International Conference of Life Sciences

The COINS 2022

Book of Abstracts

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FOREWORD

Dear Colleagues,

I'm excited to meet you at the 16th International Life Sciences Conference The COINS 2022!

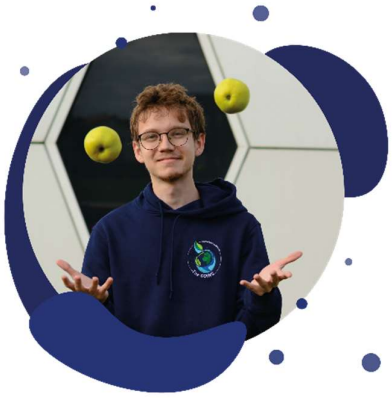
The past year has brought plenty of challenges to academia that we face to this day. Decrease of trust in science and scientists have a major impact on us that has resulted in division of our society. Living in the world of fake news and distorted facts, the scientific community should be the ones who guide everyone to think critically, observe the information and make fact based conclusions.

This year The COINS returns with an aim to bridge the gap between science and society. Since 2018 The COINS organizes activities for high school students who have a chance to participate in laboratory workshops, deepen their knowledge and be encouraged to learn and be curious. Last year for the first time high school students had an opportunity to present their scientific research and receive feedback together with students of life sciences. This year we will have this occasion once again to remind us that science is open for everyone.

Despite the importance of bringing society closer to science, it is very significant to help young people to succeed in academia. The opportunity for all the students of life sciences to present their scientific research, nonetheless of their experience, is particularly important for the students who have only begun their involvement in scientific activities. We are particularly happy that this year we have a record number of students' presentations. It shows that there are more and more people who are passionate about science.

Finally, I'd like to thank my team for all the hard work, so that everyone can enjoy the celebration of knowledge.

Spread the news and science!



Sincerely,
Daniel Šematovič
Executive Project Manager of The COINS 2022

Dear Colleagues, Honorable Guests, Ladies and Gentelmen,

It is a pleasure and great privilege to welcome The COINS 2022 conference, which is organized for the sixteenth time, by the Student representative at the Life Sciences Center. The Conference takes place in a very special time. We are about to enter into the third year of COVID-19 pandemic. The pandemic which disrupted many areas of social activities as well as many academic events. Again and again we are witnessing the power of Life Sciences to address global challenges such as COVID-19 pandemic. During very short period of time numerous vaccines, some based on novel biochemical principles, were designed, the antiviral drugs are available to patients. All this attest for the importance of scientific inquiry, and dissemination and sharing of knowledge between countries and people.

Unfortunately, just few days ago Europe and whole world was shocked by the news from Ukraine, which became a victim of the biggest military assault since the end of the second world war. The assault organized by criminal regime in Kremlin threatens to totally disrupt peaceful social and economic development, which people in Europe enjoyed for the last 75 years. The war if not stopped will inevitably affect academic life as well. It will degrade possibilities of European societies to support studies and research, including research infrastructures, many young people instead of going to universities will go to the war as it happens right now in Ukraine. Welcoming our conference in peaceful Vilnius, on behalf of the Life Sciences center community, I express full solidarity with academic communities in Ukraine. Life Sciences center is open for the assistance actions to alleviate the academic loses of Ukrainian students.

The COINS 2022 being organized for the sixteenth time will feature a large number of the most prominent speakers from all across the world with the amazing scope of scientific themes: from neuroscience to astrobiology, from computational biology to genes and molecular machines. And even though most of the sessions will take place online, I am confident that The COINS 2022 will bring together young enthusiasm and mature epxerience together, and this will provide ample of opportunities for networking, exchanging of ideas, asking questions and sharing experiences. So, ladies and gentelment, welcome to the Life Sciences Center ONLINE , welcome to the COINS 2022!

Gintaras Valinčius
Director of Vilnius University Life Sciences Centre

It is my great honor to congratulate honorable guests and participants of The COINS conference and welcome you to the one of the biggest International Conference of Life Sciences organized in Lithuania. This conference has been growing strong every year since 2004 and gathers not only the greatest scientists from all over the world, but also provides opportunities for students to present their research and build a network for their future growth. As representative of Lithuanian biotechnology association, I would like to share my admiration for Lithuanian students for organizing such a prestigious conference and creating such an important platform for researchers.

Lithuania's life science sector strength lies in the people. In the last decade Lithuania's life science sector growth was one of the fastest in Europe, with an annual growth of 22%. In 2020, in the face of a pandemic, scientists and businesses put their best effort to fight the coronavirus outbreak and the biotechnology sector reached record growth of 87% in Lithuania. Currently, the biotechnology sector generates almost 2,5% of the country's GDP and is becoming one of the most important sectors in the country. As it should be. Academic and business collaborations allow for us to seek continuous growth and international collaborations create possibilities to be a global partner for creating innovations.

During this week topics such as neuroscience, gene editing, astrobiology and others, including discussions of future of life science will be heard. I am certain that the COINS conference brings the richest content and the most distinguished speakers, who will not only share their latest achievements but also will inspire a new generation of scientists to pursue their academic and personal goals. This part I love the most about the COINS conference. Sharing that light of scientific findings that allure most of us that gathered here. Together, strengthening the network of scientists, entrepreneurs and businesses – building an international community of extraordinary people that create and deliver innovation for the benefit of the society.

I would like to wish everyone, from first year students to honorable professors, to soak in as much of professional and personal value as you can during this week, whether it is knowledge, ideas, collaborations or inspirations you seek. I hope that next year it will be possible to meet again face-to-face in Vilnius University Life Science Center and share the new achievements in Life Sciences.

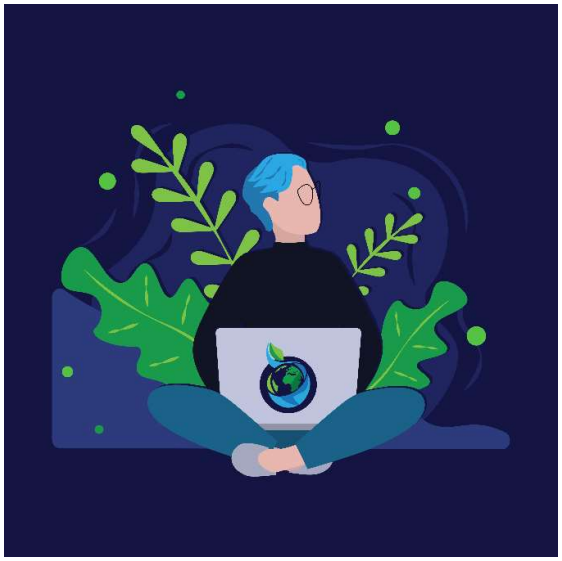
Agnė Vaitkevičienė
Executive Director and Vice-President at Lithuanian biotechnology association

Dear students, colleagues, and honored guests,

it is a great pleasure for me to welcome You all here at THE COINS 2022. First of all, I would like to thank our invited speakers for their support shown for Vilnius University students in their first steps while entering the scientific society. For many of them, this is the first, real, big, international conference. From the little seed that our students planted a long time ago, this event grew up to the one that today is attracting the greatest scientific minds from all over the world. Thank You all for letting this seed grow into a beautiful tree of science and knowledge. As a professor of the Life Sciences Center, I feel thankful for the opportunity to leave my little fingerprint in the student education and the pathway to science. In the name of the professors here at Vilnius University Life Sciences Center I can say: we are proud of You and looking forward to seeing Your achievement!

Eglė Lastauskienė
Managing Director at Vilnius University Life Sciences Centre Institute of Biosciences

PRE-CONFERENCE EVENTS

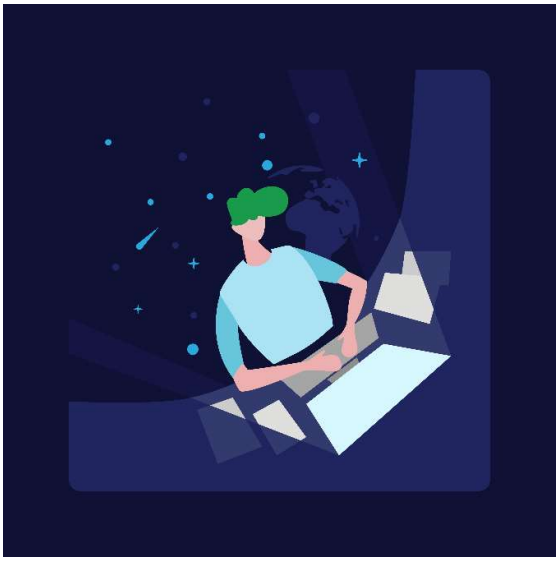


Lecture Cycle for High-School Students

We all know how stressfull high-school exams are, right? Therefore, scientists of Vilnius University Life Sciences Centre held lectures to help students prepare! The most curious and dedicated students had a chance to deepen their knowledge on topics such as neurobiology, genetics, cell biology and immunology.

Scientific writing workshop

What about University students? Those who were looking forward to writing their first paper, gathered at the Vilnius University Life Sciences Centre to learn some tips on that during an engaging lecture, followed by a workshop.



