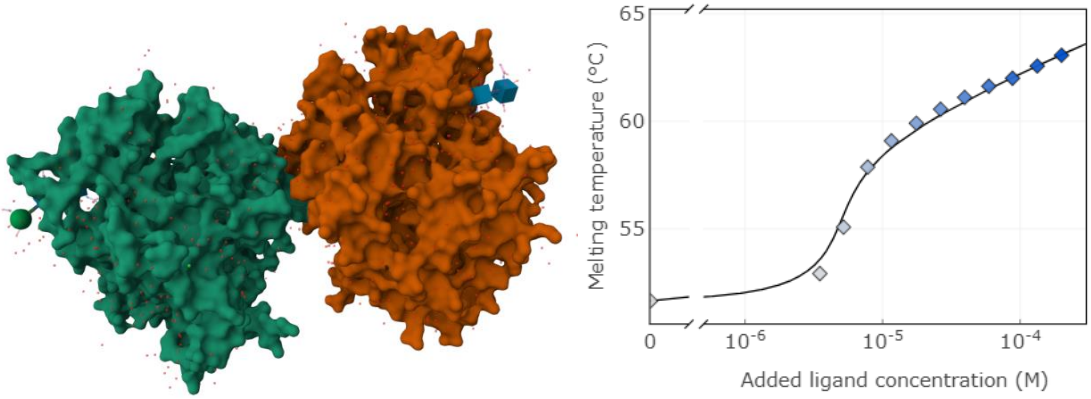

Figure 1. (Left) Crystal structure of human carbonic anhydrase IX catalytic domain (PDB id: 6Y74) (Right) Fluorescent thermal shift assay (FTSA) results of murine carbonic anhydrase IX binding to acetazolamide – symbols correspond to experimental data while line shows fitting using K_d determination model.

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ELECTROCHEMICAL BIOSENSOR FOR THE DETECTION OF MONOCLONAL ANTIBODIES AGAINST SARS-COV-2

Yana Karnitskaya[1], Maryia Drobysh[2], Viktorija Liustrovaite[1], Aurelija Zvirbliene[3], Agne Rimkute[3], Dainius Gudas[3], Indre Kucinskaite-Kodze[3], Martynas Simanavicius[3], Rimantas Slibinskas[3], Evaldas Ciplys[3], Arunas Ramanavicius[1,2]

[1]Department of Physical Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Lithuania
[2]State Research Institute Center for Physical and Technological Sciences, Lithuania
[3]Life Sciences Center, Vilnius University, Lithuania
yana.karnitskaya@chgf.vu.lt

The ongoing coronavirus disease 2019 (COVID-19) pandemic, stemming from various strains of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), resulted in a constant need to diagnose cases of this illness quickly and accurately enough to segregate infected individuals from the rest of the population and evaluate the necessity of quarantine or other public health actions. Electrochemical immunosensors and other affinity sensors offer advantages over the gold-standard polymerase chain reaction test due to their affordability, ease of use, point-of-care detection, and shortened sample analysis times, all of which are instrumental in facilitating early diagnosis of COVID-19 disease [1,2].

Here, we are reporting an electrochemical biosensor tested for the detection of different clones of monoclonal antibodies (mAbs) against the SARS-CoV-2, which are sourced from a single B cell clone and detect a particular antigen epitope. For this purpose, screen-printed carbon electrodes were covered with gold nanostructures via deposition [1] and then modified with a self-assembled monolayer (SAM) based on L-cysteine to ensure the covalent immobilization of the nucleocapsid protein (rN) [2]. The resulting system was used as a sensing platform for studying the interaction between the protein and three clones of mAbs. Employing cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques, we electrochemically characterized the working electrode at different modification stages, then captured and analyzed the response elicited by the mAbs upon interacting with the immobilized rN [2]. CV applies a triangular waveform to a working electrode to analyze reaction mechanisms from voltammogram peaks, while DPV, using fixed-amplitude pulses overlaid on step potentials, compares pre- and post-pulse currents, providing insights into electrode reactions with a consistent baseline for noise reduction and improved detection limits [2].

It was revealed, that the suggested electrochemical biosensor may provide testing options for the assessment of particular mAbs for medical monitoring that are more economically friendly. An inverse sensor for detecting SARS-CoV-2 antigens and viral particles in ambient samples may be created based on the affinity relationship between antigen and antibody.

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ACTION OF ANTI-CRISPR PROTEINS

Melita Graužinytė[1], Tomas Šinkūnas[1]

[1]Department of Protein-DNA Interactions, Institute of Biotechnology, Life Sciences Center, Vilnius University
melita.grauzinyte@gmc.stud.vu.lt

Bacteriophages and bacteria are in a constant evolutionary arms race, developing a variety of attack, defence and counter-defence tactics. In the face of phage attacks, bacteria have evolved multiple defensive mechanisms, one of which is the CRISPR-Cas system. This system encodes a ribonucleoprotein complex that destroys the invading phages by targeting their genetic material [1]. To evade this defence strategy, phages employ anti-CRISPR (Acr) proteins that disrupt the functionality of the CRISPR-Cas system, typically by interfering with its DNA-binding or hydrolytic functions [2]. CRISPR-Cas systems are used as invaluable tools for genome editing [3]. The ability of Acr proteins to modify the actions of CRISPR-Cas opens up new possibilities for their biotechnological applications [4]. More than 100 Acr families have been identified, but the molecular mechanisms are only understood for a limited number of these proteins [4].

In this study, we aim to elucidate the inhibition mechanisms of the type I-F CRISPR-Cas system by small AcrIF proteins. By combining in vivo, structural and biochemical methods, we analyse the molecular interplay between the components of the system and the AcrIF proteins.

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Cas12m EFFECTORS EXHIBIT INHERENT PROGRAMMABLE DNA BINDING CAPABILITY, FACILITATING BASE EDITING

Brigita Duchovska[1], Greta Bigelytė[1], Tautvydas Karvelis[1]

[1]Department of Protein-DNA Interactions, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
brigita.duchovska@gmc.stud.vu.lt

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) grows, attention is shifting towards alternative approaches, like base editing. This technique enables precise and predictable modifications without introducing DSBs. Adenine base editing, which converts adenine (A) to guanine (G) within the DNA sequence, offers targeted and predictable single nucleotide alterations. Although adenine base editing activity has been demonstrated with the nuclease-impaired dCas9 and dCas12a effector proteins [1, 2], 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 [3].

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. Previously, we demonstrated that Cas12m proteins provide protection against bacteriophages and plasmids through targeted DNA binding rather than DNA cleavage. Therefore, this innate programmable DNA binding could offer an alternative for dCas9 and dCas12a protein based base editing.

The objective of this study was to assess the intrinsic DNA binding capacity of Cas12m proteins for programmable base editing. We engineered base editors by fusing selected Cas12m proteins with adenine deaminase TadA-8e. Their activity was evaluated by testing the targeted A-to-G editing in human cells via next generation sequencing. Ultimately, this work contributes towards the attempts to develop a new generation of base editing tools compatible with AAV vectors.

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F6

ANALYSIS OF FUSED TUDS-DUF1722 PROTEINS

Deimantė Povilaitytė[1,2], Rapolas Jamontas[1], Audrius Laurynėnas[1], Rolandas Meškys[1], Agota Aučynaitė[1]

[1]Institute of Biochemistry, Life Sciences center, Vilnius University, Lithuania
[2]Institute of Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Lithuania
deimante.povilaityte@chgf.stud.vu.lt

tRNA is an adapter molecule required for protein synthesis. tRNAs are heavily decorated with a variety of post-transcriptional modifications. Most modifications are found in the tRNA anticodon, making them crucial for precise codon recognition and reading frame maintenance which leads to efficient protein synthesis. These modifications are introduced enzymatically [1]. Biosynthesis pathways of chemical modifications are well studied; however, little is known about the metabolic fate of chemically modified nucleosides or heterocyclic bases after RNA degradation [2].

One type of tRNA modification is enzymatic thiolation. 4-thiouridine (s^4U) is found at position 8 of tRNAs. s^4U acts as a photosensor for ultraviolet light (UV) and saves bacteria from photomutagenic effects by causing reduced growth when exposed to UV radiation [3]. Upon the degradation of bacterial tRNA 4-thiouracil monophosphates are recycled by thiouracil desulfidase (TudS) which catalyzes the abstraction of sulfide from 4-thiouracil derivatives. Although TudS domain is widespread as a stand-alone protein, about one third of known TudS domain encoding genes are coding TudS fused with the sequence of a Domain of Unknown Function 1722 (DUF1722) [4]. Our recent studies revealed that in contrast to stand-alone TudS which is active towards individual 4-thiouridine derivatives, TudS-DUF1722 protein is capable to desulfidise 4-thiouridine in the context of intact tRNA.

The aim of this study is to analyze the activity of fused Tuds-DUF1722 proteins from *Pseudomonas putida* KT2440. To achieve this goal, site directed mutagenesis was performed to detect which amino acids are crucial for in vivo activity. Phenol extraction was used to obtain tRNAs from bacteria producing various proteins, followed by tRNAs purification using Fast protein liquid chromatography (FPLC). Finally, tRNAs samples were hydrolyzed and the quantity of 4-thiouracil was determined with High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). In addition, the electrophoretic mobility shift assay (EMSA) was conducted to confirm that TudS-DUF1722 protein interacts with tRNA.

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STRUCTURAL MODIFICATIONS OF THE BACTERIOPHAGE vB_PagS_MED16 SELF-ASSEMBLING TAIL SHEATH PROTEIN GP13

Goda Stirbytė[1,2], Simona Povilonienė[1], Greta Labutytė[1], Vida Časaitė[1], Martynas Talaikis[3], Rolandas Meškys[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Saulėtekio ave. 7, Vilnius, Lithuania
[2]Institute of Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Naugarduko str. 24, Vilnius, Lithuania
[3]Department of Bioelectrochemistry and Biospectroscopy, Institute of Biochemistry, Life Sciences Center, Vilnius University, Saulėtekio ave. 7, Vilnius, Lithuania
goda.stirbyte@chgf.stud.vu.lt

The application of protein nanotubes is quickly emerging in medicine, biotechnology and pharmaceutical sector as these structures are biocompatible, biodegradable and can undergo surface modifications [1]. One of these nanotube-forming proteins are tail sheath proteins of bacteriophages. Tail sheath proteins can be modified by genetic and protein engineering, so it is possible to fuse them with enzymes, ligands or drug molecules. They possess the ability to self-assemble and form stable structures of precise geometry which makes them potential therapeutic nanocarriers that can be applied to deliver therapeutic biomolecules [2].

In this study we focused on the tail sheath protein gp13 of the bacteriophage *Pantoea* vB_PagS_MED16 which self-assembles into nanotubes. It was determined that the shape and size of these nanotubes depend on which terminus of the protein is anchored with polyhistidine-tag (His-Tag) [3]. To investigate other possible structures of the gp13 nanotubes, several protein modifications were conducted. We performed three different studies to evaluate: a) the influence of cysteine residues in gp13, b) its ability to form longer than wild-type nanotubes, c) the potential to transport a fused enzyme.

To study disulphide bond formation in nanotubes we used site-directed mutagenesis to create genes, coding mutant gp13 with additional cysteines in 114 and 132 positions or without native cysteine. After protein purification, transmission electron microscopy analysis revealed that the mutant proteins self-assemble into tubular structures, similar to the ones formed by the wild-type protein. Raman spectroscopy analysis revealed the possible disulphide bond formation in some cysteine mutants; the melting temperatures of the mutant proteins were also evaluated.

Gp13 with His-Tag at N-terminus forms short nanotubes made of several layers of hexamers, so we searched for a way to induce the formation of longer structures. This was found possible by fusing gp13 with green fluorescent protein (GFP) at the N-terminus and then specifically cleaving it with WELQut protease. The nanotubes formed by hybrid GFP and gp13 protein prior to cleavage with protease were not observed during transmission electron microscopy analysis, instead amorphous formations were visible. During the specific cleavage GFP and His-Tag were removed from the N-terminus of gp13 and the remaining protein was found capable of self-assembling into long nanotubes, up to 1.5 µm in length.

To test gp13 as a potential enzyme carrier, we fused it with amidohydrolase YqfB using one or more GS linkers. After protein purification, an activity assay was performed spectrophotometrically: the hydrolysis of *N*^{4}-acetyl-2'-deoxycytidine confirmed that YqfB fused with gp13 retains its enzymatic activity and gave prospect to use gp13 for enzyme delivery.

In conclusion, the conducted studies present ways to modify the tail sheath protein gp13 by various strategies: induce long nanotube formation and use gp13 as a potential enzyme nanocarrier.

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F8

CONSTRUCTION OF A FUNCTIONAL DIMERIC CYCLIC OLIGOADENYLATE-ACTIVATED SAVED NUCLEASE

Marija Duchovskyte[1], Dalia Smalakyte[1], Giedre Tamulaitiene[1], Gintautas Tamulaitis[1]

[1]Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
marija.duchovskyte@gmc.vu.lt

A defining characteristic of CBASS (cyclic oligonucleotide-based antiphage signaling systems) and type III CRISPR (clustered regularly interspaced palindromic repeats) antiviral defense systems is the use of cyclic nucleotides as second messengers to activate a diverse range of effector proteins [1]. These effectors degrade or disrupt key cellular components such as nucleic acids, membranes, metabolites, attenuate translation or transcription in the infected cell, thereby slowing down viral replication kinetics or leading to cell death [2]. Although effectors with cyclic oligonucleotide sensory SAVED (SMODSassociated and fused to various effector domains) domain are characteristic of CBASS defense systems, SAVED domain-containing proteins are sometimes encoded in close vicinity to type III CRISPR systems [3].

Type III-D CRISPR system of the bacterium *Clostridium novyi* encodes a SAVED nuclease containing a sensory SAVED domain fused to an endonuclease-like domain. Upon binding cyclic tri-adenylate, this effector protein oligomerizes, triggering its DNase activity. Our cryo-EM studies revealed that SAVED nuclease oligomerizes through SAVED domains, but the structure and position of the endonuclease-like domain in the helical filament are not visible. Here, we present the construction and characterization of a dimer-forming mutant SAVED nuclease to determine the arrangement of the activated endonuclease-like domains.

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F9

CALCIUM-INDUCED HETERODIMERIZATION OF S100A8 WITH S100A1 TRIGGERS AMYLOID FIBRILLATION

Viktorija Karalkevičiūtė[1], Ieva Baronaitė[1], Darius Šulskis[1], Vytautas Smirnovas[1]

[1] Sector of Amyloid Research, Life Sciences Centre, Vilnius University, Lithuania
viktorija.karalkeviciute@gmc.stud.vu.lt

S100 is a family of calcium-binding proteins, consisting of isoforms with structural similarity but functional diversity [1]. S100 proteins regulate various proteins involved in cellular functions like calcium homeostasis, cell growth, differentiation, cytoskeleton dynamics, and energy metabolism [2]. Several members are known to be important in neurodegeneration by signaling neuroinflammation and forming amyloid fibrils. One of them is S100A9, which is well-studied, but the roles of S100A1 and S100A8 remain relatively unexplored.

S100A1 is predominantly expressed in the brain, skeletal and cardiac muscles [3]. S100A1 interacts with tau, RAGE, and RyR - proteins that participate Alzheimer s disease (AD) cascade [4]. Another family member S100A8 is mostly found in neutrophils and monocytes [5] and plays a ´ role in neurological disease pathology as well. S100A8 homodimers can independently induce neuroinflammation [6] and their overexpression in AD patients leads to activation of microglia [7, 8]. However, it is known that S100A8 can form a heterodimer with S100A9 called calcprotectin [9], but intereaction with S100A1 is still not investigated. Both S100A1 and S100A8 are expressed in the cerebral cortex as per the Human Protein Atlas (https://www.proteinatlas.org/) [10] and share structural similarities [1]. Thus, our main goal was to elucidate their potential complex formation.

To explore the aggregation kinetics of the S100A1/A8 complex, we employed the Thioflavin T Fluorescence Assay, unveiling calcium concentration-dependent amyloid formation. In addition, Atomic Force Microscopy (AFM) was used to visualize the S100A1/A8 fibrils and, Differential Scanning Fluorimetry (DSF) to quantify protein stabilities. In conclusion, our research contributes new findings to the understanding of S100A1 and S100A8 aggregation dynamics, offering valuable insights into their relevance to various diseases.

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THE STUDY OF α-SYNUCLEIN AGGREGATION IN ARTIFICIAL CEREBROSPINAL FLUID

Arūnė Jocytė[1], Andrius Sakalauskas[1], Rūta Sniečkutė[1], Vytautas Smirnovas[1]

[1]Sector of Amyloid Research, Institute of Biotechnology, Life Sciences Centre, Vilnius University
arune.jocyte@gmc.stud.vu.lt

α-synuclein (α-Syn) is a naturally formed protein in the body that is involved in neurotransmission functions. However, several factors contribute to α-Syn aggregation and pathology, including aging, environmental factors, mutations, changes in α-Syn expression, interactions with other aggregated proteins and post-translational modifications [1]. During α-Syn aggregation, protein undergoes a conformational change from its normal soluble state to misfolded, resulting in the formation of fibrillar structures. The structures known as Lewy bodies and Lewy neurites are formed. These aggregates accumulate in the nervous system, disrupt normal neurotransmission (affecting dopaminergic receptor function), and may contribute to neuronal loss. Consequently, Lewy bodies and Lewy neurites are associated with a spectrum of neurodegenerative disorders, including Parkinson's disease, dementia with Lewy bodies and multisystem atrophy. Despite sharing common pathological features, each condition exhibits distinct clinical presentations and affects different brain regions, resulting in varying symptoms and effects [2].

The aim of the study is to determine the polymorphism of α-Syn amyloid fibrils in the artificial cerebrospinal fluid. To achieve this, the aggregation kinetics of α-Syn were investigated by monitoring the changes in thioflavin T fluorescence intensity over time. Additionally, the α-Syn aggregation assay was carried out by selectively removing and adding one component of artificial cerebrospinal fluid. The resulting secondary fibrillar structures were evaluated by using Fourier-transform infrared spectroscopy. Furthermore, the morphological variability of amyloid fibrils was observed by atomic force microscopy. The structural diversity of aggregates formed in the artificial cerebrospinal fluid can be compared with structures formed in disease cases. The polymorphism exhibited by α-Syn aggregates could have significant implications for disease pathogenesis and progression. Variations in the folding patterns can lead to the formation of distinct pathological species with differential toxicity profiles. By identifying disease-specific aggregates (existing polymorphism among them), further exploration can be conducted to search for potential inhibitors aimed at preventing their formation [3, 4].

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F11

Non-classical secretion of cytosolic proteins in *E. coli*

Urtė Valantinaitė[1,*], Nina Urbelienė[1], Rolandas Meškys[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Saulėtekio av. 7, Vilnius, Lithuania
*urte.valantinaite@gmc.stud.vu.lt

Escherichia coli is an extensively used expression system for producing recombinant proteins. Despite being well-characterized and possessing properties such as simple media requirements, fast growth, and high protein yields, its poor secretion of proteins into the extracellular medium remains a drawback on analytical and industrial scales. Protein secretion in the culture medium is desirable due to cheaper and simplified downstream processing in addition to improved protein stability and solubility [1]. Moreover, it is necessary to move proteins into the extracellular space in order to screen and analyze enzymes capable of degrading substrates that cannot be transported into the cell. Therefore, different approaches to enhance recombinant protein secretion in *E. coli* strains are being investigated. One of them is the non-classical secretion system. While classical secretory proteins possess a signal sequence or secretion motifs that allow them to be exported via cell's secretion systems, the non-classical system offers a distinct method of secreting proteins without requiring a signal peptide [2]. Nevertheless, it is still unclear what selection mechanism governs these enzymes' secretion pathways.

The aim of this work is to evaluate the possibility of secretion of cytosolic proteins by using the recombinant *Thermobifida fusca* PETase 075, which is known to be excreted out of the cell without an appended leader sequence [3].

In this study, fusions of an active and inactive PETase 075 variants and a bacterial amidohydrolase YqfB were engineered. Afterwards the gene encoding for the fused enzymes was expressed in *E. coli* BL21(DE3) cells. A fusion of an active PETase 075 and YqfB has been found to be secreted into the extracellular space. In addition, the hybrid protein retained both amidase (YqfB) and esterase (PETase 075) activities.

In the future, the applicability of PETase 075 to enhance the extracellular production of other recombinant cytosolic proteins of varying structures and molecular masses will be further scrutinized and optimized.

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F12

FUNCTION OF THOERIS THSB PROTEINS IN ANTIVIRAL DEFENSE

Ugnė Andrijauskaitė[1], Džiugas Sabonis[1], Giedrė Tamulaitienė[1]

[1]Department of Protein-Nucleic Acid Interactions, Institute of Biotechnology, Vilnius University
7 Saulėtekio Ave, LT- 10257 Vilnius
ugne.andrijauskaite@chgf.stud.vu.lt

Research on prokaryotic antiviral defense systems has been on the rise, especially since it was shown that the genes of bacterial defense systems are usually located in clusters called "defense islands". This observation led to discovery of new bacterial antiviral defense systems and expanded the knowledge about the mechanisms of the bacterial immune response [1]. One of the novel defense systems discovered was named Thoeris.

The Thoeris system consists of two genes, *thsA* and *thsB*. The Thoeris system can be divided into two types - type I, where ThsA is composed of SIR2 and SLOG domains, and type II, where ThsA is composed of transmembrane and Macro domains. ThsB protein contains Toll/interleukin-1 receptor (TIR) domain in both Thoeris types. Upon bacteriophage infection, ThsB produces a signalling molecule (1"-3'gcADPR for type I and histidine-ADPR for type II) which is bound by the ThsA SLOG or Macro domain, respectively [2, 3]. In type I systems, the activated ThsA forms helical filaments, that stabilize SIR2 active sites and allow rapid NAD^+ hydrolysis, resulting in NAD^+ depletion and host cell death [4]. It is yet to be discovered how ThsB recognizes bacteriophage infection and what is the ThsA Macro domain effector response.

Both ThsA and ThsB are required for protection against bacteriophages, since mutations in the active sites of ThsA or ThsB result in a complete loss of viral resistance [1]. Moreover, multiple diverse *thsB* genes can be located around the single *thsA* gene. It is predicted that differences in TIR domains serve the purpose of recognising diverse phage components increasing the range of bacteriophages the Thoeris system can protect against [1].

It has been shown that the type II Thoeris system derived from *B. amyloliquefaciens* Y2 protects against *Bacillus sp*. and *E. coli* bacteriophages [5]. In this work, we determined which proteins of the homologous Thoeris system, consisting of two *thsB* and one *thsA* genes, are essential for protection against *E. coli* bacteriophages. Determining the impact on the protection of both ThsB and ThsA proteins gives more insights into the function of the diversity of ThsB proteins and provides further perception for determining the molecular basis of bacteriophage infection recognition.

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F13

PURIFICATION OF *A. BAUMANNII* CAPSULAR POLYSACCHARIDES

Giedrė Kukanauskaitė[1], Juratė Skerniškytė[1]

[1]Department of Biochemistry and Molecular Biology, Vilnius University, Lithuania
giedre.kukanauskaite@chgf.stud.vu.lt, jurate.skerniskyte@gmc.vu.lt

Gram-negative bacterium *Acinetobacter baumannii* is an opportunistic pathogen, which causes hospital-acquired infections worldwide. The rapid spread and multiple-drug resistance have led to the breeding of this pathogen in health centers, especially in intense care units, therefore this pathogen poses an increasing threat to immunosuppressed patients. One of the main virulence factor is the capsular polysaccharides (CPS) covering the surface of the bacterium. CPS increase resistance to desiccation, disinfectants, antimicrobials, and antibiotics as well as host immune responses. The aim of this study was to obtain pure fractions of CPS without any remaining of nucleic acids, proteins, and most importantly without lipopolysaccharides.

Methods: To study the interaction of CPS with phagocytic cells of innate immunity, it was necessary to purify CPS from other cell components such as DNA, RNA, proteins, lipopolysaccharides, and others. Several purification methods were optimized to obtain the high yield of CPS. A hot phenol method was used for the purification of CPS from proteins and lipopolysaccharides. Further purification steps included Gel-filtration chromatography based on the separation of components according to their molecular size. To ensure that the purification methods gave positive results, the collected fractions were run on agarose and SDS-page gels. CPS was visualized by the staining with Alcian blue dye.

Results: After using hot phenol to separate proteins and lipopolysaccharides from CPS, Bradford assay demonstrated no proteins were present in the samples. Analysis of agarose gel showed that some DNA and RNA remaining were detected in subsequent chromatographic fractions. In contrast, SDS-page gel revealed that CPS were released in the first chromatographic fractions. Moreover, no lipopolysaccharides were observed in the first fractions. Those results indicate that the hot phenolization procedure and the Gel-filtration chromatography method helped to purify CPS from extraneous cell components such as DNA, RNA, proteins, and lipopolysaccharides.

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ENGINEERING THE PROKARYOTIC CYTIDINE DEAMINASE CDA_F14: INVESTIGATING THE EFFECT OF MUTATIONS ON ENZYME ACTIVITY

Emilija Skrodenytė [1], Nina Urbelienė [1], Rolandas Meškys [1]

[1] Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University
emilija.skrodenyte@.gmc.stud.vu.lt

Cytidine deaminases (CDAs; EC 3.5.4.5.) are enzymes belonging to the class of hydrolases that catalyse the deamination of cytidine or 2'-deoxycytidine to uridine or 2'-deoxyuridine respectively. In the Department of Molecular Microbiology and Biotechnology, prokaryotic cytidine deaminases have been discovered that are capable of hydrolyzing to uridine various derivatives of N4-acyl, N4-/O4-/S4-alkyl, and N4-/O4-/S4-arylpyrimidine nucleosides. Some of the latter compounds may be produced in organisms during damage or may be used as antiviral or anticancer drugs. Therefore, metagenomic cytidine deaminases (mCDAs) have the potential to participate in reparation processes and in the process of activation/deactivation of prodrugs [1].

One of the deaminases, the metagenomic cytidine deaminase CDA_F14, was able to deaminate wide range of 4-substituted nucleosides. As found in previous studies, this result was due to the large binding pocket of the enzyme. However, CDA_F14 deaminase did not deaminate any nucleosides with substitutions at the ribose ring. Studies on the metabolism of nucleosides with substitutes at ribose ring are important for pharmacological industry.

The aim of this study was to determine the impact of amino acids to specificity of CDA_F14 towards substrate with substituted ribose ring. Molecular docking of CDA_F14 molecule showed that Ala46 and Tyr48 form hydrogen bonds with ribose 5'-OH group, while Asn42 and Glu44 – with 3'-OH group. The site-directed mutagenesis was performed to reveal the effect of mentioned amino acids on activity of CDA_F14. As results, the replacing Ala46 and Tyr48 to glycine showed no changes in substrate specificity compared to wild-type CDA_F14. However, the Ala46Gly mutant showed impaired kinetic constants, while the Tyr48Gly mutant exhibited drastic reduction in activity towards 2'-deoxycytidine. Moreover, random mutagenesis of Tyr48 amino acid revealed, that the mutants Tyr48Phe and Tyr48Trp retain hydrolytic activity towards N4-benzoyl-2'-deoxycytidine. Additionally, these mutations altered substrate specificity compared to wild-type CDA_F14.

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F15

BIOCATALYTIC SYNTHESIS OF ASYMMETRIC WATER-SOLUBLE INDIRUBIN DERIVATIVES

Martynas Jakutis[1], Mikas Sadauskas[1], Vytautas Petkevičius[1], Martynas Malikėnas[2], Viktorija Preitakaitė[1], Justas Vaitekūnas[1], Rolandas Meškys[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Saulėtekio al. 7, Vilnius, LT-10257, Lithuania
[2]Department of Organic Chemistry, Institute of Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Naugarduko st. 24, Vilnius, LT-03225, Lithuania
martynas.jakutis@chgf.stud.vu.lt

Indigoids are natural pigments that consist of two oxygenated indole molecules. These indole derivatives can be linked in different ways, resulting in a variety of structural isomers. Indirubin is one of the possible oxygenated indole dimerization products. It is chemically a 3-2' bisindole characterized as a violet pigment that exhibits low solubility in water [1], [2]. Also, indirubin and its derivatives have been well-documented for their potent antiproliferative and anticancer properties that are mostly attributed to the inhibition of cyclin-dependent kinases and glycogen synthase kinase 3-β [1]. However, the limited solubility of non-substituted indirubin in aqueous solutions restricts its effects on proliferation [2]. Therefore, the structure of indirubin serves as a model for the synthesis of new indirubin derivatives that could demonstrate improved chemical and pharmacological properties [1], [3]. There is a published approach for indirubin organic synthesis employing indoxyl acetate and isatin derivatives [4]. Nevertheless, the recent advancements in molecular biology and biotechnology provided an opportunity to execute environmentally friendly indigoid biosynthesis driven by bacterial monooxygenases [3].

During this research, a method for the synthesis of asymmetric carboxy-substituted indirubins was developed. The approach is based on whole-cell bioconversions of substrates, such as indole-5-carboxylic acid or indole-6-carboxylic acid and 2-indolinone derivatives (Fig. 1). These bioconversions were carried out in the presence of bacterial monooxygenases expressed in the bacterial host. The bioconversion system yielded the highest monocarboxyindirubin production titer of up to 327 mgL^{-1} for 5-bromoindirubin-6'-carboxylic acid during the incubation period of 16-h. It was demonstrated that the purified monocarboxyindirubins exhibited up to a thousandfold higher solubility in water compared to indirubin. Moreover, several monocarboxyindirubins, specifically 1-methylindirubin-5'-carboxylic acid, exhibited potent antiproliferative effects on different cancer cell lines. In consequence, the applied approach for the synthesis of monocarboxyindirubins is a considerable opportunity due to the efficiency and significantly reduced environmental harm compared to organic synthesis. Besides, the synthesized monocarboxyindigoids could be attractive in the field of drug design, according to their solubility and antiproliferative activity.

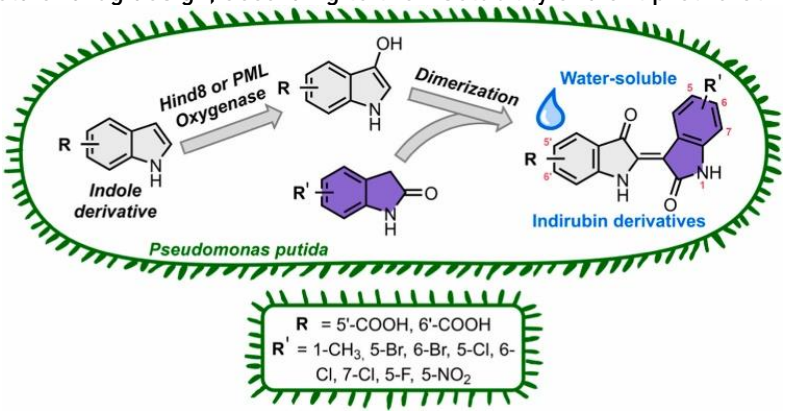

Figure 1. A schematic representation of indirubin derivative biocatalytic synthesis in *Pseudomonas putida* KT2440 cells expressing the monooxygenase activity.

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TAILOR-MADE MODIFICATIONS FOR ENZYME IMMOBILISATION USING THE CLEA METHOD

Kristupas Valaitis[1], Justinas Babinskas[1], Inga Matijošytė[1]

[1] Sector of Applied Biocatalysis, Institute of Biotechnology, LSC Vilnius University, Lithuania
kristupas.valaitis@gmc.stud.vu.lt

The increasing demands for process efficiency and sustainability for industrial production require new solutions. Biocatalysts are biodegradable, produce fewer byproducts, and have significantly higher reaction rates and substrate specificity compared to chemical catalysts. Some of the most employed biocatalysts in industrial applications are enzyme lipases, which catalyse a plethora of reversible carboxyl-nucleophile hydrolysis reactions. Nonetheless, free-state lipases exhibit limitations such as sensitivity to the reaction medium or low operational stability [1]. Enzyme immobilisation extends the application of lipases by enhancing their stability and allows enzyme recycling. Yet, currently, most applied immobilisation techniques for lipases (absorption or covalently binding to carriers) produce an insufficient increase in stability, carriers are expensive and often make up most of the enzyme-carrier mass [2]. These issues could be avoided by using carrier-free enzyme immobilisation.

One of the most promising carrier-free immobilisation methods is based on the cross-linking enzyme aggregates (CLEA). This method is fast, easy to execute and inexpensive. It entails enzyme aggregation by adding salts or organic solvents followed by aggregate cross-linking with a bi- or multi-functional linking agent. Currently, one of the most widely used cross-linkers is glutaraldehyde. However, due to its small size and high reactivity, glutaraldehyde can enter the enzyme's active site and inactivate it [3]. A potential solution to this problem could be the use of transglutaminase instead of glutaraldehyde. Transglutaminases are widely employed in the food industry to cross-link proteins, such as whey or collagen, by transamidation between lysine and glutamine residues, resulting in the formation of isopeptide bonds [4]. Yet, there is little literature describing the use of transglutaminase in the making of CLEAs.

In this project, we investigated three different approaches for lipase immobilisation by CLEA method: two-step, one-pot, and the use of transglutaminase (TGA) (Fig. 1). Lipase aggregation induced by four organic solvents (acetone, ethanol, isopropanol and 2-methoxyethyl ether) and aggregation time were optimised. The investigation of the cross-linking reaction time and glutaraldehyde concentration revealed the impact on formed CLEA catalytic properties. The range of lipase and TGA v/v ratios and their effect on the total amount of immobilised proteins and lipase activity was examined.

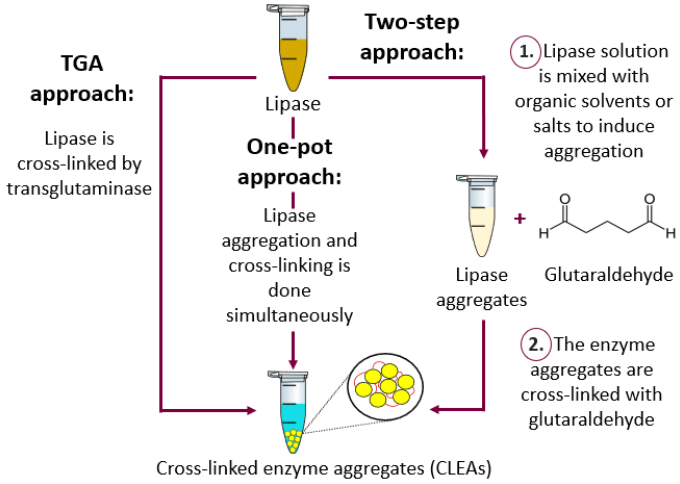

Figure 1. Three approaches of lipase immobilisation by CLEA method.

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F17

ENZYMATIC SPECIFICITY OF DEPOLYMERASE FROM *KLEBSIELLA* PHAGE P15

Rytis Jurgaitis[1], Justas Lazutka[2], Algirdas Noreika[1], Rolandas Meškys[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Sauletekis av. 7, Vilnius, Lithuania
[2]Department of Eukaryote Gene Engineering, Institute of Biotechnology, Life Sciences Center, Vilnius University, Sauletekis av. 7, Vilnius, Lithuania
rytis.jurgaitis@gmc.stud.vu.lt

Klebsiella spp. are Gram-negative bacteria commonly found in the gastrointestinal tract. As an opportunistic pathogen, it is responsible for up to 10% of all nosocomial infections, due to its emerging multidrug-resistance (MDR), and numerous virulence factors [1]. One of the most important virulence factors of *Klebsiella* spp. is the capsule composed of polysaccharides (PS), which repel the host's immune system, manifesting in the evasion of neutrophil phagocytosis [2]. The capsule also provides a spatial barrier, that hinders the attachment of bacteriophages on the outer surface components of the bacterial cell membrane. However, certain bacteriophages bear specific enzymes, namely depolymerases, which degrade capsular PS, facilitating the penetration through this defence and the infection of bacterium. While bacteriophage-borne proteins may serve as an alternative means of fighting back these MDR pathogens, they also hold potential as valuable reagents in the saccharide industry, aiding in the catalysis of novel saccharides under physiological conditions.

In this study, a bacteriophage p15 was discovered and isolated from a compost biosample (Fig. 1A) employing the double agar method with the *Klebsiella pneumoniae* KV-3 strain. The bacteriophage p15 forms 0.7–1.2 mm lysis centres, with depolymerase activity extending up to ~1.9 mm in diameter. Transmission electron microscopy (TEM) analysis revealed that phage p15 possesses a morphology corresponding to podo-type (Fig. 1B) viruses, characterized by an icosahedral head (~60 nm) and a short noncontractile tail (~13 nm). Genomic phage DNA was extracted and sequenced using Oxford Nanopore Technology, achieving a length of <40 kbp. The gDNA sequence was used to identify the potential depolymerase, gp36 (Fig. 1C). The full-sequence gene encoding gp36 was amplified by PCR and cloned into pET16 expression system for recombinant protein production. Despite the tendency of full-sequence phage-borne depolymerases to aggregate, a synthesis of gp36 was successfully induced and purified in a soluble form (Fig. 1D), using the following induction conditions: *Escherichia coli* HMS174(DE3) gene expression strain, 0.8 mM IPTG, for 17 h at 30 °C. The enzymatic specificity of gp36 was investigated through PS hydrolysis assays (Fig. 1E), using both the purified gp36 and isolated KV-3 capsular PS, with gp531 [3] serving as a reference for known enzymatic specificity. The enzymatic reaction products were analysed by TLC and HPLC-MS assays (Fig. 1F), confirming the specificity of gp36 as a β-glucosidase.

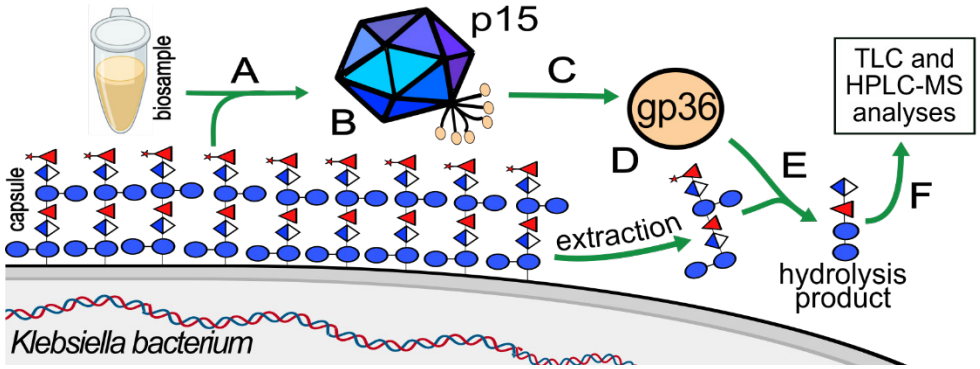

Figure 1. Klebsiella-infecting phage isolation and investigation of its depolymerase activity: bacteriophage screening from biosamples (**A**); phage morphology analysis using TEM (**B**); genome extraction and depolymerase-encoding gene identification (**C**); g36 cloning, expression and recombinant protein purification (**D**); extracted PS digestion with recombinant gp36 (**E**); TLC and HPLC-MS analyses of PS hydrolysis products to determine specificity (**F**).

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F18

A DIVERSITY OF POLYCAPROLACTONE DEGRADING CARBOXYLESTERASES

Povilas Kruopis[1], Renata Gasparavičiūtė[1], Nina Urbelienė[1], Rolandas Meškys[1]

[1]Department of Molecular Microbiology and Biotechnology, Life Science Centre, Vilnius University, Vilnius, Lithuania
povilas.kruopis@gmc.stud.vu.lt

Esters represent a major group of organic molecules used as pharmaceuticals, flavorings, odorants or chemical precursors. [1] A significant number of aforementioned applications require stereo-pure compounds, which are difficult or impossible to synthesize by chemical methods. [1] Moreover, chemical methods often involve environmentally harmful substances. [1] Therefore, efficient and stereoselective biological catalysts are necessary. Additionally, esterases have the potential to accelerate the natural biodegradation of plastics, reducing the time it takes for plastics to break down in the environment.

In this study we aim to characterize six carboxylesterases, screened from environmental microorganisms. The enzymes were identified by halo formation on polycaprolactone diol Mn530 (PCLD) agar plates. First, various cultures from environmental samples (soil, water, sludge) were collected from Vilnius region, Lithuania. Then positive hits on PCLD agar by hallo formation were purified and genomic libraries were constructed. In the second step, the clones of transformed *E. coli* DH5α, exhibiting PCLD hydrolytic activity were detected on LB agar containing 1% PCLD. Then the plasmid DNA from the positive clones was isolated, ORFs were identified by sequencing.

The sequence analysis showed, that five enzymes belong to an alpha/beta fold hydrolases (SSF53474) superfamily, and one (PCLDk83) belongs to a beta-lactamase/transpeptidase-like (SSF52266) superfamily. To further investigate their lineage, a phylogenetic tree of amino acid sequences of the enzymes was assembled using IqTree with default settings. The resulting tree clearly shows PCLDk83 as having furthest branched evolutionarily.

Various *p*-nitrophenol esters were used to determine a specific activity of the carboxylesterases. Qualitative assay determined, that all six enzymes possess a *p*-nitrophenol acetate hydrolytic activity. PCLDk32 also displayed *p*-nitrophenol butyrate, valerate, decanate and stearate hydrolytic activity. Additionally, the tested carboxylesterases revealed a hydrolytic activity towards plastics. All six enzymes were determined to hydrolyze a PCL film (Mr 80,000) and some PET monomers.

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Off - targets for good: Mispairing alters the rate and position of AsCas12a cleavage

Fausta Michnevičiūtė[1], Uzair Ahmed[1], Marius Vinogradovas[1], Stephen Knox Jones Jr.[1]

[1]EMBL Partnership Institute for Genome Editing Technologies, Life Sciences Centre, Vilnius University, Lithuania
fausta.michneviciute@gmc.stud.vu.lt

Clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 is gene-editing technology causing a major upheaval in biomedical research. It makes possible to correct errors in the genome and turn on or off genes in cells and organisms quickly, cheaply and with relative ease [1]. In addition to Cas9, researchers are actively exploring other CRISPR-Cas enzymes with therapeutic potential. One of them is AsCas12a. Cas12a, a small CRISPR-associated nuclease, uses a guide RNA (gRNA) to direct sequence-specific DNA cleavage. Cas12a recognizes DNA sequences with T-rich PAM site and cleaves DNA to produce 5' overhangs. It also tolerates small differences between the gRNA and DNA target sequences, especially in PAM-distal regions. This contributes to 'off-target' activity, where the nuclease may cut additional sequences, causing unexpected and adverse consequences in the genome. Our objective was to elucidate the kinetics and specific cleavage outcomes upon programming Cas12a with gRNA that mispairs with its DNA target. To understand how such intentional modifications to the gRNA or target DNA influence AsCas12a, we performed *in vitro* cleavage assay experiments with targets and gRNAs containing PAM-distal mispairs. We fluorescently tagged each end of the target and non-target DNA strands. Upon analysis, our data revealed their cleavage rates, initial DNA cleavage sites, and end trimming rates for each of the DNA strands and ends. We found that differences between the gRNA and target DNA sequences produced mispair-dependent cleavage products of different lengths and related trimming profiles. In conclusion, mispairing affects CRISPR-Cas enzymes' ability to accurately recognize and cut their intended target sites, which impacts their gene editing potential. Intentionally programming AsCas12a with PAM-distal mispairs could even alter gene editing outcomes simply by changing gRNA sequence. Other part of the project will be to check if the *in vitro* data aligns with *in vivo* experiments and how mispairs affect editing in cells.