KEYNOTE SPEAKERS

Michal Schwartz. The Two Decades That Revolutionized Brain Immunity: Implications for Immunotherapy to Defeat Alzheimer's Disease

For decades, the brain was considered an immune privileged organ, and as such, completely isolated from the immune system. Likewise, debilitating cognitive diseases were viewed as solely diseases of the brain, and specifically, as neuronal centric.

With the discovery that the brain needs immune cells for its maintenance and repair, and the subsequent finding of niches within the brain's borders that host immune cells, we have attained a new understanding of brain-immune relationships. Accordingly, we proposed that systemic immune aging impacts brain aging and speed of progression of neurodegenerative diseases, even if not the primary cause of any of these conditions. We further demonstrated that the choroid plexus epithelium, anatomically and functionally connecting the circulation and the brain, critically affects the brain's fate in aging and neurodegenerative diseases. Accordingly, we proposed harnessing the body's immune system as a novel target for defeating AD and Tauopathy. Specifically, we showed that reducing peripheral immune system exhaustion by blocking inhibitory immune checkpoints triggers a cascade of immunological events that activate a common mechanism of repair, regardless of the primary cause of the disease. Such activation, leads within the brain, to reduction in toxic misfolded proteins, reduced inflammation, rescue of neurons, and arrest of cognitive loss. We found that this approach is dependent on bone marrow-derived macrophages, and has the potential to overcome Trem2 polymorphism. The path towards translating this approach for therapies leading to disease modification will be described.

Laura Bojarskaitė. Nightlife of the Stars of the Brain – Astrocytes

While neuronal circuits are indisputably considered to be the computational integrators of behavior, a complete understanding of the nervous system must also incorporate the surrounding glial cells.

Astroglial function is far beyond passive support of neuronal circuits. Astroglia inextricably influences neuronal physiology from early development through guidance and synaptic shaping, and through entire life by structural and trophic support, immune function and extracellular homeostasis. With such essential functions astroglia are consequential to many different behaviors in adult animals. Sleep is no exception.

Sleep regulation has historically been admitted as a task exclusively for neurons. Changes in other types of brain cells, such as astrocytes, across the sleep-wake cycle and their role in sleep regulation are comparatively unexplored. Understanding what molecular mechanisms and signaling pathways astrocytes employ during sleep, and how they influence sleep physiology and brain rhythms, is a central outstanding question in the field of sleep research. In my talk, I will discuss our findings where we for the first time ever described astrocytic signaling during natural sleep and showed that astrocytic activity during sleep is

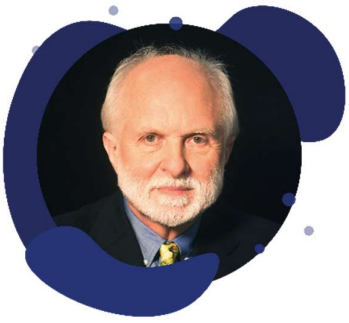
important to maintain uninterrupted slow wave sleep and to maintain proper brain rhythms during sleep, such as sleep spindles (Bojarskaite et al., Nat Comm, 2020).

Also, seminal studies from the last 10 years have demonstrated the importance of astrocytes in chaperoning brain extracellular fluid flow during sleep for clearing waste products accumulated in wakefulness in a process called glymphatic flow. In 2013 a follow-up study showed that the glymphatic system is almost exclusively active during sleep, and under certain types of anesthesia, but not in wakefulness. The regulation of glymphatic fluid flow and mechanisms that underlie its enhancement upon sleep are only rudimentarily understood. In my talk, I will also discuss our yet unpublished results regarding mechanisms that could underlie the enhancement of brain waste clearance during sleep.

Richard Youle. PINK1- And Parkin-Mediated Mitophagy in Neurodegeneration

PINK1 and Parkin, both mutated in familial PD, normally work intimately together to initiate autophagy of impaired mitochondria. When mitochondria are damaged, Pink1 senses the damage and accumulates specifically on the outer membrane of damaged mitochondria where it phosphorylates ubiquitin chains. These phosphorylated ubiquitin chains on the outer mitochondrial membrane bind to cytosolic Parkin and activate Parkin's E3 ubiquitin ligase activity yielding a feedback amplification loop that leads to autophagy of individual damaged mitochondria. Downstream of Parkin the machinery that mediates autophagosome recognition of damaged mitochondria links this pathway to genes mutated in ALS. Optineurin and the kinase TBK1, both mutated in familial ALS cases, participate in mitophagy in addition to NDP52. Optineurin and NDP52 bind to ubiquitin chains on mitochondria and also recruit autophagy machinery proteins, including the upstream kinase Ulk1 and the downstream autophagosome marker, LC3, to induce engulfment of the damaged mitochondria. NDP52 binds and recruits Ulk1 complex to initiate autophagosome biogenesis proximal to damaged mitochondria. Interestingly, in a murine model of exhaustive exercise, the product of the kinase PINK1 (phospho-S65 ubiquitin) is increased in the heart and this requires both endogenous PINK1 and Parkin expression representing a biomarker of PINK1 activity. Strategies to increase mitophagy as a defense against Parkinson's disease will be discussed.

Albert James Hudspeth. Your Biological Hearing Aid



As the gateway to verbal communication, the sense of hearing is of enormous importance in our lives. Hearing commences with the capture of sound energy by hair cells, the ear's sensory receptors, which convert that energy into electrical signals that the brain can interpret. Uniquely among our sensory receptors, the hair cell is not a passive recipient of stimuli, but instead uses an active process to enhance its inputs. The active process amplifies acoustical stimuli, sharpens frequency selectivity, and broadens the range of audible sound intensities. When the active process becomes unstable, most normal ears can even emit sound! Dr. Hudspeth will discuss the ear's normal operation, the basis of the active process, and efforts to restore damaged hearing through the regeneration of hair cells.

Armando Azua-Bustos. Searching for Life in the Atacama Desert, the Driest, Oldest and Most Martian Place on Earth

The Atacama Desert in northern Chile is by far the driest and oldest desert on Earth, showing a unique combination of environmental extremes which explain why the Atacama has been investigated as a Mars analog model for almost twenty years. In this talk I will share how it is to work in this magnificent desert, and the lessons learned on the limits of habitability of planet Earth.

Charles Cockell. Microbes in Space: Looking for Life and Human Settlement

In this talk I'll discuss the power of the microbial world to help us search for life beyond Earth and to help us implement the human exploration and settlement of space. In particular, I'll discuss some recent experiments on the International Space Station to study the use of microbes to do 'biomining' - extracting economically useful elements from the Moon, Mars and asteroids.

Daniel Angerhausen. Aliens, Exoplanets and Astrobiology

In my presentation I will give a non-expert introduction to the multi-disciplinary field of Astrobiology and in particular the science of extrasolar planets, planets orbiting stars outside our Solar System. I will describe my various projects in this emerging field using the largest ground based telescopes, the 'flying telescope' SOFIA (Stratospheric Observatory for Infrared Astronomy), the CHEOPS and JWST space telescopes the LIFE (Large Interferometer for Exoplanets) mission that I am working on at ETH Zürich. I will describe how these scientific methods and future telescopes will - for the first time in history - enable us to systematically search for life in space in the next two decades.

Alexander George Bateman. Structure Predictions Transform Protein Family Classification

In this talk I'll discuss the power of the microbial world to help us search for life beyond Earth and to help us implement the human exploration and settlement of space. In particular, I'll discuss some recent experiments on the International Space Station to study the use of microbes to do 'biomining' - extracting economically useful elements from the Moon, Mars and asteroids.

David Baker. The Coming of Age of De Novo Protein Design

Proteins mediate the critical processes of life and beautifully solve the challenges faced during the evolution of modern organisms. Our goal is to design a new generation of proteins that address current-day problems not faced during evolution. In contrast to traditional protein engineering efforts, which have focused on modifying naturally occurring proteins, we design new proteins from scratch based on Anfinsen's principle that proteins fold to their global free energy minimum. We compute amino acid sequences predicted to fold into proteins with new structures and functions, produce synthetic genes encoding these sequences, and characterize them experimentally. In this talk, I will describe the de novo design of SARS-CoV-2 candidate therapeutics, synthetic antagonists and agonists of cellular receptors, molecular machines, and recent advances in deep learning-based structure modeling and design.

Regina Barzilay. Modeling Chemistry for Drug Discovery: Current State and Unsolved Challenges

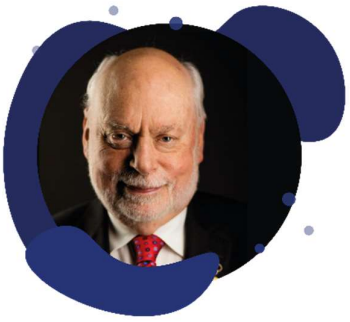
Until today, all the available therapeutics are designed by human experts, with no help from AI tools. This reliance on human knowledge and dependence on large-scale experimentations result in prohibitive development cost and high failure rate. Recent developments in machine learning algorithms for molecular modeling aim to transform this field. In my talk, I will present state-of-the-art approaches for property prediction and de-novo molecular generation, describing their use in drug design. In addition, I will highlight unsolved algorithmic questions in this field, including confidence estimation, pretraining, and deficiencies in learned molecular representations.