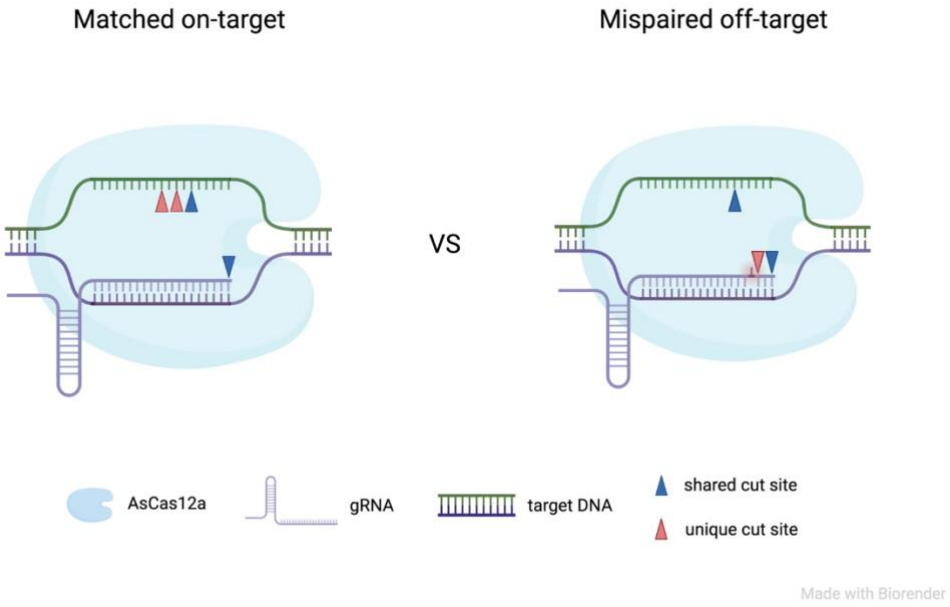

Figure 1. Mispairing in the PAM - distal region of the target DNA alters the initial cleavage sites by the AsCas12a enzyme.

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BACTERIAL CONJUGATION-BASED SCREENING PLATFORM FOR TRANSPOSABLE ELEMENTS

Bartė Žiliukaitė[1], Gytis Druteika[1], Tautvydas Karvelis[1]

[1]Department of Protein-DNA Interactions, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
barte.ziliukaite@chgf.stud.vu.lt

Mobile genetic elements (MGE), such as plasmids, bacteriophages, insertion sequences (IS), and transposons, are widespread elements that can be transmitted between the cells or can change location within the genome of the same host. MGEs are found in both prokaryotes and eukaryotes and play a crucial role in evolution and genome plasticity [1]. Recent studies and bioinformatic analysis have suggested that IscB and TnpB – proteins encoded by IS200/IS605 family insertion sequences – might be the predecessors of the CRISPR-associated proteins Cas12 and Ca9, respectively [2]. Prokaryotic CRISPR-Cas systems form an adaptive immunity against foreign DNA through RNA-dependent DNA or RNA nuclease activity and can be easily applied for programmable gene editing. However, while widely used for genome engineering, these systems lack efficiency, are prone to off-target activity, require PAM sequences that greatly limit the possible targets in the genome, and the size of the nucleases causes delivery difficulties. Furthermore, the mechanism of gene editing with Cas proteins is based on homologous recombination repair, feasible only in actively dividing cells, thus restricting application in certain cell types [3]. These limitations of CRISPR-Cas systems raise a demand for novel genome editing tools. Considering the evolutionary proximity of IS to Cas nucleases, IS could potentially offer a variety of alternative genome editing tools. In this study, we aimed to establish a bacterial-conjugation-based screening system to assess the mobility of transposable elements. Based on the previously described works [4], we constructed vectors that contain an active transposase and an antibiotic resistance gene-carrying minimal transposable element (mini-Tn). We performed selection for the bacterial strain that is most suitable for transposition assay, optimized the conditions of the bacterial conjugation, and conducted primary experiments allowing us to detect the transposon‘s mobility. These findings contribute to the development of a robust IS screening system, enhancing the ability to identify new IS elements that have the potential to be used as novel genome editing tools.

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THE EFFECT OF ENVIRONMENTAL STRESSORS ON THE EXPRESSION OF *LACTICASEIBACILLUS PARACASEI* SMALL RNAS

Odilija Safinaitė[1], Milda Mickutė[1], Giedrius Vilkaitis[1]

[1] Department of Biological DNA Modification, Institute of Biotechnology, Life Sciences Center Vilnius University, Saulėtekio av. 7, Vilnius, LT-10257, Lithuania
odilija.safinaite@gmail.com

Lacticaseibacillus paracasei is a Gram-positive, lactic acid, probiotic bacteria that is naturally found in the human body and is often used in the production of fermented foods [1]. Due to their different living locations, these bacteria can adapt to constantly changing environmental conditions, which allows them to be utilized in a wide range of industrial applications, such as production of cosmetics, food, oil and pharmaceuticals [2].

Generally, the ability of bacteria to withstand stressful situations and adjust to their surroundings is facilitated by small regulatory RNA (sRNA) molecules, which play an important role in the post-transcriptional regulation of gene expression. These small RNAs range in length from 50 to 500 nucleotides and modify the stability or expression of their mRNA targets through base pair interactions [3]. Since little is now known about these sRNA molecules in lactic acid bacteria, research on them is essential to improving their beneficial traits.

Therefore, the aim of this work was to use Northern blotting to determine how exposure to stressors affects the expression of sRNA in *L. paracasei* bacteria and to compare these findings with sequencing results. For this we selected the stressors that can be frequently encountered in medical and industrial applications: H_2O_2, lactic acid, NaCl, bile salts and penicillin G. In terms of sRNA molecules, sLCB2400-, sLCB2636-, sLCB3045+, sLCB766+ and sLCB1336+ were chosen as they showed one of the highest changes in expression after exposure to stressors, based on the sequencing results of previous work.

In order to accomplish our goal, we isolated total RNA from bacteria that were exposed to selected stressors. We then used Northern blotting, a technique in which the radioactive probes were hybridized to target sRNAs. Changes in the target sRNA expression were evaluated when the intensity of the signal between the treated and control bacteria were compared.

In conclusion, this study focused on detecting changes in *L. paracasei* sRNA expression in response to various stressors. The data acquired have the potential to enhance our comprehension of *L. paracasei's* environmental adaptation, an important aspect in the development of probiotic products.

Acknowledgements: This work was supported by funding from the Research Council of Lithuania [MIP-22-11 to GV].

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MECHANISM OF CRISPR-CAS3 HELICASE USING MAGNETIC TWEEZERS

Miglė Šarpilo, Algirdas Toleikis

Vilnius University, Life Science Center, Institute of biotechnology
7 Saulėtekio Ave, Vilnius, Lithuania
migle.sarpilo@gmc.stud.vu.lt

CRISPR-Cas provides RNA-guided adaptive immunity against invading genetic elements. CRISPR systems consist of multiple Cas proteins, which are responsible for CRISPR-dependent cell immunity mechanisms. The effector complex in CRISPR I-E consists of Cascade and Cas3. Cascade is responsible for foreign DNA targeting. Meanwhile, Cas3, which possesses helicase and nuclease activities, is a key protein of the system, necessary for crRNA-guided interference against virus proliferation.

Although single-component Class 2 CRISPR systems, such as type II Cas9 are widely used for genome-editing, the research on multi-component Class 1 proteins, including Cas3, of the same system has been less developed. Components of the I-E CRISPR system have already been used as a genome-editing tool to generate big deletions. However, the detailed mechanism by which Cas3 achieves its function is not well understood. This study aims to elucidate the mechanism of Cas3 DNA unwinding and shredding. We are using single-molecule force microscopy, namely, magnetic tweezers (MT) and RepX helicase as a model to optimise MT methodology to probe the mechanical aspects of Cas3 unwinding activity in the nearest future. Greater knowledge of the Cas3 mechanism of action would improve the application of Cas3 as a tool for genome editing.

INVESTIGATION OF DROUGHT RESISTANCE PARAMETERS IN LITHUANIAN BARLEY (*HORDEUM VULGARE L.*) CULTIVARS

Gabija Vėlavičiūtė[1], Vilius Jurgis Mensonas[2], Algė Leistrumaitė[3], Raimondas Šiukšta[2]

[1]Institute of Chemistry, Faculty of Chemistry and Geosciences, Naugarduko St. 24, Vilnius, Lithuania
[2]Institute of Biosciences, Life Sciences Centre, Vilnius University, Saulėtekis Ave. 7, Vilnius, Lithuania.
[3]Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Institutas ave. 1, Akademija, Kėdainiai, Lithuania
gabija.velaviciute@chgf.stud.vu.lt

Barley (*Hordeum vulgare L.*) is a widely cultivated cereal grain, the fourth most important, after rice, wheat and corn. Due to its rich nutritional content, including fiber, complex carbohydrates, essential vitamins and minerals, it is a key component of human diet in many developing countries, where access to more productive cereals might be limited. Additionally, a significant portion of barley production is used as livestock fodder due 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, losses and impact to the agricultural sector will only grow higher [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, moreover a convenient model organism for other cereals. In this study, correlations between various biochemical and morphological parameters in the early barley growing stages were analyzed, comparing laboratory grown barley cultivate data with field data. With the aim to determine most drought resistant Lithuanian barley cultivars and the most phenotype-representative individual parameters.

Five parameters were investigated under laboratory conditions: leaf relative water content (RWC), proline content, root cell viability, cell lipid peroxidation, chlorophyll a/b and carotenoid content. Of these, we determined RWC and proline content to be the most differentiating parameters. Accordingly, it was selected 4 of the assayed cultivars – 2 as the most resilient with 'RGT Planet' and 'Rusnė', and 2 as the most susceptible – with 'Laureate' and 'Ema'.

To compare laboratory findings with field data, five other parameters from multi-year field observations were analyzed, such as grain yield, thousand grain weight (TGW), protein content, plant height and starch content. It was observed a much-decreased drought resilience phenotype with the 'Rusnė' cultivar, on par with the susceptible 'Ema', as well as a marked improvement in the susceptible 'Laureate' cultivar. Cultivars 'Alisa' and 'Kirsna', unremarkable under laboratory conditions, were also observed to perform better in the field. Finally, the shortcomings of field and laboratory investigations were evaluated to find merit in combining the most prospective aspects of both to paint a complete picture of drought resilience in Lithuanian barley cultivars.

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DEVELOPMENTAL BIOLOGY OF OTIORHYNCHUS SALICICOLA

Liepa Valančiūtė[1,2], Sandra Radžiutė[2]

[1]Life Sciences Center, Vilnius University, Lithuania
[2]Laboratory of Chemical Ecology and Behavior, Nature Research Centre, Lithuania
liepa.valanciute@gmc.stud.vu.lt

The weevil *Otiorhynchus salicicola* was introduced to Lithuania from the Northwest of Balkans, the Alps, and other mountainous regions of central Europe through the trade of imported ornamental plants [1]. This beetle poses a significant threat to agriculture, gardening, plant trade, and general citizens as it feeds on a wide range of plants [2]. Understanding its developmental biology is essential for devising effective and sustainable pest control methods since it has not been extensively studied.

The developmental cycle of *Otiorhynchus* species typically comprises four stages: egg, larvae, pupae, and adult. During a natural generation, adults emerge in early spring and survive until autumn, laying eggs from late spring or early summer until their activity ceases. Larvae spend the winter in the final instar stage in soil until late spring when they pupate, and adults emerge once again [3].

The aim of this study is to comprehensively explore the developmental cycle of *O. salicicola*, including the duration of its various developmental stages under laboratory conditions. This understanding is crucial for initiating the process of developing effective methods to monitor and control these agricultural pests.

To implement this study, several *O. salicicola* adult weevils were collected from the wild to establish a population in a laboratory setting. Adult weevils were kept in plastic containers with host plant leaves. The beetles were closely monitored and reared, including mating and collecting eggs, measuring the head capsules of the larvae to determine the different larvae growth stages, and monitoring pupae until the adult stage. The study yielded successful results as an entire generation of the weevil was successfully cultivated in the laboratory and the durations of its various developmental stages were determined.

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F25

VISUALIZATION OF SEMA3A INTERACTION WITH NRP1 IN LIVING CELLS

Aistė Balkevičiūtė[1], Arūnas Kazlauskas[2]

[1]Laborotory of Molecular Neurooncology, Neuroscience Institute, Faculty of Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania
aiste.balkeviciute@stud.lsmu.lt

Glioblastoma tumors are highly vascularized tumors, and glioma growth depends on the formation of new blood vessels. Angiogenesis is a complex process involving the proliferation, migration, and differentiation of vascular endothelial cells under the stimulation of specific signals. It is controlled by the balance between its promoting and inhibiting factors [1]. Sema3A controls vascular pattering by interacting with membrane receptor NRP1 and functionally interfering with the VEGF-mediated pathway, which is also associated with the NRP1 complex [2]. The dynamics of Sema3A interaction with NRP1 in living cells are largely unknown, therefore, research in this field can provide new insights of cytokine-regulated mechanisms of angiogenesis in general. This research aims to determine the interaction of the receptor NRP1 with Sema3A in living cells by using a fluorescence labeling of the ligand.

During the assay, we applied several molecular engineering techniques and constructed a pTO/SCherry-Sema3A expression vector, which consist of signal sequence (required for secretion), red fluorescent protein mCherry (label), and Sema3A protein without signal sequence. Six clones were verified by restriction analysis. The functionality of pTO/SCherry-Sema3A vector was verified by Western-Blot analysis on extracts prepared from transfected HEK293FT cells with a constructed SCherry-Sema3A vector. In the functional study, medium from SCherrySema3A-expressing cells was placed by the Boyden method onto the U87MG cell line which expresses NRP1, and incubated overnight, the interaction was monitored by fluorescence microscopy.

Restriction analysis identified SCherry-Sema3A clones consisting of 3039 base pairs. Western-blot analysis confirmed the integrity of SCherry-Sema3A protein encoded by a newly constructed vector of SCherry-Sema3A. Fluorescence microscopy revealed signals of SCherry-Sema3A bound to NRP1-expressing cells.

Taken together, our data demonstrated a method for visualization of Sema3A interaction with transmembrane protein NRP1 in living cells, which, although it requires optimization in acquiring higher signals of interaction, can be a promising tool for angiogenesis studies *in vitro.*

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F26

CAPSULE-BASED TECHNOLOGY FOR SINGLE-CELL GENE EXPRESSION ANALYSIS

Emilė Pranauskaitė[1], Linas Mažutis[1]

[1]Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania
emilepra@gmail.com

Droplet microfluidics is a high-throughput technology for analyzing individual cells isolated in nanoliter-volume droplets. This technique effectively tackles the complexities of cell heterogeneity, facilitating the comparison of various cell states and types within complex samples [1]. However, traditional droplet-based systems face constraints in performing multi-step operations that are often required for preparing and analyzing complex biological samples. To circumvent these limitations our research team has pioneered a technology based on semi-permeable capsules [2,3]. These capsules are designed to selectively retain large molecules like genomic material or mRNA while facilitating the passive diffusion of smaller molecules such as enzymes or reaction components across their shells. This selective permeability enables the execution of multi-step reactions on millions of individual cells. In comparison to alternative systems possessing comparable features, our semi-permeable capsules demonstrate enhanced retention of encapsulated cells and yield greater quantities of whole-genome amplification [2].

Herein, we employed semi-permeable capsules to examine gene expression in complex clinical samples, such as cells from dense tissues or those with high concentrations of intracellular inhibitors. We devised a method to remove ambient nucleic acids or active nucleases released by dead cells, tailoring this method as highly sensitive for single-cell gene expression analysis. Taking advantage of capsule-based technology, we demonstrated the effective recovery of diverse cell types from difficult-to-analyze samples. The capsules, carrying purified cellular RNA from single cells, serve as microreactors for barcoding and subsequent library preparation. The flexibility and efficiency provided by our method, termed "CapDrop", present new avenues for exploring cellular heterogeneity and advancing single-cell RNA sequencing methodologies.

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F27

THE ROLE OF MIRNA IN LUNG CANCER PROGRESSION AND CONTROL

Laurynas Visockas[1,2], Julija Simienė[2] , Rytė Rynkevicienė[2], Kamilė Normantaitė[1,2], Stanislava Vaškevičiūtė[2], Eglė Balčiūnaitė[2], Kęstutis Sužiedelis[1,2]

[1]Institute of Biosciences, Life Science Center, Vilnius University, Vilnius, Lithuania
[2]Laboratory of Molecular Oncology, National Cancer Institute, Vilnius, Lithuania
laurynas.visockas@gmc.stud.vu.lt

Lung cancer is a leading cause of cancer death world-wide. Non-small cell lung cancer (NSCLC) is the most common form accounting for more than 80 % of lung cancer cases (Duma et al., 2019). The early diagnosis and control of cancer metastasis are critical factors in cancer treatment. Discovery of novel biomarkers and therapeutic targets have helped to increase survival rates for many types of cancer, including lung cancer. Over the past decade miRNAs have emerged as potential biomarkers and targets in lung cancer diagnosis and treatment. miRNAs are small 20-25 nucleotide long single-stranded noncoding RNAs that play an important role in tumour initiation and growth. miRNAs function in post-transcriptional regulation of gene expression can act as tumour suppressors, oncogenes or metastasis regulators. Previous studies have shown that miRNA-574, miRNA-195, miRNA-196, miRNA-328, miRNA-355, miRNA-500, miRNA-877 may be potential regulators of a number of cancers. However, a precise role of these miRNAs in lung cancer has not been elucidated.

The signatures of miRNA expression were investigated both in normal and cancerous lung tissue biopsies, as well as in lung cancer cell lines H23 and A549 in order to identify novel potential biomarkers for lung cancer. Real-time quantification of microRNAs using a stem-loop RT-PCR method (Chen et al., 2005) was used to assess changes in miRNA expression associated with lung malignancies.

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F28

APPLICATION OF COVALENT DNA LABELING FOR DNA MODIFICATION AND CHROMATIN ACCESSIBILITY ANALYSIS

Mintaute Rutkauskaite[1], Kotryna Skardziute[1], Edita Kriukiene[1]

[1]Department of Biological DNA Modification, Life Sciences Center, Vilnius University, Lithuania

mintaute.rutkauskaite@gmc.stud.vu.lt

DNA modifications, post-translational histone modifications, chromatin structure and non-coding RNA molecules are important epigenetic mechanisms that regulate gene expression without changing DNA nucleotide sequence. These mechanisms interact closely to assure a proper functioning of the cell. Therefore, more accurate predictions of gene activation potential could be achieved by simultaneously analysing multiple epigenetic regulatory layers together from one sample. [1] This prompted us to develop a new method by which cytosine methylation and hydroxymethylation patterns, as well as open chromatin regions, could be profiled from a single sample. In our Mx-TOP approach (multi-omic tethered oligonucleotide-primed sequencing), in native nuclei we perform triple labeling reactions using DNA methyltransferases eM.SssI, M.CviPI and Ado-6-alkyne cofactor to introduce a terminal alkyne group into unmodified CG and GC sites, and T4 bacteriophage β-glucosyltransferase (BGT) and UDP-6-azide-glucose cofactor to tag 5hmC residues with azide-carrying glucose moieties. [2] Such covalently-tagged sequences are then identified at a single-base resolution by the TOP-seq technology. [3]

In this study, we performed extensive validation of the different steps of the Mx-TOP procedure. We validated this method on a model DNA fragment systems, demonstrating that our triple covalent DNA labeling-based method can discriminate 5hmC modifications from unmodified CG sites. In order to enable such discrimination, in sequencing, we chemically tethered an alkyne- or azide-bearing DNA oligonucleotides that differ in their DNA sequence and chemical linker structure to the covalently-labeled unmodified CG and and 5hmC targets. To test their suitability for Mx-TOP, we performed a pilot experiment and demonstrated that DNA polymerase can amplify DNA sequences equally well from selectively conjugated azide- or alkyne-modified DNA oligonucleotides. We also assessed the potential interference of the DNA strand extension reactions from closely positioned DNA oligonucleotide-tagged sites and obtained some inhibition by the presence of 5hmC upstream to the analytical site. Finally, we investigated the potential DNA intra or inter-strand crosslinking reactions among the deposited azide and alkyne tags and determined that such cross-reactivity is absent using a high excess of alkyne- and azide-modified DNA oligonucleotides and low 5hmC levels reminiscent of those found in the native mammalian genome.

These results confirmed that Mx-TOP is able to identify different genomic targets with high specificity and efficiency and thus, is suitable for simultaneous profiling of unmethylome, DNA hydroxymethylome and chromatin accessibility in epigenomic studies.

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F29

CALIBRATION OF *ORTHOMYXOVIRIDAE* EVOLUTION IN TIME WITH ENDOGENOUS VIRAL ELEMENTS

Brygida Serebriakova[1,2], Gytis Dudas[1]

[1]Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
[2]Faculty of Medicine, Vilnius University, Lithuania
brygida.serebriakova@gmc.vu.lt

Endogenous viral elements (EVEs) are DNA sequences of viral origin that were inserted into the host germline genome and have persisted throughout evolution. Initially it was thought that all EVEs stemmed from *Retroviridae* family as one of the steps in their replication cycle involves reverse transcription and insertion of the viral genome into the host genome. It was later discovered that all groups of viruses can integrate their genomes although less frequently [1]. Over time EVEs accumulate numerous mutations and as a result barely resemble extant viruses. In some cases, a host can utilize such sequences for their needs. For instance, diverse pools of EVEs in *Aedes* mosquitoes play an important role in their immunity against viral infections [2].

The evolutionary rate of exogenous RNA viruses can reach 10-3 substitutions per site per year which is roughly a million times faster than humans. Studies have shown that calibrating molecular clocks based on available sequences can give erroneous age estimates of RNA viruses reaching at most 100 000 years when the true age can reach several million years. Dating the origins of viral lineages through EVEs gives more accurate predictions since integrated sequences evolve at host rate [3].

Orthomyxoviridae is a family of single-stranded negative-sense RNA viruses. Influenza viruses comprise four genera within the familyandare notable forcausingoutbreaks in a wide array of species including humans. Other genera include *Isavirus* which infect farmed Atlantic salmon, and *Thogotovirus* and Quaranjaviruses which are associated with ticks with occasional spillovers to humans and other animals [4]. Research on long-term evolutionary history of *Orthomyxoviridae* can reveal unprecedented viral behavior, extinct viral groups and expand our knowledge of their host range.

The aim of this research is to infer the evolutionary timescale and investigate the host range of *Orthomyxoviridae* using EVEs detected in *Arthropoda* which were derived from RNA-dependent RNA polymerase subunit PB1. To achieve this goal, publicly available protein sequences of PB1 were gathered and tBLASTn searched in *Arthropoda*. Next, a multiple sequence alignment was generated from detected integration sites and a maximum-likelihood phylogenetic tree was constructed.

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DETERMINATION OF THE MECHANISM OF ACTION OF SMALL REGULATORY RNA SLLM2- IN LACTIC ACID BACTERIA *LACTOCOCCUS LACTIS*

Loreta Stankevičiūtė[1], Milda Mickutė[1], Giedrius Vilkaitis[1]

[1] Department of Biological DNA Modification, Life Sciences Center, Vilnius University, Lithuania

loreta.stankeviciute@chgf.stud.vu.lt

Lactococcus lactis are Gram-positive, non-spore-forming bacteria, which produce lactic acid as one of their main metabolic products. These microorganisms have probiotic properties and are often used in fermentation, where their main function is to convert lactose into lactate. During this process the pH of the medium decreases, which is associated with a longer shelf life of fermented products and thus the antimicrobial properties of *L. lactis* [1]. However, these bacteria, like majority of other microorganisms, are sensitive to various environmental changes, such as temperature, nutrient availability, oxidation, and other factors which can cause them to experience stress [2]. Few genes have been identified that are involved in the expression and regulation of stress response proteins. Among them are small regulatory RNAs (sRNAs) which help bacteria to adapt to ever changing environmental conditions by either changing the amount of targeted mRNA and/or protein itself. And even though the role of sRNAs in stress response continues to be extensively studied in other bacteria, the specific functions of sRNAs in *L. lactis* remain unknown [3]. In the Department of Biological DNA Modification, it was determined that overexpression of one of *L. lactis* sRNAs sLLM2- increases bacterial resistance to lysozyme which due to its antimicrobial properties is often used in fermentation and medicine. However, the molecular mechanism behind this phenotype remained unsolved.

The aim of this study is to determine the mechanism of action of *L. lactis* small RNA sLLM2–. During this research, first, we will electroporate bacteria with two vectors: one with a cloned sLLM2– gene and an empty one for the control. Afterwards, we will grow bacteria in liquid medium and plot their growth curves to determine the early, middle, and late exponential and stationary growth stages at which bacterial biomass will be collected. After this, we will perform RNA purification and use the resulting RNA for Northern blotting. This method will help us to determine the level of sLLM2- expression in the control and experimental samples throughout separate stages of bacterial growth. Later, we will perform real-time quantitative PCR (RT-qPCR) to determine the amount of mRNA of genes potentially regulated by this sRNA. Finally, protein purification and proteomic analysis will be performed to assess how the expression of sLLM2- targets changes then sRNA is overexpressed.

We hope that the obtained results will help us to evaluate the expression of genes regulated by sLLM2- in *L. lactis* and that the mechanism of action of this sRNA will be successfully determined.

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F31

SYNTHESIS OF GEMCITABINE-LOADED ZIF-8 NANOPARTICLES AS PROMISING DRUG DELIVERY SYSTEM FOR CHEMOTHERAPY

Samira Nekoufar[1], Marjan Ghorbani[2], Masoumeh Tavakoli-Yaraki[3]

[1]Department of Biochemistry, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
[2]Iran Polymer and Petrochemical Institute, P.O. Box: 14965/115, Tehran, Iran.
[3]Department of Biochemistry, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
samiranekufar@gmail.com

Metal-organic frameworks (MOFs) are a class of crystalline materials known for their highly porous structure and potential for various applications [1]. In this study, Zn(NO3)2, a type of MOF, is examined, revealing a three-dimensional network of interconnected metal and organic components. The research presents a synthesis approach and characterization of metal organic nanoparticles using the co-precipitation method. Gemcitabine (Gem), a well-established chemotherapy agent for pancreatic cancer, is utilized in this study. In nano-drug delivery, imaging contrast is significantly enhanced owing to the increased surface area and selective accumulation in target tissues [2].

One of the prominent MOFs explored is the zeolitic imidazolate framework (ZIF-8) crystal, recognized for its potential in small molecule delivery due to its high loading ability. ZIF-8 exhibits notable biodegradability in low-pH environments, facilitating drug release in tumor sites. Following the synthesis of ZIF-8 nanoparticles (NPs), gemcitabine is added to the solution containing MOF at a 1:2 ratio and incubated. The concentration of gemcitabine is determined spectrophotometrically at 270 nm using UV/vis analysis, with the absorbance-concentration relationship established through a standard curve.

The study focuses on determining nanoparticle concentration and evaluating drug loading in nanoparticles via incubation with gemcitabine and subsequent spectrophotometric analysis. Key parameters such as encapsulation efficiency and drug loading capacity are assessed. Encapsulation efficiency measures the percentage of successfully entrapped drug within nanoparticles, while drug loading capacity quantifies the amount of drug accommodated by the nanoparticles. The study identifies an optimal drug encapsulation ratio of 1:2, resulting in an encapsulation efficiency of 99.89% and a drug loading capacity of 49.95%. Size distribution analysis of MOF and MOF-gemcitabine is conducted based on number, volume, and intensity. Additionally, zeta potential and Fourier-transform infrared spectroscopy (FTIR) are employed for further characterization.

Moreover, the concentration of gemcitabine loaded onto MOFs is determined to be 25 micrograms per milliliter, demonstrating potential for effective chemotherapy drug delivery. Overall, this research emphasizes the synthesis and characterization of metal organic nanoparticles, shedding light on their potential applications in drug delivery systems for pancreatic cancer therapy.

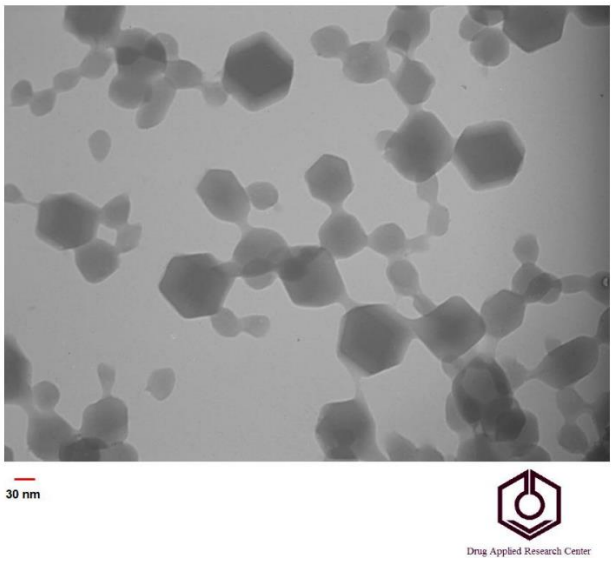

Figure 1. TEM images of MOF. Scale bar: 30 nm (A). The size distribution of MOF were determined using transmission electron microscopy (TEM) (LEO 906, Zeiss, 100KV, Germany) with a LIBRA 120 Plus Carl Zeiss microscope (Oberkochen, Germany).

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F32

R-RAS-2 AS A POTENTIAL PREDICTIVE TARGET IN TRIPLENEGATIVE BREAST CANCER

Justas Burauskas[1,2], Agnė Šeštokaitė[1,2], Monika Drobnienė[1], Rasa Sabaliauskaitė[1,2]

[1]National Cancer Institute, Vilnius, Lithuania
[2]Vilnius University, Lithuania

Neoadjuvant chemotherapy (NAC) is the standard of care for triple-negative breast cancer (TNBC) patient treatment. However, the effectiveness of treatment is unpredictable, as patients frequently develop resistance. TNBCs have a much higher recurrence and metastasis as a result of not being eligible for current treatment options due to the lack of estrogen, progesterone receptor, and human epidermal growth factor receptor 2 [1]. This aggressive cancer contributes to the overall shortened survival of patients diagnosed with TNBC [2]. For these reasons, there is a growing need to develop novel noninvasive molecular predictive approaches for treatment and monitoring [3]. It has become evident that multiple signaling pathways are responsible for treatment resistance in TNBC [2]. Recent evidence from preclinical studies have marked a pivotal role of the R-RAS-2 gene which is involved in the STAT3 signaling pathway, progression, and chemoresistance of TNBC patients [3].

The aim of this study was to evaluate the amount of STAT3, ALDH1A1, NFIB, UPF3A, BCL-2, and R-RAS-2 gene transcripts in serial plasma samples before and after NAC and determine their potential as predictive biomarkers.

In this study, we used reverse transcription quantitative PCR to determine gene expression in 84 NBC patients' paired plasma samples before and after NAC. We determined that BCL-2, R-RAS-2, STAT3 and NFIB expression was higher after NAC (all $P<0.005$, respectfully). WhileR-RAS-2 higher expression of at least 10% was associated with partial response to NAC and residual disease ($P=0.013$ and $P=0.006$, respectfully).

In conclusion, understanding how the multiple signaling pathways influence the course response to NAC in TNBC is important. To date, we found that R-RAS2 could be used as a predictive biomarker for monitoring TNBC patient response to chemotherapy and treatment effectiveness.

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Connecting the Dots: Structural Interpretation of Electrochemical Impedance Spectra in Tethered Bilayer Lipid Membranes

Filipas Ambrulevičius[1], Gintaras Valinčius[1]

[1]Institute of Biochemistry, Life Sciences Center, Vilnius University
filipas.ambrulevicus@gmc.vu.lt

Electrochemical Impedance Spectroscopy (EIS) is a powerful tool used in biosensor applications [1]. Traditional interpretation of EIS data often relies on equivalent circuit modeling, a method that provides quantitative attributes to describe the impedance response of a biosensor. However, a common challenge arises with the necessity of employing constant phase elements (CPE) to achieve a satisfactory fit to EIS data.

An alternative approach involves modeling the distribution of relaxation time, offering valuable insights into the number and timescales of different processes. Yet, both methods often lack the incorporation of structural features inherent to the biosensor under study and are difficult to interpret [2].

This research sets out to demonstrate the efficacy of a structure-based EIS model tailored for tethered lipid bilayer sensors, aiming to not only interpret but also fit experimental data while providing insights into the bilayer's structure and its interaction with the analyte of interest. It is well known that lipid bilayers are highly diverse, dynamic and often heterogeneous systems that can undergo phase transitions, induce interactions between reconstituted proteins and modulate membrane protein function. This can lead to lateral patterns in lipid composition or heterogeneity in protein affinity to the bilayer and subsequently to heterogeneous lateral distribution of conductive defects. Therefore, focus is to build on earlier works [3,4] and investigate how lateral distribution of bacterial pore forming proteins impacts EIS response and demonstrate that EIS data encodes nano scale structure. This approach relies on solution of the Helmholtz partial differential equation to impart geometric intuition for the relation of lateral distribution and qualitative features of EIS spectra, Fredholm integral equation to fit EIS spectra and recover defect density function as well as neural-network model to bridge the two, simplified diagram of the modeling approach is depicted in Fig. 1.

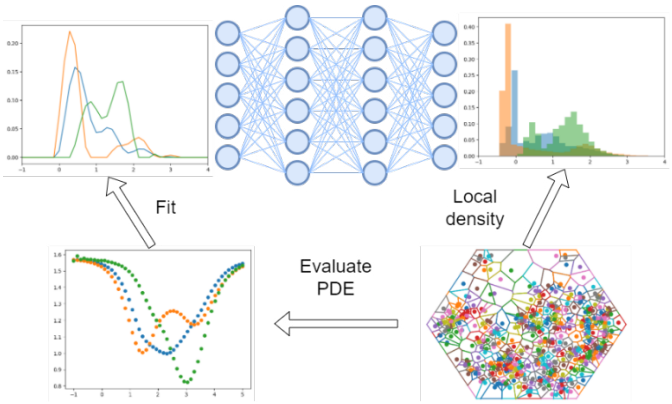

Figure 1. Modeling diagram.

By adopting this methodology, we not only achieve a comprehensive fit to the data, capturing qualitative attributes such as Helmholtz capacitance and bilayer capacitance, but also formulate geometric insights that establish a clear relationship between structural features and observed "non-idealities" in impedance spectra.

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F34

EFFECT OF STATINS LIPOPHILICITY ON THE PROLIFERATION OF HEPATOCELLULAR CARCINOMA CELLS

Goda Glebavičiūtė[1] and Giulio Preta[1]

[1]Institute of Biochemistry, Life Science Center, Vilnius University, LT-10257 Vilnius, Lithuania
goda.glebaviciute@gmc.stud.vu.lt

The HMG-CoA reductase inhibitors, statins, are worldwide used drugs for lowering the level of cholesterol in the blood [1]. Different clinical studies of statins in cancer patients have indicated a decrease in cancer mortality, particularly in patients using lipophilic statins comparing to those on hydrophilic statins [2, 3]. In this paper, we selected two structurally different statins (simvastatin and pravastatin) with different lipophilicity and investigated their effects on cell viability (MTT assay and LDH cytotoxicity assay) and apoptosis (caspase 3 activation) of hepatocellular carcinoma cells HepG2. Lipophilic simvastatin highly influences cancer cell growth and survival, while pravastatin due to hydrophilic structure and limited cellular uptake, showed minimal cytotoxic effects. Furthermore, simvastatin treatment induces concentration-dependent morphological changes in HepG2 cells as reported previously also for prostate and pancreatic cancer cells [4]. From a fusiform, fibroblast-like morphology, cell acquires a smaller rounded form. Our work underlines the importance of statin selection according to lipophilicity (Figure 1), since statins cytotoxicity, while undesirable in simple hyperlipidemia therapy, could have beneficial effects in cancer therapy.

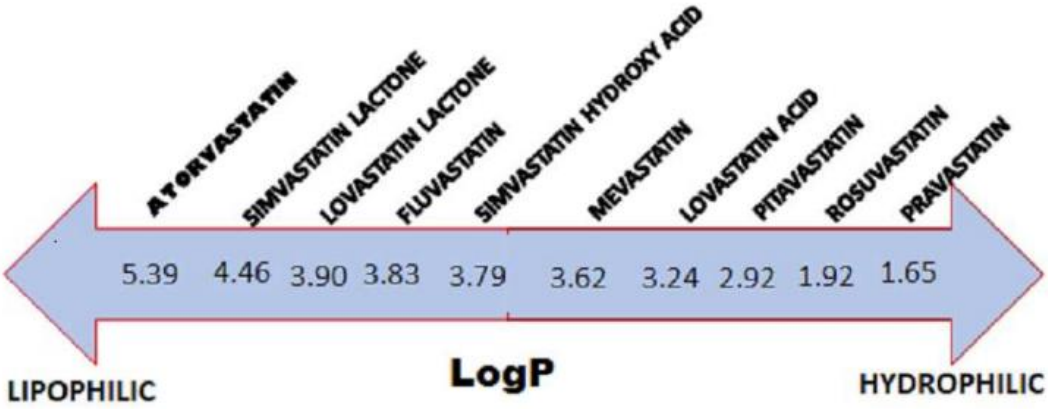

Figure 1: Statins lipophilicity, hence their ability to cross the cell membrane by passive diffusion, is expressed by the log P.

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S100A8 PROTEIN INTERACTION WITH LIPID MEMBRANES

Rimgailė Tamulytė[1], Darius Šulskis[2], Marija Jankunec[1]

[1]Institute of Biochemistry, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania
rimgaile.tamulyte@bchi.stud.vu.lt

S100 proteins are calcium-binding proteins that regulate several processes associated with Alzheimer's disease (AD) but whose contribution and direct involvement in disease pathophysiology remains not fully established. Due to neuroinflammation in AD patients, the levels of several S100 proteins are increased in the brain and some S100s play roles related to the processing of the amyloid precursor protein, regulation of amyloid beta peptide levels and Tau protein phosphorylation [1]. The number of studies on the impact of S100 family proteins in co-aggregation processes with amyloid-like proteins is increasing. However, research has yet to unravel how S100 proteins interact with neuronal membranes. The present study is focused on the pro-inflammatory calcium-binding protein S100A8 of the S100 family. We employ various biomimetic membrane models such as solid supported lipid bilayers, tethered bilayer lipid membranes and liposomes to monitor the interaction between S100A8 protein and membrane surface. For this purpose, we employed high speed atomic force microscopy (HS-AFM), fluorescence spectroscopy and electrochemical impedance spectroscopy techniques. Our results indicate that the interaction between S100A8 and the membrane is lipid-charge sensitive. The greatest membrane disruptive effect is observed in negatively charged membranes. HS-AFM data reveals that the interaction of S100A8 with negatively charged bilayer leads to the rupture of the membrane by a detergent-like effect (Fig. 1). These results might broaden the understanding of S100A8 protein interactions with neuronal membranes and potentially affect the development of new diagnostic and therapeutic approaches for AD or other related diseases.

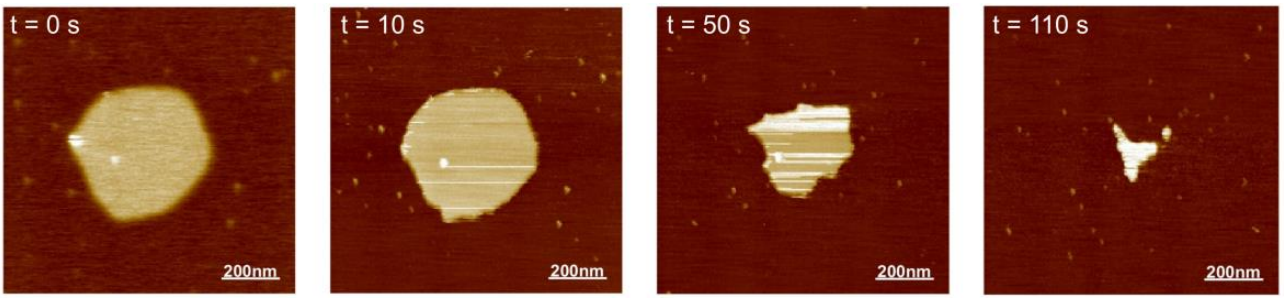

Figure 1. HS-AFM images of S100A8 induced membrane dissolution. At t = 0, the protein was injected in the fluid cell during imaging. Interaction between S100A8 and negatively charged membrane leads to a detergent-like effect.

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F36

FORMULATION OF BIOMIMETIC LIPID – BASED NANOPARTICLES FOR CRISPR/CAS9 DELIVERY

Kamilė Bužinskytė[1], Evelina Jankaitytė[1,2], Rima Budvytytė[1,2]

[1]Life Sciences Center, University of Vilnius, Vilnius, Lithuania
[2]Institute of Biochemistry, Department of Bioelectrochemistry and Biospectroscopy, Life Sciences Center, Saulėtekio av. 7, LT-10257, Vilnius
kamile.buzinskyte@gmc.stud.vu.lt

For the past years CRISPR-cas9 system is being studied as a new genome editing tool that could lead to new gene therapy strategies and improve human health. However the delivery of such system to a cell has remained a challenge. Since CRISPR-Cas9 system consists of Cas9 protein and guide RNA, it is not only important to protect both components from degradation, before they reach the cell, but also that the delivery system could transfer this gene editing tool through cell membrane and would not cause negative immunity response. Lipid based nanoparticles (LNPs) are versatile, have low imunogenity and are easy to manufacture – all these characteristics make liposomes an attractive potential delivery vector for CRISPR/Cas9 transportation through cell membrane.

Lipid nanoparticles are very flexible and can vary both in size and composition. In this research project different size and composition liposomes were investigated. Based on previous studies there were chosen 4 different LNP compositions [2]. All LNPs were made using these lipids: Dipalmitoylphosphatidylcholine (DOPC), dioleoylphosphatidylethanolamine (DOPE), cholesterol, 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) and PEG-lipids [2]. PEG-lipids‘ and cholesterol percent was altered. Also liposomes of negative charge were made, by including 1,2-dioctadecenoyl-sn-glycero-3-Phosphoserine (DOPS) instead of DOTAP.. The size and stability of LNPs were measured using dynamic light scattering technique (DLS, ZetaSizer). The results showed that concentration of PEG and cholesterol changes the stability and size of LNPs. The encapsulation efficiency was investigated using model protein BSA- Alexa Fluor 488. Protein encapsulation efficiency was above 60% percent. The encapsulation efficiency was determined by changing the size of LNPs, using diffrent pore diameter of membrane (30 nm, 200nm, 400nm) during LNPs preparation [3] . LNPs made with 200nm pores showed the best encapsulation efficiency and had the smallest polydispersity index.

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F37

THE EFFECT OF LOW-PRESSURE AND DIELECTRIC BARRIER PLASMA ON PHYTOCHEMICAL COMPOSITION OF STEVIA REBAUDIANA BERTONI PLANTS

Augustinas Andziulis, Augustė Judickaitė, Emilija Jankaitytė, Rasa Žūkienė

Faculty of Natural Sciences, Vytautas Magnus University, Lithuania
augustinas.andziulis@vdu.lt

Stevia rebaudiana Bertoni is a natural zero calorie sweetener, with Stevioside (Stev) and Rebaudioside A (RebA) being the most abundant and important secondary metabolites [1]. Stev and RebA are responsible for stevia's sweet taste. Apart from steviol glycosides, the leaves of stevia are also known for their antioxidant properties [2] Antioxidants are important in preventing free radicals from damaging proteins, DNA, lipids, and other molecules [3].

The aim of this study is to determine the effect of seed treatment with cold plasma on phytochemical composition in two *S. rebaudiana* cultivars (SHUG A3-6 and SHUG HIGH A3). Seeds prior to sowing were treated with cold plasma using low-pressure capacitively-coupled (CC) and atmospheric-pressure dielectric barrier discharge (DBD) systems for durations of 2, 5, and 7 minutes. Eight-week-old plant leaves were dried, and extracts were prepared for the analysis of steviol glycosides using HPLC, total antioxidant activity determined as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity, chlorophil *a* and *b* were determined by K. Lichtenthaler method.

Our research indicates an increase in general antioxidant activity. Both SHUG HIGH A3 and SHUG 6-A3 showed an increase in the CP2 and DBD2 groups. We noted an increase in the sum of chlorophyll *a* and *b*, which was dependent on cultivars. SHUG 6-A3 displayed an increase in the DBD2 and DBD5 groups, while HIGH A3 displayed an increase in CP5, CP7, and DBD7 groups. It looks like variations in chlorophyll levels are dependent on cultivar specific response to different plasma treatment and duration.

We noted a remarkable increase of Stev in the DBD5 group for SHUG 6-A3. Both cultivars showed a trend to increase Stev and decrease RebA biosynthesis, when comparing the RebA to Stev ratio. For SHUG 6-A3, the ratio of RebA to Stev exhibited a decrease over time affected by CP system. The DBD system showed a sharp increase for the 2-minute treatment period and a subsequent return to the control standard ratio after a 7-minute treatment, but a severe decrease for the 5-minute treatment. For SHUG HIGH A3, the ratio of RebA to Steviol revealed a general decrease post-treatment, with DBD demonstrating a poorer ratio compared to CP. Both CP and DBD systems exhibited a sharp decrease in the ratio for the 2-minute treatment, an increase for 5 and 7-minute treatments, although it was lower than the control.