Giedrius Gasiūnas. Harnessing the diversity of CRISPR-Cas proteins for genome editing

The Cas9 protein from CRISPR-Cas bacterial defense systems has been adopted as a robust and versatile genome editing tool. However, for Cas9 to bind a given target, a short nucleotide sequence, termed protospacer adjacent motif (PAM), is required. This PAM constraint as well as insufficient specificity are major obstacles for Cas9 genome editing. Natural variation afforded by CRISPR-associated (Cas) enzymes provides a rich resource for the development of RNA-guided tools with diverse and potentially beneficial properties. To explore this largely uncharacterized diversity of the Class2 nucleases for genome editing applications, we applied cell-free biochemical screens to assess DNA cleavage requirements of nearly 80 Cas9 orthologues of Type II family and Cas-beta proteins from novel type V family. We show that this set of programmable nucleases demonstrate a wide range of activities in vitro and in cellular environment. Our results indicate that the natural diversity of Cas proteins provide a source of novel gene editing tools.

Edith Heard. The Genetic and Epigenetic Regulation of X-Chromosome Inactivation in Female Mammals

X-chromosome inactivation during early female development is an essential epigenetic process that is required to achieve appropriate dosage for X-linked gene products. We are interested in understanding how the differential treatment of the two X chromosomes in the same nucleus is set up during development and how this differential expression is then maintained, or reversed in certain circumstances during development or in a disease context. The establishment of X inactivation involves the non-coding Xist RNA that triggers chromosome-wide chromatin re-organisation and gene silencing. Our lab has provided recent insights into the nature of these chromosome-wide changes, and the factors that induce them. These include the Xist-mediated recruitment of the SPEN protein to actively transcribed genes. SPEN triggers gene silencing and dampens expression of genes that escape XCI (Dossin et al, 2020). The loss of active chromatin marks as well as the global loss of topologically associating domains (TADs) are also early events during XCI (Collombet et al, 2020). I will present my lab's recent insights into the relationship between Xist RNA, RNA Pol II and chromatin, during the process of X-linked gene silencing and escape from XCI.



Sir Fraser Stoddart. Artificial Molecular Machines: From Solution to Surfaces

Stoddart performed much of his work at University of California, Los Angeles (UCLA) where his team produced a large-scale 'ultra-dense' memory device that stores information using controllable molecular switches. This is an important step toward the creation of molecular computers that are much smaller and potentially more powerful than today's silicon-based models. He also developed interlocked, self-assembling molecules called 'suitanes', named for their appearance like a limbed torso in a suit.

High School Students

E4. „NILE RED“ FLUORESCENCINIO DAŽIKLIO SINTEZĖS OPTIMIZAVIMAS

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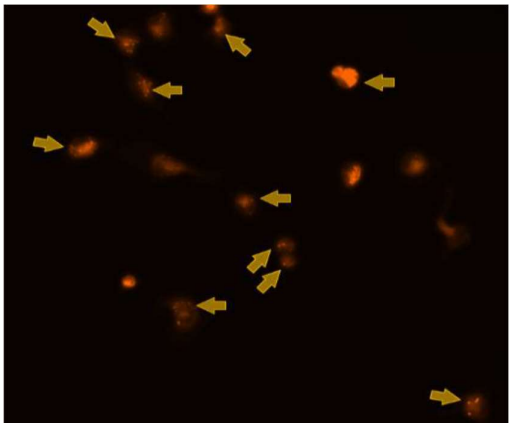
Įvadas. „Nile Red“ yra fluorescencinis, lipofilinis organinis dažiklis naudojamas fluorescencinėje mikroskopijoje mikroplastikų selektyviam atpažinimui ir biologiniuose tyrimuose norint lokalizuoti ląstelės lipidus ir kiekybiškai juos nustatyti [1]. Mikroskopijai tinkamo „Nile Red“ komercinė kaina yra apie 150 eurų už 100 mg [2]. Klasikinis „Nile Red” sintezės metodas paremtas DMF (N,N-dimetilformamidas) panaudojimu. DMF tirpiklio panaudojimo apribojimas [3] buvo patvirtintas 2021 m. lapkričio 19 d. ir turi būti taikomas visoje ES nuo 2023 m. gruodžio 12 d., todėl yra aktualu optimizuoti metodą, paremtą kitu tirpikliu, kuris galėtų pakeisti DMF „Nile Red“ sintezėje, išlaikant optimalią junginio savikainą.

Tyrimo tikslas. Atlikti fluorscenciniai mikroskopijai tinkamo grynumo „Nile Red“ sintezę ir grynimą, nenaudojant N,N-dimetilformamido.

Metodai. Sintezės metodas: nitrozinimo reakcijos metu iš 3-dietilaminofenolio gaunamas 5-dietilamino-2-nitrosofenolis – tarpinis reakcijos produktas [4]. Tarpinis produktas su 1-naftoliu ištirpdomi organiniame tirpiklyje. Atliekant „Nile Red“ sintezę naudojami penki skirtingi organiniai tirpikliai: acetonas, N,N-dimetilformamidas, dimetilsulfoksidas, metanolis, toluenas. Analizės metodas: po reakcijos mėginys analizuojamas naudojant HPLC-MS metodą. Gautoje chromatogramoje analazijuoma „Nile Red“ procentinė dalis reakcijos mišinyje. Gryninimo metodas: naudojama sausos kolonėlės vakuuminė chromatografija su pasirinkta heksano-izopropanolio sistema reakcijos mišiniui gryninti. Surinktos frakcijos tikrinamos TLC metodu siekiant patikrinti komponentų atsiskyrimą. Švariausios frakcijos sujungiamos, rotaciniu garintuvu pašalinamas tirpiklis. Nustatyti grynumui naudojamas HPLC-MS metodas.

Rezultatai. „Nile Red“ išeiga, naudojant skirtingus reakcijos tirpiklius: N,N-dimetilformamidas (26%); dimetilsulfoksidas (21%); metanolis (21%); toluenas (9%). Acetone „Nile Red“ sintezė neįvyko. Atlikus „Nile Red“ gryninimą pasiektas 92% grynumas. Naudojant išgrynintą „Nile Red“ paruoštas 1 mM dimetilsulfoksido tirpalas. Tirplas buvo testuotas fluorescenciniu mikroskopijos metodu, kas patvirtino junginio tinkamumą (1 pav).



1 pav. *Neurofibramatozės antro tipo ląstelių sukaupti citoplazmidiniai riebalų lašeliai, nudažyti naudojant šio darbo metu susintetintu 1 mM „Nile Red“ tirpalu [5].*

Išvada. Optimizuotas sintezės metodas leidžia atsisakyti N,N-dimetilformamido, kuris, atsižvelgiant į tyrimo rezultatus, gali būti pakeists į dimetilsulfoksidą ar metanolį beveik be išeigos praradimo, o gauto produkto grynumas tinkamas taikymui gyvybės moksluose. Tikslinga optimizuoti reakcijos laiką siekiant

padidinti „Nile Red“ sintezės išeigą.

[1] Rumin, J., Bonnefond, H., Saint-Jean, B., Rouxel, C., Sciandra, A., Bernard, O., ... Bougaran, G. (2015b). The use of fluorescent Nile red and BODIPY for lipid measurement in microalgae. Biotechnology for Biofuels, 8(1), 42. https://doi.org/10.1186/s13068-015-0220-4 žiūrėta: 2022-02-10
[2]Prieiga: Nile Red for microscopy 7385-67-3 (sigmaaldrich.com) žiūrėta: 2021-12-14
[3] VON DER LEYEN, U. (2021). Komisijos reglamentas (ES) 2021/2030 2021 m. lapkričio 19 d. kuriuo dėl N,N-dimetilformamido iš dalies keičiamas Europos Parlamento ir Tarybos reglamento (EB) Nr. 1907/2006 dėl cheminių medžiagų registracijos, įvertinimo, autorizacijos ir apribojimų (REACH) XVII priedas. Interneto prieiga https://eur-lex.europa.eu/legal-content/LT/TXT/?uri=CELEX:32021R2030
žiūrėta: 2021-12-01
[4] Börgardts, M., Verlinden, K., Neidhardt, M., Wöhrle, T., Herbst, A., Laschat, S., Müller, T. J. J. (2016). Synthesis and optical properties of covalently bound Nile Red in mesoporous silica hybrids – comparison of dye distribution of materials prepared by facile grafting and by co-condensation routes. RSC Advances, 6(8), 6209–6222. https://doi.org/10.1039/c5ra22736d žiūrėta: 2021-11-20
[5] Russian National Research Medical University, Department of Molecular Pharmacology and Radiobiology, Dina S Stepanova, PhD, Associate Professor.

F1. DIDESNĘ NUTUKIMO RIZIKĄ LEMIANČIO FTO GENO VIENO NUKLEOTIDO POLIMORFIZMO RS9939606 PAPLITIMO NUSTATYMAS

Nojus Jazukevičius[1], Justina Kartanaitė[1]

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Įvadas. Nutukimas yra per didelis ar nenormalus riebalų ir riebalinio audinio kaupimasis organizme. Tai sudėtinga būklė, turinti rimtų socialinių ir psichologinių aspektų, veikianti visas amžiaus grupes. Nustatyta, kad 2016 metais net 13 % pasaulio suaugusiųjų (11 % vyrų ir 15 % moterų) buvo nutukę, o 340 milijonų vaikų turėjo problemų su antsvoriu [1]. Nutukimas ne tik apsunkina kasdieninį gyvenimą, bet ir gali sukelti sunkias sveikatos problemas, įskaitant širdies ir kraujagyslių ligas, kurios yra pagrindinė mirties priežastis visame pasaulyje [2]. Nutukimą gali lemti nesubalansuota mityba, mažas fizinis aktyvumas, miego trūkumas, emociniai bei genetiniai veiksniai. Pastebėta, jog asmenys, FTO gene turintys vieno nukleotido polimorfizmą rs9939609, yra labiau linkę nutukti, nes dažniau vartoja kaloringesnį maistą, nei neturintys šio polimorfizmo [3]. Taip pat 2021 metais atliktuose tyrimuose nustatyta, kad suprastėjusiai fizinei žmonių būklei įtakos turėjo 2020 metais prasidėjusi COVID-19 pandemija [4].

Tyrimo tikslas. Ištirti didesnę nutukimo riziką lemiančio FTO geno polimorfizmo rs9939609 paplitimą 15–18 metų amžiaus mokinių grupėje ir nustatyti tiriamųjų asmenų polinkį nutukti.

Metodai. Tiriamieji (n = 67) atsakė į anoniminę apklausą, susijusią su jų mitybos ir fizinio aktyvumo įpročiais. Tiriamųjų genotipai nustatyti ištyrus biologinę medžiagą – burnos epitelį (moksleivių seiles). DNR buvo išgryninta naudojant CHELEX dervą ir padauginta polimerazės grandininės reakcijos (PGR) metodu. PGR produktai veikti restrikcijos fermentu, taikant restrikcijos fragmentų ilgio polimorfizmo (RFLP) reakciją. RFLP metu restrikcijos endonukleazės fermentas sukarpė genominę DNR ir sukūrė skirtingo dydžio fragmentus. Gautų fragmentų vizualizacija vykdyta 3 % agarozės gelyje, taikant elektroforezės metodą. Gauti rezultatai įvertinti apšvietus gelį ultravioletiniais spinduliais, naudojant transiliuminatorių. Rezultatų patikimumui nustatyti naudota statistinė analizė. Apskaičiuoti alelių bei genotipų dažniai. Pagal apskaičiuotą Chi-kvadratą ir p-reikšmę nustatytos sąsajos tarp tirto polimorfizmo ir apklausos anketos duomenų.

Rezultatai. Ištyrus FTO geno polimorfizmą (rs9939609), nustatyta, kad tirtoje 15–18 metų amžiaus mokinių grupėje dažnesnis buvo polinkį nutukti lemiantis T alelis (dažnis – 0,560) nei A alelis, ir AT genotipas (dažnis – 0,552). Didesnis genetinis polinkis nutukti (AA genotipas) nustatytas 11-ai asmenų (16 % tiriamųjų).

Išvada. Įvertinus gautus tyrimo ir anketinės apklausos rezultatus pastebėta statistiškai reikšminga sąsają tarp AA genotipo, lemiančio polinkį nutukti, ir šeimos narių kūno masės indekso (KMI). Vidutiniškai AA genotipą turinčių asmenų šeimos nariai dažniau turėjo problemų su antsvoriu nei AT ir TT genotipus turinčių asmenų. Gauti tyrimo rezultatai padeda įvertinti Lietuvos paauglių genetinį polinkį nutukti bei suteikia informacijos apie galimas nutukimo priežastis.

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F2. VIOLACEIN BIOSYNTHESIS, EXTRACTION, AND PURIFICATION FROM JANTHINOBACTERIUM LIVIDUM

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Introduction: Violacein is a water-insoluble purple pigment reported to have a wide variety of qualities including potent antibacterial, antiviral, antiprotozoal, and antiparasitic properties [1], which could be useful in medical and biochemistry fields. The current price of violacein is 463,00 €/mg of violacein and its byproduct deoxyviolacein (98 % purity) [2]. The optimization of bacterial growth for maximum biosynthesis and finding of optimal extraction and purification methods will lead to violacein being more affordable and available to processes that require antimicrobial precautions. One of the most common bacterial species that produce this purple-coloured pigment is Janthinobacterium lividum. Janthinobacterium lividum is a Gram-negative, motile, aerobic bacterium commonly found in soils, waters of rivers, lakes, and springs, and skin of salamanders [3]. These bacteria cultivated in optimal conditions produce reasonable quantities of the pigment violacein - the extraction, purification, and properties of which can be further researched.

Purpose: To extract and purify violacein pigment from Janthinobacterium lividum bacteria.

Methods: Cultivation of J. lividum: bacteria were grown in nutrient broth medium with an addition of 1% glycerol and 0.1 mg/mL ampicillin for 7 days in 26 °C temperature, with constant 180 rpm shaking.

Violacein extraction: after cultivation bacteria were centrifugated (6000 rpm) to get rid of the nutrient broth medium, then washed with 0.9% NaCl solution. The pigment was extracted with methanol by vertexing cells for 30 min and centrifuge. Supernatant with violacein was stored in the fridge.

Purification: methanol was removed from the sample by a rotary evaporator. The remaining mixture was absorbed using Celite filter agent. Dry column vacuum chromatography with a selected hexane-ethyl acetate system was used to purify the pigments [4]. Three purple-coloured fractions were collected.

Analysis: fractions were analysed by the HPLC-MS method (Fig. 1).

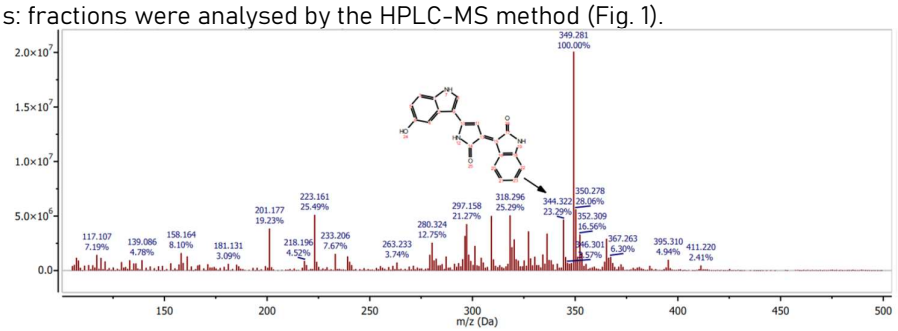

Figure 1. Before purification, the crude extract MS spectrum shows the presence of violacein (344 molar mass) and ampicillin (349 molar mass).

Results: During solvent removal by the rotary evaporator, it was determined that violacein stays stable at a temperature less than 90 °C. In higher temperature pigment would potentially oxidize and lose its colour. During HPLC-MS analysis all coloured fractions absorbed light at 585 nm light, identifying them as violacein and its metabolites (deoxyviolacein). However, the molar mass analysis showed that in all fractions ampicillin was present.

Conclusion: Cultivation of J. lividum bacteria was optimized to increase the biosynthesis of violacein. Analysis of purified pigment solution showed that ampicillin remains in the solution. This would affect violacein antimicrobial analysis and application, so in the future research will seek ways to remove ampicillin without affecting violacein.

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F3. THE IMPACT OF GUT MICROBIOTA ON THE ENDOCANNABINOID SYSTEM IN AGING MICE

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The importance of the gut microbiota, which is primarily composed of bacteria and bacteriophages, to an individual's health and disease state is becoming increasingly apparent. It is well established that the abundance of a bacteria not necessarily reflects its functional importance, as variations in both common and rare taxa increasingly are found to associate with the host's health and function. Recent research has shown that the gut microbiota participates in pathogenesis in neurodegenerative diseases, diabetes, obesity and has an impact on the aging process [1]. One of the ways through which gut microbiota exerts its marked influence on the host is the endocannabinoid system (ECS) [2].

The ECS is comprised of its receptors, enzymes and endocannabinoids. Together these components regulate and control many of the most critical organism functions e.g., learning, memory, emotional processing, sleep, temperature, pain, food demand, inflammatory and other immune responses. [3]. The exact mechanism how microbiota modulates the host's endocannabinoid system and what role this system takes in the microbiota-gut-brain axis is undetermined [4].

Knowing that diet is considered as one of the main factors in shaping the gut microbiota across the lifetime, we used C57BL/6JRj male and female mice that were subjected to different diets: control, control & prebiotics (fructooligosaccharides and galactooligosaccharides), high-fat (521 kcal/100g, 60% of energy from saturated fats) and high-fat & prebiotics. The experiment took 15 months and several tests were performed: Western Blot analyses on different components of the endocannabinoid system (diacylglycerol lipase, monoacylglycerol lipase, N-acyl phosphatidylethanolamine-specific phospholipase D, fatty acid amide hydrolase and cannabinoid receptor 1), fecal microbiota analysis and behavioral tests. The components of the ECS were examined in cerebellum and colon.