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F38

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

Tomas Stanevičius[1], Tomas Nenartavičius[1], Rimantas Daugelavičius[1,2]

[1]Department of Biochemistry, Vytautas Magnus University, Lithuania
[2]Research Institute of Natural and Technological Sciences, Vytautas Magnus University, Lithuania
tomas.stanevicius1@vdu.lt

The increasing concerns regarding fossil fuel resources and their impact on the environment are encouraging the search for alternative fuel sources. Bioethanol, which can be produced from renewable raw materials, is an excellent fuel alternative and the burning of bioethanol releases fewer nitrogen oxides as well as fewer solid particles into the environment. Specifically, second-generation bioethanol is produced from lignocellulosic biomass, which is extracted from agricultural and forestry waste, which is considered to be more suitable for biofuel production, as it is produced from non-food raw materials [1].

Xylose is the second most abundant monosaccharide in the world after glucose, and the most abundant pentose sugar on our planet. It is also the most abundant sugar found in hemicelluloses. Besides this, more than half of global agricultural plant biomass consists of lignocellulosic crop residues [2]. Xylose utilization could increase bioethanol yield from raw material, but to increase it from lignocellulose, it is necessary to maximize the uptake of xylose into yeast cells.

A thermotolerant methylotrophic yeast *Ogataea polymorpha* can ferment xylose at high temperatures. However, the transport of xylose into cells from a mixture of glucose and xylose is a bottleneck in lignocellulosic ethanol production. Xylose consumption by the wild-type strains of xylose-utilising yeasts occurs only after the depletion of glucose, which results in a prolonged fermentation process and incomplete conversion of sugars liberated by hydrolysates from lignocellulose [3]. Therefore, the delivery of xylose into the yeast cells is rather problematic because the carriers have a higher affinity for glucose than for xylose. In addition, some potential xylose carriers are removed from the yeast plasma membrane at low glucose concentrations, and it is important to ensure the stability of the carriers by modifying them [4]. For this reason, transporters found in *Saccharomyces cerevisiae* Gal2 and Hxt7 or *O. polymorpha* Hxt1 are identified as potential xylose carriers for which mutagenesis could increase the xylose uptake into cells and usage for alcoholic fermentation. This research project aims 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 for high-temperature alcoholic fermentation. As the registration of the activity of energy metabolism (respiration, glycolysis, ATP synthesis) allows us to determine the rate of glucose and xylose uptake into the cells.

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F39

POTENTIAL INHIBITORS OF A BACTERIAL ANTIVIRAL BREX DEFENSE SYSTEM

Justė Adomaitytė[1], Tomas Šinkūnas[1]

[1]Department of Protein – DNA Interactions, Institute of Biotechnology, Life Sciences Center Vilnius
juste.adomaityte@chgf.stud.vu.lt

Bacteriophages are viruses with the ability to infect and replicate in bacterial cells. Numerous bacteriophage species are virulent and therefore kill the infected cell. However, these organisms have a long history of coexistence. During their evolution, bacteria have been able to adapt by developing defense mechanisms that protect cells from the entry of bacteriophages and foreign nucleic acids. Although more than a hundred bacterial defense systems are currently known, bacteriophages can inhibit them by various mechanisms [1]. Research of defense systems and their inhibitors is crucial not only for a better understanding of microbial evolution, but also as a resource for the development of various tools for biotechnology and biomedicine.

One of the bacterial antiviral defense systems is BREX (Bacteriophage Exclusion). It is present in about 10% of prokaryotic genomes. However, the mechanism of action is still undefined. BREX systems are divided into 6 types, with our research focusing on the predominant type 1 BREX system (BREX1), which consists of 6 genes: *brxA, brxB, brxC, pglZ, brxL, pglX*. The *pglX* gene encodes the m6A DNA methyltransferase, which methylates specific sequences in the host genomic DNA to distinguish itself from foreign DNA [2]. Bacteriophages can evade the BREX1 system by two different mechanisms: (i) by epigenetically modifying (either by methylation or glycosylation) BREX1 recognition sequences in their genomes [3], or (ii) by blocking the BREX1 system with their encoded protein inhibitors [4]. In this study, we analyse some phage-encoded proteins as potential inhibitors of the *Escherichia coli* HS BREX1 system in cells.

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F40

BIOFILM FORMATION AND PREVALENCE OF BIOFILM-ASSOCIATED GENES IN CLINICAL ISOLATES OF OPPORTUNISTIC PATHOGEN *STENOTROPHOMONAS MALTOPHILIA*

Dominykas Grigorjevas[1], Laurita Klimkaitė[1], Edita Sužiedėlienė[1], Julija Armalytė[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
dominykas.grigorjevas@gmc.stud.vu.lt

Antibiotics are one of the greatest medical inventions that have saved countless lives. However, antibiotic-resistant bacteria evolved various mechanisms allowing them to escape antibiotic effect and cause life-threatening infections. *Stenotrophomonas maltophilia* is one of the rising multidrug-resistant opportunistic pathogens that causes various infections with a mortality rate of up to 37.5% [1]. This pathogen's antibiotic resistance phenotype and ability to form biofilms enables it to persist in hospitals and infect patients who are treated there [2]. Biofilms are complex microbial communities encased in extracellular polymeric substances [3]. This structure produced by bacteria helps them adhere to the surfaces and protects from desiccation, antiseptic, or immune system. *S. maltophilia* is known to be a genetically diverse species with varying abilities to form biofilms [4], therefore it is important to evaluate how the genetic difference of this bacteria affects its ability to form biofilms.

This study aimed to evaluate biofilm-associated gene distribution and biofilm formation in 44 clinical isolates of *S. maltophilia* from patients of Vilnius University Hospital Santaros klinikos. Biofilm-associated genes *pilU, rmlA, fliC, smf1, spgM, ax21, fliA, rpfF* were chosen for analysis, selected gene prevalence in isolates was evaluated by performing PCR with gene-specific primers and visualizing results using agarose gel electrophoresis. Biofilm formation at 37 °C temperature was evaluated using crystal violet dye assay [4].

Out of 44 analysed isolates, 11% were weak biofilm producers, 39% were moderate biofilm producers, and 50% were strong biofilm producers. Analysed genes were found with a varying frequency: *pilU* was detected in 100% of analysed *S. maltophilia* isolates, *rmlA* –100%, *fliC* –98%, *smf1* – 98%, *spgM* –91%, *ax21* – 91%, *fliA* – 82%, *rpfF* –64%. However, no apparent correlation was found between biofilm formation levels and biofilm-associated gene distribution. Because of this, other not analysed genes or gene regulation might be responsible for *S. maltophilia* biofilms formation level. All analysed clinical *S. maltophilia* isolates formed biofilms indicating that this ability is important to *S. maltophilia* survival in a clinical environment.

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F41

THE EFFECT OF NYSTATIN ON *CANDIDA* SPP. YEASTS IN DIFFERENT ENVIRONMENTS

Eglė Vansevičiūtė[1], Rimantas Daugelavičius[1,2]

[1]Department of Biochemistry and [2]Research Institute of Natural and Technological Sciences, Vytautas Magnus University, Lithuania
egle.vanseviciute@vdu.lt

Candida albicans and *Candida glabrata* are among the most important opportunistic pathogens that cause thousands of superficial or disseminated and fatal infections in immunocompromised individuals every year. These yeasts can acquire resistance to all four known classes of antifungal drugs, making the treatment of these infections much harder [1]. It is necessary to find new antifungal compounds or ways to strengthen the effect of those already known.

Polyene drug nystatin creates pores in the cell wall, increasing the plasma membrane permeability and osmotic disbalance [2]. This study aimed to evaluate the effect of this antifungal drug on *C. albicans* and *C. glabrata* yeasts in different environments – buffer solutions and growth media of different pH. It was determined that the deletion of efflux pump genes of ABC superfamily *CDR1* and/or *CDR2* did not have an impact on the cells' sensitivity to this drug which means that one of the popular ways to strengthen the effects of antifungal drugs by inhibiting the efflux pumps would not improve the efficiency of nystatin. Since the inhibition of efflux pumps is not a suitable way to strengthen the effect of this antifungal, the impact of different environments was tested. Effects of nystatin on *Candida* yeasts can be followed using real-time methods such as the analysis of lipophilic anion PCB^- binding to yeast cells. After evaluation of the effects of nystatin at different pHs of citrate-phosphate buffer, it was determined that *C. albicans* and *C. glabrata* wild-type cells bound the highest amount of PCB^- ions when the buffer pH was 3. Some differences between the tested yeasts were noted. At pH 6 and pH 8, *C. glabrata* cells, exposed to nystatin, bound less PCB^- than *C. albicans* cells, but at pH 3 *C. glabrata* cells bound more PCB^- compared to *C. albicans* ones. Moreover, the effect of nystatin on the yeast of both species was also stronger in growth media of pH 3 compared to pH 6.

These findings suggest that the efficiency of nystatin can be strengthened in an acidic environment, but further research on this problem is still needed.

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SYNTHETIC EXTRACELLULAR MATRIX MIMETIC BASED HYDROGELS AS A NEW TOOL FOR THE SHAPING CELL PROPERTIES

Gintarė Stankevičienė[1,3], Vytautas Cėpla[1,2], Airina Mazėtytė-Godienė[1], Tadas Jelinskas[1], Romuald Eimont[2], Tomas Rakickas[1], Vytautė Starkuvienė-Erfle[3,4&],and Ramūnas Valiokas[1,2&]

[1] Department of Nanoengineering, Center for Physical Sciences and Technology, Vilnius
[2] UAB Ferentis, Savanoriu ave. 235, Vilnius, Lithuania
[3] Life Sciences Center, Institute of Biosciences, Vilnius University
[4] Heidelberg University, 69120 Heidelberg, Germany
[&] These authors contributed equally to this work
gintare.stankeviciene@gmc.stud.vu.lt

Hydrogels are preferred scaffolds due to their tissue-like properties, including fibrous structure, softness, high water content and extracellular matrix (ECM) mimicking biochemistry. It is a powerful tool not only for the artificial tissues, but also a platform for single cell-surface interaction studies. High cost, difficult purification processes, high batch-to-batch variations and biocontamination risk of the full-length ECM proteins leads to search for alternatives such as synthetic peptides as the functional blocks for the ECM-cell interactions. By adjusting matrix stiffness and bioactivity, it is possible to engineer the desired cell morphology, orientation or functionality.

We have previously been involved in developing the following series of hydrogels that are based on collagen-derived synthetic peptides Cys-Gly-(Pro-Lys-Gly)4 (Pro-Hyp-Gly)4 (Asp-Hyp-Gly)4, hereinafter Cys-CLP, and optionally contain various functional peptide extensions: the fibronectin cell adhesion sequence RGD (Cys-CLP-RGD)[1], collagen binding sequence DGEAG (Cys-CLP-DGEAG) or laminin adhesive motif IKVAV (Cys-CLP-IKVAV)[1]. Here, using an one-pot strategy, we have been reported the synthesis of ultrathin (from a few hundred microns to the tens of nanometers) hydrogel coatings containing the above peptide blocks. Namely, we have carried out an initiator free, UV-controlled self-initiated photografting and photopolymerization reaction (SIPP)[2] on substrates such as glass and plastic. To achieve this goal, the ECM-like peptides were functionalized via Michael addition reaction with photoactive methacrylate group using the 3-(acryloyloxy)-2-hydroxypropyl methacrylate reagent, thus obtaining methacrylated peptides. We have successfully synthesized 15-50 nm thick, transparent, mechanically stable, ECM mimicking peptide hydrogels by combining the methacrylated peptides, 2-hydroxyethyl methacrylate, PEG methacrylate and methacrylic acid monomers in SIPP on the glass surface. We have been tested different cell lines (endothelial, epithelial, fibroblast and others) on these hydrogel coatings. We demonstrate the varying cell properties induced by the composition of ECM-like coatings.

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F43

CONSTRUCTION OF BACTERIOPHAGES GENOMIC LIBRARY

Miglė Plioplytė[1], Jonas Juozapaitis[1], Giedrius Sasnauskas[1]

[1]Department of Protein - DNA Interactions, Vilnius University Life Sciences Center, Lithuania
migle.plioplyte@gmc.stud.vu.lt

Bacteria have developed a wide range of antiviral defenses to protect themselves from infection by their own viruses (bacteriophages). One of the defense strategies that bacteria use against their viruses is abortive infection (Abi). The Abi system acts as a cellular response to viral infection, with virus-infected cells dying or slowing down their metabolism, thereby limiting further phage multiplication in the population [1].

Although the understanding of bacterial defense mechanisms has increased considerably, one of the main unanswered questions is how the Abi system is activated in the event of phage infection. So far, in the study of bacterial defense systems, one approach to finding factors that activate bacterial defense systems has been to study bacteriophage mutants that evade bacterial defense systems, but this approach has drawbacks [2].

Our work aims to address the shortcomings of phage mutant assays and to propose a new, faster method to simultaneously screen more bacterial defense systems for their activating factors. This will be done using libraries of random genomic DNA fragments from bacteriophages.

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April 16th

Oral Presentations

Medicine

01

FLUORESCENT VISCOSITY PROBES AS DIAGNOSTIC TOOLS FOR CANCER DETECTION

Artūras Polita[1]

[1]Department of Bioelectrochemistry and Biospectroscopy, Vilnius University, Lithuania
arturas.polita@gmc.vu.lt

Viscosity is a key characteristic of biological membranes – it governs the passive diffusion of solutes and affects the lipid raft formation and membrane fluidity. Moreover, viscosity measurements provide a convenient way to observe the compositional changes that take place in biological membranes and organelles, as the efficiency of lipid packaging and the order of lipids have a great influence on the viscosity values of lipid structures [1]. In the case of cancer, multiple mutations produce cell signaling and metabolism alterations, which change the composition of lipid membranes and allow for the identification of maligancies via viscosity measurements [2].

In this work, we explore the viscosity-sensitive dyes, called molecular rotors [3], as diagnostic tools for cancer detection. Through the use of fluorescence lifetime imaging microscopy (FLIM) in combination with organelle-specific BODIPY dyes, whose fluorescence lifetimes increase with increase in microviscosity, we investigate the order of lipids in lysosomes and lipid droplets of cancerous and non-cancerous live cells. Our results demonstrate that lipid droplets in cancerous cells have vastly different lipid packaging efficiences between different cells in the same culture. In contrast, we show that lipid packaging efficiences of lipid droplets are uniform in non-maligant cells. Additionally, we demonstrate that common antichemotherapeutic drugs – doxorubicin and etoposide – induce different lipid compositions in lipid droplets depending on the cell type. Finally, we demonstrate that both lysosomes and lipid droplets in maligant cells possess up to 3 times greater microviscosities compared to non-maligant cells.

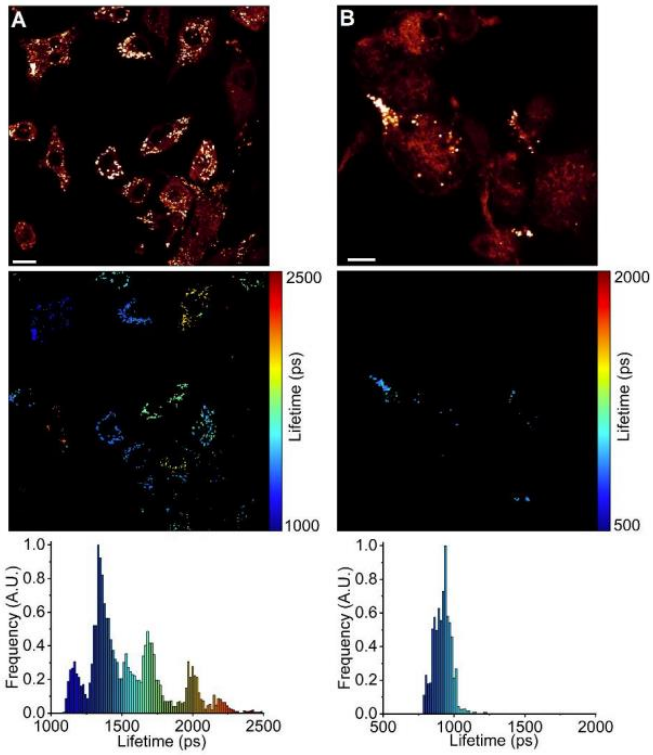

Figure 1. FLIM of BODIPY-LD in lipid droplets of human lung cancer A549 cells (A) and human embryonic kidney HEK 293T cells (B). The top panel shows images of fluorescence intensity. FLIM images are shown in the middle panel. The corresponding lifetime histograms are shown in the bottom panel. Scale bars are 10 µm.

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06

TICK-BORNE ENCEPHALITIS AND VACCINATION IN LITHUANIA

Austėja Sakalauskaitė[1]

[1]Faculty of Medicine, Vilnius University; Lithuanian Medical Students' Association (LiMSA)
sakalauskaite.austeja@gmail.com

This literature review was conducted to research tick-borne encephalitis prevalence and vaccination rates in Lithuania, the influence of financial and public education on tick-borne encephalitis vaccination. It is believed that the rise of tick-borne encephalitis, which began more than 20 years ago, is mainly related to the increase in the abundance of ticks and the expansion of their range, changing climate conditions, better diagnosis of tick-borne encephalitis, greater attention of specialists to this disease, but this problem continues to persist in Lithuania.

Although the morbidity of tick-borne encephalitis has been decreasing since 2020 in Lithuania, the country is still an endemic zone compared to other countries in the EU/EEA. Research shows that people living in villages are more prone to getting tick-borne encephalitis than people in cities. This can be explained by frequent visits, work, living in or near forests, having more contact with ticks, and sometimes ingesting the food that is not thermally treated. From 2020 to 2022, there have been 4 deaths and 1 176 hospitalizations in Lithuania (every year, more than 80% of people infected with tick-borne encephalitis get hospitalized). [1]

Currently, two vaccines in Europe are used for vaccinating individuals from 1 year of age which contain inactivated virus and are protective from all subtypes of the virus. Research shows that after 3 doses of the vaccine, more than 98% of subjects who followed the vaccination schedule were protected from tick-borne encephalitis. It is recommended to get the vaccine before the start of the warm season to get the full immunity, but analysis shows that people usually get it too late. The side effects of tick-borne disease vaccines are mild, quickly pass, and are more prevalent after the first dose. Short-term fever occurs more often in children after vaccination, severe systemic reactions and post-vaccine neuritis are very rare side effects. [2]

The biggest issue with tick-borne encephalitis is the prevalence of residual effects after the illness. Research shows that a third of patients have post-encephalitis syndrome with the most common residual effects being headache, fatigue, difficulty concentrating and sleep disorders. Rare but serious residual effect is muscle paralysis. [3]

Research shows that there has been a rise in vaccine hesitancy across 90% of countries since 2014. Vaccine confidence is built by medical staff, government, media, and other non-state actors. There is a lack of time to effectively discuss vaccination in healthcare institutions. In addition, negative media reports can a create lack of confidence in healthcare policies, and anti-vaccine groups are negatively impacting vaccine confidence.

Currently, the minimum wage in Lithuania is 633 euros after taxes. With the high inflation rate, the cost of living is increasing rapidly. Therefore, people are not able to afford additional vaccines that are not funded. In addition, research shows that every 1 euro invested in vaccination has a return of 4 euros of economic revenue, consequently providing beneficial economic consequences, economic growth, and savings in healthcare costs. [4]

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April 17th

Biophysics and Neurobiology

H1

MATERNAL HIGH-FAT DIET IMPACT ON THE OFFSPRING'S RETINA MORPHOLOGY AND INFLAMMATION RESPONSE

Patricija Čepauskytė[1], Gintarė Urbonaitė[1], Neda Ieva Biliūtė[1], Guoda Laurinavičiūtė[2], Urtė Neniškytė[1,3]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
[2]Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Lithuania
[3]VU-EMBL Partnership Institute, Life Sciences Center, Vilnius University, Lithuania
patricija.cepauskyte@gmc.stud.vu.lt

Aim: The increased high fat content of today's normal diet is one of the factors contributing to the rising obesity rates in society. Many studies indicate that maternal high-fat diet (mHFD) is the cause of systemic inflammation, which may lead to the development of neurodevelopmental disorders in the offspring [1, 2, 3]. Female estrous cycle stages appeared to also have different response to inflammation [4, 5, 6]. In the context of inflammatory conditions, CD68 is used as a microglia activation marker that is often associated with immune cells of the central nervous system, including the retina [7]. Studies have shown that a high-fat diet can impact the retina, however, there's little research done investigating its effects on the offspring retina [8]. This study aims to evaluate area changes of microglia and CD68 in the peripheral retina of mHFD offspring and assess how microglia and CD68 area depend on the stages of the estrous cycle.

Methods: Female C57Bl/6J mice from weaning to lactation were fed with control diet (CD, 10% fat) or high-fat diet (HFD, 60% fat). The offspring were weaned to CD. The eyeballs of the offspring were collected, fixed with 4% PFA, cryoprotected and sliced using cryotome. Microglia and activated microglia cells were labeled immunohistochemically using anti-RFP and anti-CD68 antibodies respectively, while cell nuclei were labeled with DAPI. The estrous cycle stages were determined by vaginal cytology in female offspring on the day of tissue collection (22 weeks old).

Results: We evaluated the area of microglia and CD68 in the peripheral retina and compared the measurements between the groups of offspring. mHFD significantly increased area of microglia and CD68 in female peripheral retina compared to maternal control diet offspring but had no significant effect on male retina. In addition, during assessment of microglia area and CD68 area in microglia, alterations were observed in female offspring estrous cycle stages due to mHFD.

Conclusion: Our findings demonstrated that mHFD had a gender-specific effect on the area of microglia and CD68 in offspring peripheral retina as well as revealed microglia and CD68 area changes in mHFD female offspring during estrous cycle stages.

Funding: This work was supported by the Science Promotion Fund of Vilnius University

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A non-tethered system for long-term electrophysiological analysis

Martynas Arbačiauskas[1], Vytautas Jonkus[2], Osvaldas Rukšėnas[1]

[1]Department of Neurobiology and Biophysics, Life Sciences Center, Vilnius University, Lithuania
[2]Institute of Applied Electrodynamics and Telecommunications, Vilnius University, Lithuania
martynas.arbaciauskas@gmail.com

Various disorders, including mental and neurodegenerative, have been shown to correlate with circadian disruptions [1]. Long-term electrophysiological evaluation allows the extraction and analysis of circadian and ultradian components, yet traditional long-term recording options are either limited in recording duration (battery life) or restrict the animal (tethered approaches). With non-tethered research systems emerging [2], we present such a system for electrophysiological signal recording in rats, and a specific application in sleep analysis.

Our system can record data from 4 channels simultaneously, with a battery life of ~30 hours at a 1 kHz sampling rate. Recording equipment is placed on the animal by using a harness and connected to implanted electrodes. Data is saved to an SD card. The signal that is obtained contains more noise yet is comparable to that obtained from a tethered system.

Using this approach, we were able to record a three-week-long signal, with daily ~5-minute interruptions for a battery change. Furthermore, we show that sleep-like states can be extracted in a completely unsupervised manner by a combination of clustering and rule-based analysis.

The sleep state results that are obtained fit the parameters found in literature – rat sleep is polyphasic, with more occurring in the light phase of the day. In total, non-REM sleep was detected in 49% of data, wake in 47%, and REM in 4%. Extracted sleep episodes were short (median durations <1 min for all states) but highly variable in duration, with non-REM and wake states lasting longer than REM. A clear circadian pattern can be observed in all parameters (Fig. 1) and extracted circadian or ultradian components of sleep partially match those extracted from locomotor activity data, occurring periodically at ~24, ~6 and ~4 hours.

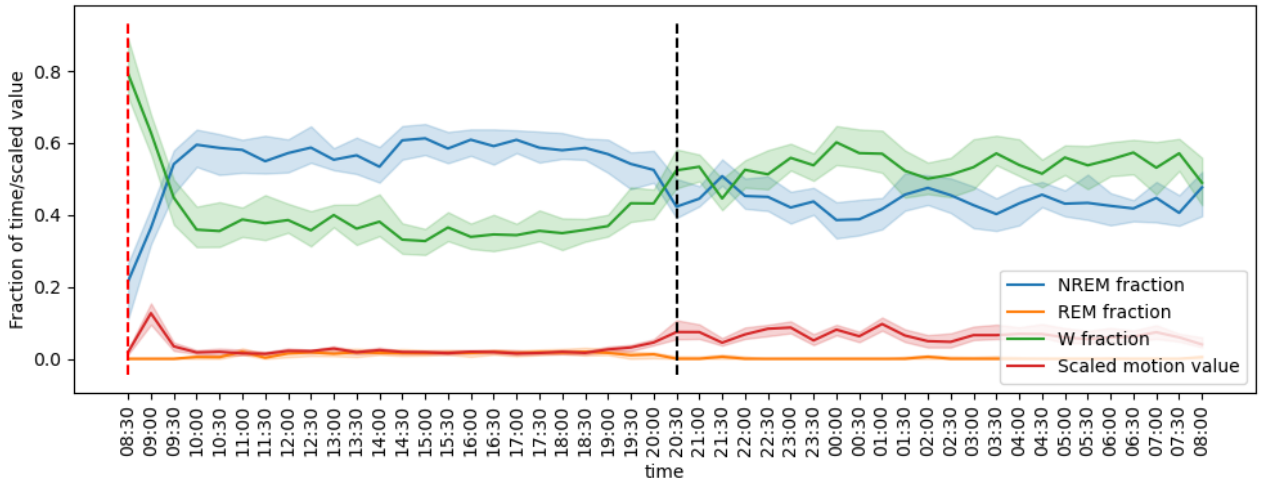

Figure 1. Median 24-hour parameters of sleep-like states (non-REM, REM and wake) extracted from electrophysiological data and scaled locomotor activity values. A clear circadian pattern is visible, with the beginning of the dark phase indicated as a black line.

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THE ROLE OF CIRCADIAN RHYTHMS IN BRAIN ACTIVITY

Julija Arbačiauskaitė[1], Robertas Guzulaitis[1]

[1]Department of Neurobiology and Biophysics, Vilnius University, Lithuania
julija.arbaciauskaite@gmc.stud.vu.lt

It is widely acknowledged that circadian rhythms regulated bodily processes are by over a 24-hour period [1]. Circadian rhythms may influence brain functions, as seen in the variation of internal states such as arousal, vigilance, and attention throughout the day. This has a direct impact on the brain, where low-frequency oscillations are prevalent during rest, while high-frequency oscillations occur during activity [2]. However, research is often conducted without considering the time of day, which can significantly affect the results. Therefore, it is necessary to investigate how brain activity changes under the same recording conditions but at different times of the day.

In this study, we tracked the activity of mice over a 24-hour period. The results showed that the mice were significantly more active at night, with maximum activity occurring about an hour after the lights were switched off. Based on these results, brain activity was monitored in head-fixed mice through electrocorticography (ECoG) recordings. ECoG recordings were taken at two time-points: one hour after the lights were switched off (8 PM, night) and one hour after the onset of the light period (8 AM, day). Both, spontaneous and evoked brain activity were assessed during the recordings. Differences were observed in almost all frequency bands during both spontaneous and evoked oscillations. The study shows a significant variation in brain activity during the day, highlighting the importance of noting the time of experiments.

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MIGRAINE MODEL: DIFFERENCES IN MALE AND FEMALE RATS

Eva Marija Petrauskaite, Ieva Poceviciute, Valentina Vengeliene

Department of Neurobiology and Biophysics, Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
eva.petrauskaite@gmc.stud.vu.lt

A migraine is a neurological condition characterized by recurrent, intense headaches typically accompanied by symptoms such as nausea, vomiting, and sensitivity to light or sound. These exhausting headaches often appear as throbbing or pulsating pain on one side of the head and can last anywhere from a few hours to several days. Despite their prevalence and impact, migraine remains poorly understood [1,2]. Current understanding suggests several interacting factors, including genetics, neurological conditions and environmental influences. Migraines can be triggered by a variety of stimuli, such as certain foods, drinks, hormonal imbalance, stress, fatigue, or even the weather changes. Migraine-type of headache can also be pharmacologically induced, for instance by use of nitroglycerine therapy.

Trinitroglycerin (NTG), a vasodilator that relaxes and widens blood vessels, can trigger events in the brain mimicking certain aspects of migraines not only in humans but also in laboratory animals [1,2]. Thus, administering NTG to rodents has become a well-established animal model of migraine. Since it is known that women are more likely to develop migraine-type of headache than men, the aim of this study was to compare if administration of NTG would induce different effect in male and female rats. To this end, rats received total of 8 NTG (10 mg/kg) injections every second day. Ultrasonic vocalizations (USV), loss of righting reflex (LORR) and hot plate tests were performed to measure migraine-like pain in both sexes.

From the results obtained during the experiment, it is clear that a migraine-like pain develops following 5 administrations of NTG in female rats, whereas in male rats expression of pain was not statistically significant even after 8 administrations of NTG. Specifically, our data demonstrated that the number of 22 kHz vocalizations (demonstrating negative emotional state of rats) in females increased significantly, whereas in male rats there was no significant difference between vehicle and NTG groups. LORR test (this test is used to measure the sensitivity to the sedative effect of substance), showed that NTG induced sedation in all rats. In male rats, this effect was still present even after 90 minutes. In female rats, LORR was restored within 90 minutes. Hot plate test showed in NTG treated male rats pain-sensitivity threshold was lower compared to controls. Contrary, females were more sensitive to the thermal pain than controls.

In conclusion, our data support that administration of NTG may cause headaches resembling a migraine-like pain, however female rats are a more suitable sex for modeling of NTG-induced migraine in animals.

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INFLUENCE OF ATTENTION ON THE LOW AND HIGH GAMMA-BAND AUDITORY STEADY-STATE RESPONSE

Giedrė Matulytė[1], Evaldas Pipinis[1], Inga Griškova-Bulanova[1], Marek Binder[2]

[1] Department of Neurobiology and Biophysics, Vilnius University, Vilnius, Lithuania
[2] Institute of Psychology, Jagiellonian University, Krakow, Poland
giedre.matulyte@gmc.stud.vu.lt

Auditory steady-state response (ASSR) is an electrophysiological brain response to periodic auditory stimuli and is characterized by having a constant frequency and phase for an infinitely long time period [1]. ASSR has been increasingly researched as a potential biomarker of neuropsychiatric diseases such as schizophrenia [2]. Furthermore, in recent years it has also been investigated for a possible application in brain-computer interface technologies [3]. The most frequently researched is 40 Hz ASSR while studies of higher gamma band ASSR are still scarce. In addition, attention has been investigated as one of the factors that could impact the ASSR [1]. However, studies of attention role in ASSR provide inconsistent results. Therefore, to further expand ASSR applications, it is important to focus on understanding how attention can affect both low and high gamma band ASSR.

The aim of the study was to evaluate the influence of attention on the 40 Hz and 80 Hz ASSRs. Electroencephalography (EEG) was used to record ASSRs elicited to 40 Hz and 80 Hz click trains in nineteen healthy subjects, during two experimental conditions: 1) concentration - counting presented stimuli while watching a video, 2) distraction - ignoring presented stimuli while watching a video. ASSRs obtained over the frontocentral region of interest were evaluated using two measures: phase-locking index (PLI) and evoked amplitude (EA). Both measures were averaged across time period starting from 0 ms to 500 ms. The averages of PLI and EA were compared between two attentional conditions. Significance of results was evaluated by applying paired samples t-test with 10 000 test repetitions.

Study results revealed significantly increased PLI of 40 Hz ($p = 0.04$) and 80 Hz ($p = 0.04$) ASSRs with attention to presented stimuli. Moreover, evoked amplitude measurement showed tendency for increased response power during concentration condition for 40 Hz ($p = 0.06$) ASSR.

In conclusion, our findings reveal increased phase locking of 40 Hz and 80 Hz ASSRs when attention is directed to click stimulation.

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EVALUATION OF MALES' PSYCHOPHYSIOLOGICAL PARAMETERS AFTER THE SHORT SING-A-SONG STRESS TEST

Kotryna Paulauskaitė, Dominyka Kontrimaitė, Ramunė Grikšienė, Erik Ilkevič

Department of Neurobiology and Biophysics, Vilnius University, Vilnius, Lithuania
kotryna.paulauskaite@gmc.stud.vu.lt

In the most studies evaluating the effects of social stress, participants are asked to provide a speech in front of the audience, which is known as the Trier Social Stress test [1]. However, this test is costly for studies with larger samples due to the constant need of having audiences of several people. Therefore, the short Sing-a-Song Stress Test (sSSST) was developed [2]. In this test, participants are suddenly cued to sing a song in the presence of a video camera. Although it was shown that the sSSST reliably elicits subjective and physiological stress, the dynamics of different physiological parameters during this test is still understudied. The aim of the present study was to establish the sSSST procedure and to evaluate how it affects males' subjective arousal and parameters of heart rate, heart rate variability (HRV), and breathing rate.

A total of 20 males participated in the study. Prior to the sSSST procedure, electrocardiography (ECG) electrodes and a respiratory belt were attached to participants. Males sat in front of the computer while different tasks followed by a 60 s countdown were shown. The tasks consisted of neutral messages that had to be read silently or out loud. After three control tasks, males were informed that they have to sing the National Anthem of Lithuania within 90 s. Before starting the 90 s countdown, the camera was turned on and the image of the participants briefly appeared on the screen. This manipulation was used to increase social stress and make males believe that their performance will be recorded. When 90 s passed, participants were informed that they do not need to sing and could relax (see Fig. 1 for the study design). The 90 s period of preparation to sing was regarded as a stress condition. Baseline (before the task) and stress-recovery (after the task) values of ECG and respiration were also measured for 90 s. Additionally, males had to evaluate their subjective arousal by filling in the Visual Analog Scale (VAS), both before and after the task. The leftmost side of VAS states "I feel completely calm" and the rightmost "I feel aroused to the maximum".

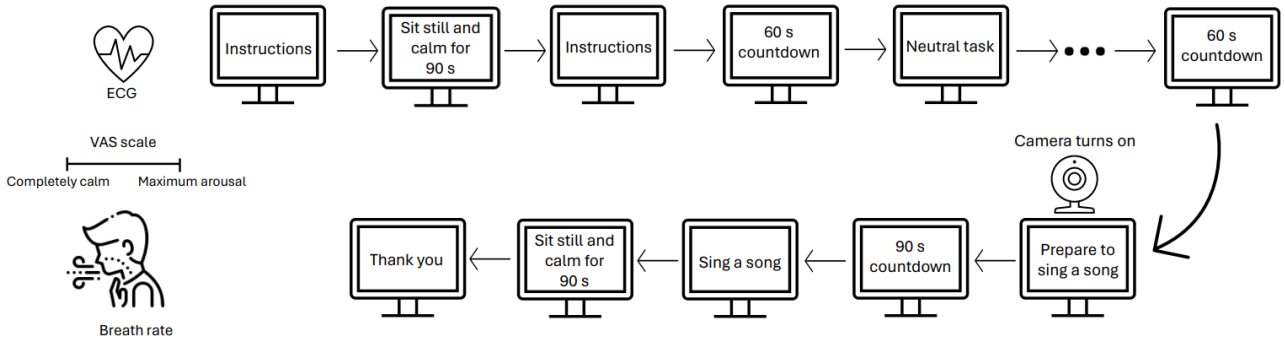

Figure 1. Study design.

The analysis of study results revealed that participants' heart rate was higher and R-R intervals were shorter during stress condition compared to both baseline and stress-recovery. Although the baseline did not differ from values during stress, the low frequency component of the HRV was lower during stress-recovery compared to stress condition. Furthermore, high frequency components did not differ between conditions. Males' breathing rate during stress was higher than during baseline but not stress-recovery conditions. Moreover, stress-recovery subjective arousal ratings were higher compared to baseline. These results suggest that the sSSST reliably induces mild stress, which is reflected in heart rate, heart rate variability parameters and subjective ratings.

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DIFFERENTIATION OF HUMAN DENTAL PULP MESENCHYMAL STEM CELLS

Greta Žvinakytė[1,2], Giedrius Steponaitis[2]

[1]Faculty of Medicine, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Laboratory of Molecular Neurooncology, Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania
greta.zvinakyte@lsmu.lt

Introduction: Glioblastomas, recognized as the most fatal form of human brain tumors, typically lead to a median patient survival duration of 15 months [1]. A minor fraction of cells within these tumors, termed as Glioma stem cells (GSCs), are predominantly accountable for resistance to treatment, tumor recurrence, and progression [2]. Hence, despite their limited presence within the tumor, GSCs are the primary cells to target. Neural stem cells (NSCs), present in the brains of adult organisms, are instrumental in repairing and regenerating damaged or aging tissues [3]. Considering the high similarity between GSCs and NSCs, it's vital to include NSCs when identifying GSC-specific markers for targeted treatment. Additionally, it has been shown that stem cells can exert a therapeutic effect on gliomas by inhibiting tumor growth through extracellular signaling [4-5]. To explore the therapeutic influence of extracellular vesicles and GSC-specific markers, we first need to establish a NSCs model. This can be accomplished in several ways, such as isolating them from embryos or de-differentiating stem cells or iPSC. The latter approach is safer and aligns with current EU standards. We therefore suggest that MSCs obtained from dental pulp could potentially dedifferentiate into NSCs, similar to those sourced from the human brain. To validate this proposition, it is essential to verify that the cells extracted from dental pulp are indeed MSCs with the ability to differentiate.

Aim: To confirm the capacity of extracted MSCs to develop into adipocytes, osteoblasts and evaluate the expression of stemness markers after the differentiation.

Methods: The extracted MSCs were validated by inducing adipogenic and osteogenic differentiation. For adipogenic differentiation was used 100nM Dexamethasone, while the StemPro Osteogenesis kit was used for osteogenic differentiation. After 4 weeks cells were stained to assess the differentiation results, employing AlizarinRed for osteogenic and OilRedO dye for adipogenic differentiation. RNA was isolated from the cell pellets using TRIzol reagent and transcribed with the High-Capacity RNA-to-cDNA kit. The expression levels of MSCs markers were measured using RT-qPCR, the expression difference was calculated using the 2-dCT method. [6]

Results: AlizarinRed staining, conducted after a four-week period of differentiation into osteoblasts, demonstrated the existence of calcium deposits in the monolayer cell cultures. Similarly, OilRedO staining indicated small presence of lipid droplets in the cells cultivated in conditions of adipogenic differentiation. The mRNA analysis of differentiated cells showed an increased expression of RUNX, BGLAP for osteogenic and PPARG for adipogenic differentiation compared to the control. The expression of stemness markers such as SOX2, OCT4, KLF4, NES, MYC, NANOG, TUBB3 was aberrated in differentiated cells compared to control cells.

Conclusion: The findings of the study demonstrate that previously derived dental pulp stem cells have the capability to differentiate into both osteoblasts and adipocytes, confirming their identity as MSCs. Next, these cells can be used for de-differentiation into NSCs for furher GSCs studies.

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H8

ASSESING AXON BOUTON DYNAMICS AND MICROGLIA CHANGES DURING MOUSE VISUAL CORTEX PERIOD

Monika Kuseliauskyte[1], Daina Bujanauskiene[1], Igor Nagula[1], Urte Neniskyte[1,2]

[1]Life Sciences Center, Vilnius University, Sauletekio al. 7, 10257 Vilnius, Lithuania
[2] VU-EMBL Partnership Institute, Lithuania
monika.kuseliauskyte@gmc.stud.vu.lt

Genetic factors and environment together shape the architecture and functionality of the developing brain. The development of a neural network comprises several stages, a critical development period occurs when plasticity in specific brain regions is especially heightened.

The ocular system is crucial in studies of neuronal plasticity during this critical period, as the visual area's critical period is well-defined and easily manipulated by visual stimuli. In particular, layers 2 and 3 of the primary visual cortex form cortico-cortical connections, where plasticity is most evident (Hooks & Chen, 2020). Research indicates that during the critical plasticity period of the mouse visual cortex (from the 21st to the 35th postnatal day), visual stimuli and changes in the synapses leads to optimized connectivity. Throughout the intricate process of cerebral maturation, glial cells assume a pivotal role in coordinating the connectivity among neurons. This underscores the importance of meticulously assessing the dynamic alterations that transpire.

During neuron network development, microglia mediate synaptic pruning and facilitate neuronal circuit formation through the phagocytosis of synaptic connections. Activity-dependent changes prompt microglial cells to phagocytose excess synapses through various signaling receptors, thus contributing to the maturation of the neuronal network (Andoh & Koyama, 2021). Synapses exhibiting lower activity are targeted for phagocytosis via "eat me" signals. A thorough understanding is crucial, as the mechanisms behind pruning are not fully known across all brain regions.

High-resolution fluorescent microscopy and quantitative optimized binary-processed image analysis enabled us to collect detailed images of microglia and neuronal axon branches in the visual cortex's layer 2/3. By analyzing these images, we have investigated axon bouton size/density and microglia area changes in the immature cortex (P21), during the peak of the critical period (P28) and in the cortex of mature adult mice (P35).

We have discovered that during the critical development period in layers II/III of the visual cortex, changes in the microglia-occupied area, as well as bouton density and size. During the peak of the critical period, both the area of microglia and the size of axon boutons increase, while bouton density decreases compared to before and after the critical development of the visual cortex. The study reveals the essential functions, thereby shedding light on the sophisticated, activity-dependent mechanisms facilitating neural circuit optimization and underscoring the temporally constrained nature of cerebral maturation processes.

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EXTRACELLULAR VESICLE ASSOCIATED MIR-16-5P, MIR-103A-3P, MIR-497-3P, MIR-22-3P EXPRESSION IN PARKINSON'S DISEASE

Violeta Belickienė[1], Gintarė Autukaitė[2], Mantas Andriuškevičius[2], Edvinas Višinskas[2], Aistė Pranckevičienė[3], Andrius Radžiūnas[4], Paulina Vaitkienė[1]

[1]Laboratory of Molecular Neurobiology, Lithuanian University of Health Sciences, Lithuania
[2]Facaulty of Medicine, Medical Academy, Lithuanian University of Health Sciences, Lithuania
[3]Health Psychology Department, Faculty of Public Health, Lithuania University of Health Sciences, Lithuania
[4]Neurosurgery Department, Lithuanian University of Health Sciences, Lithuania
violeta.belickiene@lsmu.lt

Parkinson's disease (PD) is a neurodegenerative disorder that results in tremor and bradykinesia due to the death of dopaminergic neurons. PD is difficult to diagnose, it progresses for several years before a clinical diagnosis is made and its treatment isn't curative (1). Recently, miRNA interference has been extensively studied due to it's affects in many biological processes including neurodegenerative diseases (2). MiRNA molecules formed in various body cells can be packed into extracellular vesicles (EV), then infiltrate the extracellular space and travel through biological fluids providing long lasting effects of disease related genes. EV associated miRNA's are expected to useful in clinical practice to facilitate diagnosis, prognosis and treatment (3).

The aim of this study was to evaluate EV miR-16-5p, miR-103a-3p, miR-497-3p, miR-22-3p expression levels in the serum of patients with PD and check for relations with patient's age, sex, the onset of the disease, its duration, severity of symptoms and selected method of treatment.

EV miRNA's were isolated from blood serum, transcribed into cDNA and its expression was measured by RT-PCR. Statistical analysis was performed using Student's t test, ANOVA criteria and Pearson's correlation coefficient in GraphPad Software Inc. Prism 8. 3 groups of PD patients were tested. 36 control group patients received medicamental treatment, 39 underwent deep brain stimulation and 13 had gamma knife surgery. miR-126 expression was evaluated before and after the surgical treatment.

The results reveled, that miR-16, miR-103a and miR-22 expression was higher in patients receiving medicamental treatment when comparing patients between different treatment groups ($p < 0,05$; $p < 0,01$; $p < 0,001$). Statistical analysis showed that miR-16, miR-22 and miR-103a levels were down regulated ($p < 0,05$; $p < 0,01$) and miR-497 was upregulated as intensity of bradykinesia increases. As symptoms of tremor become more intense miR-16 levels decrease and miR-497 expression increases. No statistically relevant changes in miRNA expression were observed after assigned treatment, but it seemed, that patients had lower miR-103a and miR-497 expression levels after receiving gamma knife surgery. However, more patient samples are needed to confirm this pattern. Association between miRNA expression levels were observed regarding gender of the patients. Men had higher miR-103a and miR-22 levels in their blood ($p < 0,05$). Also, no statistically relevant correlation was found between miRNA expression and patients age, duration of the disease and age of diagnosis.

Primary data suggest that patient groups with downregulated EV miR-16, miR-22, miR-103a expression levels and elevated miR-497 experience stronger PD related symptoms and require surgical treatment. However miRNA profile did not change significantly after treatment.