We showed that changes in gut microbiota composition, varying expression of ECS components in different tissues and behavioral tests' results were influenced by the aforementioned diets during the aging. A better understanding of the impact of microbiota on ECS could suggest new therapeutical targets for neurodegenerative diseases and possible biomarkers for microbiota-gut-brain axis related disorders.

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F4. PROSTATE CANCER RNA BIOMARKERS TEST IN URINE SAMPLES

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Prostate cancer is the most common cancer among men in Lithuania which gives rise to a significant number of cancer death. To improve on this, early diagnosis is crucial These days the diagnosis of prostate cancer usually involves serum prostate-specific antigen (PSA) testing [1]. However, the increase of PSA levels is not specific for prostate cancer leading to a number of unnecessary prostate biopsies. To improve the diagnosis of prostate cancer and avoid unnecessary invasive procedures there is a need of additional specific prostate cancer biomarkers [2]. Therefore our aim is to find markers which could be detected in non-invasive material – urine, and thus make the diagnosis of prostate cancer easier.

In this study 200 urine samples collected from patients before and after prostate massage were analyzed by quantitative RT-PCR using SYBR-green. Firstly, based on the research results from other scientists and previous investigations in Diagnolita laboratory, we chose 21 potential prostate cancer biomarkers. In addition, 5 reference genes were selected. Using reference gene PAPOLA, which expression is stable in different human tissues [3], we examined how biomarkers quantity in urine altered due to prostate massage. The analysis was based on the fact that massage increases the proportion of transcripts from prostate. Accordingly, 16 markers with increased relative quantity after prostate massage ($p<0.05$) were selected as this shows that a large fraction of biomarkers RNA got into urine from prostate and not other male urogenital organs. Futhermore using NormFinder method [4] we compared prostate-specific reference genes AR-FL, ACPP, PSA and SPDEF on their suitability for normalization. The comparison was performed separately for samples collected before and after prostate massage and included grouping by Gleason grade: healthy, Gleason 6 and Gleason 7 and higher. ACPP was identified as the best single reference gene for normalization in both datasets. Next, ACPP alone and various combinations of ACPP with other reference genes were investigated. Combination of ACPP and PSA was chosen for normalization as the resulting biomarker AUC values were the highest and significantly better than normalizing only by ACPP ($p<0.05$). Finally, using LASSO (least absolute shrinkage and selection operator) method we selected which biomarkers from the rest of 12 were the best for prediction of clinically significant (Gleason 7 and higher) prostate cancer. With samples collected before massage the selected markers were ONECUT2, PCAT1, ERG, TDRD1, PRCAT42, PRCAT122 and after – PCA3 (Fig. 1).

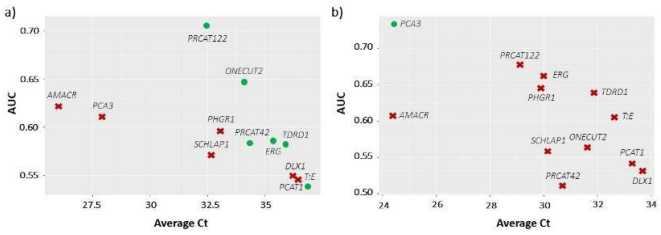

Figure 1. AUC and average Ct values of prostate cancer biomarkers in samples before massage (a) and after (b). Markers selected by LASSO are shown in green color.

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Neurobiology and Biophysics

G1. Functionality of L-type Ca2+ channels in human mesenchymal stem cells during chondrogenic differentiation

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Human mesenchymal stem cells (hMSCs) derived from bone marrow have a potential for differentiation to the multiple lineages and acquiring the specific sets of ion channels. As was reviewed in [1], in 15% of undifferentiated hMSCs an inward L-type calcium current was observed. It is expected that the expression of L-type calcium channels, depends not only on the cell type, but also on the state of its differentiation. It still remains unclear if the number of functional L-type calcium channels increase during chondrogenic differentiation, if this is influenced by pharmacological and physical factors.

In this study we used the whole-cell patch-clamp to directly investigated the changes of functional activity of L-type calcium ion channels in hMSCs during chondrogenic differentiation. hMSCs were obtained from bone marrow after post-trauma surgical procedures. hMSCs cultured in the Dulbecco's Modified Eagle Medium (DMEM) were used as the control group. Chondrogenic differentiation was induced by cultivating of hMSCs in chondrogenic medium [2]. We tested if Transforming growth factor (TGF) and Interleukin added to the chondrogenic media influence the functional activity of L-type calcium channels. The effect of electrical stimulation on activity of L-type calcium channels was tested also.

As published before [1], there were no noticeable inward current in hMSCs in control. The hMSCs in chondrogenic media did not have any inward current as well. Adding of TGF to the chondrogenic media activated a noticeable inward current that was blocked by nifedipine –a specific blocker of L-type calcium channels. Adding the interleukin to the chondrogenic media also induced inward current, that was blocked with nifedipine. The electrical stimulation applied to the hMSCs in chondrogenic media did not induced any inward current.

We conclude that cultivation of hMSCs in chondrogenic media alone do not increase the activity of L-type calcium channels. The electrical stimulation used was not sufficient to induce any noticeable inward currents. However, the TGF or Interleukin added to the chondrogenic media increases the functional activity of L-type calcium channels in hMSCs.

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G2. VALUABLE COMPOUNDS EXTRACTION FROM MICROALGAE USING COLD PLASMA AND PULSED ELECTRIC FIELDS

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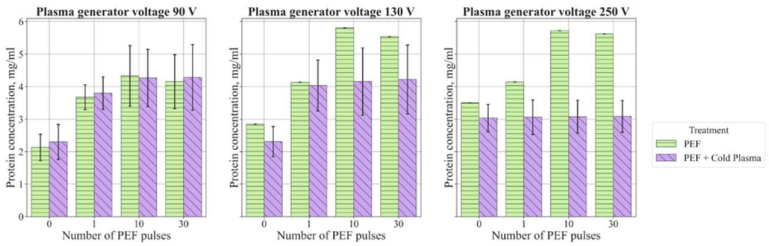
Microalgae are a very promising and sustainable source of biofuels, food and feed. These microorganisms effectively fix CO2 and synthesize highly valuable compounds. During extraction with the most effective method - high-pressure homogenization, different fractions of algae compounds are mixed, thus there is a need for an additional purification step. Therefore, other effective extraction methods are investigated [1].

Pulsed electric fields (PEF) is an intensively researched extraction method. It is based on usage of short (μs-ms) and strong (5-80 kV/cm) electric pulses, which cause an increase of cell membrane permeability. Advantages of this method are selectiveness and purity of extracted substances [2]. Nevertheless, extraction with PEF isn't effective enough, hence researchers are trying to improve it. Cold plasma treatment could possibly be a solution. During the treatment, generated radicals may cause lipid oxidation, leading to membrane weakening and pore formation [3]. A combination of these techniques could be a useful strategy to increase extraction yields of valuable compounds from microalgae and they could be adapted to an industrial scale.

Fig. 1. Protein concentration extracted from *C. vulgaris* (cultivated in BG-11) after cold plasma and/or PEF treatments.



For protein extraction experiments, suspension of Chlorella vulgaris microalgae was washed once and concentrated up to 100 g/L wet biomass with BG-11 medium (σ = 1,96 mS/cm). Then they were treated with cold plasma or PEF only, or firstly by cold plasma and then by PEF. Cold plasma treatment lasted for 5 minutes, and plasma generator voltages were 90 - 250 V. PEF parameters were U = 3,5 kV, t = 10 μs, f = 1 Hz, n = 1;10;30, d = 0,1 cm. Conductivity of the suspensions was measured after cold plasma treatment. Further, after selected treatment, samples were incubated for 2h at room temperature. Finally, protein concentration in the supernatant was determined with Bradford method.

Firstly, conductivity changes were observed after cold plasma treatment. For instance, after treatment with plasma generator voltage of 250 V, suspension's conductivity has risen by 53,5 %. This indicates that cells' membranes were permeabilized. Additionally, when samples were treated with a combination of cold plasma and PEF (plasma generator voltage 90 V, n (PEF) = 1; 10; 30), protein extraction has increased, compared to only plasma treatment control. However, the results (Fig. 1) have shown that PEF alone is more efficient for protein extraction than combination of PEF and cold plasma. For example, obtained protein concentrations after PEF (n = 10) and cold plasma + PEF (plasma generator voltage 250 V, PEF n =10) treatments were respectively 5,72 mg/ml and 3,07 ± 0,72 mg/ml. Therefore, further investigation on how cold plasma and PEF together affect microalgae cells is needed.

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G3. THE POTENTIAL USE OF HUMAN DERMAL MESENCHYMAL STEM CELLS AND MULTILAYER NANOPARTICLES FOR CANCER THERANOSTICS

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Cancer is one of the most important issues facing society, science and medicine because morbidity and mortality rates continue to rise. For example, 19.3 million new cancer cases and 10 million deaths were recorded in 2020 [1]. The search for innovative solutions is increasingly focused on nanotechnology, especially upconverting nanoparticles (UCNPs). These nanoparticles are promising tool for cancer diagnostics and therapy because they have properties such as excitation and emission in the optical tissue transparency window, low noise and autofluorescence signal, photochemical stability, low toxicity. The incorporation of the photosensitizer chlorin e6 into UCNPs could provide therapeutic effect with 980 nm laser excitation and diagnostic effect with 793 nm excitation. Thus such nanoparticles could be used as theranostical agent. However, the selective delivery of nanoparticles to the tumor site remains one of the biggest challenges. This problem could be solved with mesenchymal stem cells (MSCs), which due to their properties tend to migrate to the lesion site, including tumor tissues. Therefore, mesenchymal stem cells could be used as nanoparticle carriers toward cancer cells [2].