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DEVELOPMENT OF AN ALGINATE HYDROGEL SYSTEM FOR 2D CELL CULTURE: APPLICATIONS IN BV-2 MOUSE MICROGLIA STUDIES

Paulius Liauba[1], Monika Iešmantaitė[1], Egidijus Šimoliūnas[1], Daiva Baltriukienė[1]

[1]Department of Biological Models, Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania
paulius.liauba@chgf.stud.vu.lt

Microglia, innate immune cells in the central nervous system, not only respond to chemical signals but are also influenced by the mechanical properties of their microenvironment. Microglia display a preference for stiff materials and the degree of stiffness directly correlates with their inflammatory activity. Although this response has a physiological role in vivo (e.g., microglia are recruited to stiff amyloid plaques in Alzheimer's disease) [1], microglial behavior changes while cultured in vitro, as cells are typically cultured on non-physiologically rigid materials. Studies suggest that culturing microglia on substrates with brain-like elasticity promotes an anti-inflammatory activation. However, the impact of surface elasticity on microglial function is still largely unexplored. The field of biomaterials offers cell culture systems allowing precise control over mechanical and compositional cues to mimic the properties of natural tissue. [2] Alginate hydrogels, with their biocompatibility and tunable mechanical properties, have emerged as promising biomaterials. Despite their extensive use in 3D cell culture, their application in 2D neural cell culture remains underexplored.

In this study, we developed alginate hydrogels with a stiffness of approximately 1 kPa and a functionalized surface for adequate cell adhesion. Initially, we demonstrated that BV-2 cells exhibit higher phagocytosis efficiency when cultured on alginate hydrogels compared to plastic, regardless of lipopolysaccharide (LPS) treatment. It may be linked to the M2 (anti-inflammatory) phenotype, prompting us to evaluate and compare the expression of pro-inflammatory and anti-inflammatory genes following LPS treatment on both stiff plastic surfaces and the alginate hydrogel system. Our results indicate that BV-2 cells cultured on alginate hydrogels express lower levels of pro-inflammatory genes and higher levels of anti-inflammatory genes compared to those cultured on plastic.

Nevertheless, further experiments will show whether this effect is solely attributed to the stiffness of the substrate or if alginate itself plays a role in modulating microglial response.

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H11

DEEP LEARNING-BASED DENDRITIC SPINE SEGMENTATION AND QUANTIFICATION

Aušrinė Mikelionytė[1], Lina Saveikytė[1,2], Urtė Neniškytė[1,2]

[1]Department of Neurobiology and Biophysics, Life Sciences Center, Vilnius University, Lithuania [2]EMBL-VU LSC Partnership Institute, Vilnius University, Lithuania
ausrine.mikelionyte@gmc.stud.vu.lt

Dendritic spine plasticity includes the changes of spine number and morphology and has the central role in brain development, physiology and pathology. Density and structure of dendritic spines changes dynamically with experience during development and healthy adulthood as well as can reflect pathogenesis of major neurological disorders, such as autism, schizophrenia or Alzheimer's. Thus, the quantitative analysis and classification of dendritic spines is important towards understanding neuronal function and bases of brain pathology. Although many methods for automated spine segmentation and evaluation have been developed, manual analysis still obtains better results, even though being time consuming and subjective. Therefore, we are developing deep-learning based automated tool for precise dendritic spine segmentation and quantification in light microscopy images obtained by different techniques.

Two-photon microscopy is prominent method to analyze spine changes and dynamics in various types of brain preparation including *in vivo*. Therefore, we trained and evaluated models for semantic spine segmentation in two-photon microscopy images of pyramidal excitatory neurons expressing iGluSnFR from mouse organotypic hippocampal slices*. We used deep learning platform APEER and open source FIJI software. To facilitate the recognition of the objects, images first were pre-processed with custom-designed FIJI plugins and used as a data set for training and segmentation in APEER. Then images were pixel-wise annotated and models were trained for semantic segmentation of spines, shaft and background using deep learning U-net architecture. After that, models were validated in independent datasets. Finally, we quantitatively determined how the performance of different models in pixel and spine segmentation depends on training data set size. To select optimal number of images for model training, we used regressive analysis to select threshold values. The analysis showed that our model required small training data set for successful spine detection. Also we showed that increasing training data set size spine segmentation performance on different images improves.

In conclusion, our method suggested a quick automated tool for the quantification and segmentation of dendritic spines from two-photon microscopy images. This study is a part of project to create universal deep-learning model to segment spines from two-photon, confocal and STED microscopy. This tool could assist further research on brain plasticity, development and diseases.

** shared by Sébastien Marais from Bordeaux Imaging Center*

BIOCHIP TO STUDY MECHANICAL STRESSES IN CELLS

Andrius Pečiulis[1], Airina Mazėtytė-Godienė[2], Tomas Rakickas[2], Tadas Jelinskas[2], dr. Martynas Gavutis[2]

[1]Vilnius University, Center of life Sciences, Saulėtekio av. 7, LT-10257, Vilnius, Lithuania
[2]Department of Nanoengineering, Center for Physical Sciences and Technology, LT-02300 Vilnius, Lithuania
andrius.peciulis@gmc.stud.vu.lt

Mechanobiology is a new interdisciplinary field that studies the mechanisms by which cells sense and respond to mechanical signals from the environment. Mechanical forces and stresses have been shown to be important in morphogenesis, cell migration, polarization and proliferation [1]. Knowledge of the changes induced by mechanical forces at the molecular, cellular and tissue levels would provide new insights into tissue physiology, disease development and lead to new regenerative medicine approaches. In particular, mechanobiological research of heart tissue, could contribute immensely to drug testing, disease modelling and regenerative medicine, given development of standardized experimental methods and platforms [2], [3].

Our main goal is to create hydrogel based platform for myoblast growth and differentiation, that would allow further research of heart tissue. For this purpose we chose to fabricate a biochip with hydrogel of tunable stiffness and create different patterns of fibronectin on top of this gel, to serve as binding sites and physical barriers for myoblast cell attachment, growth and spreading. Hydrogel chosen for this task was polyacrylamide, as it is one of the mostly utilized hydrogels worldwide, mimicking extracellular matrix environment, with well defined properties, low cost, plethora of preparation protocols and prospects for functionalization [4].

Within the framework of this project polyacrylamide chips of 4 different Young's modules values were fabricated, to confirm literature data AFM measurements of several chips from each batch have been taken: Target value - 10kPa (experimental value: 11,87 ± 2,13 kPa), 50kPa (experimental value: 54,71 ± 22,87 kPa), 75kPa (experimental value: 69,03 ± 10,48 kPa), 100kPa (experimental value: 126,8 ± 2,83 kPa). Additionally, a different type of chip was fabricated, with hydrogel containing carboxyl groups via introduction of methacrylic acid. This allowed us to employ NHS/EDC reaction to allow for covalent binding of fibronectin to hydrogel surface. These chips had stiffness of ~32kPa. During cell seeding experiment, mouse myoblast (cell line: C2C12) cells were seeded on these patterned polyacrylamide chips and left to grow and differentiate, resulting in formation of muscle fibers on fibronectin patterned areas. Cell growth and differentiation has been tracked on a weekly basis, by brightfield microscopy. Wide field fluorescent microscopy has been utilized to confirm presence of fibronectin patterns on biochips.

Use of patterned polyacrylamide surfaces for cell growth and differentiation offers a different approach for biomimetic tissue engineering. Main advantages of such system are control of cell growth area and substrate stiffness. Although such type of platform requires further investigation and refinement, it holds promise as system for drug screening and disease modelling.

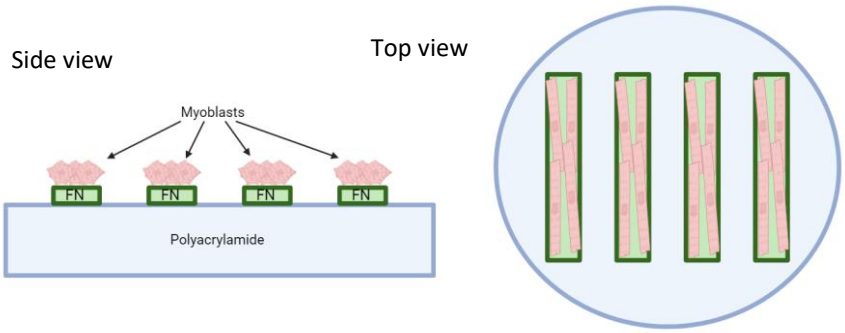

Figure 1. A schematic overview of polyacrylamide biochip, for myoblast growth. FN – fibronectin.

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THE EFFECT OF THE ANESTHETIC PROPOFOL ON CONNEXINS OF CARDIAC AND VASCULAR SYSTEM

Orestas Makniusevičius[1], Tadas Kraujalis[1,2], Mindaugas Šnipas[1,3], Lukas Gudaitis[1], Lina Kraujalienė[1]

[1]Lithuanian University of Health Sciences, Medical Academy, Institute of Cardiology, Kaunas, Lithuania
[2]Kaunas University of Technology, Department of Applied Informatics, Kaunas, Lithuania
[3]Kaunas University of Technology, Department of Mathematical Modelling, Kaunas, Lithuania
orestas.makniusevicius@lsmu.lt

Propofol is a widely used general anesthetic, which causes a rapid induction of anesthesia and rapid recovery after it. The most prominent side effect of propofol is the decrease of systemic vascular resistance that leads to hypotension [1]. Moreover, in some cases, propofol has been shown to inhibit cardiac conduction and cause bradycardia that can result in cardiac arrest. The propofol effect on the induction of bradycardia has been known for more than 30 years, but its pathophysiological mechanism is still not fully understood. One of the putative mechanisms for the conduction block could be the ability of propofol to change the activity of human atrial muscarinic cholinergic receptors [2]. It is also known that propofol may directly inhibit the sinoatrial (SA) node cells. On the other hand, propofol was shown to have a protective effect against ventricular arrhythmias during myocardial ischemia [3]. Thus, the influence of propofol on the heart rhythm remains controversial.

The side effect of propofol may manifest itself through its action on different targets. Indeed, several studies have shown that propofol may regulate cell coupling through gap junction (GJ) channels formed of connexin-43 (Cx43). As known Cx43 and Cx45 form gap junction channels in nervous system and, together with Cx37 and Cx40, are also expressed throughout cardiac and vascular system. These connexins are required for coordination of vascular responses and play a key role in ensuring propagation of action potential in cardiac tissue.

The aim of this study was to get a better understanding of the capacity of cardiovascular connexins to participate in side effect caused by propofol. First, we compared the effect of propofol concentrations on GJs formed by Cx40, Cx43 and Cx45, which were expressed exogenously in human cervix epithelial adenocarcinoma cells (HeLa). The junctional conductance was measured using double whole-cell patch clamp method. Our data show that Cx40 and Cx43 channels exhibit similar sensitivity to propofol, while Cx45 channels are only sensitive to much higher propofol concentrations (above 60 µM) than Cx40 and Cx43 channels.

It was suggested that propofol may affect Cx43 GJ channels through activation of protein kinase C (PKC), which in turn phosphorylates Cx43 and reduces coupling through GJs [4]. In our study, the kinase inhibitor GF109203X was used to assess this putative pathway of propofol action on GJs. It is established that low concentrations of GF109203X specifically inhibit PKC while higher concentrations inhibit both PKC and protein kinase A (PKA). Our data showed that low (40 nM) concentration of GF109203X significantly reduced inhibition of Cx43 channels conductance by propofol, indicating that propofol regulates Cx43 channels in PKC-dependent manner, which is in good agreement with already published studies. In contrast, GF109203X had no effect on inhibition of Cx40 channels by propofol, indicating that neither PKC nor PKA is involved in regulation of these channels by the anesthetic. This preliminary data suggest that propofol can have a prominent, connexin specific effect on GJs.

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BI-ENZYMATIC ELECTROCHEMICAL BIOSENSOR FOR THE DETECTION OF NEUROTRANSMITTERS

Elžbieta Ragauskaitė[1,2], Diana Macaitytė[1], Gintautas Bagdžiūnas[1]

[1]Group of Supramolecular Analysis, Department of Bioanalysis, Institute of Biochemistry, Vilnius University
[2]Department of Physiology, Biochemistry, Microbiology and Laboratory Medicine, Vilnius University
elzbieta.ragauskaite@gmc.stud.vu.lt

Sensors have emerged as powerful tools for the neurochemical monitoring and detection of neurotransmitters, specifically serotonin, epinephrine and norepinephrine in our previous works [1]. Enzymatic electrochemical biosensors operate on the principle of utilizing enzymes as recognition elements, ensuring specificity and efficiency in detecting neurotransmitters [2]. The catalytic activity of enzymes facilitates the conversion of neurotransmitters into measurable signals. Serotonin, epinephrine and norepinephrine are often detected in the central nervous system and play important roles in mood regulation, stress response and cognitive function. Therefore, the high sensitivity and specificity of these biosensors make them promising candidates for diagnostic purposes, allowing for the identification of neurotransmitter imbalances associated with various neurological and psychiatric disorders [3]. On the other hand, challenging bioanalytical issues, including specificity, stability and selectivity, are resolved by combining nanomaterials with nanotechnological methods. As a result, the electron transfer rate between the active center of enzymes and electrodes has been improved by using gold nanoparticles (AuNPs) as electric wires and mediators for electron transfer. Enzymatic biosensors present a flexible framework for investigating different aspects of neurotransmitter release, and personalized approach to neurochemical assessment could revolutionize treatment strategies, enabling interventions based on individual neurotransmitter profiles.

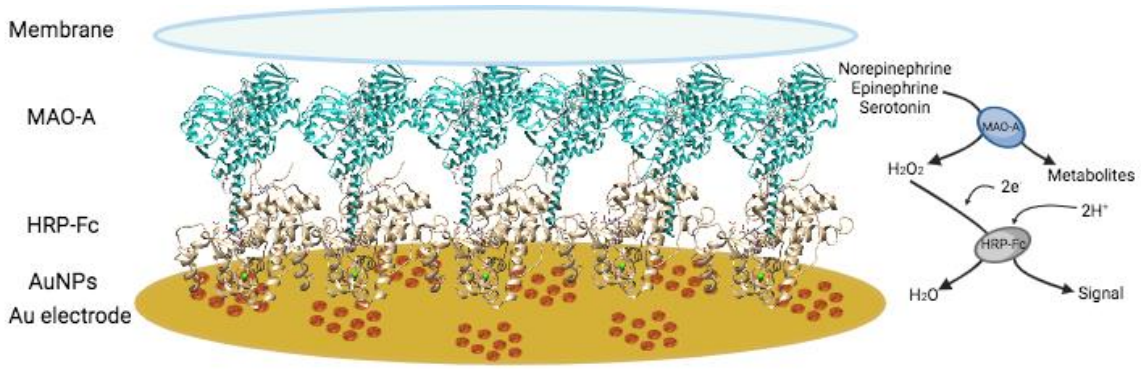

Figure 1. Schematic representation of the bioelectrode based on HRP-Fc and MAO-A. The matrix relies on the oxidation of norepinephrine/epinephrine/serotonin by catalyzing MAO-A, which involves molecular oxygen. The reaction produces metabolites and hydrogen peroxide as end products. HRP-Fc acts as a mediator, taking up the hydrogen peroxide along with two protons and two electrons from a gold electrode.

The goal of this study is to develop a versatile matrix for H_2O_2-releasing enzymes suitable for biosensors (Fig. 1). Therefore, the optimal conditions for modifying horseradish peroxidase (HRP) in an organic solvent with a redox-active ferrocene group and immobilizing it on an electrode decorated with AuNPs were investigated. After characterizing and comparing the bioelectrodes based on the original and modified enzymes, a biosensor for the detection of neurotransmitters based on monoamine oxidases A and B (MAO-A and MAO-B) and HRP-Fc was developed, and its sensitivity, selectivity, and stability were evaluated.

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H15

Antimicrobial Peptide Colistin Interaction with Bacterial Membrane Mimicking Models

Modestas Mažerimas[1], Rima Budvytyte[1,2]

[1]Life Sciences Center, University of Vilnius, Vilnius, Lithuania
[2]Institute of Biochemistry, Department of Bioelectrochemistry and Biospectroscopy, Life Sciences Center, Sauletekio av. 7, LT-10257, Vilnius
modestas.mazerimas@gmc.stud.vu.lt

The discovery of antibiotics in the 20th century marked a significant breakthrough in combating bacterial infections, leading to a substantial reduction in morbidity and mortality. However, the widespread use of antibiotics accelerated the development of resistance in bacteria, rendering once life-saving drugs ineffective. This challenge has underscored the urgent need for new approaches to combat antibiotic-resistant strains1,2. One promising avenue is the use of antimicrobial peptides (AMPs), also known as host defense peptides3,4. Colistin belongs to the polymyxin antibiotic family. The hydrophobic nature of colistin molecules' N-terminal fatty acyl segment underlies both the intrinsic toxicity and the substantial impact on the antimicrobial potency of colistin5. However, the exact mechanism of colistin action against microbes is yet to be known. Some of the postulated mechanisms include negative membrane surface charge neutralization, inner membrane perturbation leading to membrane permeabilization increase, dimer formation mediating the contacts between periplasmic leaflets of inner and outer membranes, and oxidative burst that produces a reactive hydroxyl radical5,6.

Bacterial membranes mimetics, composed of synthetic lipid analogs mimicking natural membranes, known as tethered lipid membranes (tBLM), enable us to quantitatively assess the impact of colistin acting on tBLMs by using electrochemical impedance spectroscopy (EIS)7,8. Besides that, atomic force microscopy (AFM) - a high-resolution imaging technique is used to study morphological surfaces changes at the nanoscale level9. Our experimental results revealed that tBLMs, when exposed to colistin, exhibited reduced conductance within the systems. These conductance changes indicate that the membrane exhibits increased stiffness. Complementing our findings with AFM revealed the structural changes and mechanical changes. Lately, we also accompanied Laurdan dye to investigate changes in liposome fluidity.

Taken together, our research demonstrates that tBLMs, in combination with EIS, AFM and Laurdan dye, serve as a powerful tool for screening and characterizing the activity of AMPs against bacterial membranes. This innovative approach holds great promise for addressing the challenge of antibiotic resistance and advancing the development of effective antimicrobial therapies.

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INVESTIGATING FARNESENE: A NOVEL Cx43 INHIBITOR

Gabrielė Zuokaitė, Vytautas Raškevičius, Vytenis Arvydas Skeberdis

Laboratory of Cell Culture, Institute of Cardiology, Lithuanian University of Health Sciences, 50162 Kaunas, Lithuania
gabriele.zuokaite@vdu.lt

Understanding how cells communicate opens the door to new medical therapies. Connexin 43 (Cx43) is detected in most human cell types including cardiac tissue and certain tumours cells. Those cells communicate through gap junctions made by Cx43. Limited signalling between cells can lead to heart diseases. Terpenes and Terpenoids, synthesized via the mevalonate pathway through the condensation of isoprene units, are renowned for their broad biological activities, encompassing anti-cancer, anti-inflammatory, and cardio-protective effects [1]. Recent work by Mickus and colleagues in 2021 has confirmed Terpene's direct effect on the conductivity of gap junctions [2]. This research aims to extend these findings by characterizing the influence of selected Terpenes on Cx43 gap junctions, evaluating their role as either inhibitors or activators in a pharmacological context.

The methodology encompasses experimental studies on Novikoff-hepatoma cells originally obtained from a rat liver. This cell line is endogenously expressing Cx43, maintained in the LSMU, Institute of Cardiology, Laboratory of Cell Culture. Farnesene is an acyclic sesquiterpene from the Terpenes family selected for investigation. It was chosen based on its documented biological activities with a particular focus on showing the impact that Farnesene has on gap junction's conduction. Additionally, there was an interest in exploring the Terpene's linear structure and examining how its biological activity correlates with the length of the chain.

We investigated the effect of Farnesene on the Cx43 gap junction's conductivity, exploring its potential as a chemical modulator. This study aims to disclose the specific ways Farnesene influences Cx43 gap junctions, broadening our understanding of its role in intercellular communication. By assessing how Farnesene adjusts Cx43's activity, and determining its IC_{50} values and Hill coefficients we highlight its therapeutic promise for treating Cx43-linked conditions, such as cardiac arrhythmias and other pathologies associated with Cx43 dysregulation. We also carried out molecular docking of Farnesene into Cx43. This research is a step towards understanding how nature's bounty can be harnessed for our health, emphasizing the need for further exploration into the biological effects of natural compounds.

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CD2AP ASSOCIATION WITH THE ENDOSOMAL ARPC1A - CONTAINING ARP 2/3 COMPLEX

Eglė Pociūtė[1,2], Tiago Piconêz[1], Cláudia Guimas Almeida[1]

[1] NOVA Medical School - Universidade Nova de Lisboa, Lisboa, Portugal
[2] Life Sciences Center, Vilnius University, Lithuania
egle.pociute@nms.unl.pt

Alzheimer's disease (AD) is a devastating neurodegenerative disease that affects 1 in 9 people aged 65 and increasing with aging. The principal component of extracellular plaques found in the brains of AD is beta-amyloid (Abeta). Abeta generation occurs mainly in early endosomes where the amyloid-precursor protein (APP) is cleaved by BACE1 and the γ-secretase complex. AD includes significant loss of neurons, loss of memory, and cognitive impairment. In the face of the large scale and lack of solutions to tackle this disease, it is vitally important to study and reveal the mechanisms associated with this pathology [1,2].

In the most common late-onset AD (LOAD), which represents 95% of all AD cases, several endocytic regulators were identified as risk genes, including CD2AP. CD2AP is a protein implicated in the trafficking of APP and BACE1 into and out of early endosomes. Previous research has shown that the knockdown of CD2AP reduces APP endosomal sorting for degradation increasing Abeta generation [3]. However, it is unknown how CD2AP controls APP sorting. Since CD2AP is an actin-binding protein that controls actin stability, which helps to drive membrane invagination for endocytosis and shape membrane tubules for secretory trafficking [4], we hypothesized that CD2AP regulates endosomal F-actin, required for APP sorting.

Recent results show that CD2AP loss of function reduces actin dynamics associated with early endosomes. Moreover, interference with ARPC1A, an important subunit of the endosomal F-actin polymerizing Arp2/3 subcomplex, mimics CD2AP-dependent defects in APP endosomal sorting and increases Abeta levels.

This work aims to investigate whether CD2AP is associated with the endosomal ARPC1A–containing ARP2/3 complex. The techniques used in this research were siRNA knockdown, Immunoblotting, and high-resolution microscopy with immunofluorescence. Results were analyzed with ImageJ software.

Results show that knocking down the ARPC1A isoform results in reduced levels of CD2AP, and the effects on the perinuclear F-actin observed after CD2AP downregulation can also be observed in the case of ARPC1A isoform downregulation. Results support that CD2AP might be part of the endosomal ARPC1A–containing ARP2/3 complex.

We identified a CD2AP partner, ARPC1A, as being required for APP endosomal sorting and amyloidogenesis, which should be targeted to prevent Alzheimer's disease.

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Generation of SH-SY5Y cell line stably expressing EGFP and mRuby2 reporter genes via CRISPR-mediated HDR

Vilius Kasparas Kasparavičius[1], Simonas Kutanovas[2], Neringa Daugelavičienė[2], Urtė Neniškytė[1,2]

[1]Institute of Bioscience, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]VU LSC-EMBL Partnership for Genome Editing Technologies, Life Sciences Center, Vilnius University, Vilnius, Lithuania
kasparas.kasparavicius@gmc.stud.vu.lt

Genome editing is making its way towards therapeutic applications. However, genome editing tools are usually researched using cancerous, undifferentiated cell lines as research models, which do not accurately represent living organisms. The commonly used neuronal model, SH-SY5Y human neuroblastoma cell line, is often employed in undifferentiated state, which is not representative of mature neurons [1]. Differentiated SH-SY5Y neural-like cells are distinguished from undifferentiated ones by their neural marker expression, neurite formation, and halt of proliferation.

We established a dual-reporter SH-SY5Y cell line expressing EGFP (green fluorescent protein) and mRuby2 (red fluorescent protein), which could be used for gene editing tools, such as prime editors, screening, and evaluation of tool efficiency in both differentiated and undifferentiated cell states at the single-cell level. The dual-reporter system was generated by template knock-in via CRISPR-mediated HDR into the safe harbor AAV1 locus of SH-SY5Y cells. The donor template and CRISPR-Cas were transfected via lipofection. In the template plasmid, mRuby2 was positioned near its promoter, for easy visualization upon successful transfection. EGFP, along with the puromycin resistance gene, was driven by the cell's endogenous promoter, ensuring EGFP expression only after a successful integration into the genome. Monoclonal populations of edited cells were obtained by single-cell dilution, and cells expressing EGFP and mRuby2 were identified and selected by fluorescent microscopy.

Furthermore, we optimized the conditions of SH-SY5Y differentiation [1], by selecting Corning© Matrigel extracellular matrix, which enabled cell fixation with paraformaldehyde for antibody staining and visualization by immunofluorescence microscopy. Throughout the 18-day differentiation course, cells have been exposed to a low-serum environment with retinoic acid, resulting in a halt of proliferation and development of neurites. Following terminal differentiation cells were exposed to environment supplemented with neural factors. Neuronal features were highlighted using immunostaining with axon marker SMI312 and postsynaptic marker PSD95.



Figure 1. SH-SY5Y stable cell line, expressing EGFP seen in the nucleus and mRuby2 seen in cytoplasmic membrane.

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AFFINITY OF HUMAN CARBONIC ANHYDRASES CAI, CAII, AND CAXIII IN BUFFERS OF DIFFERENT COMPOSITION

Tomas Ragauskas, Lina Baranauskienė

Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Centre
Vilnius University, Saulėtekio av. 7, Vilnius, Lithuania

tomas.ragauskas@chgf.stud.vu.lt

Carbonic anhydrases (CA's) are metalloenzymes found in human cells. They catalyse the reversible reaction of carbon dioxide hydration ($CO_2 + H_2O \rightleftarrows HCO_3^- + H^+$). These enzymes contribute to the efficient transport of the product released during cellular respiration away from the site of formation, participate in pH regulation, fluid secretion, and other physiologic processes. By selectively regulating activity of carbonic anhydrases, i.e. by inhibiting them, it is also possible to control diseases such as glaucoma, edema, epilepsy, mountain sickness, etc [1].

The observed affinity (the dissociation constant K_d) for human carbonic anhydrase binding to sulfonamide inhibitors depends on the experimental conditions, such as pH of the buffer [2]. The affinity dependence on pH in this system is due to linked (de)protonation reactions of ligand sulfonamide group and Zn^{2+}-bound water in the protein active centre. In this study, we analysed how the observed affinity of human carbonic anhydrases is affected by presence of different salts. The carbonic anhydrases CAI, CAII and CAXIII were tested with 15 different small-molecule ligands and the values of the ligand dissociation constants were measured using fluorescent thermal shift assay (FTSA). Two different conditions were used in this study: phosphate buffer with NaCl and HEPES buffer with Na_2SO_4 at pH 7.0-7.5. The experimental data were processed using "Thermott" open access online tool to determine ligand affinity for hCAs [3].

A systemic difference of ligand affinity for carbonic anydrase isozymes in different composition buffers was observed. In most cases, dissociation constants were by up to 2-3 times lower when Na_2SO_4 has been used in buffer, as shown in figure 1.

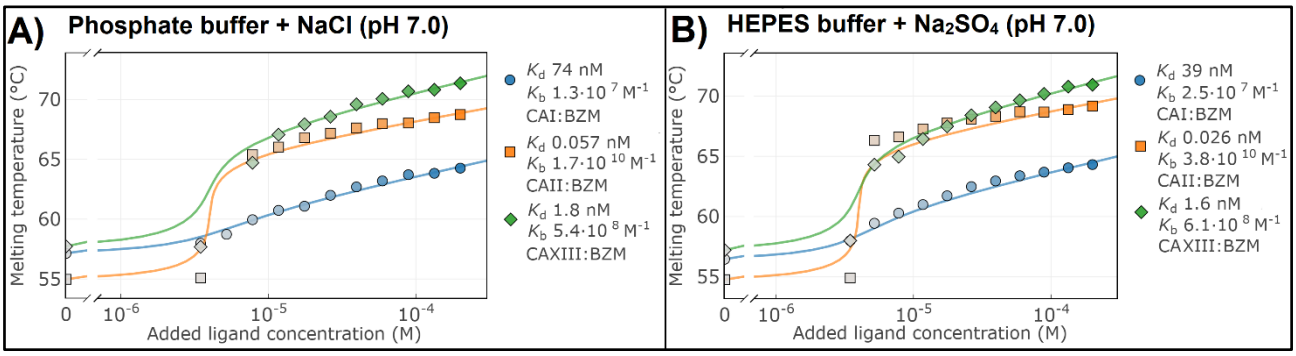

Figure 1. CAI, CAII and CAXIII binding affinity with brinzolamide (BZM) under two different conditions at the same pH (7.0): A) phosphate buffer with sodium chloride and B) HEPES buffer with sodium sulphate.

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DESIGN AND EVALUATION OF TARGET-SPECIFIC COVALENT INHIBITORS OF SARS-CoV-2 PAPAIN-LIKE PROTEASE

Martynas Bagdonas, Laimonas Stančaitis, Audrius Zakšauskas, Aurelija Mickevičiūtė, Asta Zubrienė

Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio 7, Vilnius, Lithuania
martynas.bagdonas@gmc.stud.vu.lt

According to the World Health Organization the outbreak of COVID-19 caused by the SARS-CoV-2 virus has resulted in over 7 million related deaths by March 2024. Continuous SARS-CoV-2 genome mutations have led to insufficient efficacy of vaccines and have not ensured versatile control of the situation. For this reason, combinations of vaccination and antiviral drugs could be the key to more effective treatment strategy. Papain-like protease (PL^{pro}) is a 316 amino acids length protease encoded by the SARS-CoV-2 genome. PL^{pro} is responsible for the proper virus polyprotein cleavage and the release of non-structural proteins (NSPs), which are essential for viral replication, transcription, and maturation. In addition, PL^{pro} plays a significant role in the suppression of host innate immune system by removing post-translational ubiquitination and ISG-ylation, which are central protein modifications of the antiviral immune response. Therefore, the PL^{pro} is identified as a promising target for antiviral drug development [1,2]. However, there are currently no potential PL^{pro} drug candidates in clinical trials.

In this study, a structure-guided ligand synthesis strategy was used to design peptidomimetic, site-specific covalent inhibitors. A series of compounds, bearing 2-oxyacetohidrazide as an analogous Gly-Gly amino acids fragment, which enables to penetrate to the active site of the enzyme, were synthesized. In addition, several electrophilic „warheads“, capable of reacting with the catalytic cysteine (Cys111), were incorporated into the compounds structures. The binding affinities of all compounds were evaluated using a FRET-based enzymatic assay. The covalent binding mode and direct interaction with catalytic Cys111 were confirmed by mass spectrometry and site-directed mutagenesis of full-length SARS-CoV-2 PL^{pro}. The most potent compound **AZ 23-6** showed nanomolar dissociation constant toward SARS-CoV-2 PL^{pro} (**Fig.1**) and a very weak inhibitory effect on structurally similar human ubiquitin-specific proteases (USPs).

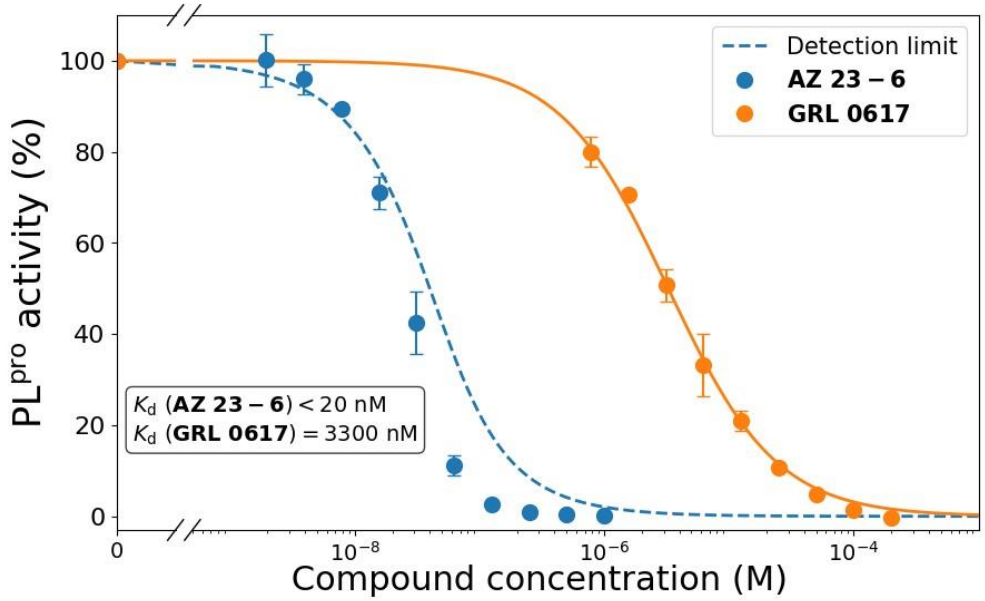

Figure 1. Dose-response curve of WT PL^{pro} inhibition with compound AZ 23-6 and commercial compound GRL 0617.

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ANTIMICROBIAL PHOTODYNAMIC INACTIVATION USING MAGNESIUM CHLOROPHYLLIN-CHITOSAN COMPLEX

Gabrielė Vasiliauskaitė [1], Irina Buchovec[2]

[1] Department of Neurobiology and Biophysics, Vilnius University, Lithuania
[2]Institute of Photonics and Nanotechnology, Vilnius University, Lithuania
gabriele.vasiliauskaite@gmc.stud.vu.lt

Food deficiency is a global concern additionally, up to 40% of the crops are lost due to plant pathogens [1]. In addition, the growing resistance of microorganisms to antibiotics and antimicrobial technologies is a noteworthy challenge. Nevertheless, antimicrobial photodynamic inactivation (API) shows promising outcomes in deactivating microorganisms [2]. API is based on using photosensitizer (PS), light, and molecular oxygen [3]. When PS absorbs a quantum of light, it initiates the generation of reactive oxygen species (ROS) through molecular interactions with oxygen present in the environment. Changing the PS charge can increase microbial inactivation due to ROS-induced oxidative stress. The natural PS magnesium chlorophyllin (Chl) is an anionic compound, which limits its binding or cell penetration. However, when PS is combined with the non-toxic cationic chitosan (CHS): the resulting Chl-chitosan (Chl-CHS) complex can more easily penetrate intracellular spaces, leading to more efficient inactivation of microorganisms [4]. The aim of this study was to evaluate the ability of the photoactivated Chl-CHS complex to inactivate the plant pathogen *Pseudomonas syringae*.

P. syringae is a plant pathogen affecting the leaves and stems of cucumbers, cantaloupe, watermelons, kiwifruit, and tomatoes. P. syringe cells in the exponential growth phase (optical density (OD_{600}) = 0,55) were used for the experiments. The antibacterial efficiency of photoactivated 0,001 % Chl-0,1 % CHS complex (2 pH) was tested in 0,9 % NaCl solution (Fig.1). In parallel, the effects of 0,1 % CHS (2 pH) without irradiation, photoactivated 0,001 % Chl (7 pH), and 402 nm light alone on P. syringe planktonic cell viability were evaluated (colony forming units (CFU)/mL). The samples were irradiated at 63 J/cm^2 (402 nm) after incubation for 30 min in the dark at 28 °C (170 rpm). In addition, the photostability of the cell-free Chl-CHS complex was determined by spectroscopic measurements after an irradiation dose of 63 J/cm^2.

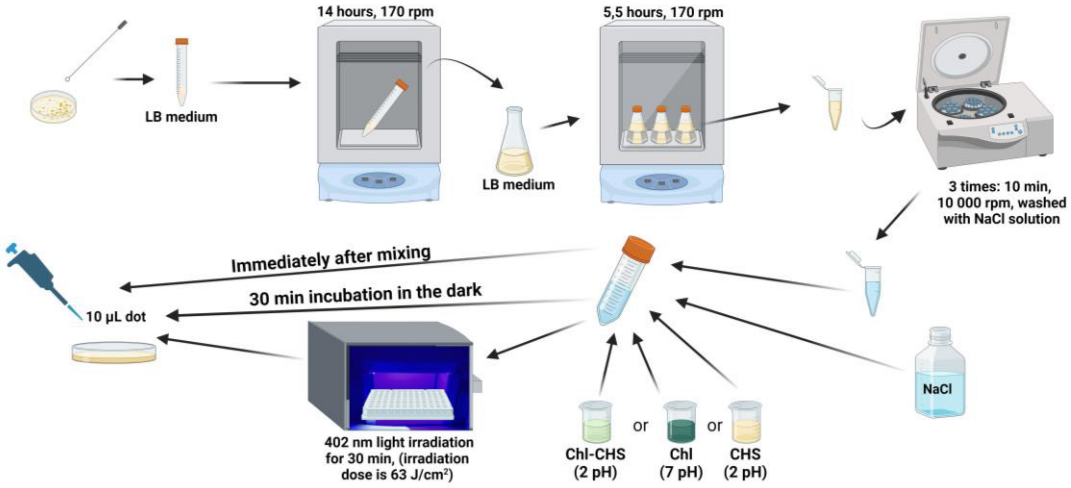

Figure 1. Experiment scheme.

This study showed that the 402 nm light photoactivated Chl-CHS complex is a suitable PS for API and can effectively control plant pathogens such as *P. syringae*.

This study aims to further evaluate the ability of Chl-CHS-based API to inactivate plant pathogens biofilms and investigate the generation of ROS species after this treatment application.

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APPLICATION OF COPPER CHLOROPHYLLIN-BASED PHOTODYNAMIC INACTIVATION AGAINST THE PLANT PATHOGEN PSEUDOMONAS SYRINGAE

Laura Kaziūnaitė[1], Irina Buchovec[2]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
[2]Institute of Photonics and Nanotechnology, Faculty of Physics, Vilnius University, Lithuania
laura.kaziunaite@gmc.stud.vu.lt

The spread of various plant pathogens in agriculture reduces product quality, threatens food security, and can lead to significant economic losses for farmers. One such plant pathogen is the Gram-negative bacterium *Pseudomonas syringae*, which causes diseases in a wide range of crops including tomatoes, kiwifruit, wheat, and many other plant species [1]. Pesticides are commonly used to control plant pathogens. However, intensive use of chemicals is known to cause adverse effects on the plant itself, nearby animals and humans. Therefore, other effective strategies to combat plant pathogens are increasingly needed.

Antimicrobial photodynamic inactivation (API) is an alternative technology that can help fight a wide range of microorganisms, including plant pathogens. API is based on the use of a photosensitizer (PS), which generates reactive oxygen species (ROS) when activated by light at a specific wavelength in an oxygen-saturated environment (Fig. 1) [2]. In order to develop an efficient API, it is necessary to select a suitable PS that remains stable under light irradiation. The aim of this study is to investigate the inactivation of the plant pathogen *P. syringae* by copper chlorophyllin (CuChl) induced API.

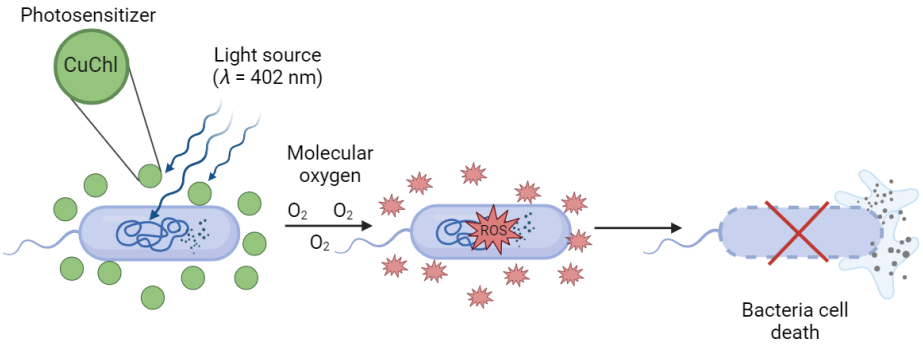

Figure 1. Antimicrobial photodynamic inactivation mechanism of action (CuChl - copper chlorophyllin, ROS - reactive oxygen species).

During this study, the CuChl photostability analyses were conducted. Two solutions of CuChl were prepared at concentrations of 0,015 mM and 0,0075 mM, respectively. These solutions were illuminated using a specialized LED light source. The selected parameters included an optimal PS excitation wavelength of 402 nm, irradiance of 35 mW/cm^2, and exposure times ranging from 1 min to 30 min (light doses from 2,1 J/cm^2 to 63 J/cm^2). In addition to spectroscopic measurements, the CuChl induced API was investigated. Two solutions were prepared: a control solution and a dark solution. The control solution consisted of PBS buffer and *P. syringae* bacterial cells, while the dark solution additionally contained a 0,015 mM or 0,0075 mM concentration of CuChl. Both solutions were exposed to the same specialized LED light source, under identical parameters as used in the photostability studies, but different exposure times were chosen: 15 min, 30 min and 45 min (light doses: 31,5 J/cm^2, 63 J/cm^2 and 94,5 J/cm^2). The CuChl induced API effect was determined using a bacterial plating technique by counting the number of culturable microorganisms present.

The measurements of the photostability study show that CuChl remains photostable even when applying the maximum light dose of 63 J/cm^2. The API results showed efficient inactivation of *P. syringae* bacteria using the 402 nm light and CuChl. However, bacteria was more susceptible to only using 402 nm light, than to using both CuChl and light.

The further prospect of this research is to perform CuChl induced API studies against the plant pathogen *P. syringae* using a larger concentration of PS.

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SPECTROSCOPIC STUDY OF PHOTOOXIDATIVE STRESS IN CHLOROPLASTS OF MACROALGAE

Rasa Miliukaitė[1,2], Vilmantas Pupkis[2], Agnė Kalnaitytė-Vengelienė[1]

[1]Biophotonics Group, Laser Research Center, Faculty of Physics, Vilnius University, Saulėtekio av. 9, bld. 3, LT – 10222, Vilnius, Lithuania
[2]Department of Neurobiology and Biophysics, Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania
rasa.miliukaite@ff.stud.vu.lt

Oxidative stress is a significant factor influencing various physiological processes in plants. It can arise from unfavorable lighting conditions such as UV exposure and excessive light, as well as nutrient deficiencies and exposure to various chemical compounds (e.g. hydrogen peroxide) [1]. While intact plant and algae cells benefit from protective mechanisms provided by the cell membrane and cytoplasm, which aid in regulating and adapting to external stressors, isolated chloroplasts lack these defenses and are more vulnerable to direct impacts from environmental stressors. Therefore, it is important to assess stressors-induced responses in organelles. Furthermore, there is a lack of comprehensive studies explaining the autotroph fluorescence and photosynthetic parameter changes induced by different types of oxidative stressors, which could prove invaluable in the development of biosystem-based sensors for environmental monitoring purposes.

The aim of this study is to spectroscopically determine changes of autofluorescence and photosynthetic parameters in isolated chloroplasts of macroalgae *Nitellopsis obtusa,* induced by white light, hydrogen peroxide and photosensitizer chlorophyllin. Furthermore, comparing responses to different types of stressors would allow to distinguish photooxidative stress from other types of effects. The study was conducted using steady state and kinetic (pulse-amplitude-modulated (PAM)) spectroscopy. Isolated chloroplasts were kept in buffer solution pH 7.5, in dark and refrigerated (4°C) environment and used for experiments within 24-hour timeframe. During experiments three doses of white light (exposure to 829 µmol photons/(m^2s) for 5, 10 and 20 min) and H_2O_2 (1, 2.5 and 5 mM) were chosen to determine dose-dependent changes in chloroplast responses. Chlorophyllin was chosen as photosensitizer, potentially causing oxidative stress, therefore samples were illuminated with violet light (LED max. em. at 404±9 nm) for more efficient excitation of the photosensitizer.

PAM results revealed that exposure to H_2O_2, chlorophyllin, and white light leads to a decrease in effective PSII quantum yield Y(II) during dark adaptation, with the impact of light being dose-dependent (Figure 1 A). Fluorescence excitation spectra (λ_{em}=683 nm) of samples affected with white light (Figure 1 B), as well as chlorophyllin revealed relative decrease of intensity in 460-500 nm spectral region, although no significant change was seen for H_2O_2 exposure. These results revealed sensitivity of isolated chloroplast system to variable conditions, therefore more statistical studies are needed to confirm the findings.

Acknowledgement. This research is funded by Research Council of Lithuania, agreement No. P-ST-23-111.