The aim of our study was to evaluate the effect of multilayer upconverting nanoparticles and their complex with chlorin e6 on mesenchymal stem cells and breast cancer cell lines.

We investigated multilayer lanthanides-doped nanoparticles with an embedded photosensitizer chlorin e6 (Ce6) (UCNPs-Ce6) and as controls we used chlorine-free UCNPs. Confocal microscopy images demonstrated that UCNPs and UCNPs-Ce6 accumulate in MSC and human breast cancer cells (MCF-7, MDA-MB-231) cytoplasm and around the nucleus. The lactate dehydrogenase (LDH) cytotoxicity test showed no statistically significant dark toxicity on cell viability. Moreover, a wound healing assay revealed that MSCs migrate toward the scratch site despite being incubated with nanoparticles. Finally, we showed that UCNPs-Ce6 under 980 nm light induced damage to MSC and cancer cells, while UCNPs alone are non-toxic.

Overall, UCNPs-Ce6 loaded human dermal MSCs could be a useful tool in cancer theranostics.

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G4. INVESTIGATION OF pH BANDING IN NITELLOPSIS OBTUSA VIA FLUORESCENCE SPECTROSCOPY

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Photosynthesis is a physicochemical process which sustains virtually all life on Earth. The performance of photosynthetic activity can be monitored by the use of fluorescence spectroscopy, as part of the excitation energy can be emitted instead of being used in in photochemical reactions [1]. Characean algae represent a unique model to study photosynthesis. Upon irradiation with actinic light characean internodes produce alternating acid and alkaline bands along their surface [2] whose relationship with photosynthetic activity still needs to be investigated.

An optical fibre system paired with controlled stepper motor enabled registration of chlorophyll fluorescence signals along Nitellopsis obtusa internodes in 1 mm intervals. A low intensity (< 1 mW) LED light source emitting at 405 nm was used for excitation. Two types of experiments were carried out to evaluate the effect of illumination on photosynthetic activity - signals were registered in dark-adapted internodes and in cells that were illuminated with a 18 W white LED lamp for 30 min. after dark adaptation.

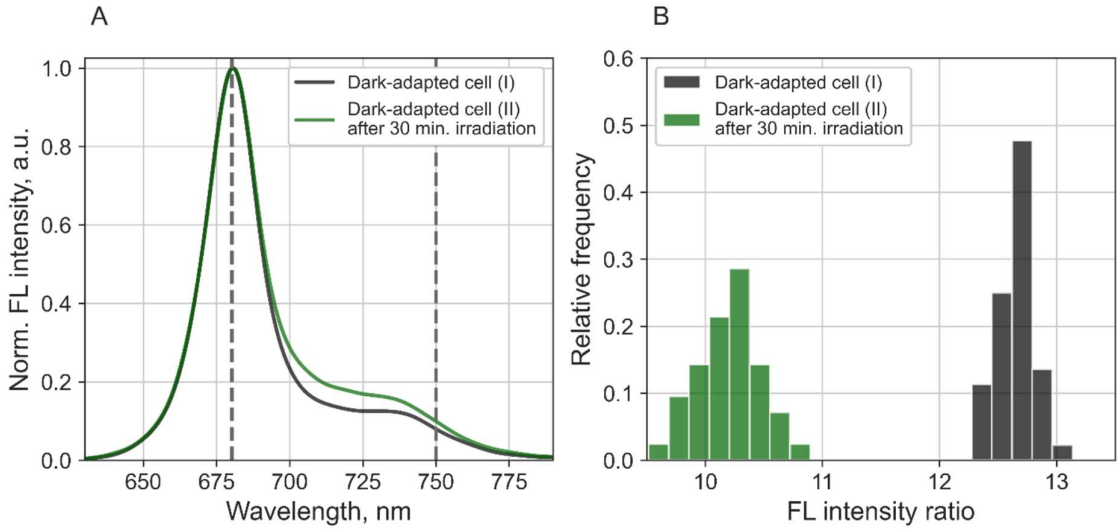

Figure 1. Typical chlorophyll fluorescence (FL) spectra (A) and distributions of intensity ratios between FL intensity values registered at 680 nm and at 750 nm (B) along algae cells.

The fluorescence emission spectrum of Nitellopsis obtusa exhibited two maxima around 680 nm and 739 nm (Fig. 1A). Variation of fluorescence intensity at these wavelengths as well as changes of spectral shape were observed along the cell. Moreover, 30 min. illumination of previously dark-adapted cells resulted in lower maximal fluorescence values and greater variance of fluorescence intensity ratio between values registered at 680 nm and at 750 nm (Fig. 1B). These results indicate different photosynthetic activity zones within the cell. As the fluorescence ratio reflects changes of spectral shape, the greater variance after illumination suggests its dependence on photoinduced alterations of photosynthetic performance in Nitellopsis obtusa internodes.

Hence, the sensitivity of the chosen optical non-invasive method is sufficient to detect light-dependent physiological patterns. However, further research of pH banding should be carried out to determine the relationship between photosynthetic performance and the banding phenomenon.

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H1. AUDITORY STEADY-STATE RESPONSES DO NOT DEPEND ON SEX DIFFERENCES AND ESTROUS CYCLE IN MICE

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Brain is always active no matter if we sense the environment, perform a task, rest or even sleep. Brains activity is reflected in electrical oscillation within neural networks. These oscillations not only contribute to various functions, but also is a subject of change during multiple neural system disorders. Auditory Steady State Response (ASSR) is used as a method to assess brain's ability to oscillate. Due to the changes during schizophrenia, ASSR is increasingly used as a biomarker to diagnose or even predict schizophrenia [1]. Recent studies suggest that brain changes during schizophrenia may be highly dependent on gender and/or sex hormones [2]. First, the incidence and severity of schizophrenia vary between men and women [3]. Second, women have exacerbations of schizophrenia during the menstrual cycle [4]. Therefore, it is very likely that gender and sex hormones may affect the schizophrenia biomarker ASSR.

The aim of this study was to evaluate ASSR sex differences and its' variation throughout the estrous cycle. The experiments were performed on mice (14 females and 12 males). ECoG electrodes were implanted for chronic ASSR registration in auditory cortex. ASSR evoked by providing 2 ms white noise 'clicks' at 40 Hz, 70 dBA. ASSR was evaluated as power increase in 35-45 Hz range of power spectrum and as phase-locking index (PLI). Phases of estrous cycle were determined from cytological evaluation of vaginal smears taken right before ASSR registration.

Preliminary results show that ASSR does not differ between males and females (PLI p=0.432, power ratio p=0.403; Mann-Whitney test). Furthermore, ASSR remains similar across different phases of estrous cycle in females (PLI p=0.366, power ratio p=0.194; Friedman test). In conclusion, our results suggest that ASSR does not vary between male and female mice and ASSR does not change during estrous cycle.

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H2. REDUCTION OF CELL LOSS IN MICROFLUIDIC CELL-ENCAPSULATION EXPERIMENTS

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Droplet microfluidics is a high throughput technology for analyzing individual cells isolated in nanoliter volume droplets. Single-cell technologies also solve cell heterogeneity problems and allow scientists to compare cell states and types in complex samples [1]. During the encapsulation step, the density mismatch between the cells and solution in which cells are dispersed can cause an undesirable cells sedimentation and loss. To prevent this from happening density matching solutions (e.g., Optiprep) are typically being used. In this work, we investigated how cell sedimentation can be prevented by altering solution viscosity. Our work consists of two parts: first, we tested cell count change inside the microfluidic device during encapsulation step by flowing cells in various density-adjusted or viscosity-adjusted solutions. In the second part, we derived the theoretical model explaining the experimental results.

We investigated 9e10 mouse hybridoma cell distribution in higher density or viscosity solutions, passing through the microfluidic chip, and imaged at defined time intervals. The obtained images were analyzed using the Ilastik machine-learning algorithm [2] and Python script. We found that cell behavior in the fluid can be described following the Stokes law for the terminal velocity. Stokes' law states that the particle sedimentation velocity is directly proportional to the density difference between the particle and the liquid and inversely proportional to the viscosity of the liquid [3]. The time-dependent diffusion equation was solved to account for osmotic pressure change and the cell-shrinking.

Our results show that adjusting the solution density (a common practice in field), leads to a loss of large fraction of cells due to uncontrolled cell sedimentation and floating. On another hand, the use of higher viscosity solutions (e.g., dextran) reduced cell loss by 3-6-fold and allowed us to keep more cells in a suspension during the course of experiment.

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H3. INFLUENCE OF DIET ON THE IMMUNE SYSTEM OF THE BRAIN IN AGEING MICE

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Neuroinflammatory processes in the animal brain increase with age, which can lead to various cognition-weakening neurodegenerative diseases, e.g., Alzheimer's disease [1]. Neuroinflammation can occur due to the dysfunction of microglia. Microglia are the resident immune cells (macrophages) of the brain that play a crucial role in maintaining homeostasis. Microglia cells are constantly scavenging the CNS for various potentially hazardous structures, e.g., amyloid plaques, damaged or unnecessary synapses and neurons, infectious agents [2].

In recent years, numerous studies have shown that the gut microbiota plays a key role in regulating brain functions via the gut-brain axis. Microbiota is an ecological community of commensal microorganisms that live symbiotically and pathogenically in the digestive tracts of vertebrates [3]. Metabolites produced by the gut microorganisms can affect the function of microglia as well as regulate the inflammation in CNS.

Diet has a major impact on the composition and well-being of the gut microbiota, and thus could affect the microglia. Furthermore, prebiotics are known be effective at supporting the growth of healthy gut bacteria [4]. The aim of this study is to understand how different diets can influence the function of microglia.

The C57BL/6JRj male mice, which were used for this research, were divided into two age groups - aged and young. Additionally, animals were subdivided into four different diet groups – control (387 kcal/100g), control + prebiotics (fructooligosaccharides and galactooligosaccharides), high-fat (521 kcal/100g, 60% of energy from saturated fats) and high-fat + prebiotics. After 10 months of diet (aged group) and 1 month of diet (young group) the microglial cells were isolated from the brain of the animals in order to perform phagocytosis, senescence and ROS experiments, which directly correlate to microglial activation and functioning in the CNS of aged and young animals.

We observed that microglial cells exhibited decreased capabilities in aged animals when compared to young animals. Similarly, microglia from animals fed with high-fat diet showed a bigger decline in functionality when compared to other diets. Finally, preliminary results suggest that prebiotics might have a positive effect on microglial cells.

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H4. PERCEPTION OF CROSS-SECTIONED 3D OBJECTS: WHAT FACTORS CAN INFLUENCE OUR PERFORMANCE?

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Spatial abilities are an important part of human intelligence. It is known that these abilities can be influenced by sex and sex hormones. However, biological factors are not the only source of influence, socio-cultural, cognitive, and individual differences play a role as well [1]. In this study we aimed to evaluate the influence of biological and non-biological factors on participants' spatial abilities assessed using Cross Section Task (CST) [2].

We recruited 138 women (26.3 ± 4.6 years) and 39 men (25.8 ± 4.4 years). Women were divided into four groups according to their hormonal status: oral contraceptives users (OC, n = 35), hormonal intrauterine device users (IUD, n = 29), naturally cycling women in early follicular phase (NCF, n = 37) and in mid-luteal phase (NCL, n = 37). Saliva samples were taken to assess concentration of sex hormones (testosterone, progesterone and estradiol). Participants performed paper-pencil Cross Section Task and Visual Working Memory task. Gender-Related Attributes Survey (GERAS) was used to evaluate participant's masculinity and femininity in personality, cognition, and interests' domains. Other individual characteristics were evaluated using Self-reported Cognitive Assessment Questionnaire.

On CST task men outperformed naturally cycling women in mid-luteal phase ($p = 0.004$) and OC users ($p = 0.012$) but not naturally cycling women in early follicular phase and IUD users ($p > 0.05$). There was a significant negative correlation between progesterone concentration and Cross Section Task performance ($r = -0.49$, $p = 0.003$) in OC users group. Higher GERAS cognition domain masculinity score was positively related with CST performance ($r = 0.265$, $p = 0.001$), femininity cognition score - negatively ($r = -0.204$, $p = 0.01$). Higher self-assessed spatial and mathematical-logical abilities were linked positively with performance ($r \geq 0.321$, $p \leq 0.012$). No difference between groups was found in working memory task performance and no correlation between two tasks (all $p > 0.05$).

Study results suggest that social factors such as gender related cognitive attributes, individual differences, namely self-report overall spatial and mathematical-logical abilities, as well as biological factors including sex and hormonal status can be linked with the Cross Section Task performance.

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I1. ULTRA-FAST 150 PICOSECOND ND: YAG LASER FOR TATTOO INK FRAGMENTATION

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Tattoos are a permanent mark or design made on the body by the introduction of pigment through ruptures in the skin. Due to various reasons, a great demand for safe and effective removal has developed. With the discovery of interaction between laser and specific chromophores made the treatment of tattoos possible. Nowadays, laser treatment is widely accepted as the gold standard for tattoo removal [1].

Although laser tattoo removal is regarded as safe, both temporary and permanent side effects do occur. Temporary side effects include pain, erythema, crusting, pinpoint bleeding, blistering, swelling, infection, and pigmentary disorders. Permanent side effects include scarring, hypo- or hyper-pigmentation, and color change of tattoo pigment [2].

Even with the advent of novel laser technology, complete clearance of tattoos remains difficult. Prior to treatment, patients are informed that complete tattoo removal is heavily influenced by ink characteristics, skin type, and age of the tattoo, as treatment of older tattoos is often more successful. Understanding the identity and dye composition of tattoo ink is beneficial in improving treatment planning and predicting response to laser therapy [3].

Q-switched nanosecond (ns) laser have been the modality of choice up until recently. However, the pulse duration range is still too long to effectively break ink into small enough particles, while reducing the collateral thermal damage to the surrounding tissue. Picosecond (ps) lasers have emerged at the forefront of laser tattoo removal due to their shorter pulse lengths showing better performance than ns lasers in tattoo clearance or pain level in preclinical and clinical studies [4].

The photoacoustic effect is the basis of this tattoo removal method. The administration of energy in such a short pulse leads to a rapid thermal expansion of the target, that is, subsequently fragmented by the release of an acoustic wave that leads to the mechanical destruction of neighboring. The disintegration of pigments into smaller fragments allows macrophages to phagocyte fragments and to carry them away via the lymphatic system. Also, the formed endothermic steam carbon reactions alter the optical properties of the tattoo inks by producing shell-like structures that in turn reduce the tattoo visibility [5].

As most commercially available lasers excel at 300-600 ps, in our study we aimed to investigate the laser-tissue interactions of a Nd: YAG laser, that can generate 150 ps pulses. The effects of 150 ps ultrashort pulses are not currently described in the literature, and the area is lacking in animal studies that offer great translational value. Thus, we conducted our in vivo study on porcine skin, that is very close to human skin in many regards. As our laser device operates at switchable wavelength (1064/532 nm) and spot size (2/4 mm), we analyzed the ink pigment fragmentation efficiency of five different colors: black, blue, green, red, and yellow after 3 treatment seasons. The tattoo removal was measured using clinical macroscopic examination (siascopy) and light microscopy after histochemistry staining.

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I2: MELATONIN REDUCES ALCOHOL DRINKING IN RATS WITH DISRUPTED FUNCTION OF THE SEROTONERGIC SYSTEM

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The reason for limited treatment success of substance-use related problems may be a causal heterogeneity of this disorder, which, at least partly, is manifested as differences in substance use motives between individuals. Both animal and human studies identified low sleep quality as one of the drinking motives due to its association with increased probability of alcohol consumption. The aim of the present study was to assess if rats with pharmacologically-induced differences in function of serotonergic system, would respond differently to melatonin treatment compared to their control counterpart rats with respect to voluntary alcohol consumption. Serotonergic system is responsible for many physiological functions, such as sensory processing, cognition, emotion regulation and sleep. Melatonin is a hormone that synchronizes behaviors and physiological functions of the body to the presence/absence of day-light, and it has been demonstrated that compounds targeting the melatonergic system reduced drug-related behaviors in animals [1, 2].

Disrupted function of the serotonergic system is achieved by exposing rodents to monoaminergic antidepressant treatment during early postnatal days (PD) for approximately two weeks. Hence, rat pups received once daily administration of selective serotonin reuptake inhibitor (SSRI) escitalopram between post-natal day PD8 to PD21. In adulthood, rats were given free access to alcohol for several weeks. Thereafter, both control and escitalopram treated rat groups were exposed to repeated administrations (a total of 3 daily injections just before the onset of the dark phase) of either vehicle or 40 mg/kg of melatonin. Preference for sucrose and quinine solutions were tested in all animals to control for differences in reward- and/or taste-sensitivity.

The present study demonstrated that adult outbred rats treated with selective serotonin reuptake inhibitor escitalopram during early postnatal weeks had similar reward- and/or taste-sensitivity but tended to drink higher amounts of alcohol than their control counterpart rats. Repeated administration of melatonin before the onset of the dark phase caused clear but not significant reduction in voluntary baseline home-cage alcohol consumption by control rats and a significant decrease in alcohol intake by rats treated with escitalopram.

In conclusion, our data support the therapeutic potential of melatonin as a treatment for alcohol use disorder. However, individual differences between alcohol users may considerably modify the outcome of the melatonin treatment, whereby patients that manifest lower sleep quality due to disruption of serotonergic activity are more likely to benefit from this treatment.