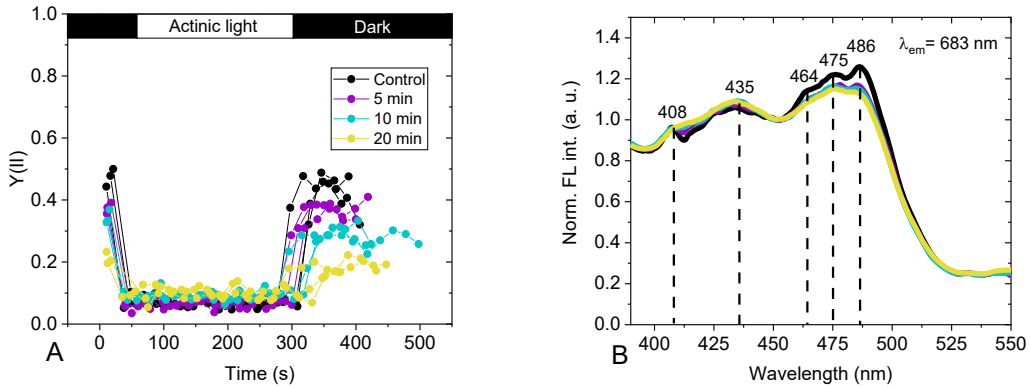

Figure 1. Dependency of (A) the effective quantum yield of PSII (Y(II)) and (B) autofluorescence excitation spectra of *Nitellopsis obtusa* isolated chloroplasts on different doses of white light. Fluorescence registered at 683 nm and normalised at around 455 nm.

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INVESTIGATION OF HERBICIDE EFFECT ON *NITELLOPSIS OBTUSA* AUTOFLUORESCENCE

Aušrinė Navickaitė[1], Vilmantas Pupkis[1], Saulius Bagdonas[2]

[1] Department of Neurobiology and Biophysics, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Laser Research Center, Faculty of Physics, Vilnius University, Vilnius, Lithuania
ausrine.navickaite@gmc.stud.vu.lt

In recent decades, environmental conditions have been changing at an accelerating rate, affecting the photosynthetic processes of plants and algae. Chlorophyll fluorescence (FL) measurements provide a non-invasive and effective method for monitoring a plant's physiological state - increased FL intensity indicates disruptions in photosynthesis [1]. Nevertheless, the response of plants to different environmental stressors can result in indistinguishable changes in the recorded FL signals. To enhance the diagnostic potential of FL measurements in practical applications, it is essential to determine and select appropriate FL parameters.

Herbicides such as diuron and paraquat, which inhibit photosynthetic electron transport through established mechanisms of action [2], are often utilized to validate FL-based techniques that aim to detect impaired photosynthetic performance. To investigate the effects of diuron and paraquat on the single cell level, we employed the internodal cells of the macroalgae *Nitellopsis obtusa*. Pairing an optical fiber system with a controlled stepper motor enabled us to record autofluorescence (aFL) spectra at 1 mm intervals along the cell. For excitation, a low-intensity (< 1 mW) LED light source emitting at 405 nm was used.

Fig. 1A shows the aFL spectrum of *N. obtusa*, which has two peaks: the main one at 680 nm and the second one at 740 nm. FL intensities measured at these wavelengths varied along the cell, correlating with changes in the FL intensity ratio between 680 nm and 750 nm (F680/F750). Diuron increased the peak intensity value at 680 nm (F680), while no significant changes were observed after paraquat treatment. Investigation of the relationship between F680 and the F680/F750 ratio showed a distinct separation of algal cells treated with either of the herbicides (Fig. 1B). These results confirm that diuron and paraquat interfere with photosynthetic performance, and that the combination of the selected FL parameters is sufficient to detect the effect of herbicides in *N. obtusa* cells. If future studies confirm that the proposed parameters adequately describe the influence of other external factors on photosynthetic processes, the applied method could be used to monitor the physiological state of plants.

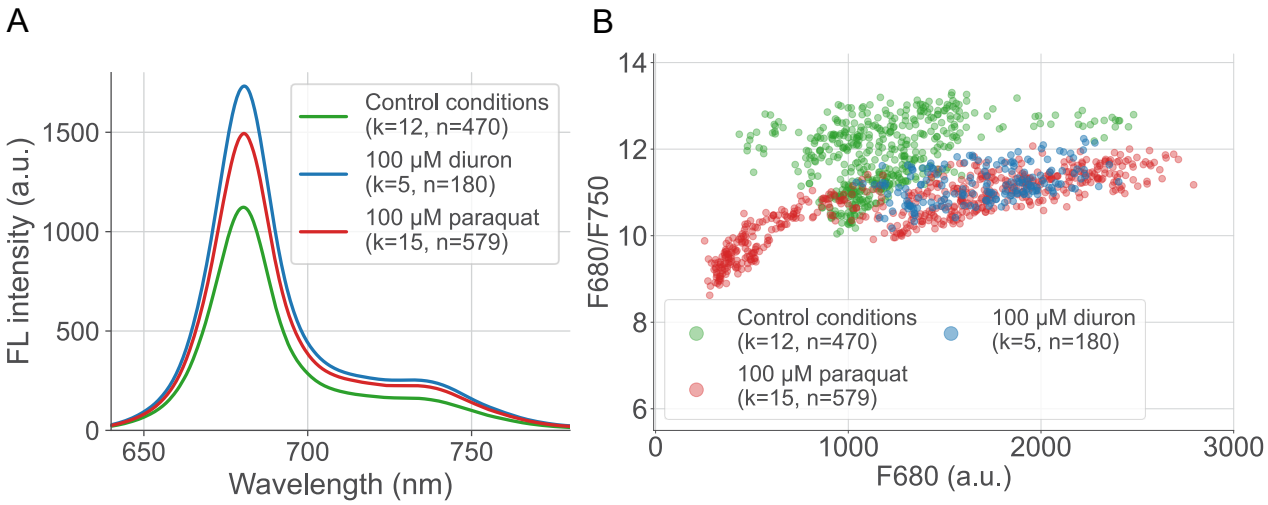

Figure 1. Herbicide effect on *N. obtusa* median aFL spectrum (A) and the relationship between F680 and the F680/F750 ratio in algae cells kept under different conditions (B); k – number of cells, n – number of recorded spectra.

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STEADY-STATE FLUORESCENCE QUANTUM YIELD OF A FLUCTUATING LIGHT-HARVESTING ANTENNA

Dominykas Borodinas[1], Jevgenij Chmeliov[1,2]

[1]Institute of Chemical Physics, Faculty of Physics, Vilnius University, Lithuania
[2]Department of Molecular Compound Physics, Centre for Physical Sciences and Technology, Vilnius, Lithuania
dominykas.borodinas@ff.stud.vu.lt

Over billions of years of evolution, various photosynthetic organisms have developed different photosynthetic apparatus. Despite their vast diversity, all these apparatus are designed in a very similar way: the so-called light-harvesting antenna is composed of pigment molecules usually bound to a protein scaffold. The mutual arrangement of these pigment-protein complexes, as well as their spectroscopic properties, ensures optimal absorption of the incoming photons and can lead to extremely efficient (up to ~99%) delivery of the generated electronic excitations to a reaction center [1]. Despite the ever-growing knowledge about the structural organization of these complexes and excitation energy transfer dynamics in photosystem II, specific molecular mechanisms responsible for such high efficiency of excitation energy transfer are still not fully understood. To explain fluorescence measurements, fluctuating light-harvesting antenna model was formulated, which takes into account the continuous spatial rearrangement of the pigment-protein complexes within the photosynthetic membrane [2].

This work aims to expand the previously suggested fluctuating light-harvesting antenna model, introducing constant excitation generation and dissipation parameters depicting constant illumination conditions and molecular relaxation. The time evolution of the excitation in such a system can be described by a diffusion equation

$$\frac{\partial}{\partial t}p(\boldsymbol{r},t|R) = D\nabla_{\mathrm{d}}^2 p(\boldsymbol{r},t|R) + G - k_{\mathrm{dis}}p(\boldsymbol{r},t|R),$$

with the initial condition $p(\boldsymbol{r},t=0|R) = \delta(\boldsymbol{r})$ and boundary condition given by $p(\boldsymbol{r},t|R)_{|r|=R} = 0$. Here $p(\boldsymbol{r},t|R)$ is the density of the survived excitation at the time moment t, parametrically depending on R, the distance to the reaction center; D is the diffusion constant; ∇_d^2 is the Laplacian in a d - dimensional system; d represents the effective dimensionality of the antenna during the transfer of excitation energy.

An average steady fluorescence quantum yield $\langle F\left(Dc^{2/d}\right)\rangle_x$ was obtained by taking into account the average concentration of excitation traps, c. Numerical solutions demonstrated that with the same number of excitation traps, the intensity of the steady-state fluorescence decreases faster in systems with higher dimensionality (Fig. 1).

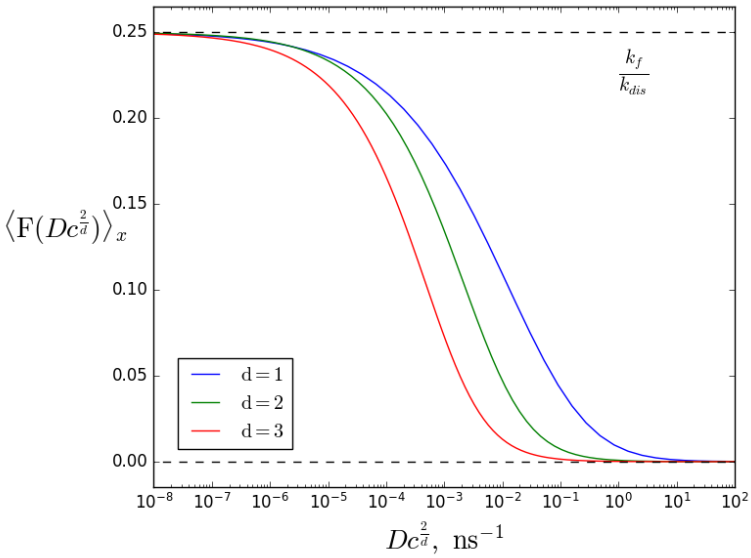

Figure 1. Steady-state fluorescence quantum yield in various dimensions.

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NON-LINEAR MICROSCOPY FOR CERVICAL CANCER DIAGNOSTICS

Elena Osteikaitė[1], Viktoras Mažeika[1], Mykolas Mačiulis[1], Martynas Riauka[1], Edvardas Žurauskas[3], Rūta Čiurlienė[4] and Virginijus Barzda[1,2]

[1]Laser Research Center, Vilnius University, Saulėtekio Ave 9 corp. III, LT-10222, Vilnius, Lithuania
[2]Department of Physics, University of Toronto, 60 St. George St, Toronto, M5S 1A7, Canada
[3]Department of Pathology, Forensic Medicine and Pharmacology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
[4]Oncogynecology Department, National Cancer Institute, Santariškių St. 1, Vilnius, Lithuania
elena.osteikaite@ff.stud.vu.lt

Non-linear microscopy is a relatively new tool in the field of microscopy for histopathology, that could potentially improve the process of cancer diagnostics and prognostics. A second harmonic generation (SHG) can be observed from the non-centrosymmetric collagen molecules present in the extracellular matrix. The signal can provide further insight into the orientation and the ultrastructure of the collagen fibers. Similarly, a third harmonic generation (THG) response is evoked simultaneously from the membranes and nanostructures present in the cells. Such signal allows to inspect the cell's nuclei, present in the entire tissue of the sample that is being measured [1]. The goal is to analyse the non-linear tissue response at the margin of the tumor, as well as to inspect the changes in the structure of the collagen, comparing the results of cancerous and normal tissue. The detected changes in the collagen fibers could be used in the betterment of clinical histopathology.

The method of the study includes the imaging of collagen using SHG microscopy, while illuminating the samples with different states of laser light polarization. The two methods in practice are the Double Stokes-Mueller Polarimetry (DSMP) and Polarization-in, polarization-out (PIPO) methods. The recorded signals were used to calculate and generate the distribution maps of the C ratio (orientation of collagen fibers out of the image plane [1]) and the R ratio (structural organization of fibers in the focal volume [2]). These maps can be compared with the recorded and visualized signals of the THG imaging response, as well as images of fluorescence imaging and bright field microscopy (Fig. 1).

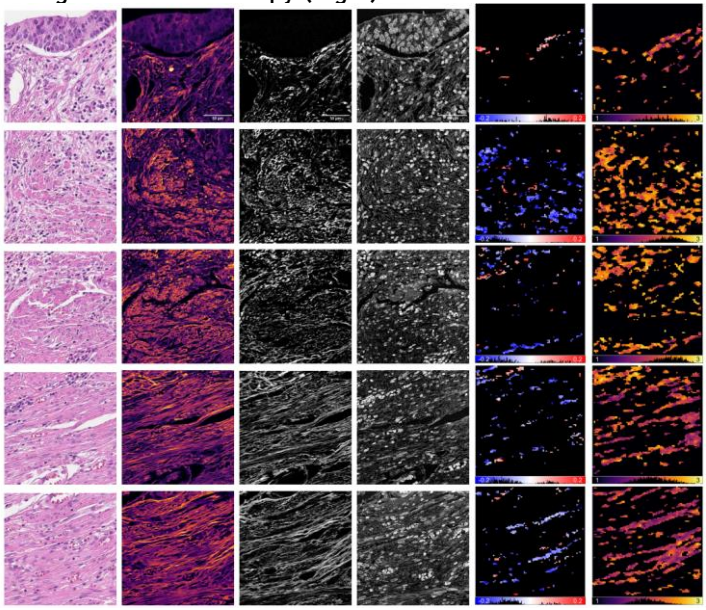

Figure 1. Non-linear microscopy results of the human cervix serous carcinoma. Right to left: bright view microscopy images, fluorescence, SHG and THG images, C and R ratio distribution maps (DSMP measurements). Top to bottom: images of the margin of the tumor, moving further away to the healthy tissue.

The results from the polarimetric measurements showed an increase of the R ratio when approaching the tumor margin. The THG images showed enlarged nuclei of the cancerous cells. The increased R ratio could signal a higher-level of disorder for collagen fibers. The non-linear multimodal microscopy provides valuable information about the structure of the collagen fibers and can potentially be integrated into the process of clinical H&E histopathology.

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Polyethylene Glycol Impact on Upconverting Nanoparticles And Chlorin E6 Complex For Enhanced Cancer Therapy

Emilė Pečiukaitytė[1,2], Simona Steponkienė[1], Eglė Ežerskytė[1,3], Vaidas Klimkevičius[1,3], Vitalijus Karabanovas[1,4]

[1]Biomedical Physics Laboratory, National Cancer Institute, Vilnius, Lithuania
[2]Life science center, Vilnius University, Vilnius, Lithuania
[3] Institute of Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Vilnius, Lithuania
[4] Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania
emile.peciukaityte@nvi.lt

In 2024, it is predicted that over 2 million new cancer cases will occur in the United States alone, with 1.2 million deaths projected in the European Union [1]. These predictions underscore cancer's enduring status as a leading cause of death worldwide, significantly affecting human well-being. In recent years, nanoparticles and nanomedicine have generally broadened perspectives and possibilities, becoming the basis for more precise and novel techniques, both for diagnostics and cancer therapy. Theranostics, which combines diagnostic and therapeutic procedures within a single system, promises expedited and more accurate cancer treatment alongside diagnosis. It is essential that the theranostic complex be biocompatible, and phospholipids (PL) are ideal candidates since PLs are the main cell membrane components. Additionally, polyethylene glycol (PEG) is known to extend the longevity of nanoplatforms in the bloodstream. [2]

This study aims to assess the impact of PEG amount in phospholipid (PLs) coating on $NaGdF_4:Yb^{3+}$, $Er^{3+}@NaGdF_4:Yb^{3+},Nd^{3+}$ upconverting nanoparticles (UCNPs), and Chlorin e6 (Ce6) complex formation and efficacy. Hydrogenated soybean phosphatidylcholine (HSPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine phospholipids conjugated with polyethylene glycol (DSPE-PEG) were used for the investigation. Firstly, UCNPs were transferred from organic solvent to water by surface modification. Each of 3 different PL coatings had various amounts of PEG. This transfer from the organic medium into an aqueous environment is essential prior to using UCNPs in biomodels. After that, complex formation between differently coated UCNPs and photosensitizer Ce6 was performed to select the best ratio of HSPC and PEG. Because of UCNPs' extraordinary emission properties, phospholipid-coated UCNPs function as diagnostic agents, while Ce6 is a photosensitizer with exceptional singlet oxygen generation capabilities, which serve in cancer therapy. The therapeutic effect was evaluated indirectly using a probe singlet oxygen sensor green and, on the MDA-MB-231 cancer cell line.

The collected results show that all three variations of surface modification do not compromise the spectroscopic properties or optical stability of UCNPs. All 3 UCNPs-Ce6 complexes had emission peak changes in the regions of Ce6 absorbance, which indicate energy transfer from UCNPs to Ce6. All 3 complexes resulted in singlet oxygen generation; however, HSPC:DSPE-PEG with a ratio of 2:1 exhibited the most effectiveness. Singlet oxygen generation validates that all three complexes were formed successfully. Further investigations on accumulation, toxicity, and viability on MDA-MB-231 cancerous cells using the most effective HSPC:DSPE-PEG 2:1 UCNPs-Ce6 complex were conducted to prove the clinical efficacy of theranostic complex. We anticipate that the PLs-modified UCNPs-Ce6 complex will emerge as a promising candidate for optical diagnostics and impactful cancer therapy. This research enhances our broader comprehension of PLs coatings' significance in improving nanoplatforms for cancer therapy.

This study was supported by the funds of Lithuania. Grant No. S-MIP-22-31 and Grant No. P-ST-23-224.

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DIRECT MEASUREMENT OF DNA MECHANICAL PROPERTIES

Laura Milaknytė, Algirdas Toleikis

DNA Motors Laboratory, Vilnius University, Life Sciences Center, Lithuania
laura.milaknyte@chgf.stud.vu.lt

Magnetic tweezers have emerged as invaluable tools for probing the mechanical properties and behaviors of biomolecules at the single-molecule level. By exerting controlled magnetic forces on paramagnetic beads tethered to molecules of interest, researchers can unravel the intricate dynamics of biological systems with unprecedented precision. The following sections include methodology and findings of investigating DNA mechanical properties using magnetic tweezers technology.

DNA mechanical properties were investigated using magnetic tweezers technique [1]. Magnetic beads attached to DNA substrate can be controlled using 2 cube-shaped magnets and all of the measuring is done from the view bellow attachment surface (fig. 1). This technique is significant in the realm of biophysics, as it provides insights into the mechanical properties, interactions, and behaviors of biomolecules with incredible precision. Moreover, their non-destructive nature and high spatial resolution make them invaluable tools for testing the intricate workings of biological systems. Thus, magnetic tweezers represent a valuable technique in modern biophysical research, facilitating the exploration of the intricate mechanisms at the molecular level.

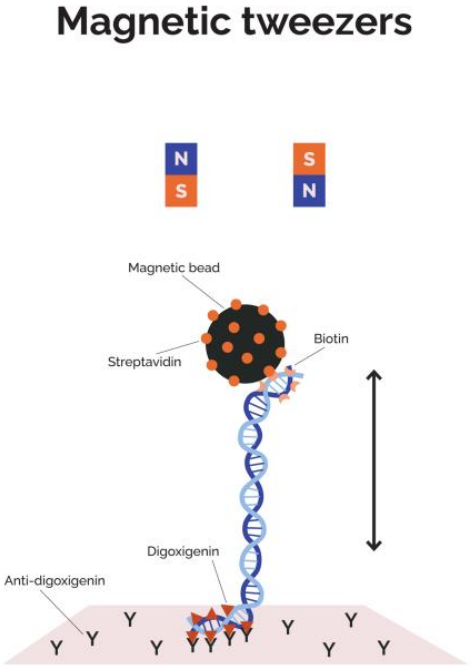

Figure 1. A scheme of magnetic tweezers experiment setup

Three different positions of magnets were used in the experiment and using the technique described in the paragraph above measurements were made. Force calculations were done using the equipartition theorem [2]. Then the DNA force-extension curve was plotted using the calculated force as well as z displacement and Worm-Like-Chain model [3] was applied to the data. The highest force generated was around 24 pN when the magnets were around 2 mm away from the sample. Later on this data was used to perform supercoiling experiments at different forces and to determine the optimal conditions for maximal superspiralization.

Accurate calculating of force applied to the DNA substrate at different magnet heights and optimal conditions for DNA superspiralization are extremely important for further experiments of interactions between DNA and DNA motor proteins. By knowing the exact conditions applied to the experiment it is easier to repeat it and also deduce a more precise mechanism of the protein action.

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RESEARCH OF THE ANNEXIN-A4 PROTEIN FUNCTION IN CELL PLASMA MEMBRANE RECOVERY AND CELL VIABILITY POST-ELECTROPORATION

Dominykas Makarovas[1], Baltramiejus Jakštys[2], Saulius Šatkauskas[3]

[1]Biochemistry Cathedral, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania
[2]Cell and Tissue Biotechnology Research Group, Research Institute of Natural and Technological Sciences, Vytautas Magnus University, Kaunas, Lithuania
dominykas.makarovas@stud.vdu.lt

When short, high-voltage electric pulses are applied, the cell plasma membrane becomes permeable. This process is known as electroporation (EP). It is still unclear how exactly electropermeabilization works.[1] The duration, quantity, and intensity of electric pulses are critical characteristics that impact the effectiveness of EP; high levels of any of these parameters may have a detrimental effect on cell viability.[2] Cell survival post-EP depends on the plasma membrane recovery, underscoring the function of the annexin family proteins, especially annexin A4, which is known to be involved in the processes of cell plasma membrane repair following activation by Ca^{2+} ions. Although there have been many publications on the influence of calcium on cell plasma membrane recovery after EP, but there is a lack of knowledge regarding the role of proteins in the restoration of cell plasma membrane post-EP. The purpose of this research was to investigate how Ca^{2+} ions affect the cell plasma membrane's ability to recover after EP. The response of MCF7 wild-type cells, which have an intact annexin A4 gene, was compared to MCF7-AnxA4-KO cells, which have defective annexin A4 gene expression.

The MTS assay was utilized to assess the vitality of the cells, and flow cytometry was employed to determine the dynamics of electropermeabilization and plasma membrane repair by quantifying the amount of propidium iodide permeable cells. Fluorescence microscopy was used to monitor ANX-A4 activity. A single 100 µs electric pulse was used to operate the electroporation at different intensities, using a 2 mM concentration of $CaCl_2$.

As a result, MCF7-WT cells are less susceptible to the negative effects of electroporation in comparison to MCF7-AnxA4$^-$ (KO) cells. Furthermore, although calcium had no observable effect on cell electropermeabilization, the data indicated that calcium had a negative effect on the survivability of both cell lines. On the other hand, 35 minutes after EP, the amount of permeable cells in both cell lines decreased, which signified cell plasma membrane restoring process that was more pronounced in MCF-WT cell line. Microscopy images indicate that cells electroporated in calcium demonstrate higher AnxA4 activity and faster translocation from cytosol to plasma membrane and nucleus proving that AnxA4 protein is involved in cell plasma membrane recovery after EP.

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April 17th

Genetics

FUNCTIONAL LINKAGE OF BACTERIAL BREX DEFENCE COMPONENTS IN THE CELL

Aistė Petrauskaitė[1], Tomas Šinkūnas[1]

[1]Department of Protein – DNA Interactions, Institute of Biotechnology, Life Sciences Center Vilnius University, Vilnius
aiste.petrauskaite@gmc.stud.vu.lt

Bacteriophages are the most abundant biological entity in the biosphere, and they are responsible for the destruction of 20-40 % of bacterial cells every day [1]. This evolutionary pressure drives the emergence of diverse bacterial defence systems, one of which is the BREX (Bacteriophage Exclusion). This system is present in about 10 % of known prokaryotic genomes, yet its detailed defence mechanism remains to be elucidated [2].

Our study focuses on the type I BREX (BREX1) system, which is encoded by a cluster of six genes: brxABCXZL. This system methylates host genomic DNA at specific sequences, thereby protecting it from autoimmunity. The non-methylated DNA of bacteriophages triggers BREX1, which blocks phage proliferation (Fig. 1). We have previously shown that deletion of certain BREX1 genes results in cytotoxicity [3]. This suggests that some BREX1 proteins are involved in the autoregulation of the immune response, while others may act as effectors that interfere with the vital process of the cell. Here, we are analysing different compositions of BREX proteins to find a link to their function in the cell.

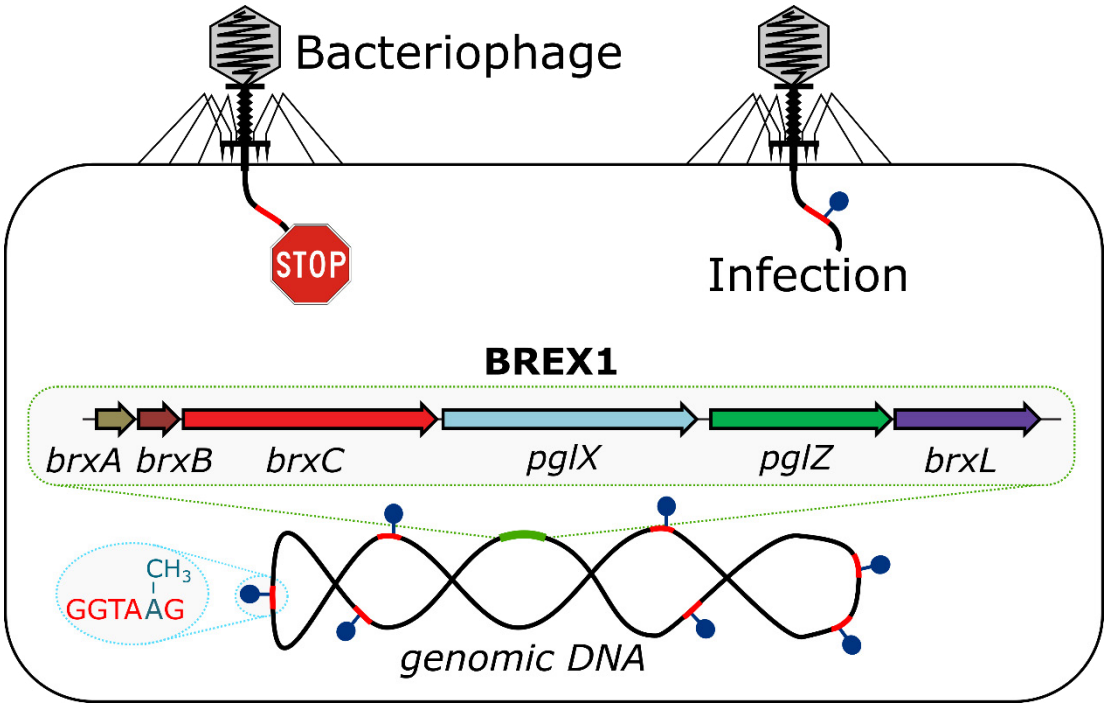

Figure 1. The BREX1 defence system protects bacteria from bacteriophage infection.

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ISOLATION OF T2 BACTERIOPHAGE MUTANTS EVADING BACTERIAL DEFENSE SYSTEMS

Toma Liegutė[1], Jonas Juozapaitis[1], Monika Jasnauskaitė[2], Patrick Pausch[2], Inga Songailienė[1]

[1] Vilnius University, Life Sciences Center, Department of Protein-DNA Interactions
[2] Vilnius University, Life Sciences Center, European Molecular Biology Laboratory Partnership Institute
toma.liegute@gmc.stud.vu.lt

Bacteria, the most diverse organisms on the planet, have been in an arms race against their main threat – bacteriophages for billions of years. Eons of co-evolution resulted in an abundance of bacterial defense systems that prokaryotes utilize to avoid viral infection and counter-attack mechanisms in bacteriophages. The bacterial viral defense arsenal includes restriction-modification, CRISPR-Cas, and abortive infection mechanisms which are effectively used to avoid bacteriophage takeover. Bacteriophages in response develop escape mutations that help evade bacterial defense but lead to a decrease in fitness. Bacterial defense systems, except for CRISPR-Cas, are mainly found in genomic clusters called bacterial defense islands [1]. Many new defense mechanisms have been discovered using bioinformatic tools to probe these bacterial defense islands. Our focus is on recently discovered prokaryotic antiviral systems that contain reverse transcriptases – retrons and DRTs (defense-associated RTs) [2].

Each bacterial defense system has a mechanism for sensing the infection. Potential bacterial defense triggers include bacteriophage capsid proteins, viral DNA or RNA and viral gene products. For example, the retron Ec48 defense system is triggered by Gam protein of phage λ. Frameshift mutations in λ *gam* gene abolished the antiviral effect of the bacterial defense system [3].

The main goal of this study is to isolate bacteriophage mutants that evade the bacterial defense system due to spontaneously occurring mutations and determine which genes are responsible for the detection by the antiviral mechanism. Bacteria co-transformed with reverse transcriptase were challenged with Enterobacteria phage T2 to test plating efficiency. The promising hits containing mutated bacteriophages were multiplied in selective pressure conditions to introduce spontaneous phage escape mutations and checked if they lyse *E. coli* cells containing the defense system. Promising bacteriophage isolates were sequenced, and results were compared to reveal a gene potentially responsible for the activation of the antiviral defense system.

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POPULATION STRUCTURE ANALYSIS OF SOSNOWSKY'S HOGWEED (*Heracleum sosnowskyi*) IN LITHUANIA: INSIGHTS FROM MICROSATELLITE (SSR) POLYMORPHISM ANALYSIS

Gelminė Vanseviciūtė[1], Jolanta Patamsytė[1], Donatas Žvingila[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekio Av. 7, 10257 Vilnius, Lithuania
gelmine.vanseviciute@gmc.stud.vu.lt

Sosnowsky's hogweed (*Heracleum sosnowskyi* Manden.) is an invasive species originating from the Caucasus region [1]. This monocarpic perennial herbaceous flowering plant, belonging to the carrot family Apiaceae, has spread widely throughout the Baltic region [2], Belarus [3], Russia [3], and Ukraine [4] primarily as a result of its introduction as a silage plant in the Soviet Union. The plant's juice can cause skin photosensitivity and burns, posing a danger to humans, although animals are less susceptible due to the protective barrier provided by their fur. In Lithuania, the species is very abundant and is primarily studied botanically and ecologically, with limited molecular research conducted thus far. Unlike its counterpart, *H. mantegazzianum*, which is the focus of molecular research in Europe, *H. sosnowskyi* has received little attention. Therefore, it is important to investigate this species through molecular perspective.

This study aims to investigate the genetic structure of seven Lithuanian populations (Sirutiškis, Girelė, Pakruojis, Šiauliai, Želva, Cirkliškis, Ignalina) using microsatellite (SSR) polymorphism analysis. Specifically, the objectives are: 1) to assess the polymorphism of 10 SSR loci in *H. sosnowskyi*, and 2) to examine genetic relationships within and among the populations.

The analysis involved a total of 56 samples, comprising 7 populations with 8 individuals each. SSR-PCR was initially conducted for 10 markers, followed by DNA fragment analysis by capillary electrophoresis (Nanodiagnostika). Bioinformatic analysis was performed using Peak Scanner Software (v1.0) (Applied Biosystems). Potential genotyping errors were assessed using Micro-Checker (v2.2.3) (Cock van Oosterhout et al., 2004) [5]. Subsequently, the data underwent analysis using the GeneAlEx Microsoft Excel add-on (Peakall and Smouse 2012) [6].

Analysis of molecular variance revealed that most of the genetic variation was attributed to within-population differences, while 17% of the variance was observed among populations. Statistically significant ($p = 0.001$) genetic differentiation was found among regions within Central Lithuania, including Sirutiškis and Girelė, as well as in the Northern region, covering Pakruojis and Šiauliai, the Eastern region comprising Cirkliškis and Ignalina, and the Želva area. The medium polymorphism level was estimated to be 80 %.

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G4

DNA POLYMORPHISM STUDY IN GOLDENRODS POPULATIONS OF DIFFERENT SPECIES COMPOSITION

Karolina Šablinskaitė[1], Jolanta Patamsytė[1], Julija Danilovienė[2]

[1]Institute of Biosciences, Vilnius University, Lithuania
[2]Nature Research Centre, Lithuania
karolina.sablinskaite@gmc.stud.vu.lt

Alien plant species are one of the most dynamic and rapidly changing components of flora. Studies about their immigration, naturalization and spread are essential to diminish potential negative consequences. Nowadays, large numbers of intentionally introduced plants, formerly being only in cultivation, are increasingly found escaped and occur in various human-made, disturbed or seminatural habitats [1]. *Solidago canadensis* (Canadian goldenrod) was introduced to Europe as ornamental plant in the 17th century. After a short time from the introduction, it escaped from cultivation and became naturalized in many European countries. In Lithuania, *S. canadensis* was mentioned in 1934 only as cultivated ornamental plant, while its occurrence in the wild was noted in 1954. Today, *S. canadensis* is listed as invasive species posing the greatest threat to native species and ecosystems in Europe [2], because of tremendous consequences for native biodiversity due to the direct effects of interspecific competition, disease, or herbivory. Also due to indirect effects of hybridization and introgression between alien and native species. The hybridization between alien and native plants should be considered as a risk to biological diversity [3]. *S. × niederederi* is a natural hybrid between the alien *S. canadensis* and the native *S. virgaurea* (European goldenrod). In recent years, hybrids have been increasingly found in several European countries. In Lithuania, *S. × niederederi* is quite frequent in mixed populations of *S. canadensis* and *S. virgaurea* [1].

In this study, we examined 5 populations of goldenrods in Lithuania. The genetic diversity was investigated by SSR (microsatellite) markers, which are a valuable tool, allowing for the estimation of kinship, the identification of invasive genotypes, and the estimation of gene flow among populations [4]. We compared the genetic similarity of the hybrid type and the parent species by using 6 different SSR oligonucleotide primers, which have been developed specifically for the genus *Solidago*.

Principal Coordinates analysis (PCoA) showed that the lowest polymorphism occurs in the local *S. virgaurea*. Another two species showed higher genetic diversity level and are genetically more similar to one another, regardless of the population. Hybrid and invasive species had unique (private) alleles while native type had none. For further analysis individuals were divided into 13 groups according to their species and population. Analysis of molecular variance (AMOVA) showed 13% difference between the species and 7% among populations. 3 out of 5 populations had unique alleles.

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G5

Genetic analysis of *tweaky spike(tw)* locus in independent allelic *tw* mutants

Ineta Karosaitė[1], Vėjūnė Pukenytė[1], Raimondas Šiukšta[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekis ave. 7, LT-10257 Vilnius, Lithuania

ineta.karosaite@gmc.stud.vu.lt

Barley (*Hordeum Vulgare*) is a widely cultivated cereal crop, ranking fourth in global production [1]. With the world's population expanding and environmental conditions deteriorating the need for more adaptable/fertile cereal crops is increasing sparking interest in barley mutants [2]. An example of such mutants would be chemically induced barley *tweaky spike* mutants, they exhibit notable morphological alterations in inflorescence and parts of the spike, accompanied by genetic instability, reversions, and changes in amino acid composition [3]. These features indicate that mutation at *tw* gene interferes with normal development of barley inflorescence and the genetic mapping and analysis of this locus will provide new insight into barley spike development.

This study aimed to explore the genetic basis of *tw* phenotype in barley by investigating mutations in 8 genes found in a non-recombinant region of F_2 (All x tw_2) generation of individuals with the *tw* phenotype (tw_2-$rF_{2)}$. Initially, PCR reactions were performed with the first six genes, visualized by electrophoresis, extracted from the agarose gel and sequenced using Senger technology. After reviewing gene sequences with comparative analysis, we determined that the six genes were not associated with *tw* mutations since the alterations did not recur in other *tw* allelic mutants or were not detected at all. However, the remaining two genes hold more promise one gene has a full gene deletion and the other a deletion of an exon in tw_2-rF_2. We used regular PCR procedures to amplify fragments of these two genes in various allelic *tw* mutants and two *WT* cultivars (All and A3) to determine if deletions are present. Our findings suggest that in *WT* barley these deletions are absent, but deletions are present in most of allelic *tw* mutants. In a nutshell, this study investigates potential candidate genes for the *tw* mutation, however further gene sequence analysis is required to determine the precise changes that occur.

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PHENOTYPIC CHARACTERIZATION OF BARLEY *TW* MUTANTS

Greta Kaupaitė[1], Vėjūnė Pukenytė[1], Vytautas Čepas[1], Virginija Vaitkūnienė[2], Raimondas Šiukšta[1]

[1]Institute of Biosciences, Life Sciences Centre, Vilnius University, Saulėtekis Ave. 7, LT-10257, Vilnius, Lithuania.
[2]Botanical Garden of Vilnius University, Kairėnai Str. 43, LT-10239, Vilnius, Lithuania
greta.kaupaite@gmc.stud.vu.lt

Over the last few decades, areas of arable lands had suffered from an unexpectedly rapid climate change, which limits the yield of edible crops, especially cereals, which are the main food and forage source all around the world. Common crop varieties cannot adapt sufficiently to extreme weather events and creation of new more productive, and stress-resistant varieties is crucial to satisfy the continuously increasing demand for crops [1,2]. Barley is the fourth most important cereal crop in the world, having one of the smallest genomes in the tribe *Triticeae*, making barley suitable for the identification of mutations compared to other evolutionary relatives, wheat and rye [3]. In conventional breeding, which is predominant in the European Union due to strict GMO regulatory policy, great attempts are paid to identify genes related to grain yield and/or adaptation [4]. Barley pleiotropic developmental *tweaky spike* (*tw*) mutants have an abnormal flower/inflorescence structure, possibly caused by disturbances in auxin physiology [5]. Considering these properties, *tw* mutants have no direct economic value, but some features of their pleiotropic complex are directly related to fertility and product quality, therefore, the exploration of *tw* mutation genetic determination and regulation may expand the range of targets for genetic manipulations.

This study aims to describe *tw* phenotype in more detail and for this purpose field studies were used, during which data were collected on morphometric parameter as well in response to exogenous phytohormones (auxin (IAA), cytokinin (CK) and gibberellin (GA)). The characteristics of five allelic *tw* mutants were observed: *tw*, tw_1, tw_2, tw_9, tw_{10}, two non-allelic *tw* mutants: *tweaky no.18* (*n18*) and *tweaky and missing kernel* (*tmk*) and two Wild Type (WT) cultivars: 'Auksiniai II' (AII) and 'Auksiniai 3' (A3).

Morphometric data analysis showed that the mutants have shorter spikes, less grains per spike, are taller than WT and are more prone to tillering, and non-allelic *tw* mutants (*n18* and *tmk*) differ from both WT and allelic *tw* mutants in terms of morphometric parameters. Exogenous phytohormones analysis showed that auxin in wild-type plants promotes the formation of crowns and cracks, while in *tw* mutants, a reduction in the frequency of crowns, lodicule transformations and changes in the number of flower organs was observed. After exposure to gibberellin, an increase in the frequency of changes in the number of flower organs was found, while cytokinin caused a decrease in the frequency of cracks and crowns in *tw* mutants. GA and CK did not significantly affect flower and inflorescence development in wild-type plants.

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G7

Evaluation of Genotoxic and Antigenotoxic Properties of Reishi (*Ganoderma lingzhi)* and Lion's Mane (*Hericium erinaceus*) Mushroom Extracts

Romina Ashrafi[1], Milda Babonaite[1], Juozas Rimantas Lazutka[1]

[1]Department of Botany and Genetics, Vilnius University, Lithuania
romina.ashrafi@gmc.stud.vu.lt

The medicinal mushrooms *Ganoderma lucidum* (Lingzhi or Reishi) and *Hericium erinaceus* (Lion's Mane) have been used for generations in Asia to promote health and are said to have numerous health advantages such as antioxidant, antimicrobial, and anticancer properties [1, 2, 3].

In this study, lymphocytes from five healthy donors were treated with Reishi and Lion's Mane mushroom extracts, manufactured by Lithuanian company "MISHKAY ", to evaluate their cytotoxic, genotoxic, and antigenotoxic effects. Cytotoxicity was evaluated using a dual acridine orange/ethidium bromide staining technique and no significant reduction in cell viability was observed. Genotoxicity and antigenotoxic effects were assessed by the alkaline comet assay. Although both mushroom extracts induced DNA damage, compared to the negative control, no statistically significant changes were observed. However, it was notable that treatments with 10, 20, and 40 µLl of Lion's Mane and 40, 50, and 80 µLl of Reishi mushroom extracts, resulted in less DNA damage compared to other tested concentrations. Therefore, these concentrations were used in the assessment of the antigenotoxic properties of mushroom extracts and two protocols were employed, pre-treatment and post-treatment. In the pre-treatment phase, cells were initially treated with the selected mushroom extracts and then subjected to a 20-minute exposure to H_2O_2 to evaluate the genoprotective properties of the extracts. Conversely, in the post-treatment phase, cells were first exposed to H_2O_2, following the 30-minute treatment with mushroom extracts to investigate its impact on the DNA repair system. While the pre-treatment with 80 µLl of Reishi extract decreased the DNA damage slightly, compared to the positive control, it had no effect. As though with Lion's Mane, 10 µLl concentration slightly decreased DNA damage compared to positive control, but 20 and 40 µLl concentrations induced more damage. Post-treatment interventions demonstrated an improvement in DNA damage repair. In the case of the Reishi mushroom extract, all tested concentrations reduced DNA damage, induced by h_2o_2. 80 µLl treatment being the most efficient. For the Lion's Mane mushroom extract 20, 10, and 40 µLl concentrations induced less DNA damage respectively.

Overall, our findings suggest that both Reishi and Lion's Mane mushroom extracts possess potential antigenotoxic properties, which could be attributed to their bioactive components. Further evaluation is required to test these mushroom extracts thoroughly and determine their effectiveness in repairing DNA damage conclusively.

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APPLICATION OF BIODOSIMETRY TO ESTIMATE THE DOSE OF ACUTE IONIZING RADIATION

Gabija Rožėnaitė[1], Emilija Gruškaitė[1], Aleksandra Samozvanova[1], Eglė Kubiliūtė[1], Juozas Lazutka[1], Jonas Venius[2]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
[2]Biomedical Physics Laboratory of National Cancer Institute, Vilnius, Lithuania
gabija.rozenaite@gmc.stud.vu.lt

The threat of radiation exposure from nuclear events is always plaguing public health. To combat it there is a growing need of medical counter measurements to manage the exposure to radiation. Radiation biodosimetry is used to approximately estimate the dose of acute ionizing radiation a specific person has been exposed to based on a relative biological effect. This way, biodosimetry diagnostics can be used to present patient-specific information of the radiation-induced damage or biological effect to the tissue, which consequentially can be used to provide the best medical treatment. [1]

The aim of this study is to construct the dose-response curves and to analyze if are accurate and correctly estimate the actual acute ionizing radiation doses the person has been exposed to.

Biodosimetry assays are used to correlate the approximate received ionizing radiation dose with radiation-induced tissue damage. To obtain estimated radiation values, dose-response curves are constructed firstly. In this study, they were created by analyzing the frequency of micronucleus (MN) formation in peripheral blood lymphocytes *in vitro* exposed to varying doses of acute ionizing radiation (Fig.1). The blood samples obtained from 3 volunteers (subject 1- male, aged 36; subjects 2 and 3- women, aged 23 27, respectively) were irradiated at doses 0.1-5 Gy (dose rate 0.3 Gy/min) using Varian TrueBeam 6MV linear accelerator. In total, 143209 cells were analyzed between 15 different ionizing radiation doses and 4 different, individual for the subjects 1-3 and combined, dose-response curves (Fig. 2) were created using Biodose Tools software [2]. To test the accuracy of the dose-response curves a blind test was conducted with 4 unknown ionizing radiation doses using blood samples of two additional subjects, women aged 21-66.

All 4 calibration curves were used to estimate each of the 4 unknow acute radiation exposure doses with the addition of the control dose between two subjects. In total 40 different ionizing radiation dose estimations were made using the whole-body exposure model. The dose estimation was deemed to be correct, if the actual dose fell within the estimated dose range 95% confidence interval. Out of the 4 dose-response curves, the first curve had the highest, 60% accuracy and the second dose-response curve had only 10% accuracy.

In conclusion, the preliminary results of our study reveal that not all dose-response curves based on MN analysis are able to correctly reconstruct the actual ionizing radiation dose, therefore, further analysis is required to improve the reliability of the dose estimation curves.

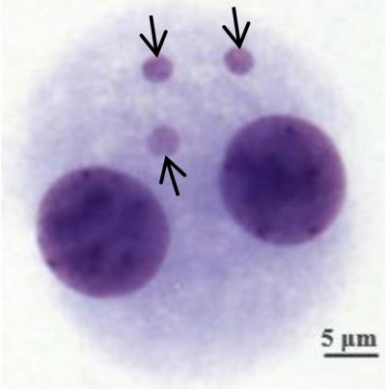

Fig. 1. Binucleated cell with three micronuclei

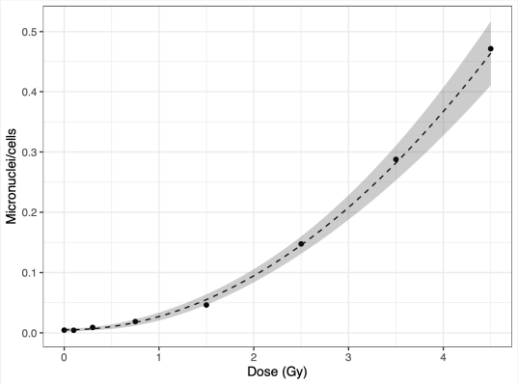

Fig. 2. Example of the dose-response curve

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G9

GENE EXPRESSION ANALYSIS IN 4-NITROQUINOLINE 1-OXIDE TREATED LEUKOCYTES OF PATIENTS WITH TYPE 1 DIABETES MELLITUS

Aiste Popandopula[1], Laura Siauliene[2], Zydrune Visockiene[2], Kristina Daniunaite[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Centre of Endocrinology, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania
aiste.popandopula@gmc.stud.vu.lt

Type 1 diabetes mellitus (T1DM) is a T-cell–mediated autoimmune disease that destroys pancreatic β-cells and causes insulin deficiency leading to hyperglycemia. Health and life-threatening complications are caused by chronic hyperglycemia which promotes the generation of reactive oxygen species (ROS) [1]. Oxidative stress (OS) caused by an excess of ROS and impaired antioxidant defense mechanisms leads to DNA damage and changes in gene expression [2]. A detailed understanding of the pathogenesis of T1DM and its association with OS would allow the identification of biomarkers that could be used for diagnostic or prognostic purposes.

This study aimed to evaluate the expression of 30 selected genes (*CAT*, *ERN1*, *HIF1A*, *NUDT1*, *OGG1*, *SOD2*, etc.) in leukocyte samples treated with 4-nitroquinoline 1-oxide (4NQO), imitating OS effects, and their respective controls. The samples were collected from 23 T1DM cases and 10 non-diabetic patients (NDP; 33 paired samples in total). The expression levels of the selected genes were quantified by means of real-time PCR using custom TaqMan assay plates.

H19, *HOTAIR*, *MT1E*, *NEAT1*, and *SOD2* baseline expression levels were higher in T1DM leukocytes compared to NDP (all $p < 0.050$), but no differences were observed in the expression of the other genes (all $p > 0.050$). After treatment with 4NQO, the expression of the OS response-related genes *ERN1* and *NUDT1* was lower in the T1DM group than in the NDP group ($p = 0.004$ and $p = 0.042$, respectively). In the latter group, 4NQO increased *ERN1* and *MT1E* expression ($p = 0.010$ and $p = 0.010$, respectively), however, no differences were observed in the T1DM group. The expression of DNA repair-associated genes (*FOXO3*, *HIF1A*, *MLH1*, etc.) did not differ between 4NQO-treated and untreated T1DM samples (all $p > 0.050$). However, 4NQO treatment increased *HIF1A* expression ($p = 0.049$) in NDP samples. Among the long noncoding RNA genes, treatment with 4NQO also resulted in increased *HOTAIR* expression in both T1DM and NDP groups ($p = 0.032$ and $p = 0.014$, respectively) as compared to the 4NQO-untreated samples, while increased *GAS5* and *NEAT1* expression was observed only in NDP.

In conclusion, our preliminary data revealed elevated expression levels of certain genes, with differential responses to OS induction. While the study sheds light on the gene expression changes in T1DM, larger-scale studies integrating clinical data are crucial to comprehensively understand the underlying molecular pathways and identify robust biomarkers.

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G10

NOTCH SIGNALING PATHWAY COMPONENTS AS BIOMARKERS FOR OVARIAN CANCER DIAGNOSIS

Paulina Kazlauskaitė[1,2], Ieva Vaicekauskaitė[1,2], Rūta Čiurlienė[1], Rasa Sabaliauskaitė[1,2]

[1]National Cancer Institute, LT-08406 Vilnius, Lithuania
[2]Institute of Biosciences, Life Sciences Center, Vilnius University, LT-10257 Vilnius, Lithuania
paulina.kazlauskaite@gmc.stud.vu.lt

Ovarian cancer is one of the most common gynecologic cancers, exhibiting the highest mortality rate. In 2020, 313 959 women worldwide were diagnosed with ovarian cancer, leading to 207 252 confirmed deaths [1]. Typically, epithelial ovarian cancer is diagnosed at an advanced stage, known as high-grade serous ovarian cancer (HGSOC). The elevated mortality is linked to the absence of specific symptoms for ovarian cancer and lack of well-established biomarkers [2]. Currently utilized biomarkers in the clinic, CA-125 and HE4, have limitations. For example, CA-125 is elevated in less than 50% of early-stage tumors, and HE4 testing is not recommended in routine practice due to contradictory studies [3-4]. Addressing the critical need for improved diagnostic tools, the NOTCH signaling pathway emerges as a potential biomarker, demonstrating a pivotal oncogenic role in HGSOC and contributing to the occurrence and development of ovarian cancer [5].

The aim of this research was to investigate gene expression changes of the NOTCH signaling pathway ligands *JAG2*, *DLL1* and signaling pathway target *HES1*, to evaluate these genes as potential biomarkers for the diagnosis of ovarian cancer.

This study included tissues from 66 patients suspected of ovarian cancer, including 42 HGSOC, 15 other gynecological cancers, and 9 benign gynecologic tumors. Genes expression changes were examined using RT-qPCR. Results were normalized to the reference gene *GAPDH*, and the $\log_2(2^{-\Delta CT})$ method was used to calculate genes relative expression.

We detected that all studied genes were downregulated in HGSOC when compared to benign gynecologic tumors tissues, with significant downregulation of *DLL1* and *HES1* genes. The combined ROC curve panel of all three genes for distinguishing the class with HGSOC risk from benign cases showed an AUC of 0.99, $p<0.0001$, sensitivity of 92.86%, and specificity of 100%. Furthermore, significant differences in relative expression of *DLL1* and *HES1* genes were found between HGSOC and other gynecological cancers groups. Finally, patients with higher CA-125 serum biomarker values showed statistically significant differences ($p=0.02$) in *HES1* gene relative expression compared to the normal CA–125 level.

In conclusion, our pilot study suggests that components of the NOTCH signaling pathway hold potential as biomarkers for ovarian cancer diagnosis. However, further comprehensive studies, including non-invasive samples, are essential to validate these genes as suitable biomarkers for the development of novel cancer screening assays.

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G11

METHYLATION PROFILING OF HOMEOTIC AND CHROMATIN REMODELING GENES IN CANCEROUS OVARIAN TISSUES

Rugilė Gineikaitė[1,2], Ieva Vaicekausakitė[1,2], Milda Urbonaitė[1,2], Rūta Čiurlienė[1], Rasa Sabaliauskaitė[1,2]

[1]National Cancer Institute, Vilnius, Lithuania
[2]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius Lithuania
rugile.gineikaite@gmc.stud.vu.lt

Ovarian cancer is the third most common gynecologic cancer in the world and has the highest mortality rate of all gynecologic cancers [1]. Due to asymptomatic progression, ovarian cancer is diagnosed in 4 out of 5 cases in advanced stages (III or IV), when cancer has spread and control of the disease is difficult [2]. Moreover, ovarian cancer is heterogenic disease, which complicates the diagnosis of the disease and the selection of the optimal treatment strategy. However, approximately 70% of ovarian cancer cases are highgrade serous carcinomas, which is the deadliest type of ovarian cancer [3]. Current diagnostic methods for ovarian cancer lack specificity and sensitivity, thus it is important to search for new modern diagnostic tools. In recent years, promoter hypermethylation of tumor suppressor genes has gained a lot of attention for its potential to be applied as cancer biomarker. Methylation studies of homeotic and chromatin remodeling genes attracted clinicians because they are important for the development of various organs, including ovaries and are critical for maintaining normal body functions and homeostasis. Therefore, dysfunctions of homeotic and chromatin remodeling genes are associated with various diseases, including development and progression of ovarian cancer.

This study aimed to evaluate promoter methylation profiles of homeotic (*ALX4*, *CDX2* and *HOPX*) and chromatin remodeling (*ARID1A*) genes that act as tumor suppressor genes in cancerous ovarian tissues as potential ovarian cancer biomarkers for more accurate and specific diagnostics. In total methylation profile was evaluated in 56 tissue biopsy samples using methylation-specific PCR (MSP).

The results demonstrated that methylation profile of *HOPX* gene significantly differ ($p = 0.017$) between benign gynecologic tumors and high-grade ovarian carcinoma patients, while methylation profiles of *ALX4* and *CDX2* genes showed tendency between the same two groups of patients. Furthermore, methylation profile of *CDX2* gene significantly differ ($p = 0.013$) between benign gynecologic tumors and other types of gynecologic tumor patients, whereas methylation profile of *HOPX* gene displayed tendency between the same groups. Methylation profile of *ARID1A* gene showed no significant differences between any of the groups.

Thus, homeotic genes have the potential to be used for early detection of ovarian cancer and various development of cancer diagnostics, improving the quality of patients' lives. However, more extensive analysis must be performed to validate the studied biomarkers.

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G12

THE PILOT STUDY OF GLOBAL CCFDNA METHYLATION LEVEL OF PANCREATIC CANCER PATIENTS

Vakarė Gruodytė[1], Romualdas Riauka[1], Aldona Jasukaitienė[1]

[1]Laboratory of surgical gastroenterology, Institute for Digestive Research, Lithuanian University of Health Sciences, Lithuania
vakare.gruodyte@stud.lsmu.lt

In 2020, 495,000 cases of pancreatic cancer (PDAC) were recorded worldwide, with 466,003 resulting in death [1]. These numbers are on the rise annually, indicating a rapidly growing problem. Pancreatic cancer is challenging to treat due to late-stage diagnosis and surgical complexity, prompting ongoing research into new treatment methods and risk reduction strategies. Early diagnosis methods are crucial, with emerging hypotheses suggesting a potential link between circulating cell-free DNA (ccfDNA) concentration and methylation level with PDAC stages [2].

The aim of our study is to elucidate the concurrent alterations in cell-free circulating DNA (ccfDNA) and methylation levels across successive stages in plasma samples obtained from individuals diagnosed with pancreatic cancer.

The study was approved by the Kaunas Regional Biomedical Research Ethics Committee. Blood samples were collected in 10 ml EDTA-containing purple-top tubes, and plasma was separated via ultracentrifugation before being frozen at -80°C. After thawing, cell-free circulating DNA (ccfDNA) was isolated using the "MinElute ccfDRA Kit". Methylation detection was performed on the ccfDNA samples using the procedures outlined in the "Global DNA Methylation Assay Kit" with ELISA. Differences between groups were analyzed using the Kruskal-Wallis test.

Our study involved individuals diagnosed with pancreatic cancer (n=59), pancreatitis (n=10), and a control group (n=9). We aimed to assess ccfDNA concentration. Patients with pancreatic cancer had significantly higher ($p>0.05$) ccfDNA levels than the control and pancreatitis groups. Specifically, the control group averaged 27.3 ng/ml, while pancreatitis averaged 42 ng/ml. Pancreatic cancer concentrations varied across stages: second stage (122.394 ng/ml), third stage (135.325 ng/ml), and fourth stage (129.8 ng/ml). These concentrations laid the groundwork for further investigation. Additionally, a global methylation analysis was conducted on ccfDNA samples from a subset of n=33 due to low ccfDNA concentrations. Methylation data were categorized based on cancer stage, metastasis, vascular invasion, degree of differentiation, extent of cancer spread, and cancer localization.

The highest methylation average among cancer stages was observed in the third stage at 0.468%, contrasting with a control average of 0.215%. The second stage had a level of 0.136%, and the fourth stage was 0.111%. However, differences between these groups were not significant ($p>0.05$). Noteworthy results were observed in cancer spread, with an average of 0.468% for systemic spread compared to 0.03% in the highest spread group ($p>0.05$). Additionally, there was a 4.7-fold difference in methylation levels between the first and second differentiation stages ($p=0.05$). Minimal variation was observed in cancer localization, with levels remaining comparable across the head, body, and tail.

This study concludes our pilot study. We can conclude that there were no significant changes between our study groups, but it remains interesting to continue our work and analyze this data in a higher sample of patients.

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IDENTIFICATION OF GENETIC BIOMARKERS IN CLEAR CELL RENAL CELL CARCINOMA

Milda Urbonaitė[1], Ieva Vaicekauskaitė[1,2], Rugilė Gineikaitė[1,2], Rasa Sabaliauskaitė[1,2]

[1]Institute of Biosciences, Life Science Center, Vilnius University, Vilnius, Lithuania
[2]National Cancer Institute, Vilnius, Lithuania
milda.urbonaite@gmc.stud.vu.lt

The most common histological subtype of kidney cancer is called clear cell renal cell carcinoma (ccRCC) [1]. In Lithuania, kidney cancer ranked 7th in terms of incidence and mortality in 2022 [2]. This type of cancer is often detected at late stages due to the absence of signs of disease at early stages. Even after detection, treatment is difficult because ccRCC is resistant to chemotherapy, and therefore surgical treatment is the most common treatment, which does not always remove the tumor completely and can lead to future recurrence of kidney cancer [1].

The WNT and MYC pathways are important in tumorigenesis because they regulate essential cellular processes that are often dysregulated in cancer, leading to uncontrolled cell growth, survival, and progression of tumors. WNT pathway key gene is *CTNNB1* and *C-MYC* is the main gene in MYC [3][4].

The absence of available non-invasive tumor markers for early clinical diagnosis is one of the primary causes of late cancer detection and high mortality. A liquid biopsy sample from ccRCC patients would be useful for the diagnosis of kidney cancer, allowing not only faster but also low-cost detection of malignant kidney cancer. Therefore, the main goal of this study is to investigate whether *C-MYC* and *CTNNB1* could be novel genetic biomarkers of ccRCC.

In this study we evaluated 29 blood plasma samples of malignant ccRCC patients and 11 blood plasma samples of non-malignant kidney tumor by using quantitative reverse transcription PCR (RT-qPCR) method. -deltaCt method was used for normalization with *GAPDH*.

Analysis of the results shows that the expression of the *C-MYC* and *CTNNB1* genes is different in ccRCC cases when compared to control samples. *CTNNB1* separates ccRCC cases from benign carcinoma with AUC 0.69, while *C-MYC* AUC was 0.68. Also, gene expression showed correlations with other clinical-pathological features.

Changes in *C-MYC* and *CTNNB1* gene expressions detected in blood plasma samples may be useful for the early detection of ccRCC. Additional analysis is required to confirm the *CTNNB1* and *C-MYC* gene expressions as putative non-invasive kidney cancer biomarkers.

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EXPLORING THE LINK BETWEEN *STAT4* RS7574865 AND LARYNGEAL SQUAMOUS CELL CARCINOMA

Enrika Pileckaite[1], Alvita Vilkeviciute[1], Vykintas Liutkevicius[2], Rasa Liutkeviciene[1]

[1]Neuroscience Institute, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Department of Otorhinolaryngology, Lithuanian University of Health Sciences, Kaunas, Lithuania
enrika.pileckaite@lsmu.lt

Laryngeal squamous cell carcinoma (LSCC) originates from the squamous epithelial cells of the mucosa and develops in several stages. The exact pathogenesis of LSCC is still unclear, although the onset of the disease is characterized by complex genetic, architectural, and cytological changes in the squamous epithelium [1]. Throughout the tumorigenesis process, cells lose their normal ability to repair DNA damage and regulate the cell cycle [2]. Although the Signal Transducer and Activator of Transcription 4 (*STAT4*) is not directly involved in the regulation of the cell cycle checkpoint or DNA repair, it plays a role in the activation of various immune cells and the production of inflammatory cytokines. In addition, the *STAT4* is associated with growth factors, apoptosis, and angiogenesis, suggesting a possible contribution to tumorigenesis [3]. In this study, we investigated how *STAT4* rs7574865 is involved in the development of LSCC.

This research aims to identify the SNP of STAT4 associated with LSCC patients and healthy volunteers.

A total of 312 patients diagnosed with LSCC and 320 individuals with no known health problems participated in the study. DNA extraction from venous blood samples from both LSCC patients and healthy individuals was performed using the salting-out method. Genotyping of *STAT4* rs7574865 was performed using real-time polymerase chain reaction (RT-PCR). The subsequent statistical analysis of the data was carried out using the" software program "IBM SPSS Statistics 29.0.1.0".

The distribution of GG, GT and TT genotypes of *STAT4* rs7574865 is statistically significantly different in LSCC patients compared to the control group (p=0.012). In addition, the T allele of rs7574865 was found to be statistically significantly more common in patients with LSCC than in the control group (p=0.003). To evaluate the influence of rs7574865 on the manifestation of LSCC, a binomial logistic regression analysis was performed. The analysis revealed that the TT genotype of *STAT4* rs7574865 is associated with 2.5-fold increased odds of developing LSCC according to the codominant model (p=0.007), and 2.2-fold increased odds of LSSC occurrence according to the recessive model (p=0.016). *STAT4* rs7574865 TT and GT genotypes are associated with 1.5-fold increased odds of developing LSCC according to the dominant model (p=0.018). Each T allele of *STAT4* rs7574865 increases the odds of developing LSCC by 1.5-fold according to the additive model (p=0.004).

Results of the present study showed that *STAT4* rs7574865 TT genotype is associated with LSCC occurrence.

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MIR-155 AND MIR-137 MODULATE EXPRESSION OF HISTONE METHYLATION REGULATORY GENES IN PROSTATE CANCER CELLS

Ruta Maleckaite[1], Marta Tamosiunaite[1], Kristina Daniunaite[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania.
ruta.maleckaite@gmail.com

Prostate cancer (PCa) together with other malignancies accounts for 54.2 % of all new cancer cases and 50.2 % of cancer deaths in the European Union in 2022 [1]. In recent years, inhibitors of histone methylation (HM) regulatory genes, for example, KDM1A, brought attention as a possible target for PCa treatment [2]. While HM genes themselves are epigenetic regulators, they can undergo regulation by various molecular mechanisms in both normal and cancerous cells. Understanding regulatory interplay between HM genes and their regulatory microRNAs (miRNAs) may illuminate new approaches for treating PCa and other malignancies with emerging epigenetic drugs.

Our previous study showed that miR-155 and miR-137 are downregulated in clinical PCa samples, accompanied by higher *KDM1A* and *KDM5B* expression levels, which correlate with methylated miR-155 and miR-137 host genes promoter status, altogether indicating a potential regulatory mechanism [3]. Significant associations between miR-137 and several HM genes (*KDM1A*, *KDM5B*, etc.) have already been established in preclinical PCa models (like PC-3 cell line) [4], while data regarding the association of miR-155 with HM genes is scarce [5]. In the present study, we selected nine HM genes and constructed a hypothetical miRNAHM gene regulatory network based on data obtained using in silico approaches. Following an assessment of baseline miRNA and HM gene expression levels, as well as DNA methylation status of miRNA host gene promoters, we conducted gain-of-function / loss-of-function experiments in PC-3 and LNCaP cell lines using miRNA-specific mimic and inhibitor assays, respectively. Gene and miRNA expression was quantified by means of real-time PCR. Cell biology assays were performed for validated interactions.

The baseline expression of *KDM1A* was notably higher compared to other HM genes in both PC-3 and LNCaP cells lines, while *KDM5D* showed no expression in PC-3 cells. The low to absent expression of miR-155 was linked to the methylated status of its host gene promoter, whereas the low levels of miR-137 could not be accounted for by an unmethylated promoter. Next, transfection conditions (i.e. duration, assay concentration) were optimized before proceeding with functional analyses. In PC-3 cells, the mimic of miR-137 downregulated *KDM1A* expression, whereas decrease in *KDM5B* levels did not reach statistical significance ($P = 0.0027$ and $P = 0.0980$, respectively). Unexpectedly, the mimic led to increased expression of *KDM3A* and *KDM5A* ($P = 0.0170$ and $P = 0.0280$, respectively). Furthermore, the transfection with miR-155 mimic resulted in decreased KDM5B expression ($P = 0.0478$), while downregulation of its other direct targets (i.e. *KDM1A*, *KDM3A*, and *KDM5A*), was not significant (all $P > 0.0500$). Preliminary data showed that transfection with miR-155 decreased cell viability by 36% compared with the cells transfected with scrambled control. Consistent results of transfection experiments were observed in preliminary data from LNCaP cells, suggesting reproducibility across different cellular contexts; however, ongoing experiments are under way to thoroughly validate these findings.

To conclude, our study demonstrates the novel finding that miR-155 regulates *KDM5B* expression in PCa cells, impacting cell viability. Also, we confirmed miR-137 as a negative regulator of *KDM1A*. Further experiments should follow to evaluate these regulatory connections in other cell lines, thereby providing deeper insights into the broader relevance of these miRNA-HM gene interactions in PCa progression.

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G16

DIFFERENTIAL EXPRESSION OF COPPER-DEPENDENT CELL DEATH ASSOCIATED GENES IN PROSTATE TUMORS

Dalia Vektaryte[1], Kristina Daniunaite[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
dalia.vektaryte@gmc.stud.vu.lt

Prostate cancer (PCa) is the second most prevalent cancer among men worldwide [1]. Its incidence is higher in Western countries and tends to increase due to advanced age and hereditary factors. Despite improvements in longevity for men diagnosed with PCa, a substantial 40% of survivors endure a marked decline in their quality of life [2]. Early diagnosis, coupled with the ability to identify specific disease subtypes at its early stage, could result in more cost-effective, targeted treatment strategies, potentially alleviating the burden associated with PCa.

Recent studies indicate that copper ionophores have an inhibiting effect on tumor growth. Cuproptosis is a recently described form of programmed cell death that is caused by accumulation of intracellular copper and its altered homeostasis [3]. Therefore, this study aimed to investigate the role of cuproptosis in PCa by identifying potential tumor subtypes according to the expression patterns of cuproptosis and copper homeostasis-related (CCH) genes.

The PCa cohort (PRAD; N = 464) from The Cancer Genome Atlas (TCGA) database was used for the analysis [4]. CCH genes (54 in total) were identified based on literature review. Differential expression was observed in 37 genes (*ATP7B*, *CDKN2A*, *DLAT*, *SLC31A1*, etc.) between PCa and noncancerous tissue samples. After conducting clustering analysis on tumor samples from PCa patients, the samples were classified into three distinct subtypes based on CCH gene expression profile (Fig 1). No correlations were observed among PCa samples based on clinical-pathological indicators such as tumor stage pT and differentiation grade (ISUP group). Further exploration of these PCa clusters is warranted. This entails ongoing clustering analysis to identify a minimal set of CCH genes for characterization of PCa subgroups. Furthermore, CCH genes will be evaluated as potential prognostic markers of PCa.

In conclusion, our study of cuproptosis in PCa, demonstrated by clustering analysis of CCH gene expression profiles, paves the way for further research on targeted therapies for PCa subgroups.

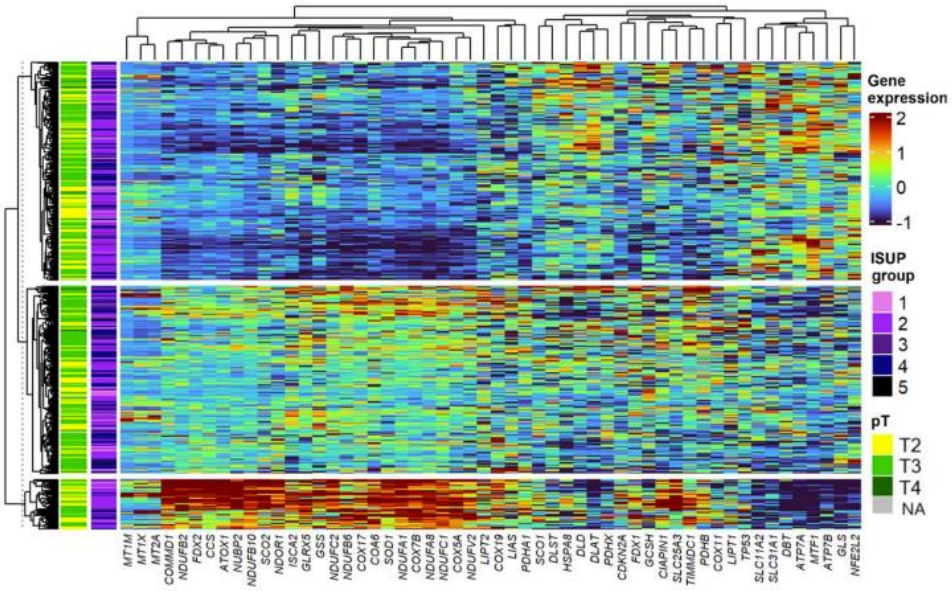

Figure 1. Prostate cancer (PCa) subtypes based on cuproptosis-associated gene expression profile. ISUP - PCa tissue differentiation groups defined by the International Society of Urological Pathology; pT - tumor stage according to TNM classification, NA - not available.

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G17

ASSOCIATIONS BETWEEN *TERF2* RS251796 AND LEUKOCYTE TELOMERE LENGTH IN MALES WITH OPTIC NEURITIS

Monika Duseikaite[1,2], Greta Gedvilaite[1], Dalia Zaliuniene[1], Loresa Kriauciuniene[1], Rasa Liutkeviciene[1]

[1] Laboratory of Ophthalmology, Institute of Neuroscience, Lithuanian University of Health Sciences, Lithuania
[2]Faculty of Pharmacy, Lithuanian University of Health Sciences, Lithuania
monika.duseikaite@lsmu.lt

Background. Optic neuritis (ON) is the most common cause of subacute optic neuropathy in young adults [1]. It is thought to be an immune-mediated disease and research indicates a link between telomeres and inflammation, as studies demonstrate that inflammation can lead to increased telomere shortening [2]. Telomere-related genes and telomere dysfunction in diseases can lead to persistent chronic low-grade inflammation [3]. Telomeric repeat binding factor 2 (TERF2) functions as an inhibitor of telomerase and thus plays a role in the regulation of telomere length [4].

Aim. The present study aimed to determine the associations of telomere-related *TERF2* rs251796 polymorphism and relative leukocyte telomere length (LTL) with the occurrence of ON in male subjects.

Methods. Men diagnosed with optic neuritis (ON) were analyzed, while the control group comprised men without any health problems. The DNA samples were obtained from peripheral blood leukocytes and purified using the DNA salting-out technique. Real-time polymerase chain reaction (RT-PCR) assessed single nucleotide polymorphism (SNP) and relative leukocyte telomere lengths (LTL). The data obtained were processed and analyzed using the "IBM SPSS Statistics 29.0" program.

Results. *TERF2* rs251796 (AA, AG, and TT) statistically significantly differed between the long and short telomeres group, with frequencies of 65.7%, 22.9%, and 2.0% in long telomeres, compared to 35.1%, 56.8%, and 8.1% in the short telomeres group (p=0.013). *TERF2* rs251796 CT genotype, compared to CC, under the codominant genetic model, was associated with a 4.7-fold decreased odds of telomere shortening (p=0.005). Meanwhile, CT+TT genotypes, compared to CC under the dominant genetic model, were associated with a 3.5-fold decreased odds of telomere shortening (p=0.011). Also, CT genotype, compared to CC+TT, under the overdominant genetic model, is associated with a 4.4-fold decreased odds of telomere shortening (p=0.004).

Conclusions. The current findings suggest a protective role of *TERF2* rs251796 in the occurrence of ON in men.

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G18

URINARY MIRNA-BASED BIOMARKERS FOR THE DETECTION OF RENAL CELL CARCINOMA

Laura Juozepavičiūtė[1*], Emilija Stepukaitė[1], Algirdas Žalimas[2], Kristina Šnipaitienė[1,2], Raimonda Kubiliūtė[1,2]

[1]Life Sciences Center, Vilnius University, Vilnius, Lithuania
National Cancer Institute, Vilnius, Lithuania
*laura.juozepaviciute@gmc.stud.vu.lt

The most distinctive hallmark of Renal Cell Carcinoma (RCC) is the highest mortality rate amongst all genitourinary cancers. Timely diagnosis is currently difficult to tackle due to the asymptomatic onset of cancer and should be the key element to focus on. Currently, apart from conventional imaging methods and invasive biopsies, there are no dependable biomarkers to aid these clinical needs. The potential solution to this problem might be non-invasive liquid biopsies such as urine samples containing tumor-derived epigenetic markers, more specifically, microRNAs (miRNAs). These molecules are short, non-coding nucleotide sequences that participate in the regulation of many signaling pathways, involving cell behavior in stressful environments along with many other processes that handle proliferation and differentiation. Recent research indicates that miRNAs are strongly linked to tumor formation in Renal Cell Carcinoma [1]. The purpose of this study is to determine the differences in the level of four miRNAs, particularly miR-148a, miR-365, miR-375, and miR-429 in the urine of patients diagnosed with Renal cell carcinoma compared to asymptomatic controls.

In total, 85 urine samples collected from RCC-having patients, alongside 85 urine samples sourced from asymptomatic individuals were investigated. Firstly RNA from the urine samples was isolated by using miRNeasy Mini Kit (Qiagen) followed by cDNA synthesis with TaqMan™ MicroRNA Reverse Transcription Kit (Applied Biosystems). Reverse Transcription Quantitative PCR (RT-qPCR) was used to evaluate the relative amount of the particular miRNA in each sample.

The analysis revealed a significant increment in the urinary miR-429 level among patients having RCC in comparison to asymptomatic individuals ($FC = 26.3$, $P < 0.001$). The same tendency was determined with miR-148a ($FC = 9.07$, $P < 0.001$), miR-365 ($FC = 7.09$, $P = 0.002$), and miR-375 ($FC = 3.53$, $P = 0.035$).

The current study suggests that alterations in miR-148a, miR-365, miR-375, and miR-429 levels in urine could be an indicator of tumor presence, providing a potential biomarker for diagnosis and prognosis in Renal Cell Carcinoma.

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G19

ANALYSIS OF *IN VITRO* CYTOTOXICITY AND GENOTOXICITY OF POLYSTYRENE NANOPARTICLES IN HUMAN HEPATOMA CELL LINE HEPG2

Emilija Striogaitė[1], Milda Babonaitė[1], Juozas Rimantas Lazutka[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
emilija.striogaite@gmc.stud.vu.lt

Due to the widespread use of plastics, there is a rising serious ecological problem because they remain in the environment for hundreds of years. Plastics may be degraded into microparticles and further into nanoparticles (NPs). The most common NPs in the environment are polystyrene nanoparticles (PS-NPs). They are used in a vast variety of applications: as thickening agents in cosmetics, food packaging, bioimaging and electronics [1], so it is significant to do genotoxicity studies to avoid the potential damage that can be caused by these nanoparticles.

In this study, we evaluated the cytotoxicity and genotoxicity of PS-NPs in the human hepatoma cell line HepG2 *in vitro*.

In this experiment, HepG2 cells were tested at different concentrations of polystyrene nanoparticles (5-100 µg/ml). To investigate the potential toxic effects of these nanoparticles, firstly, they were characterized, and then the cellular uptake and levels of reactive oxygen species were assessed by performing flow cytometry. The cytotoxicity was evaluated using the AlamarBlue assay [2]. The potential single-stranded and double-stranded DNA breaks by the exposure of PS-NPs were tested by alkaline comet assay and potential induced oxidative damage by enzyme-modified comet assay. The comet is created when damaged DNA with a negative charge moves from the nucleus towards a positively charged anode, meanwhile intact DNA remains in the nucleoid [3]. To observe the results of these experiments, we used ethidium bromide and examined the samples under a fluorescence microscope.

The results from flow cytometry analysis of HepG2 cells that were exposed to PS-NPs indicated that there was an efficient uptake of the nanoparticles into the cells. Additionally, it was observed that an increase in the concentration of PS-NPs resulted in a corresponding rise in the levels of reactive oxygen species. Tested concentrations were not cytotoxic in HepG2 cells, but an increase in DNA damage was found to be statistically significant at most concentrations. The enzyme-modified comet assay results also revealed that the concentrations of PS-NPs studied induced oxidative damage in the cells.

These findings indicate that polystyrene nanoparticles can be absorbed effectively and have genotoxic potential, raising concerns about plastic safety.

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EXPLORING THE ROLE OF OXYTOCIN RECEPTOR GENE POLYMORPHISMS (rs53576, rs13316193) IN ALCOHOL USE DISORDERS: INSIGHTS FROM A LITHUANIAN POPULATION STUDY

Kornelija Bekėžaitė[1], Miglė Kaminskaitė[1]

[1] Laboratory of Behavioral Medicine, Neuroscience institute, Lithuanian University of Health Sciences, Kaunas, Lithuania
kornelija.bekezaite@mf.stud.vu.lt

Harmful alcohol consumption is a significant global issue, contributing to over 3 million deaths annually and impacting health, society, and economies worldwide. In 2019, while the European region averaged 7.8 liters of alcohol per person per year, Lithuania stood out with a notably higher consumption rate of 12.78 liters per person [1].

Despite ongoing efforts, the mechanisms underlying alcohol addiction remain incompletely understood. Addiction is recognized as a complex disorder influenced by a multitude of external and internal factors, and understanding the individual contributions and interactions of these factors is an ongoing focus of research [2]. Endophenotypes such as impulsivity, recurrent in substance use disorders, warrant thorough examination [3]. Additionally, alterations in the mesocorticolimbic system, which involves neurotransmitters like dopamine, serotonin, and oxytocin, play a crucial role in understanding addiction's biological basis [3]. Investigating changes in these neurotransmitter receptors, particularly on a genetic level, is essential for assessing susceptibility to alcohol use disorders (AUDs).

Furthermore, considering the absence of a universally effective treatment approach for addictions, a detailed understanding of the mechanisms and changes occurring in individuals with substance use disorders is vital. This knowledge, combined with an appreciation of individualized patient characteristics, facilitates the development of tailored treatment or prevention strategies to meet the specific needs of each individual.

Oxytocin, a neuropeptide involved in stress regulation and the reward system, has emerged as a potential target for addiction treatment [4]. This study aimed to investigate the association between single nucleotide polymorphisms (SNPs) of the oxytocin receptor gene (*OXTR*), specifically rs53576 and rs13316193, and AUDs in the Lithuanian population.

Biological specimens were obtained from a cohort of 333 individuals, including volunteers and patients undergoing addiction treatment. Genomic DNA extracted from saliva samples underwent genotyping using real-time polymerase chain reaction (RT-PCR) employing Taqman technology. Simultaneously, participants completed validated questionnaires, including the Alcohol Use Disorders Identification Test (AUDIT), Substance Abuse Risk Profiling Scale (SURPS), and Barrat Impulsivity Scale-11 (BIS-11).

While no direct correlation was observed between *OXTR* gene polymorphisms and risky alcohol consumption or dependence, certain genotypic variations were linked to specific personality traits conducive to risky alcohol behaviors. Notably, carriers of the AA genotype of rs53576 exhibited heightened anxiety sensitivity ($p=0.042$), while individuals with the TT genotype of rs13316193 displayed increased impulsivity ($p=0.011$) and anxiety susceptibility ($p=0.027$). Moreover, significant associations were noted between the rs13316193 genotype and the propensity towards despair ($p=0.027$).

In conclusion, this comprehensive investigation sheds light on the intricate interplay between genetic factors, neurobiological mechanisms, and behavioral phenotypes in the context of AUDs. These findings provide valuable insights for the development of personalized prevention and intervention strategies tailored to individual susceptibilities, contributing to more effective approaches for addressing AUDs.

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BFP TO GFP CONVERSION SYSTEM TO EVALUATE PRECISE GENOME EDITING IN ZEBRAFISH

Kotryna Paulikaitė[1], Justas Lazutka[1], Danguolė Žiogienė[1], Patrick Pausch[2], Darius Balčiūnas[1,3]

[1]Institute of Biotechnology, Life sciences Center, Vilnius University, Vilnius, Lithuania
[2]Life Sciences Center - European Molecular Biology Laboratory (LSC-EMBL) Partnership for Genome Editing Technologies, Vilnius University, Vilnius, Lithuania
[3]Department of Biology, Temple University, Philadelphia, PA, USA
kotryna.paulikaite@gmc.stud.vu.lt

Genome editing tools have potential to be used as therapy to treat genetic diseases or cancer [1]. We can use these targeted tools to introduce modifications in DNA sequence which result in gene knock-in or knockout. Knowing how effective and precise they are helps develop new genome editing tools and techniques. To this date various assays have been created to evaluate genome editing tools. One of them employs the green fluorescent protein (GFP) as it can be converted to blue fluorescent protein (BFP) after a targeted modification [2]. This system can be integrated into *Danio rerio* to create a new assay for genome editing tool analysis *in vivo* as zebrafish is a convenient model organism to observe fluorescence because their embryos are transparent and develop rapidly [3]. Since BFP fluorescence is relatively weak and suffers from high background, we have modified the system to switch from BFP to GFP after editing.

Two transgenic *Danio rerio* lines have been created in this work. The first line (vln20) expresses a BFP* transgene that was obtained by introducing Y66H mutation into eGFP. The second line (vln22) has BFP** gene that is a BFP* gene with an additional mutation expected to improve BFP fluorescence. The blue fluorescence in the vln22 *D. rerio* line is visible under the fluorescent microscope while it can not be detected in the vln20 fish line. This data suggests that the additional mutation in the BFP** makes the blue fluorescence of the expressed protein more intense and visible.

Embryos obtained from these lines were injected with Cas9 mRNA, gRNA and HDR template to convert BFP coding gene back to the GFP gene *in vivo*. The injected embryos were screened at 3 days post fertilization (dpf) to evaluate the editing efficiency as well as observe possible differences between the two lines. The blue fluorescence becomes barely visible in vln22 embryos after injection, indicating high Cas9 activity after injection. Also, most of the embryos from both lines that were injected with Cas9 mRNA, gRNA and HDR template had varying amounts of green fluorescence at 3 dpf. This shows that BFP to GFP editing in zebrafish is working and can potentially be used to determine how effective and precise editing tools are.

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COMPARATIVE ANALYSIS OF ZEBRAFISH *(DANIO RERIO)* LINES ENCODING HA EPITOPE TAGGED TRANSCRIPTION FACTOR TCF21

Vaiva Slatkevičiūtė[1], Miglė Kalvaitytė[1], Darius Balčiūnas[1,2]

[1]Department of Eukaryote Gene Engineering, Institute of Biotechnology, Vilnius University, Lithuania
[2]Department of Biology, Temple University, Philadelphia, USA
vaiva.slatkeviciute@gmc.stud.vu.lt

Myocardial infarction stands as a leading cause of mortality, not just in Lithuania but worldwide. In 2022, approximately 13 500 cases were recorded in Lithuania, attributed to ischaemic heart disease, often culminating in myocardial infarctions [1]. This condition inflicts damage upon heart tissues, resulting in scarring that significantly impairs heart function and often leads to death. Thus, research of regenerative mechanisms becomes imperative. Zebrafish (*Danio rerio*) showcases a remarkable ability to regenerate damaged heart tissues. Extensive research using this model organism underscores the significance of the epicardium during cardiac regeneration, with a transcription factor 21 (Tcf21) serving as a prevalent marker specific to this tissue during development and adulthood. Moreover, studies indicate elevated levels of Tcf21 in epicardial tissue following heart damage in adult zebrafish, suggesting its potential significance in this process [2].

This study aims to explore the functions of Tcf21 and pinpoint its targets, aiming to deepen the comprehension of its involvement in zebrafish heart development and regeneration. Unravelling the precise genes targeted by Tcf21 requires the use of various techniques, including chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq), or analogous methodologies. Nonetheless, the scarcity of specific and effective antibodies against Tcf21 poses a considerable challenge for analysing its binding sites in zebrafish. To overcome this, the study endeavours to develop a transgenic zebrafish line expressing Tcf21 tagged with a triple HA epitope, compatible with widely available antibodies suitable for antibody-based methods.

The creation of this transgenic zebrafish line involved employing Tol2 transposase to facilitate random insertion of a *ubb:loxP-mRFP-loxP-tcf21-3xHA* transgene into the zebrafish genome. This gene configuration permits the conditional activation of Tcf21-3xHA expression by removing the *loxP-mRFP-loxP* cassette using CreERT2 recombinase. Subsequent comparative analysis of three single-copy *Tg(ubb:loxP-mRFP-loxP-tcf21-3xHA)* F1 generation fish involved crossbreeding with CreERT2 recombinase-expressing Tg(ubb^{R}:CreERT2*)^{vIn2} zebrafish line, initiating *loxP-mRFP-loxP* cassette excision, and analysing larval phenotypes by Alcian Blue staining. This enables the investigation into whether Tcf21-3xHA is functional and can rescue the mutant *tcf21*$^{\Delta}$ phenotype. Following comparative analysis, line *Tg(ubb:loxP-mRFP-loxP-tcf21-3xHA)*^{vIn14} emerges as the most proficient in rescuing the mutant *tcf21*$^{\Delta}$ phenotype by expressing Tcf21-3xHA. This selected line will serve as the focal point for further investigations into Tcf21 regulatory targets and its pivotal role in heart development and regeneration.

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TBX5A GENE ENHANCERS INFLUENCE CARDIAC DEVELOPMENT IN ZEBRAFISH (*DANIO RERIO*)

Greta Muravska[1], Justas Lazutka[2], Darius Balčiūnas[1,2]

[1]Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Department of Biology, Temple University, Philadelphia, USA
greta.muravska@gmc.stud.vu.lt

Tbx5a (T-box transcription factor 5a) is a gene essential for zebrafish (*Danio rerio*) cardiac and forelimb development. An ortholog of this gene is found in humans – *TBX5* gene which is also responsible for heart and upper limb development. Mutations in *TBX5* gene cause Holt-Oram syndrome – an autosomal dominant disorder, also known as 'Heart-Hand' syndrome [1]. This makes zebrafish an ideal model organism for researching the genetic and developmental aspects of this condition. Furthermore, regulatory elements of the *tbx5a* gene, specifically enhancers, may have a bigger role in this process than we initially thought. Using genetic engineering methods, such as CRISPR/Cas9, on zebrafish is an efficient system for manipulating the genome and conducting knock-out and knock-in experiments of individual DNA regions of choice – all by using a customizable RNA guide molecule [2]. By using this approach, it is possible to remove the entire enhancer region of the *tbx5a* gene and observe embryogenesis and cardiac development, providing valuable insights about these regulatory elements.

Zebrafish that have a *heartstrings* (*hst)* recessive lethal mutation in the *tbx5a* gene display evident cardiac edema and an absence of pectoral fins – the swelling of the heart caused by fluid accumulation [3]. In our laboratory, the viability of these fish is sustained by the bacterial artificial chromosome (BAC) which 'saves' the phenotype and thus makes studying these mutants more feasible. Despite the gene's vital importance, our understanding of its enhancers is currently limited – which is why the aim of our work is to study how they may influence cardiac development in zebrafish.

The experiments were started by crossbreeding the genetically engineered zebrafish, heterozygous for the deleted *tbx5a* enhancer region, with a homozygous *hst* mutant. The offspring phenotypes were analysed and were screened 5dpf not only for YFP (yellow fluorescent protein) signal, indicating BAC presence, but also for cardiac edemas. Embryos with a negative YFP signal were collected and were sorted by phenotype – in about 50% of them cardiac edema was present. Afterwards, genomic DNA was extracted and amplified using the triple primer PCR (polymerase chain reaction) method. Lastly, electrophoresis in a 1% agarose gel was performed to analyse PCR product length and determine the genetic differences in the enhancer region.

Results have shown that offspring with only one *hst* copy present exhibit cardiac edema phenotype if enhancers in the homologous chromosome are removed. Conversely, embryos expressing a wild-type phenotype (which also had one *hst* allele), had functional *tbx5a* enhancers. Both groups had one wild-type copy of the *tbx5a* gene and one copy of the *hst* allele – the only difference was the presence of the enhancer region which could have caused the phenotype differences. Since one of the three primers used in the PCR reaction was in the middle of the enhancer region, we could easily detect that the sequence has been knocked-out if a particular PCR product is not present in the gel.

In summary we have identified the first essential enhancer region of the *tbx5a* gene, highlighting its crucial role in heart development.

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Cell Biology

I1

THE ROLE OF HUMAN CHORIONIC GONADOTROPIN IN EPIGENETIC REGULATION OF ENDOMETRIAL STROMAL CELLS DECIDUALIZATION

Erika Girniūtė[1,2], Deimantė Žukauskaitė[1], Rūta Navakauskienė[1]

[1]Department of Molecular Cell Biology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania
[2]Institute of Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Lithuania
erika.girniute@chfg.stud.vu.lt

Endometrial stromal cells (ESCs) are one of the main components of endometrium, a dynamic tissue that undergoes a periodic cycle of cell proliferation, differentiation, apoptosis, and regeneration. The differentiation process of ESCs, called decidualization, includes cell cycle arrest, inhibition of proliferation, and various molecular alterations that transform fibroblast-like cells into rounder secretory decidual stromal cells [1]. *In vivo* decidualization is regulated by progesterone and cyclic adenosine monophosphate (cAMP) via activation of protein kinase A (PKA) signaling pathway, and other ovarian hormones such as luteinizing hormone (LH) and human chorionic gonadotropin (hCG) which stimulate ESCs transformation via luteinizing hormone/choriogonadotropin receptor (LHCGR) and initiation of adenylyl cyclase and PKA pathway [2]. Decidualization-induced reprogramming of ESC cell functions is fundamental to creating an appropriate environment for blastocyst implantation, placenta formation, and preparing endometrium tissue for successful pregnancy [3].

In this study, we aimed to analyze the role of hCG in epigenetic regulation primarily post-translational histone modifications in non-treated and induced to decidualization ESCs. To determine differences in short and long-term response to hCG, decidualized and non-decidualized ESC cells were additionally induced with 10 IU/ml concentration of hCG for 6 and 24 hours. Using western blot analysis changes in histone modification as well as histone-modifying enzyme levels were determined. To further investigate hCG effects on decidualization-related genes, chromatin immunoprecipitation assay (ChIP) was employed and alterations of H3K27Ac epigenetic modification levels were analyzed on selected gene promoter regions.

Western blot analysis results revealed that short-term induction of hCG takes place in processes by leading to alterations in post-translational histone modifications related to transcriptionally active chromatin – H3K27Ac, H3K4Me3, and H4hyperAc. However, longer treatment of hCG and decidualization results in an increase in H3K27 tri-methylation, a mark of transcriptional silencing. Furthermore, long-term decidualization and hCG induction appeared to raise H3K27 methyltransferase complex protein (EZH2, EED) levels. Immunoprecipitation of H3K27Ac marked chromatin revealed the specific increment of this modification in promoters of transcription factor genes (*FOXO1, HAND2, HOXA10*) that are known to mediate signaling and regulatory pathways related to progestin-induced decidualization [4]. Changes in activation marks on gene promoters were observed after induction with hCG alone or with hCG in the presence of decidualization inducers. Overall, these findings suggest that hCG may take part in processes of ESC transcription activation and regulation of signaling pathways involved in ESC decidualization.

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I2

MOLECULAR CHARACTERISTICS AND FACTORS INFLUENCING BLASTOCYST IMPLANTATION AND ENDOMETRIAL RECEPTIVITY – IMPACT OF THREE-DIMENSIONAL CULTURE MODELS

Aistė Zentelytė[1], Vaiva Vaišnoraitė[1], Rimantas Gricius[2], Rūta Navakauskienė[1]

[1]Department of Molecular Cell Biology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania
[2]Clinic of Obstetrics and Gynaecology, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
vaiva.vaisnoraite@gmc.stud.vu.lt

Endometrium is the inner lining of the uterus where the attachment and implantation of a blastocyst occur. Endometrial tissue contains several cell populations, including a population of mesenchymal stromal cells (enMSCs) [1]. These cells not only can be used to study the biology of endometrial receptivity and processes of embryo implantation, but also be applied for therapeutic use. Endometriosis is a disease in which tissue similar to endometrium grows outside of the uterus and the migration of enMSCs is hypothesised to be one of the causes [2]. Significant number of women suffering from endometriosis may experience difficulties to conceive and even infertility.

Decidualisation of endometrium is required for successful blastocyst implantation and development, this process involves functional and morphological changes of endometrial stromal cells, Thus, studying decidualisation potential of endometrial and endometriotic MSCs (enMSCs and ezMSCs, respectively) could bring new insights into the pathophysiology, prevention, and treatment of endometriosis and infertility.

To study the process of blastocyst implantation, the knowledge of molecular communication between implanting embryo and endometrium is crucial. Conditioned culture media of blastocysts is full of secreted signalling molecules that could participate and impact the implantation process, therefore the analysis of secreted molecules, such as proteins, could provide new knowledge about embryo development and implantation potential [3].

Our study aims to determine differences in enMSCs and ezMSCs comparing routinely used 2D (monolayer) culture and more physiologically relevant 3D (spheroid) conditions in terms of stem cell characteristics and decidualisation potential. Also, to establish proteomic profiles of conditioned culture media of blastocyst in cases of successful conception and failure to conceive.

Isolated primary enMSCs and ezMSCs presented similar spindle shaped morphology. Cells were positive for typical mesenchymal markers CD44, CD73, CD90, CD105, CD146, CD140b and negative for hematopoietic and endothelial markers CD34 and CD31. Under appropriate conditions these stem cells proved to be capable to differentiate towards adipogenic, osteogenic, and chondrogenic lineages. 3D culture was induced by seeding endometrial and endometriotic cells into low attachment cell culture plates. During 9-day culture period enMSCs and ezMSCs grown in 2D and 3D conditions were characterised by the diameters of formed spheroids, and by the expression of genes linked to stemness (*OCT4, NANOG*), paracrine signalling (*VEGFA, FGF2, TGFB1*), cell death and hypoxia (*BAX, BAK1, BCL2, HIF1A*). Decidualisation of 2D and 3D cell cultures was characterised by morphological changes and by the expression of differentiation associated genes (*IGFBP1*, *PRL*, *FOXO1, PCR*).

182 differentially secreted proteins were determined in blastocyst culture media. The most pronounced changes were found in the secretion of proteins encoded by *JUP* and *DSG1* genes (significantly more proteins in cases of conception) and in the amounts of proteins encoded by *SUGP2* and *MUC5B* genes (more proteins in cases of non-pregnancy). The proteins encoded by the JUP and DSG1 genes are involved in epithelial cell-to-cell communication and cell adhesion, and could be directly involved during blastocyst implantation.

In summary, our study demonstrates that endometrial tissue homeostasis and secreted proteins of blastocysts are required for molecular communication and are critical for successful conception.

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THE ROLE OF AUTOPHAGY IN THE RESPONSE OF ENDOMETRIAL CANCER CELLS TO CHEMOTHERAPEUTIC AGENTS

Dominyka Florinaitė[1], Aušra Sasnauskienė[1]

[1]Department of Biochemistry and Molecular Biology, Institute of Biosciences,
Vilnius University Life Sciences Center, Lithuania
dominyka.florinaite@chgf.stud.vu.lt

Endometrial cancer (EC) is the second most common gynaecological malignancy and the seventh most frequent cancer among women worldwide and both the incidence and mortality rates have been rising over the last decade. Poland and Lithuania are among the world's leading countries in terms of the incidence of EC. Most patients with EC benefit from initial surgical treatment, but one fifth of patients may still relapse, even if there were no signs of metastatic disease. The rising number of EC cases has encouraged to search for novel treatment strategies which effectiveness would surpass currently used conventional chemotherapy and surgery, as these approaches become less advantageous in later stages of EC. Currently, there is no approved targeted therapy for EC treatment. Hence, ongoing research is focused on targeted therapies specific for EC.

Autophagy (also known as macroautophagy) is a specialised mechanism that maintains cellular homeostasis and cell survival through the lysosomal degradation pathway by degrading and eliminating misfolded proteins and dysfunctional organelles. It is a vital process for cell adaptation to starvation, development, cell death and tumour suppression [1]. However, studies have shown that autophagy is known to exert a dual effect on cancer development, either promoting or inhibiting tumorigenesis, depending on the stage of tumour development [2]. When malignancy is present, autophagy prevents degradation of damaged components and helps cells to overcome stressful conditions, such as hypoxia, oxidative stress and nutrient deprivation, leading to the development of cancer and chemoresistance. Therefore, regulation of autophagy presents as a promising and potential strategy to enhance cancer treatment [1].

Overactivated PI3K/AKT/mTOR signalling pathway has been found to be associated with EC pathogenesis. This pathway plays an important role in cell growth and survival, so inhibition of the PI3K/AKT/mTOR pathway is of therapeutic interest. This signalling pathway plays an important regulatory role in autophagy. In this study, we have used several small-molecule mTOR inhibitors and their combination with conventional chemotherapy drugs. We have analysed the effects of these inhibitors on the intensity of autophagy and cell survival of an EC cell line. The impact of mTOR small-molecule inhibitors was compared with specific inhibitors of autophagic process. These results will uncover the role of autophagy for EC cell survival and resistance to chemotherapeutic drugs.

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EFFECTS OF MICROGRAVITY ON YEAST DEATH TYPE AND RESISTANCE TO REACTIVE OXYGEN SPECIES

Irmantas Čiužas[1], Gerda Anužienė[2], Justina Versockienė[3], Eglė Lastauskienė[4]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Chemical Physics Institute, Faculty of Physics, Vilnius University, Vilnius, Lithuania
irmantas.ciuzas@gmc.stud.vu.lt

A change in gravity, in either direction, can cause many changes in organisms, from metabolism to conservative systems. Microgravity is a reduction of the gravitational force, and studies of its effects on living organisms are very important for long-term life support systems and work in space stations, lunar and planetary bases, where the gravitational forces are significantly different [1]. Long flights of space missions alter the length of astronauts' telomeres. Telomeres are nucleoprotein structures located at the ends of linear chromosomes of eukaryotic organisms, which maintain the stability and integrity of the genome, protect the ends of linear chromosomes from recombination, splicing and the effects of nucleases [2]. Telomeres ensure the replication and distribution of chromosomes to daughter cells, but during each cycle of replication, telomeres shorten, and shorter and shorter chromosomes enter the daughter cells. Telomere shortening is known to be physiologically associated with aging processes, and telomere dynamic changes also play a role in cancer cells and the development of various disorders [3].

The study attempted to determine how microgravity conditions affect reactive oxygen species (ROS) resistance and type of death (apoptosis/necrosis) in *Saccharomyces cerevisiae* yeasts. Microgravity was simulated using a rotary cell culture system and acetic acid was used to induce apoptosis. The cells then were exposed to specific ROS-detecting dyes and the appearance of ROS in the cells was quantified by fluorescence microscopy. Also, the cells were stained with annexin-V and propidium iodide and the type of cell death (apoptosis/necrosis) were assessed using fluorescence microscopy. The method of an attenuated total reflection of infrared radiation (ATR IR) spectroscopy was applied too for the type of cell death analysis.

The early results show a slight difference between *S. cerevisiae* cells grown under normal gravity and microgravity. In microgravity conditions, ROS staining was higher compared to normal gravity when using a lower concentration of acetic acid, and there was a significantly larger count of apoptotic and necrotic cells.

This study suggests that microgravity influences the type of cell death, leading to variations in yeast cell demise under such conditions. To gain a comprehensive understanding of microgravity's effects, further research involving diverse cell types and prolonged exposure to microgravity is crucial.

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15

THE ROLE OF P62 IN CELL SURVIVAL OF CHEMORESISTANT HUMAN COLORECTAL CANCER CELLS

Guoda Vaitiekaitė[1], Andrius Jasinevičius[1], Aušra Sasnauskienė[1], Vilmantė Žitkutė[1]

[1]Department of Biochemistry and Molecular Biology, Institute of Biosciences, Life Sciences Centre, Vilnius University, Lithuania
guoda.vaitiekaite@gmc.stud.vu.lt

Colorectal cancer (CRC) is the third most common diagnosed and second leading cause of cancer death worldwide. CRC is treated using a combination of surgery and radiotherapy or chemotherapy. Chemotherapy uses a combination of chemotherapeutic agents 5-fluorouracil (5-FU) and oxaliplatin (OxaPt) [1]. 5-FU and OxaPt cause DNA damage and induce programmed cell death. During chemotherapy, cancer cells can acquire resistance to chemotherapeutic drugs, which reduces the effectiveness of cancer treatment [2]. Currently, the causes of chemoresistance mechanisms are not sufficiently understood.

One possible cause of chemoresistance is the multifunctional protein selective autophagy receptor p62, also known as SQSTM1 (Sequestosome 1). Higher levels of p62 protein are detected in the cancerous tissues of colorectal cancer patients compared to healthy tissues. Increased levels of p62 in CRC patients predict poor survival prognosis [3]. p62 has multiple functions in cells due to its multidomain protein structure. p62 is best known as an autophagic receptor that targets ubiquitinated organelles or proteins to autophagosomes for degradation. Also, p62 regulates the NF-κB and NRF2 signalling pathways which are important for tumorigenesis. NF-κB and NRF2 transcription factors activated by p62 cause oncogenic mutations and have an impact on cancer cell proliferation, differentiation and viability. p62 also regulates cell survival and initiates angiogenesis and cytokine expression in the tumour environment [4]. The impact of p62 on the molecular causes of chemoresistance in CRC cells is poorly understood.

Therefore, we evaluated the impact of the multifunctional protein p62 on the survival of chemoresistant human CRC cells. As an *in vitro* model, we used colorectal carcinoma cell line HCT116 and its chemoresistant sublines HCT116/FU and HCT116/OXA which had acquired resistance to chemotherapeutic drugs 5-FU and/or OxaPt. First, we evaluated the impact of the p62 expression silencing on HCT116, HCT116/FU and HCT116/OXA cell viability. Further, we identified p62 interactions with cell death-regulating proteins and established the effects of p62 silencing on apoptosis. Finally, we assessed the effects of p62 downregulation on interleukin 8 (IL-8) and its receptor CXCR2 levels in HCT116 cells and their chemoresistant sublines.

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16

DEVELOPMENT OF ENDOMETRIAL CANCER CELL LINES FOR PERSONALIZED THERAPY

Laura Marija Račytė[1], Aistė Avižaitė[1], Veronika Dedonytė[2], Evelina Šidlovska[3], Margarita Montrimaitė[4], Gediminas Januška[4], Rūta Čiurlienė[4], Eglė Žalytė[1]

[1]Department of Biochemistry and Molecular Biology, Institute of Biosciences, Vilnius University Life Sciences Center, Vilnius, Lithuania
[2]Department of Botany and Genetics, Institute of Biosciences, Vilnius University Life Sciences Center, Vilnius, Lithuania
[3]National Center of Pathology, Vilnius, Lithuania
[4]National Cancer Institute, Vilnius, Lithuania
laura.racyte@gf.stud.vu.lt

Endometrial cancer is the sixth most common cancer in the world, with Lithuania and Poland leading in Europe [1], [2]. In addition, type II endometrial carcinomas account for the majority of endometrial cancer-related deaths due to their aggressiveness, late-stage detection, and high tolerance to standard treatment [3]. Common risk factors of endometrial cancer include older age, hormone therapy, obesity, hyperglycemia, diabetes, and some genetic disorders. Paclitaxel, cisplatin, and carboplatin are the first-line chemotherapy drugs that are used to treat endometrial cancer. However, new targeted therapy agents are constantly under development [3]. Cell lines are standard *in vitro* model systems of cancer. Cell lines are easy to grow, allow direct comparison of experimental results, and are widely used to study molecular mechanisms of tumor cell biology. Unfortunately, most of the commercially available endometrial cancer cell lines are derived from the tumors of Asian patients. What is more, established lines that have been cultivated for a long time adapt to an artificial *in vitro* environment and lose their original phenotype due to genetic drift. For instance, more than 40 years have passed since the Ishikawa cell line, a well-differentiated human endometrial adenocarcinoma cell line, was established [4]. Therefore, each cancer patient and each disease is different and cannot be properly represented by a defined set of cell lines. The extent of genetic and epigenetic diversity between and within patient tumors is increasingly detailed. Finally, despite our growing understanding of this disease, the exact molecular and genetic processes that cause an increased predisposition to endometrial cancer among European women remain unknown. Thus, further clarification of the molecular pathogenesis of this disease is necessary, and the establishment of different endometrial cancer cell lines will be essential.

In this study, we present novel endometrial cancer cell lines, derived in 2023 from the tumor tissue of Lithuanian endometrial cancer patients. We characterized the cells by determining their growth rate, detecting the expression of cancer markers, analyzing colony-forming efficiency, karyotype and cell sensitivity to paclitaxel, cisplatin, carboplatin and targeted therapy drugs. We believe that these novel cell lines will be an effective tool for preclinical endometrial cancer studies in the future.

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17

ASSESSMENT OF STEMNESS INHIBITORS IN OVARIAN CANCER CELL LINES

Akvilė Zareckaitė[1], Eglė Žymantaitė[2,3], Agata Mlynska[1,2]

[1] Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania
[2] Laboratory of Immunology, National Cancer Institute, Vilnius, Lithuania
[3] Life Sciences Centre, Vilnius University, Vilnius, Lithuania
akvilezareckaite23@gmail.com

Introduction. Ovarian cancer is the eighth most common cancer among European women and accounts for 5% of all cancers worldwide. Unfortunately, ovarian cancer is diagnosed in the late stages due to the lack of specific symptoms and causes 207,000 deaths per year globally out of the 314,000 new cases [1]. Therefore, it is essential to understand the molecular mechanisms that contribute to tumor metastasis and chemoresistance. Elimination of cancer stemness properties is thought to be a strategy to increase therapeutic response. Therefore, there is a crucial need to identify and characterize these properties using reliable markers and to identify their vulnerabilities that can be exploited for therapeutic intervention [2]. Drug repurposing could be a valuable approach for developing novel cancer management strategies by exploring existing drugs. However, it requires extensive analysis and application in different 2D and 3D cell culture models to get promising results that could increase the effectiveness of ovarian cancer treatment.

Objective. In this study, three drug candidates (JIB-04, Napabucasin, and Salinomycin) were tested for their ability to inhibit stemness properties in ovarian cancer cell lines SKOV3 and COV362.

Methods. The expression of selected stemness-associated transcription factors - *SOX2*, *OCT4*, and *NANOG* - was examined in 2D cell culture of SKOV3 and COV362 using RT-qPCR. Both cell lines were tested for establishing the IC_{50} of selected inhibitors. The effect of stemness inhibitors on gene expression was evaluated after 48 and 72 hours. Next, the analysis was extended to a 3D spheroid model. The effectiveness of inhibitors was evaluated based on the spheroid diameter after treatment with IC_{50}, IC_{50} x2, IC_{50} x5, IC_{50} x10 and IC_{50} x20 drug doses.

Results. IC_{50} values of JIB-04, Napabucasin, and Salinomycin were determined in SKOV3 and COV362. Decrease in the expression of *SOX2*, *OCT4*, and *NANOG* was noted in both cell lines after 72 h incubation with stemness inhibitors in 2D setting. However lack of the effect of the same concentrations in the 3D model led to optimization of the protocol by adding drugs to the spheroid culture medium every two days and additionally using higher drug dosage. Starting from IC_{50} x5 dose, growth of cells in 3D models were inhibited and spheroid diameter was significantly reduced compared to the control samples. However, IC_{50} and IC_{50} x2 were less effective in 3D models which confirms that selected inhibitors are more sensitive in 2D models.

Conclusion. The comprehensive assessment of stemness inhibitors in ovarian cancer cell lines, integrating both 2D and 3D culture approaches, provides valuable insights for optimizing drug efficacy and advancing the development of novel therapeutic strategies for ovarian cancer treatment.

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18

FORECASTING NEOADJUVANT CHEMOTHERAPY RESPONSE IN TRIPLE-NEGATIVE BREAST CANCER THROUGH MicroRNA SIGNATURES

Domas Štitilis[1, 3], Adomas Vasiliauskas[3], Linas Kunigėnas[1], Monika Drobnienė[1, 2], Eglė Strainienė[1, 3], Kęstutis Sužiedėlis[1, 2]

[1]National Cancer Institute
[2]Life Sciences Center
[3]Vilnius Gediminas Technical University
domas.stitilis@gmail.com, adomas.vasiliauskas@stud.vilniustech.lt

Breast cancer remains the most prevalent malignancy and the primary cause of cancer-related mortality among women worldwide. Among its subtypes, Triple-Negative Breast Cancer (TNBC) stands out as an exceptionally aggressive form, constituting 10-20% of all breast cancer cases. Characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), TNBC presents a significant challenge in terms of treatment due to the lack of targeted therapy options, leading to a generally poor prognosis. [1]

MicroRNAs (miRNAs), small non-coding RNA molecules, play a crucial role in regulating gene expression. Dysregulation of miRNAs can significantly influence cancer development and progression by altering the expression of genes involved in critical pathways. [1], [2]

This study aims to provide insights into the role of selected miRNAs in TNBC progression. Bioinformatic case study analysis of the cancer genome atlas (TCGA) datasets revealed 195 differentially expressed miRNAs targeting 57 genes linked to the platinum drug resistance pathway. Analysis of patient survival data showed 13 of those to be directly related to patient survival rate. In this research, we examine the following miRNAs: miR-340-5p, miR-1307-3p and miR-937-3p.

To further investigate the function of these miRNAs, we selected a subset for quantitative analysis. We measured their expression levels in TNBC patient samples using quantitative reverse transcription PCR (qRT-PCR). Specifically, we analyzed patient samples before and after neoadjuvant chemotherapy. Patient samples were divided into two groups based on their Residual Cancer Burden (RCB) classification, distinguishing between RCB-0-I and RCB-II-III to reflect varying treatment outcomes. Statistical analysis of miRNA expression levels was conducted using the Student's t-test to compare the pre- and post-neoadjuvant chemotherapy patient groups. To evaluate the effect of miRNA expression on patient outcomes, Kaplan-Meier curves were utilized, showcasing the influence of miRNA levels on overall survival and progression-free survival before and after treatment. This approach identified significant changes in miRNA expression associated with the response to neoadjuvant chemotherapy in triple-negative breast cancer. The comparative analysis of how miRNA expressions are altered by chemotherapy potentially sheds light on their roles in mediating the therapeutic response and resistance mechanisms in TNBC.

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19

LINE-1 PROMOTER METHYLATION AS A DIAGNOSTIC TOOL FOR CLASSIFICATION OF PRE-CANCEROUS CRC LESIONS

Rima Miknaitė[1], Rytė Rynkevičienė[2], Inga Kildušienė[3,4], Kęstutis Sužiedėlis[2,5], Giedrė Smailytė[3,6]

[1]Faculty of Chemistry and Geosciences, Vilnius University, Lithuania
[2]Laboratory of Molecular Oncology, National Cancer Institute, Lithuania
[3]Laboratory of Cancer Epidemiology, National Cancer Institute, Lithuania
[4]Department of Abdominal and General Surgery and Oncology, National Cancer Institute, Lithuania
[5]Life Sciences Center, Vilnius University, Lithuania
[6]Department of Public Health, Institute of Health Sciences, Vilnius University, Lithuania
rima.miknaite@chgf.stud.vu.lt

Colorectal cancer (CRC) is the second most common cause of cancer-related deaths and the third most frequently diagnosed cancer worldwide [1]. It is also the fifth most common frequently occurring cancer localization in Lithuania. Hyperplastic polyps that grow on the intestine mucosa surface are the first sign of presumable CRC. Subsequently, an increasing amount of mutations and/or changes in genome methylation status leads to conventional adenomas or serrated lesions. Finally, if these polyps of serrated lesions or conventional adenomas continue to grow, they can develop into adenocarcinoma [2]. Serrated adenomas are major precursor of colorectal cancer however, their identification is challenging thus there is a need for additional tools – biomarkers [3]. LINE-1 retrotransposon is widely used as epigenetic marker which shows the methylation status of the genome. LINE-1 expression and LINE-1 promoter hypomethylation are associated with genomic instability, indicating it as one of potential marker of cancer development [4].

The present study aimed to assess LINE-1 promoter methylation status in tubular adenoma, serrated lesions and adenocarcinoma, and corresponding health tissue. Adenocarcinoma (n=20), tubular adenoma (n=32), serrated adenoma (n=15) and corresponding adjacent normal tissue have been collected (NCI, Vilnius, Lithuania). Quantitative determination of LINE1 promoter three CpG islands methylation status in bisulfite-converted DNA was performed by pyrosequencing (Qiagen) method, and averages of the three CpG islands data were analyzed (GraphPad).

The findings of this study show a statistically significant difference in LINE-1 promoter methylation status between tubular adenoma and adenocarcinoma and adjacent healthy tissues. Also, the fact that there are no changes in LINE-1 promoter methylation status in serrated adenoma in comparison to healthy tissue, rises the hypothesis that epigenetic alterations do not play the main role in serrated adenoma development. Analysis of ROC curves shows that all three pathological groups have statistically significant different methylation profiles so methylation status of LINE-1 promoter can be used as an additional diagnostic biomarker.

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I10

INVESTIGATION OF THE CYTOTOXIC PROPERTIES OF CHIMERIC ANTIGEN RECEPTOR-EXPRESSING JURKAT CELLS

Mantas Špakovas[1,2], Nadežda Dreižė[1], Algirdas Kaupinis[1], Mindaugas Valius[1]

[1]Proteomics center, Institute of Biochemistry, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Life Sciences Center, University of Vilnius, Vilnius, Lithuania
mantas.spakovas@gmail.com

B-cell lymphomas are usually treated with chemotherapy, radiotherapy and autologous or allogeneic stem cell transplantation, but there are some cases where the disease is resistant to treatment, leading to a poor prognosis. For example, after unsuccessful treatment of relapsed and refractory lymphoma, a patient's survival can be as low as 6 months. This is a worrying prognosis, but it has improved significantly with the introduction of a new treatment modality, CAR-T therapy. This form of treatment is based on targeting the patient's immunity against cancer cells. CAR-T is a type of autologous T cell that has been genetically modified to express the chimeric antigen receptor (CAR), a protein that determines the specificity of T cells in killing cells expressing specific surface biomarkers. CAR-T therapy is currently used to treat chemotherapyresistant malignant lymphoblastic leukemia, diffuse large B-cell lymphoma and follicular lymphoma. In these diseases, cancer cells have higher levels of the B-cell antigen CD19, and CAR-T cells are targeted to specifically recognize this protein and eliminate these cells [1].

Jurkat is a cell line derived from an acute leukemia patient. It belongs to the T-cell group, however, it is not considered to be a killer cell due to the surface markers it expresses, yet this cell line is widely used in CAR studies [2, 3]. To study CAR signaling pathways, we generated a CD19-targeted CAR-expressing cell line Jurkat-CAR (J-CAR). The cell line was established by introducing into the cells a gene encoding a CAR consisting of the FMC63 CD19 recognition domain, the CD8 and CD28 hinge and transmembrane domains, the 4-1BB co-stimulation domain, and the CD3ζ activation domain, together with the gene of a selection marker - a Green Fluorescent Protein (GFP) which is used for cell sorting and tracking. In this study, we found that expression of the CAR protein in cells reprograms them, altering the transcriptomic and proteomic profile of the cells, resulting in the emergence of cells with cytotoxic features. To activate J-CAR via ligand-receptor connection, CD19-expressing B-cell lymphoma cell line SU-DHL-4 was used in co-culture. Cell co-culture revealed an unexpected phenomenon: cytotoxic effects of J-CAR cells.

To confirm that J-CAR cells function in a CD19-dependent manner, this surface biomarker was introduced into HL60 cells that did not previously express CD19 - even though the effect was weaker than in the case of SU-DHL-4, the changes in cell viability confirmed the specificity of CAR protein. In addition, J-CAR activation was observed to reduce cell viability. This is supported by data obtained by high-throughput differential phosphoproteomic analysis, which showed an increase in the activity of cellular stress associated kinases. The study shows that there is a possibility to adapt CAR therapy to allogeneic cell conditions; however, this requires an optimization of the method, whereas the data obtained in a prototype system using Jurkat-CAR cells will be presented in a poster.

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COMPARATIVE STUDIES COMBINING CHEMOTHERAPEUTIC APPROACHES AND TARGETING OF SIGNALING PATHWAYS FOR PERSONALIZED CANCER TREATMENT

Monika Tenkutytė, Aurimas Stulpinas, Audronė Kalvelytė

Department of Molecular Cell Biology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania
monika.tenkutyte@gmc.stud.vu.lt

Numerous signaling pathways regulating cell proliferation and survival are affected during cancer development. Cancer cells have oncogenic rearrangements (driver mutations) which make cells proliferative, invasive, and resistant to cell death induction. Intracellular signaling pathways extending from plasma membrane receptors to the nucleus, including the mitogen-activated protein kinases (MAPKs) and AKT, contribute substantially to the malignancy of cancer cells [1]. While oncogenes are involved in the generation of dysregulated signals, multi-level signal transduction cascades, however, remain fully functional and are responsive to chemotherapy treatment. This dynamic signaling network, together with compromised oncogenic signaling, provides a fantastic potential to explore efficient interventions that may translate into successful cancer treatment. Investigation and understanding of molecular mechanisms by which intracellular signals are transmitted are important in improving targeted and combination therapies.

Here, by Western blot method, we demonstrate that cells from lung cancer tumors have phosphorylated – activated – MAP and AKT kinases. The basal phosphorylation level of studied kinases varies among the cell lines, depends on extracellular contacts, and increases after chemotherapy treatment. Drugs targeting MAP kinase ERK and AKT signaling pathway effectively inhibited their target phosphorylation in all cell lines studied, however, the anticancer effect was modest, as determined by the MTT method. The effect of inhibitor and conventional drug cisplatin combinations on cell proliferation and cell death was not uniform between lung cancer cell lines. However, regardless of different cell genotype or phenotype, all investigated lung cancer cell lines showed cross-talk between PI3K/AKT and MEK/ERK signaling pathways with a negative feedback mechanism upon treatment with various and distinct inhibitors of these kinases, with or without chemotherapeutic agents. Therefore, drug resistance to these types of targeted therapies arises, at least in part, from negative feedback interactions between these two signaling pathways. Thus, it can be said that combination therapy using both ERK and AKT inhibitors is more beneficial for cancer treatment rather than targeting different kinases alone or in combination with chemotherapy.

The role of the MAP kinase JNK in determining cell fate varied depending on cell line (genotype), chemotherapeutic drug, and the dosage of the drug. In the A549 cell line, which has mutated *K-RAS* and intact *TP53*, JNK was proapoptotic at high cisplatin concentrations, and antiapoptotic at low (sublethal) concentrations. Cisplatin treatment alone increased AKT, ERK1/2, JNK, and TP53 phosphorylation. However, treatment with cisplatin in combination with JNK inhibition differently affected the phosphorylation of TP53 and AKT, correlating with their role in regulating cell death. The studies showed that combining JNK inhibition with low cisplatin concentrations could be a promising approach to overcome drug resistance and reduce systemic toxicity.

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I12

EXTRACELLULAR AGGREGATES OF AMYLOID PROTEIN S100A9 INDUCE MICROGLIAL ENERGY METABOLISM SHIFT

Jovita Gružaitė[1,2], Katryna Pampuščenko[3], Danielius Umbrasas[1], Vytautas Smirnovas[4], Vilmantė Borutaitė[1], Ramunė Morkūnienė[1,2]

[1]Laboratory of Biochemistry, Neuroscience Institute, Lithuanian University of Health Sciences, Lithuania
[2]Department of Drug Chemistry, Lithuanian University of Health Sciences, Lithuania
[3]Laboratory of Pharmacological Regulation of Pathological Processes and Drug Metabolism, Neuroscience Institute, Lithuanian University of Health Sciences, Lithuania
[4]Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
jovita.gruzaite@lsmu.lt

Mounting evidence suggests that chronic neuroinflammation driven by microglia, the resident macrophages of the brain parenchyma, plays a pivotal role in the development and progression of neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD) [1]. When challenged with pathological and proinflammatory stimuli, including endogenous and exogenous amyloid aggregates, microglia undergo programmed metabolic alterations to exhibit diverse functions and phenotypes. These metabolic adaptations are necessary for proper immune action in the brain; however, this can also lead to amplified inflammatory response and altered microglial phagocytic activity, which, in turn, may further exacerbate neurodegeneration [2,3]. Recent studies have shown that highly amyloidogenic and proinflammatory protein S100A9 is abundantly expressed in microglia and neurons intracellularly as well as deposited extracellularly in both AD and PD patients [4,5]. However, limited understanding exists regarding the cytotoxic effects of S100A9 and its molecular mechanisms regulating complex immune response and metabolic reprogramming of microglia cells. Thus, our research aimed to investigate the direct effect of S100A9 aggregates on microglial bioenergetics.

Experiments were carried out on cultured murine microglial cell line BV-2. BV-2 cells were exposed to nanomolar concentrations of pre-aggregated recombinant amyloid protein S100A9 for 24 h. After incubation, oxygen consumption rates of intact and permeabilized BV-2 cells were measured using a high-resolution respirometry system Oroboros O2k, while glycolytic activity was assessed by measuring the real-time proton flux in intact BV-2 cells using O2k-pH ISE-Module.

We found that exposure of BV-2 cells to S100A9 negatively affected the capacity of the mitochondrial electron transfer system, which resulted in reduced oxidation of mitochondrial complex I- and II-linked substrates, leading to a decrease in ADP phosphorylation rate. Meanwhile, BV-2 cells exhibited markedly enhanced glycolytic activity in response to S100A9, suggesting a compensatory switch to anaerobic glycolysis to maintain adequate ATP levels.

Overall, our results imply that S100A9 causes dysfunction of the mitochondrial electron transfer system and likely contributes to neurodegeneration by promoting a shift from oxidative energy metabolism to glycolysis. Therefore, targeting altered microglial energy metabolism could be an emerging therapeutic strategy to treat neurodegenerative diseases.

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I13

MORPHOLOGICAL AND FUNCTIONAL CHARACTERIZATION OF THE *Fhl1* KNOCKOUT IN MOUSE CARDIAC FIBROBLASTS

Rusnė Uzielaitė[1], Emilija Baltrukonytė[1], Daiva Baltriukienė[1]

[1]Department of Biological Models, Institute of Biochemistry,
Life Sciences Center, Vilnius University, Lithuania
rusne.uzielaite@gmc.stud.vu.lt

Fibrosis is a pathological process that can affect many organs, and, in most cases, it leads to organ failure. Deaths from fibrosis account for about 35% of all deaths worldwide [1]. Fibrosis can occur after tissue damage or various diseases. The result of it is the formation of excessive amounts of extracellular matrix components such as collagen, fibronectin, and laminin. These matrix components can form a scar that replaces the functional tissue [2]. There is also no effective treatment for the disease, as the processes involved in fibrosis are very complex and have not yet been fully explored.

To better understand the mechanisms of fibrosis, various signalling pathways are being explored as potential targets for the treatment of this disease. Fibroblasts are the main cells used in fibrosis research as they are responsible for maintaining the homeostasis of the extracellular matrix components. However, working with primary cells such as cardiac fibroblasts is difficult because they differentiate rapidly, have poor viability and are contaminated with other cell types [3]. Therefore, development of cell models for research purposes is necessary.

In this study, immortalized mouse cardiac fibroblasts are used as a potential model for cardiac fibrosis research. In addition, the effect of the *Fhl1* gene knockout on immortalized mouse cardiac fibroblasts is investigated. The FHL1 protein is associated with cytoskeletal patterning processes and functions as a transcription factor in the nucleus [4]. Mutations in this gene are also associated with various alterations in muscle function and structure, but data related to fibrotic diseases are lacking [5]. Therefore, the present study aims to compare primary mouse cardiac fibroblasts and immortalized mouse cardiac fibroblasts and to investigate possible changes in morphology and functionality of immortalized mouse cardiac fibroblasts after *Fhl1* gene knockout.

This study may demonstrate the potential application of immortalized mouse cardiac fibroblasts in future studies and stimulate consideration of the importance of *Fhl1* relevance for further studies within context of fibrosis.

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April 17th

High School Students

ASSESSING THE PREVALENCE OF ANTIBIOTIC-RESISTANT BACTERIA IN LITHUANIAN SOILS

Indrė Skyriūtė[1], Vaiva Knabikaitė[1], Vladimir Orlov[1]

[1]7th Fort of Kaunas Fortress, Educational Life Science Laboratory, Kaunas, Lithuania
iskyriutė@gmail.com vaiva.kn@gmail.com

The overuse of antibiotics in medicine and agriculture has fueled the emergence of antibiotic-resistant bacteria, posing a significant threat to global public health [1]. These resistant bacteria are not only found in hospitals but also persist in environmental reservoirs like soil [2]. This research investigates the prevalence of antibiotic-resistant bacteria in Lithuanian soil and explores the potential link to human activities. Soil samples were collected from ecologically clean and contaminated areas across Lithuania.

Clean areas included national parks and forests with minimal human intervention. Contaminated areas were identified based on past military activity, livestock farming, and fertilizer use.

The research employed a serial dilution technique to quantify the bacterial populations and assess their antibiotic susceptibility. One gram of each soil sample was diluted tenfold six times, resulting in a series of dilutions from 10-1 to 10-6 . A portion of each dilution was then plated onto agar media. Two sets of plates were prepared for each sample: one set without antibiotics (control) and two sets containing tetracycline, a common antibiotic, at low (3 µg/ml) and high (30 µg/ml) concentrations. After incubation, the colonies on each plate were counted to estimate the total bacterial count (CFU/g of soil) and the percentage resistance to tetracycline at different concentrations.

Bacterial identification was performed on colonies exhibiting low and high tetracycline resistance isolated from contaminated and clean areas. This involved isolating the bacterial DNA, amplifying 16S rRNA gene segment using a Polymerase Chain Reaction (PCR) technique, and then sequencing the amplified DNA by the Sanger method. The specific bacterial species were identified by comparing the obtained sequences to SILVA database.

The findings suggest a possible link between anthropogenic activity and antibiotic resistance. Soil samples from contaminated areas exhibited a higher percentage of bacteria resistant to low and high tetracycline concentrations (3 µg/ml and 30 µg/ml, respectively) compared to samples from clean areas (Figure 1). Furthermore, bacterial species identification revealed the presence of *Stenotrophomonas maltophilia* – a bacterium known for complex drug resistance – in a highly tetracycline-resistant sample from a contaminated area [3] [4]. These findings highlight the potential public health concerns associated with increased antibiotic resistance in the environment.

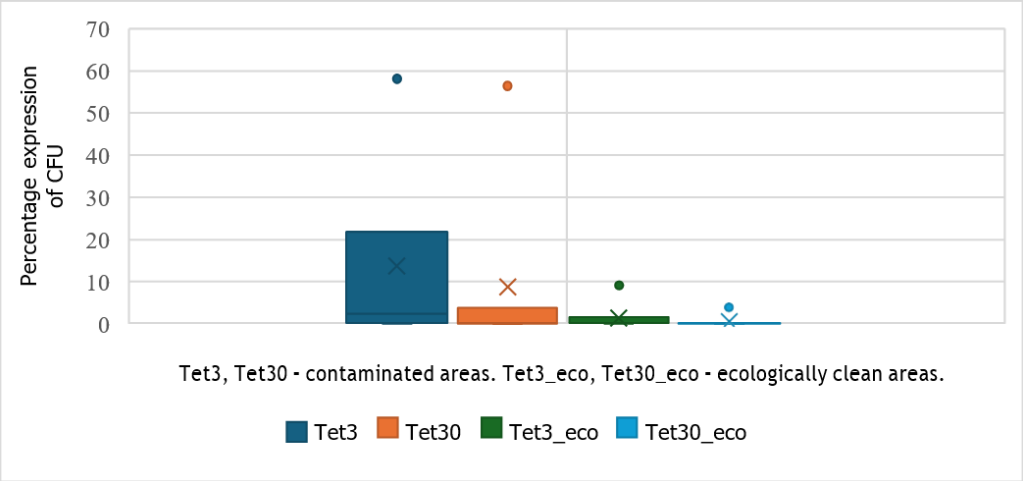

Figure 1. Prevalence of tetracycline-resistant bacteria in contaminated and clean areas

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EVALUATION OF *ELOVL6*, *FTO*, *MC4R*, AND *PPARG* POLYMORPHISMS AS PROGNOSTIC BIOMARKERS OF INSULIN RESISTANCE IN TYPE 2 DIABETES PATIENTS

Liepa Raudoniūtė[1], Gabija Paulauskaitė[1], Ugnė Meškauskaitė[1,2]

[1]Educational Life Science Laboratory, Seventh Fort of Kaunas Fortress, Kaunas, Lithuania
[2]Institute of Endocrinology, Lithuanian University of Health Sciences, Kaunas, Lithuania
liepa.raudoniute@gmail.com, gabija.paulauskaite2005@gmail.com

Introduction. Type 2 diabetes (T2D) is one of the most prevalent chronic metabolic diseases worldwide [1]. Insulin resistance (IR), impaired glucose tolerance and absorption, and hyperglycemia are common characteristics of T2D patients [2]. Although a sedentary lifestyle, a Western diet, and obesity all contribute to the development of IR and T2D, scientists are increasingly focusing on genetic variations [3]. SNPs in genes responsible for energy homeostasis, glucose and lipid metabolisms, such as *ELOVL6* rs17041272, *FTO* rs9939609, *MC4R* rs17782313, and *PPARG* rs1801282, have been found to increase the risk of developing the two main risk factors for T2D: insulin resistance and obesity [4–7].

Aim. The aim of this study was to investigate the prevalence of *ELOVL6* (rs17041272), *FTO* (rs9939609), *MC4R* (rs17782313), and *PPARG* (rs1801282) SNPs in T2D patients (with and without severe insulin resistance) and healthy individuals (control), and to assess the relationship between these SNPs and T2D and insulin resistance.

Methods. Genomic DNA was extracted from 164 peripheral blood samples collected from 52 patients with T2D, 58 patients with T2D and severe IR, and 54 subjects without T2D (control). *ELOVL6* rs17041272 and *FTO* rs9939609 SNPs were genotyped by PCR-RFLP method, while *MC4R* rs17782313 and *PPARG* rs1801282 SNPs were genotyped by ARMS-PCR. Amplified DNA fragments were separated on 2% agarose gel. The results were evaluated using the Chi-square, Fisher`s exact test, One-Way ANOVA, t-test, correlation, and multiple linear and logistic regression. Results were considered statistically significant with a p-value ≤ 0.05.

Results. Association tests showed that the *MC4R* (rs17782313) polymorphism is significantly associated with T2D and severe IR (p = 0.013). In the case of this polymorphism, the C allele and CC genotype were statistically significantly more frequent in T2D patients with severe IR. Blood HbA1c level was significantly higher in subjects with CC genotype (10.1 ± 2.5%) than with TT+CT genotypes (7.9 ± 2.2%) (p = 0.02). Although the prevalence of *FTO* (rs9939609) polymorphism did not differ between the studied groups (p = 0.517), blood HbA1c level (AA: 8.6 ± 2% vs. TT+AT: 7.8 ± 2.3%) and HOMA-IR index (AA: 18.9 ± 25.8 vs. TT+AT: 17.7 ± 38) were significantly higher in subjects with AA genotype than with TT+AT genotypes, respectively p = 0.026 and p = 0.036. Meanwhile, statistically significant associations between *ELOVL6* (rs17041272) and *PPARG* (rs1801282) SNPs and T2D as well as severe IR were not found.

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ANTIBIOTIKAMS ATSPARIŲ BAKTERIJŲ UPĖJE ŠALČIA PAPLITIMO IR ĮVAIROVĖS TYRIMAS

Emilija Kasparovičiūtė[1], Vladimir Orlov[1]

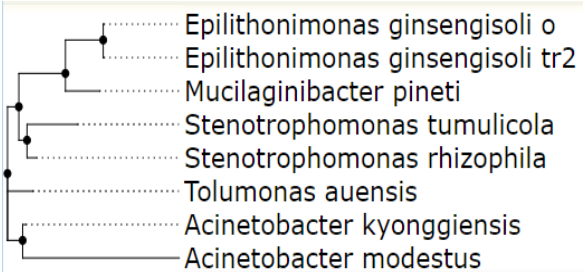
[1]Kauno tvirtovės VII fortas, Archyvo g. 61, Kaunas, LT50157 Lietuva
emilijakasparoviciute@gmail.com

Įvadas. Bakterijų atsparumas antibiotikams yra sparčiai progresuojanti problema ir iššūkis visame pasaulyje. Šis reiškinys kelia nerimą tiek medicinos specialistams, tiek visuomenei [1]. Jeigu nespręsti šios problemos, gydymas antibiotikais gali tapti neveiksmingas. Esant atsparioms bakterijoms paprastos infekcijos gali tapti nevaldomos ir pavojingos [2]. Bakterijos tampa atsparios antibiotikams dėl natūralių evoliucijos procesų, žmonių veiksmų, tokių kaip neteisingas vaistų vartojimas ar pernelyg didelis jų naudojimas, piktnaudžiavimas jais ūkiuose. Antropogeninis poveikis vandens telkiniams gali paskatinti antibiotikams atsparių bakterijų plitimą.

Tyrimo tikslas. Ištirti antibiotikams atsparių bakterijų paplitimą upėje Šalčia bei jos užtvankose (viešuose tvenkiniuose) bei nustatyti jų genetinę įvairovę.

Metodai. Iš skirtingų tiriamųjų vietovių surinkti vandens mėginiai skiedžiami serijiniu būdu ir užsėti ant Petri lėkštelių su R2A terpe be antibiotikų, su maža koncentracija tetraciklino ir su didele koncentracija tetraciklino. Po trijų parų inkubavimo nustatomas kolonijas formuojančių vienetų kiekis ir procentalus bakterijų atsparumas skirtingoms antibiotikų koncentracijoms. Siekiant atlikti antibiotikams atsparių bakterijų rūšies nustatymą, atrenkamos antibiotikui atsparios bakterijų kolonijos, iš jų išgryninta DNR ir pagausintas 16S rRNR genas taikant polimerazės grandininės reakcijos (PGR) metodą. Su tikslu patvirtinti amplikono ilgį bei koncentraciją, atliekama elektroforezė ~1,5 % agarozės gelyje. Gauti rezultatai įvertinti naudojant transiliuminatorių. Amplikonai, kurių 16S genas pasigausino ir vizualiai įvertintas gelyje kaip 1500 bp fragmentas, sekvenuojami Sangerio metodu. Gavus sekoskaitos duomenis, analizuojamos sekoskaitos chromotogramos, sekos sutvarkomos ir lygiuojamos į SILVA duomenų bazę [3].

Rezultatai. Kiekviename mėginyje buvo aptiktos mažai (3 ug/ml) antibiotikų (tetraciklino) koncentracijai atsparios bakterijos. Rečiau buvo aptinkamos didelei (30 ug/ml) antibiotikų (tetraciklino) koncentracijai atsparios bakterijos. Nustatyta, kad mažai antibiotikų koncentracijai yra atsparios 5,4% bakterijų, tuo tarpu didelei koncentracijai iki 0,15%. Sudarytame filogenetiniame medyje (1 pav.), matyti, kad nemaža dalis aptinkamų vandenyje bakterijų yra giminingos.



1 pav. Tyrimo metu nustatytų antibiotikams atsparių bakterijų rūšių filogenetinis medis.

Išvada. Įvertinus esamus tyrimo rezultatus pastebėta, kad tiriamajame tvenkinyje yra aptinkamos antibiotikams atsparios bakterijos. Didžiausią atsparumą turinčios bakterijos buvo aptinkamos užtvankose (viešuose tvenkiniuose). Iš gautų sekvenavimo duomenų, galima teigti, jog ištirtuose telkiniuose dominuoja tam tikros bakterijos, turinčios bendrą kilmę. Gauti duomenys gali būti svarbus norint suprasti bakterijų atsparumo antibiotikams paplitimo dinamiką ir jų įvairovę aplinkoje.

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CONCENTRATION OF ANTIBIOTIC-RESISTANT BACTERIA IN NEMUNAS AND THEIR CONNECTION TO OXYGENASE ENZYMES: A PILOT STUDY

Saulė Pakrosnytė[1,2], Augustė Čekanavičiūtė[1,2], Vladimir Orlov[1,2]

1 - 7th Fort of Kaunas Fortress, Educational Life Science Laboratory, Kaunas, Lithuania
2 - Kaunas Jonas Jablonskis gymnasium
saulepak7@gmail.com, auguste.cekanaviciute@gmail.com

Introduction. Antibiotic resistance is an urgent global public health threat, killing at least 1.27 million people worldwide and associated with nearly 5 million deaths in 2019 [1]. It needs to be regulated and the source of it needs to be studied. It is known that antibiotic resistance is mainly caused by pollution, thus to solve the issue first we need to know what kind of pollution causes it and where it comes from [2].

Initial mechanisms of resistance to antibiotics among pathogens are mostly via chromosomal mutations leading to the overexpression of efflux pumps. High-level resistance is achieved only after the acquisition of flavin-dependent monooxygenase-encoding genes from the environmental microbiota [3]. This is a reason for researching oxygenase enzymes alongside antibiotic resistance. In addition, these enzymes also have the potential to be a biomarker of pollution [4]. However, there is a lack of research on this topic, creating a gap of knowledge that narrows our insight into the antibiotic resistance issue. The above-mentioned arguments prompted us to plan this pilot study to see if we can claim the link between antibiotic resistance and water pollution in Nemunas river.

Goal. To determine the concentration of antibiotic-resistant bacteria in different locations of the river Nemunas and to compare the data to levels of multiple pollutants in the same areas. To assess the relationship between the production of different oxygenase enzymes and antibiotic resistance in bacteria.

Methods. The sampling locations are based on the research on pollution levels in the river that is done by the municipality of Kaunas. Antibiotic resistance is measured by planting the samples on Petri dishes with no antibiotic, 3 µL/ml, and 30 µL/ml of tetracycline after serial dilutions. The colonies are calculated using the "ImageJ" application. The oxygenase enzymes are recognized by growing samples on Petri dishes containing Indole (purple pigment made), 2-methylindole (yellow pigment made), 5-bromoindole (blue pigment made), and 5-nitroindole (brown-red pigment made). DNA from the bacteria is extracted using the Zymo research Quick-DNA Miniprep Kit. The 16s region is duplicated using the PCR method and then tested by electrophoresis with a 1,5% gel and sent off to be sequenced by Sanger's method. The sequencing results are compared to the SILVA 16S database to determine the species.

Results. Analysis of this pilot study data showed that the concentrations of antibiotic-resistant bacteria colonies were different throughout the river and the concentration at the start of the city of Kaunas was lower than at the end of the city (medium-resistant bacteria went from 0,65% to 9,01%). When compared with the accessible pollution data of the same locations, no strong connection could be found, so we do not know yet what pollutant is responsible for this resistance. In the future, we are planning to also put the indole substrates with the antibiotics, to see what percentage of the resistant bacteria use the tet(x) mechanism of resistance.

The bacteria that exhibited oxygenase-producing properties were determined to be: *Janthinobacterium lividum*, *Pseudomonas poae*, *Paenibacillus pabuli,* and *Flavobacterium panacisoli.* None of these bacteria grew on a Petri with 30 µL/ml of tetracycline. However, we did not manage to do any further experiments with these bacteria.

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ETERINIŲ ALIEJŲ ANTIBAKTERINIŲ SAVYBIŲ PRIEŠ KARIESO SUKĖLĖJĄ *STREPTOCOCCUS MUTANS* TYRIMAS

Lijana Airapetian[1], Vladimir Orlov[2]

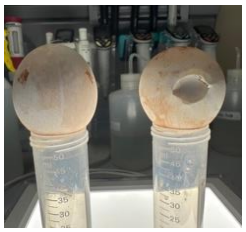
[1]Kauno tvirtovės VII fortas, Edukacinė biochemijos laboratorija
Archyvo g. 61, Kaunas, LT 50157, Lietuva
lijanaairapetian@gmail.com

Įvadas. Pasaulio sveikatos organizacijos (PSO) duomenimis (2022 m.) apskaičiuota, kad burnos ertmės ligomis serga beveik 3,5 mlrd. žmonių visame pasaulyje, o 3 iš 4 sergančiųjų gyvena vidutines pajamas gaunančiose šalyse. Apskaičiuota, kad pasaulyje 2 mlrd. žmonių kenčia dėl nuolatinio dantų ėduonies, o 514 mln. vaikų - dėl pieninių dantų ėduonies [1]. Nors burnos ertmės ligų iš esmės galima išvengti, jos yra didelė sveikatos našta daugeliui šalių ir paveikia žmones visą gyvenimą, sukeldamos skausmą, diskomfortą, subjaurojimą ir net mirtį. Svarbu paminėti, jog burnos sveikatos sutrikimų gydymas yra brangus ir nėra įtrauktas į visuotinio sveikatos draudimo (VSS) sistemą [2]. Streptococcus mutans - pagrindinė karieso sukėlėja, prisitaikanti prie rūgščios aplinkos ir fermentuojanti sacharozę, dėl to demineralizuoja dantų emalį [3]. Antibiotikų naudojimas prieš šią bakteriją - neefektyvus ir rizikingas dėl atsparių štamų atsiradimo. Tačiau eteriniai aliejai ne tik efektyviai slopina Streptococcus mutans augimą, bet ir gerokai mažesnėse koncentracijose už MSK paveikia šių bakterijų metabolizmą eliminuojant jų patogeniškumą

Tyrimo tikslas. Ištirti eterinių aliejų antibakterinį poveikį prieš Streptococcus mutans ir nustatyti jų galimybę slopinti kariesą sukurtoje modelinėje sistemoje.

Tyrimo metodai. Eterinių aliejų antibakterinis poveikis buvo nustatomas naudojant serijinį skiedimo metodą, matuojant minimalią slopinančią koncentraciją (MSK). Buvo sukurta karieso modelinė sistema, kurioje buvo naudota Todd-Hewitt-Bouillon mitybinė terpė, Streptococcus mutans bakterijos, kininių cinamonų žievės eterinis aliejus bei kiaušinis. Sukurtas modelis buvo stebimas tris savaites ir buvo vertinami gauti vizualūs tyrimo rezultatai.

Rezultatai. Tarp visų šešių ištirtų eterinių aliejų, tyrimų rezultatai atskleidė, kad kininių cinamonų žievės eterinis aliejus efektyviai veikia prieš Streptococcus mutans bakterija net 0,015625% koncentracijoje (Lentelė Nr. 1.). Karieso modeline sistema parodė, kad šis aliejus efektyviai apsaugo kiaušinio lukštą nuo bakterijų sukeliamos erozijos. Kiaušinio lukštas buvo stipriai pažeistas, augant mitybinėje terpėje be eterinio aliejaus (1 pav.). Tačiau terpėje su eteriniu aliejumi, net esant dešimt kartų mažesnei nei minimali slopinamoji koncentracija (MSK) eterinio aliejaus koncentracijai, buvo indukuotas bioplėvelės formavimasis. Tai atskleidžia bakterijų adaptaciją prie nepalankių sąlygų (2 pav.).



1 pav. Kiaušiniai po 3 savaičių inkubacijos. Kairėje yra kiaušinis, kuris buvo terpėje su eteriniu aliejumi, o dešinėje – buvęs terpėje be eterinio aliejaus.



2 pav. Vaizdas mitybinėje terpėje po 3 savaičių inkubacijos su eteriniu aliejumi ir be jo.

Trivialus Pavadinimas	Taksonominis Pavadinimas	Minimali slopinanti koncentracija (MSK)
Kvapniųjų bazilikų	Ocimum basilicum	0.125%
Tikrųjų citrinžolių	Cymbopogon citratus	0.25%
Burboniškų pelargonijų	Pelargonium graveolens	0.5%
Raudonėlių	Origanum vulgare	0,03125%
Gvazdikėlių	Syzygium aromaticum	0,03125%
Kininių cinamonų žievės	Cinnamomum aromaticum	0,015625%

Lentelė Nr. 1. Įvairių eterinių aliejų minimali slopinanti koncentracija (MSK) Streptococcus mutans atžvilgiu.

Išvada. Kininių cinamonų žievės eterinis aliejus efektyviai veikia prieš *Streptococcus mutans* bakteriją, rodanti stiprų antibakterinį potencialą karieso prevencijai ir gydymui. Eterinis aliejus veikia bakterijų metabolizmą ir vietoj to, kad ardytų kiaušinio lukštą, mikroorganizmas pradeda sintetinti bioplėvelės susidarymui būtinus polimerus.

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Blue light emitting diodes synthesis pathway search and performance using „Synthia“ (artificial intelligence)

Viltė Butkevičiūtė[1], Marta Marčiulionytė[1]

[1]Kauno tvirtovės VII fortas, Archyvo g, 61 Kaunas

vilebutk@gmail.com

Background: Artificial intelligence – modeling of human intelligence in machines programmed to perform tasks that usually require human intelligence.

„Synthia” – retrosynthesis software that allows scientists to quickly and efficiently find innovative ways to create new molecules.

Aim(s): Find the most suitable synthesis path using AI and perform a blue light emitting molecule synthesis according to the found pathway.

This molecule (Figure 1) was chosen because of carbazole property to emit blue light [1]. Also, nitrogen heteroatoms deepen the color, pushes the shift towards the short waves. This is determined by the ingress of nitrogen into the conjugated system. Compounds with a low molar mass were chosen, as they are more stable [2].

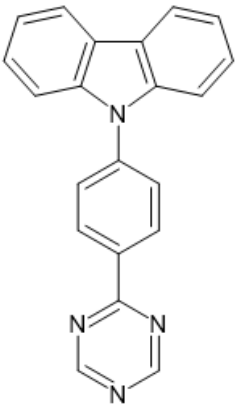

Figure 1. 9-[4-(1,3,5-triazin-2-yl)phenyl]-9H-carbazole.

Modified structure (Figure 2) has longer conjugated carbon atom chain with supporting nitrogen atoms. The structure of molecule suggests its ability to effectively emit blue light.

The suggested synthesis path for modified structure described in Figure 3.

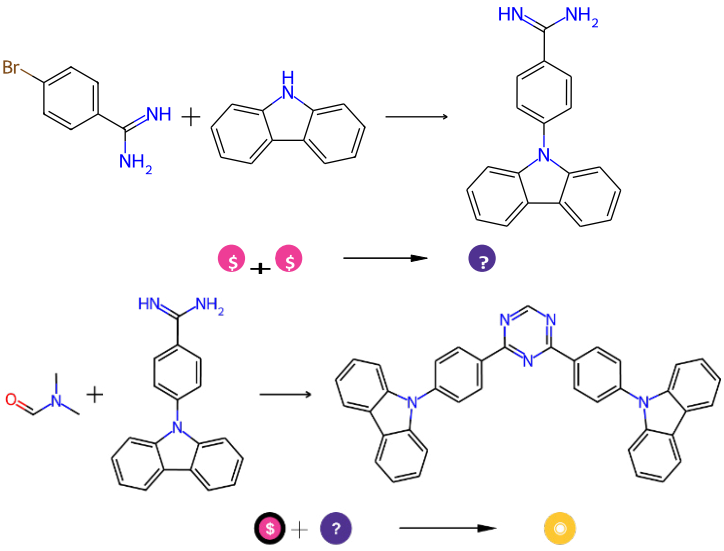

Figure 3. Synthesis path for modified structure generated by "Synthia".

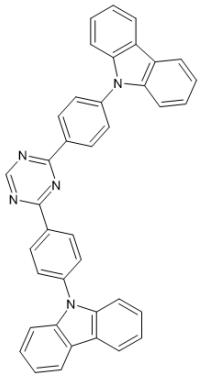

Figure 2. Modified structure.

HPLC-MS analysis confirmed the successful synthesis of the modified structure. Intermediate compound with a molar mass of 285 Da ([M+H]=286 Da; [M-H]=284 Da). The modified structure's mass was successfully measured at 594 Da ([M+CH_3O^-]) as the real molar mass is equal to 563 Da.

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ANTHOCYANINS-ENRICHED INDICATOR BIOPLASTIC FOR ASSESSING THE QUALITY OF DAIRY PRODUCTS

Gertrūda Narauskaitė[1], Marta Marčiulionytė[1]

[1]Kauno Tvirtovės VII fortas, Archyvo g. 61, Kaunas
gertruda.n44@gmail.com

Aim: to develop bioplastic that alerts the consumer when the dairy product begins 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, a lot of meat and dairy products spoil quickly, and the expiration date becomes inaccurate. In this case, anthocyanins-enriched indicator bioplastic would change its color according to the pH level of the product. Anthocyanins are mixed into a biopolymer made out of glycerol and starch. Bioplastic naturally degrades in the environment, avoiding the formation of microplastics or their particles.

Anthocyanins are extracted from 50 grams of red cabbage, beetroot, hibiscus, or red rose using Soxhlet extractor (solvent – methanol or water). 2 grams of starch, 4 grams of glycerol, 1 milliliter of anthocyanin extract, and 19 milliliters of distilled water are mixed and heated. 3 milliliters of 0.1M HCl solution is added. The mixture is heated until it is thick and is neutralized (pH=7) using 0.1m NaOH. The mixture is spread onto the baking paper and left to dry. Anthocyanins are immobilized using sodium alginate and calcium chloride.

To determine the indicator properties of determine the indicator properties of the extracts, phosphate buffer solutions with pH 7.0-6.0 were prepared. 0.1 milliliter of buffer and 0,1 milliliter of extract were pipetted into wells, and color changes were observed (Figure 1). Red cabbage extracted showed promising results.

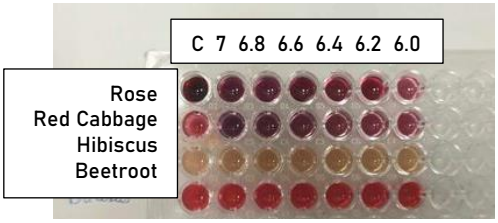

Figure 2. Indicator properties of different extracts using phosphate buffer.

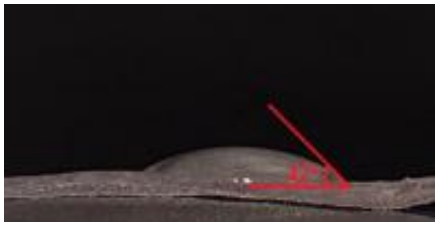

Figure 1. Contact angle.

To determine the hydrophobic properties of the bioplastic, a contact angle measurement was conducted (Figure 2.). Seven bioplastic films of different compositions were made. The highest contact angle was determined in "c2.2." film, where anthocyanins were immobilized using sodium alginate and calcium chloride.

The swelling degree of the c2.2. film was determined to be 146% based on the change of mass. Additionaly, anthocyanin imobilization was investigated, revealing that 0.016 grams of anthocyanins per 1 gram of film migrate into the solvent enviroment.

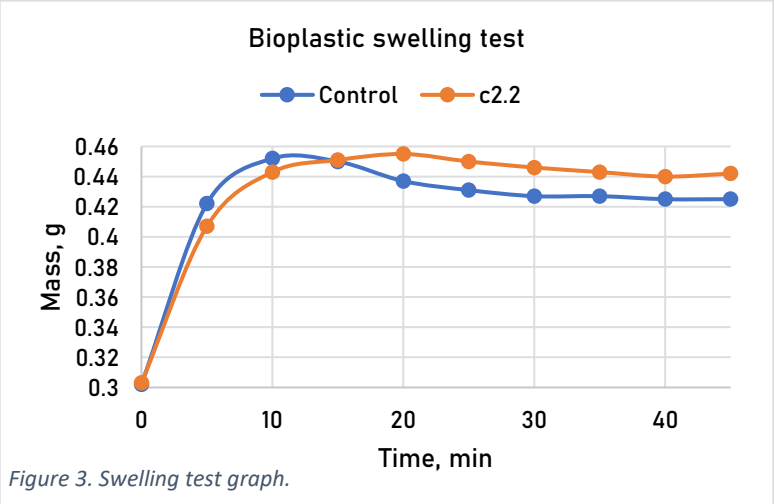

Figure 3. Swelling test graph.

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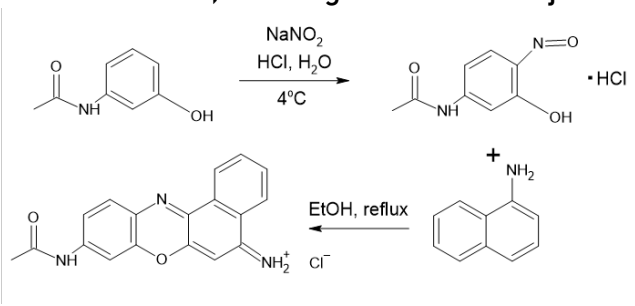
„DARROW RED“ DAŽO SINTEZĖS KELIO OPTIMIZACIJA IR PRITAIKYMAS DAŽANT SKIRTINGŲ GYVŪNŲ NERVINES LĄSTELES

Taja Zuozaitė; Marta Marčiulionytė
Kauno tvirtovės VII fortas, Archyvo g. 61, Kaunas
Kaunas, Lietuva

Mokslo sritis: chemija

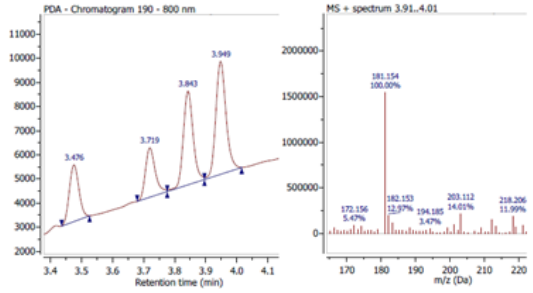
Įvadas: „Darrow Red“ yra paprastasis oksazino dažas, kuris gali būti naudojamas neuronų ląstelių kūnams dažyti[1][2]. Jo ypatumai panašūs į pagrindinius mėlynus dažus, taip pat šis dažas pasireiškia metachromazija. Priešingai nei gimininių junginių, šio dažo sintezės kelias nėra tiksliai aprašytas.

Darbo tikslas: atrasti efektyvų Darrow Red dažo sintezės kelią bei gryninimą



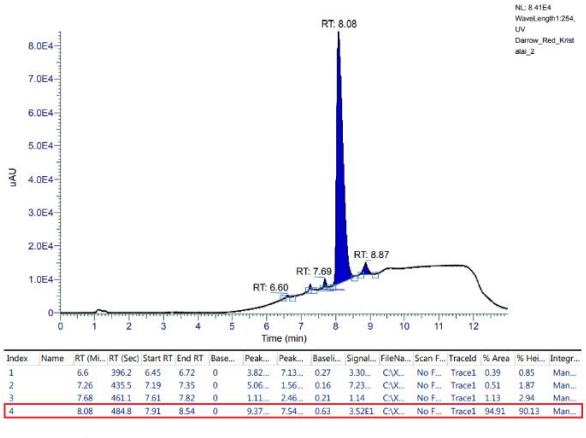
Pav. 1. Tarpinio junginio sintezė tiesioginio nitrozinimo būdu ir “Darrow red” sintezė iš tarpinio junginio

Metodai: Tyrimo metu buvo išbandytos 3 skirtingos reakcijos siekiant gauti tarpinį Darrow Red junginį - sintezės su T-BOC, naudojant skirtingas sąlygas ir tiesioginis nitrozinimas. Nustatyta, jog iš bandytų metodų tiesioginis nitrozinimas buvo efektyviausais būdas susintetinti tarpinį junginį. Toliau buvo vykdyta sintezė su gautuoju tarpiniu junginiu ir 1-naftilaminu, vykdant refluksą (pav. 1). Gautas mišinys buvo filtruojamas vakuuminiu filtru ir gauti kristalai surenkami. Norint labiau išgryninti kristalus jie buvo pakartotinai plaunami šiltu heksanu tol, kol tirpiklis po praplovimo išliko skaidrus. Atlikus sintezę buvo apskaičiuota, jog išeiga = 50%



Pav. 2. Tarpinio junginio HPLC-PDA-MS analizė

Rezultatai: Norint įsitikinti, jog buvo susintetintas norimas tarpinis junginys, buvo atlikta HPLC-PDA-MS analizė (pav. 2). Gautoje chromatogramoje: Rt=3,4 min – metacetamolis, Rt=3.9min - tarpinis junginys, kurio molinė masė yra 181,15. Gauto tarpinio junginio molinė masė sutinka su jo teorine moline mase, taigi gautas norimas tarpinis junginys. Susintetintam „Darrow red” buvo atlikta HPLC-MS analizė, siekiant patvirtinti, jog tikslinis junginys buvo susintetintas. Teorinė junginio masė atitinka 303 molekulinę masę, gautą HPLC-MS analizės metu. Remiantis gauta „Darrow Red“ po gryninimo chromatograma ir spektrograma nustatytas 95% grynumas (pav. 3). Susintetintas dažiklis gali būti pritaikomas gyvūnų nervinių ląstelių bei vėžinių ląstelių dažymui. Taigi, iškeltas tyrimo tikslas atrasti efektyvų Darrow Red dažo sintezės kelią bei šio dažiklio gryninimą buvo pasiektas.



Pav. 3. gauto „Darrow Red“ grynumo analizė naudojant HPLC-UV/Vis

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EPITRANSCRIPTOMIC STUDIES OF BRAIN TUMOR: ANALYSIS OF GENE EXPRESSION IN TUMOR CELLS AND TUMOR TISSUES

Kamilė Milkintaitė[1,2], Rūta Urbanavičiūtė[3]

[1] Kaunas University of Technology Gymnasium, Kaunas, Lithuania
[2] Seventh Fort of Kaunas Fortress, Kaunas, Lithuania
[3] Lithuanian University of Health Sciences, Kaunas, Lithuania
k.milkintaite1@gmail.com

Introduction. Glioma, a highly aggressive brain tumor with a median survival of just 14.6 months, lacks effective treatment options, leading to high mortality rates [1]. A new focus of research is RNA methylation and its regulating genes that are believed to be potential targets for early diagnosis and targeted gene therapy, offering the possibility of earlier cancer detection and more effective treatment for patients [2]. While gliomas were traditionally thought to develop from astrocytes, there is a theory that stem cells can acquire astrocyte-like properties [3]. The pluripotency and ability of these cells to maintain an undifferentiated state prevent the complete eradication of tumors, contributing to the high mortality of this cancer [4]. The transcription factor SOX2 is considered essential for the development of stem cells, and its expression increases in cases of glioblastoma [5-6]. However, there is limited data on the influence of such biomolecules determining cell stemness on the epitranscriptome and the epigenetic changes occurring in gliomas that drive their development or progression. Therefore, understanding RNA methylation regulators' molecular mechanisms and their impact on cellular processes is crucial for treatment tailoring.

Aim. To investigate the differences in the expression of *ALKBH5* and *FTO* demethylases, as well as *BUD23* and *METTL1* methyltransferases, in postoperative glioma tumor tissues samples with varying degrees of malignancy, and in a modified glioblastoma U87 cell line. To determine the effects of inhibition of the *ALKBH5* methylation regulator on biological processes using RNA-sequencing data analysis.

Methods. The methodology involves extracting iRNA using Trizol, synthesizing cDNA from RNA using reverse transcriptase enzymes. Additionally, primer design was performed using bioinformatics tools such as the "Ensemble" database and the "PerlPrimer" program. Gene expression analysis was conducted using real-time PCR with SYBR Green detection, allowing for quantitative assessment of gene expression. Results were statistically evaluated using the Kolmogorov-Smirnov test, Mann-Whitney criterion, t-test, Kaplan-Meier method, and chi-square criterion. Statistically significant results were considered when the p-value was ≤ 0.05. Furthermore, RNA sequencing data analysis from "NCBI SRA" database was performed to assess the impact of disrupting the methylation process by suppressing *ALKBH5* gene expression on glioblastoma cells, their gene expression, and biological processes.

Results. The *ALKBH5*, *FTO*, and *BUD23* gene expressions were directly linked to the malignancy level of brain tumors, suggesting their potential as biomarkers or targets for glioma treatment. Patients with higher expression levels of these genes tended to survive longer. However, *METTL1* expression showed no such association with tumor aggressiveness or patient survival. Additionally, significant correlations were observed between the expression of these genes and various factors such as IDH mutation, patient gender, age, and survival duration. The SOX2ΔC protein's expression significantly impacted the regulation of RNA methylation modifiers, particularly *ALKBH5* and *BUD23*. Furthermore, suppressing *ALKBH5* gene expression in glioblastoma cells led to significant changes in the expression of numerous genes involved in crucial biological processes such as DNA replication, mismatch repair, Fanconi anemia, cell cycle and homologous recombination, suggesting a profound influence on tumor biology.

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Al^{3+}, Hg^{2+} ION DETECTION USING AN ORGANIC DYE

Kotryna Ašipauskaitė[1], Marta Marčiulionytė[1]

[1]Kauno tvirtovės VII fortas, Organinės chemijos ir instrumentinės analizės edukacinė laboratorija
Archyvo g. 61, Kaunas, LT 50157, Lietuva
kotrynaas10@gmail.com

Transition metals, including aluminum and lead, are extremely toxic. Even small concentrations in an organism can damage organs and disrupt metabolism and the immune system. In addition, these elements are carcinogens. It enters the body primarily through the air, water, soil pollution, and food.[1] That is why research on detecting these metals is of great significance. Currently the most widely used are spectroscopic and electroanalytical methods, such as atomic absorption/emission spectroscopy, and inductively coupled plasma mass spectroscopy (ICP-MS). The high cost, complex sample preparation, and limited availability of these methods is a significant drawback [2]. However, using an organic dye Brooker's Merocyanine is a cheaper, quicker, and more accessible way of detecting heavy metals.

This research aims to create a method of Al^{3+}, Pb^{2+}, and Hg^{2+} ion detection using an organic dye Brooker's Merocyanine.

First, a three-stage Brooker's Merocyanine synthesis was carried out. Next, to determine the interaction of Brooker's Merocyanine dye with metal ions spectrophotometric analysis was used. Once the dye is mixed with various transition metal solutions, the absorption shifts bathochromically or hypsochromicaly (Figure 1). The color intensity of the dye and metal ion complex can help determine the concentration of heavy metal ions in the test sample. It was noticed, that Al^{3+}, Hg^{2+} together with the dye form clear complexes. Brooker's Merocyanine water solution absorption maximum was determined at a wavelength of 374 nm. It was used later in developing the calibration curve of 1-25 mg/l aluminum, and 1-10 mg/l lead solutions. This metal detection method was applied heavy metal detection in plants. With Brooker's Merocyanine, it was possible to determine, how much metal ions were absorbed by plants from the soil (Figure 2 and Table 1)..

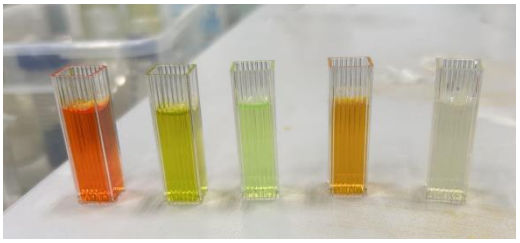

Figure 1. *Brooker's Merocyanine complexes with metal solutions. From left to right: 0,1 mmol/l Brooker's Merocyanine solution; 0,1 mmol/l Ni^{2+}, Cu^{2+}, Co^{2+}, Al^{3+} solutions combined with dye 1:1 ratio.*

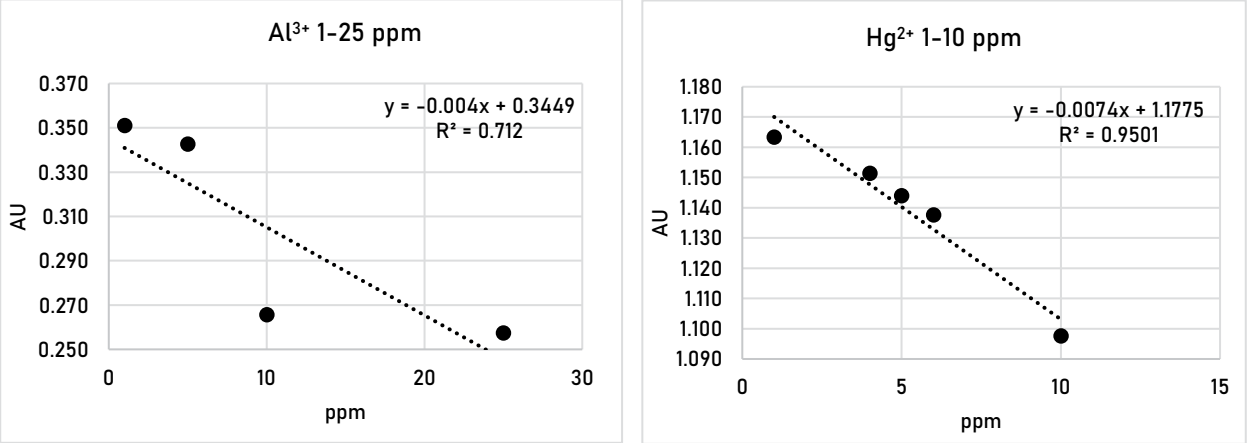

Figure 2. *Calibration curve for the calculation of Al^{+3} and Hg^{2+} ion concentration in plants.*

Table 1. *Results of determining Al+3 and Hg+2 ions concentration in plants.*

Plant	Ion	Absorbance, Au	Concentration, ppm	Plant	Ion	Absorbance, Au	Concentration, ppm
Pea	-	1.174	0	Cucumber	-	1.169	0
	Al^{+3}	0.328	4,2		Al^{+3}	0.286	14,7
	Hg^{+2}	1.153	3.3		Hg^{+2}	1.102	12,2

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April 17th

Oral Presentations

Enviromental Sciences

WHAT DOES IT FEEL TO BE A STRESSED PLANT?

Vilmantas Pupkis[1], Indrė Lapeikaitė[1], Vilma Kisnierienė[1]

[1]Department Neurobiology and Biophysics, Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
vilmantas.pupkis@gmc.vu.lt

Plants constitute an integral part of the majority of both terrestrial and aquatic ecosystems. Consequently, any external stressors may exert a profound effect not only on the functioning of a particular plant species but also on a variety of other ecologically interconnected organisms, which is particularly important considering the overarching effects of the global climate change [1].

When a plant is locally stressed by an external non-damaging stimulus, an action potential (AP) is generated. This electrical signal propagates along the plant body and modulates various physiological functions such as the synthesis of stress hormones or the modulation of the photosynthetic organisms. During the AP generation in photoautotrophs, the initial plasma membrane depolarization is caused by Ca^{2+} influx into the cytoplasm. In higher plants, this is achieved via glutamate receptor-like (GLR) channels. However, these channels are absent in aquatic Characean macroalgae that exhibit prominent electrical excitability. Therefore, Ca^{2+} channels of an unknown molecular identity must participate in the electrogenesis.

In order to investigate the initiation of excitation in this evolutionary taxon, we investigated the effects of various putative Ca^{2+} channel modulators on the electrical excitation parameters of the internodal cells of *Nitellopsis obtusa*. Using classical two-electrode current/voltage clamp techniques, we recorded APs and excitation current transients.

Our results demonstrate that Ca^{2+} channel blockers verapamil, tetrandrine, and NED-19 depolarized the AP excitation threshold. Additionally, they prolonged the temporal characteristics of the electrical excitation, revealing non-specific inhibitory effects of these pharmacological agents. In contrast, putative activators inositol trisphosphate (IP_3) and inositol hexakisphosphate (IP_6) hyperpolarized the AP excitation threshold, pointing to the activation of Ca^{2+} channels.

While classical animal IP_3 receptor homologues are absent in plants and algae, our research revealed that Ca^{2+} channel modulation by inositol phosphates plays a significant role in the unique Characean electrogenesis. Insights provided by this research refine the fundamental understanding of the mechanism of plant electrical signalling, which can be applied in artificially controlling plant stress levels.

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03

DETECTION OF *SARCOCYSTIS* PARASITES IN THE ENVIRONMENTAL SAMPLES

Agnė Baranauskaitė, Petras Prakas,
Dalius Butkauskas, Elena Servienė, Živilė Strazdaitė-Žielienė

[1]Nature Research Centre, Akademijos st. 2, Vilnius, Lithuania
agne.baranauskaite@gamtc.lt

Parasites of the genus *Sarcocystis* are unicellular microorganisms that infect mammals, birds, and reptiles, and are characterized by an obligatory life cycle of two hosts, intermediate and definitive. *Sarcocystis* parasites can enter the animal or human body with food or by drinking or bathing in sporocyst-contaminated water [1]. To date, research on *Sarcocystis* parasites has mainly been carried out by analyzing carcass samples using morphological or molecular methods. Until recently, there was very little data on *Sarcocystis* studies from environmental samples [2,3]. Therefore, the aim of our research was to optimize the molecular identification methodology for *Sarcocystis* parasites targeting the *cox1* gene and apply it to fast and accurate diagnostics of environmental samples.

During initial studies, 114 water samples were collected and used to optimize the process of sporocyst collection and molecular identification. Filtration of water samples and nested PCR using four different species-specific primers were selected as the most specific method for the detection of *Sarcocystis* parasites DNA in water bodies. Subsequently, 150 samples from 5 groups of water bodies were analyzed and it was found that the type of water body does not affect the prevalence of these parasites. The DNA of nine *Sarcocystis* species was detected in the tested samples, and it was found that species infecting cattle and sheep were most often found in the samples, species infecting goats with an average frequency, and species infecting pigs and horses the least often. Moreover, it was decided to investigate whether the cysts in water bodies settle to the bottom and can be detected in sediment samples. During this study, 99 sediment samples collected in the Baltic States and Poland were analyzed. Based on molecular methods, parasites were detected least often in samples from Estonia (50%), while in other study countries detection rates were comparatively high and ranged from 88% to 100%.

Considering the results of these two studies, it was decided to carry out research with environmental samples collected from domestic animal farms in Lithuania. Ten livestock farms were selected, and water, hay and soil samples were collected from them. Before genomic DNA isolation, sporocysts in water and hay samples were concentrated by filtration, while soil samples were used for direct gDNA isolation. Based on results, the highest occurrence rate of Sarcocystis parasites' DNA was found in the hay (43%) and water (40%) samples, meanwhile the lowest number was detected in samples from soil (31%). Mostly, five (40%) or six (30%) different species were identified in individual farms, rarely three (20%) or seven (10%) species. To summarize, the most common *Sarcocystis* species in livestock farms in Lithuania were *S. cruzi* (53%), *S. arieticanis* (50%), and *S. bertrami* (50%).

These studies are the first not only in Lithuania, but also in the whole world, during which species of the *Sarcocystis* genus infecting domestic animals were identified in environmental samples. It is important to develop and optimize research methodologies for detection of these parasites in the environment. If there is a possibility to examine animal's environment, it is not necessary to kill animal itself to carry out carcass examinations. Carrying out such tests would be an excellent prevention of the spread of infection in farms and economic losses.

This project has received funding from the Research Council of Lithuania (LMTLT), agreement [S-MIP-23-7].

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ERYTHROCYTES SIZE AND SHAPE CHANGES IN FISH EARLY-LIFE STAGES AFTER EXPOSURE TO MICROPLASTICS

Agnė Bučaitė[1,2], Milda Stankevičiūtė[1]

[1]Laboratory of Ecotoxicology, Nature Research Centre, Vilnius Lithuania,
[2]Vilnius University Life Sciences Centre, Institute of Biosciences, Vilnius Lithuania
agne.bucaite@gamtc.lt

Vehicle traffic is often associated with air pollution in urban areas and exhaust emissions have been thoroughly investigated which led to their progressive decrease after many countries adapted effective mitigation strategies. Meanwhile, little attention has been paid to non-exhaust emissions of vehicles such as tire particles (TP) generated due to the contact and friction with the road. According to OECD non-exhaust particulate emissions from road transport will not decrease even after rise in electric vehicles and the issue can be considered as "an ignored environmental policy challenge" [1]. For aquatic ecosystems, the inevitable entry of TP into surface waters is a cause for concern due to their potential toxicity since TP are one of the most frequently observed microplastics (MPs) in areas heavily polluted by traffic [2].

The aim of this work is to evaluate changes in erythrocyte size and shape in rainbow trout (*Oncorhynchus mykiss*) early life-stages after the exposure to TP. *O. mykiss* embryos were exposed directly to the particles (size ≤ 0.250 mm) for one month. After the exposure blood smears were prepared and stained slides were analyzed using Olympus BX51 (Tokyo, Japan) light microscope. *O. mykiss* erythrocytes were evaluated with the ImageJ 1.52av software [3]. Analysis was carried out according to procedure described by De Oliveira et al. (2020) [4]. In brief, random photomicrographs were taken and one hundred cells were analyzed for each fish. Analyzed morphometric variables were erythrocyte area, perimeter, aspect ratio and circularity. Statistical analysis was performed using R (version 4.3.2) software.

We observed a significant increase in erythrocyte area, perimeter, as well as a significant change in cellular circularity. However, there were no significant changes in aspect ratio. Hematological parameters play a crucial role in assessing the physiological state of vertebrate animals. Therefore, changes in erythrocyte size (area and perimeter) as well as shape (aspect ratio and circularity) could prove to be useful biomarkers of exposure to xenobiotics. Moreover, morphometric measurements of red blood cells, along with physiological and biochemical indices in blood, can serve as effective tools for prognostic and diagnostic purposes in assessing fish health status. Additionally, they can function as early warning signs of pollution and physiological biomarkers of organ dysfunction in biomonitoring surveys.

Study was funded by the Research Council of Lithuania through the project S-MIP-22-51 (ARFA).

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THE IMPACT OF AVIAN MALARIA INFECTION ON THE GUT MICROBIOTA OF THE VERTEBRATE HOST

Justė Aželytė[1], Apolline Maître[2,3,4], Lianet Abuin-Denis[2,5], Elianne Piloto-Sardiñas[2,6], Alejandra Wu-Chuang[2], Rita Žiegytė[1], Lourdes Mateos-Hernandez[2], Dasiel Obregon[7], Alejandro Cabezas-Cruz[2], Vaidas Palinauskas[1]

[1]P. B. Šivickis Parasitology laboratory, Nature Research Centre, Lithuania.
[2]UMR BIPAR, French Agency for Food, Environmental and Occupational Health & Safety, France.
[3]National Institute for Agricultural Research, Food and Environment, France.
[4]Laboratory of Virusology, Université de Corse, France.
[5]Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology, Cuba.
[6]Direction of Animal Health, National Center for Animal and Plant Health, Cuba.
[7]School of Environmental Sciences, University of Guelph, Canada.
juste.azelyte@gamtc.lt

Recent studies have shown that parasite infections can affect the immune system and physiology of the vertebrate host through induced changes in the microbiota. Although avian malaria is a frequently encountered infection in wild birds, capable of causing severe illness and sometimes host mortality, there is still a lack of information regarding the impact of *Plasmodium* parasites on avian gut microbiota. In this study, bacterial network analysis was employed to investigate whether the avian malaria parasite *Plasmodium relictum* influences the resistance of canaries' microbiota to colonization (Figure 1).

A study was carried out in which a group of canaries was experimentally infected with the *P. relictum* parasite. Blood and fecal samples were collected throughout the experiment. Microbiome analysis was performed using 16S rRNA amplicon units.

Preliminary results of the study revealed that avian malaria infection does not have a significant impact on the alpha diversity of avian microbiota, but significant differences in beta diversity metrics were observed between infected and control birds. Microbiome analysis identified specific taxa characteristic of each group and differences in the abundance of certain bacteria. Compared to the control group, interactions between bacteria affecting network structure and stability were identified in the microbiota of infected birds.

The obtained results indicate that avian malaria parasites modulate the host's gut microbiota, causing deviations from the normal microbiota state. Changes in avian microbiota during *Plasmodium* infection may be related to resistance to colonization.

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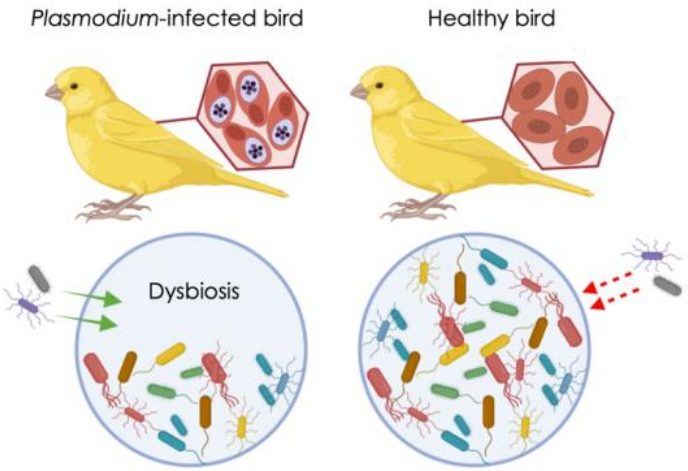

Figure 1. A potential impact of Plasmodium on avian gut microbiota.

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