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I3. PEF ASSISTED PREPARATION OF FROZEN COMPETENT E. COLI CELLS

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Escherichia coli is a thoroughly researched and widely used gram-negative bacteria for gene modification[1]. Because of its rapid growth rate, high protein production and easy cultivation conditions, it is considered a model procaryotic organism and is widely used in biotechnology. There are many protocols describing the preparation of E. coli for transformation, and many of them include freezing to preserve competent E. coli for later use. Though freezing preserves the cells, it also damages them, causing a decline in viability, hence optimization of the preparation protocols as well as improvement in the efficiency of cryoprotectant introduction into the cells is needed. It is known that some organisms can produce cryoprotectant trehalose which prevents freeze-induced damage by stabilizing membranes, inhibiting ice crystal growth and preventing cells from dehydration. Yet, E. Coli cells do not naturally synthesize it, and their membranes are impermeable to it.

The method of electroporation is widely used and well researched for introducing different molecules through pores in the membrane, formed by the effects of pulsed electric fields (PEF). Short (□s-ms) square wave electric pulses field strength of 5-30 kV/cm are used to induce permeability of the cell membrane for direct molecule transfer[2].

E. coli cells were prepared by growing them in LB medium 400ml, until OD600= 0.5 and washing them with ice-cold MilliQ water afterwards. Cells were resuspended in electroporation buffer (MilliQ+trehalose) of different trehalose concentrations (0.1M, 1M) and placed in an ice bath. The suspension was then treated with pulsed electric field (PEF) of different parameters in samples of 100µl and freezed in -20℃. After 7 days, samples were placed in an ice bath to thaw, diluted and spreaded out on Petri dishes on solid LB medium. Results were collected by counting grown colonies and multiplying by the dillution.

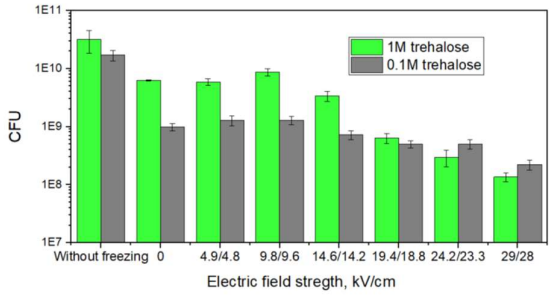

Fig. 1. E. coli viability after 7 days freezing in -20℃, depending on trehalose concentration and PEF parameters.

A slight difference in conductivity between samples was observed, visible by the differences in electric field strength (EFS) – larger trehalose concentration yielded slightly stronger electric field. Furthermore, E. coli viability increased 5.5 times with EFS 4.8-14.6 when using 1M trehalose buffer when compared to 0.1M trehalose buffer with field strength of 4.8-19.4 kV/cm. However, viability was greater (~1.6 times) when using 0.1M trehalose buffer with field strength of 23.3-29 kV/cm. The highest viability was achieved using 1M trehalose buffer with field strength of 9.75 kV/cm. While investigating incubation time and conditions before freezing, no significant change in viability after freezing was determined nevertheless, less fluctuation was observed when samples were kept in an ice bath after adding trehalose buffer. Incubation time after treatment with PEF and before freezing did not provide changes in viability after freezing. The goal of this study was to use electroporation for introduction of the cryoprotectant trehalose into E. coli cells (prior to freezing), this technology would assist with longer E. coli cell preservation.

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I4. EVALUATION OF ELECTROPORATION EFFICIENCY: TITANIUM AGAINST STAINLESS-STEEL ELECTRODES

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During electroporation (EP) short electrical pulses are applied to increase cell membrane permeability and are typically used to transfer substances into cells or extract molecules from cells [1, 2]. Electroporation is a perspective process, that is used in medicine, biotechnology or food processing. Further development of the method requires new insights on material designs. Based on past research it is known, that materials from which electrodes are made, have different outcomes after electroporation. There are investigations dedicated to aluminum, copper, stainless steel, gold, platinum and carbon electrodes, but very few investigations could be found of titanium electrodes used for electroporation [2]. This investigation is based on the idea that titanium electrodes are more inert than stainless steel electrodes, thus the use of titanium electrodes should provide higher cell viability without a sacrifice in the efficiency of electrotransfer and electropermeabilization level of cells' plasma membrane.

Experiments were carried out using the Chinese hamster ovary (CHO) cells. Electroporated was performed between two different electrodes: titanium (BT-20, composition of alloy: 5.5-7 % aluminum, 0.5 - 2 % molybdenum, 0.8 - 2.5 % vanadium, 1.5 - 2.5% zirconium) and stainless steel (AISI 304: 18.1 % chromium, 8.3 % nickel). A 50µl of cells in a low conductivity electroporation medium (0.1 S/m, 270 mOsmol, 7.2 pH) was electroporated between electrodes separate with a 2 mm gap. Electric field pulses strength varied from 0.6 kV/cm to 2.2 kV/cm, cells were electroporated with 1 or 9 high voltage (HV) pulses of 100 µs duration, repeating at 1 Hz frequency. Levels of cell membrane permeability and amount of irreversibly electroporated cells were determined using flow cytometry while measuring fluorescence intensity and the number of PI-positive (PI+) cells. Cell viability was evaluated with MTS 24 and 48 hours after exposure and colony formation assays 6 days post-treatment.

Our results showed that the intensity of intracellular PI fluorescence and the number of PI+ cells using titanium or stainless steel electrodes increased identically with amplification of electric field intensity. Results of cell viability showed that titanium and stainless steel electrodes affect the CHO cell viability similarly with some exceptions using MTS at 48h after EP. Here, cell viability using 1.8 kV/cm 1 HV was 30 % ± 14 % and 54 % ± 5 % for titan vs stainless steel respectively and sing 0.8 kV/cm 9 HV 24 % ± 12 % and 44 % ± 23 % for titan vs stainless steel respectively. Using titanium or stainless steel electrodes resulted in the same amounts of irreversibly permeabilized cells.

In conclusion, no significant differences in cell viability and membrane permeability under most of the electroporation parameters tested were observed using stainless steel vs titanium electrodes. However, in some conditions cell viability was lower using titan electrodes, but these discrepancies will be reevaluated. All in all, our results stand for stainless steel electrodes as the best price/electroporation efficiency ratio providing electrodes, however, suggest that in specific cases, if needed, titanium electrodes will provide the same efficiency as stainless steel.

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Oral Presentations

INTERPLAY BETWEEN EPIGALLOCATECHIN-3-GALLATE AND IONIC STRENGTH DURING AMYLOID AGGREGATION

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Amyloid fibrils formed during the aggregation of amyloidogenic proteins are a hallmark of neurodegenerative diseases such as Alzheimer's or Parkinson's [1]. Although scientists around the world have been searching for drugs or treatments for these diseases for many years, few have been discovered, and the field of research analyzing the mechanisms of protein aggregation and what factors may influence them is still unclear. Studies show that various environmental factors such as temperature, pH, or protein concentration can affect not only the rate of aggregation but also the structure and morphology of the formed fibrils [2,3]. For this reason, analyzing the efficacy of inhibitory compounds under different environmental conditions may give incorrect results. This work investigated how the ionic strength of a solution changes the inhibitory effect of an anti-amyloid compound epigallocatechin-3-gallate (EGCG), using three different amyloid proteins.

To elucidate the influence of ionic strength, aggregation kinetic parameters of the three selected proteins with different amino acid sequences were first analyzed. Two proteins / peptides associated with neurodegenerative diseases, amyloid β (Alzheimer's disease) and α-synuclein (Parkinson's disease), and one model protein - recombinant human insulin, were selected for the study. Fourier-transform infrared spectroscopy (FTIR) was used to verify that different structures of amyloid fibrils were formed in the reaction mixture with EGCG during the aggregation under different ionic strength conditions.

In the study, it was observed that each amyloid protein has its own characteristic ionic strength value in the solution at which the EGCG inhibition efficiency is highest. In the case of insulin and α-synuclein, it was observed that the inhibition efficiency is higher at lower NaCl concentrations and increasing the ionic strength of the solution significantly reduced its effectiveness. For amyloid β, the inhibition remained similar with increasing NaCl concentrations from 100 mM. Additionally, analysis of the FTIR spectra of fibrils demonstrated that the ionic strength of the solution affects not only aggregation kinetic parameters but also the structure of the resulting fibrils.

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MARCH 1st

Biochemistry and Molecular Biology

A1. PROTEINS FOR HOST INFECTION OF vB_PagM_AAM22 PHAGE VIRION

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Bacteriophages (phages) are viruses that specifically infect bacteria in a variety of microbial ecosystems. Like other viruses, phages have a DNA or RNA genome protected by the proteins of virion. In nature, phages face a variety of physicochemical factors (UV radiation, fluctuations of pH value and temperature). Because of that, virions of phages are composed of highly robust and stable complexes of their structural proteins. This property is attractive for engineering of self-assembled nanostructures formed by the recombinant proteins of phage virions.

Phage virion-derived nanostructures have a great potential as the environment-friendly biomaterials for the development of safe and efficient devices that can be used in nanotechnologies and medicine. However, the potential of suitable proteins of a variety of sequenced phages remains unknown. The most abundant and widespread phages have complex virions designed to recognize and infect host cells. Because of that, prediction of protein function by only bioinformatic methods is difficult, and experimental confirmation of the predicted function is needed. Identification of virion proteins that are responsible for host infection may help understand the processes of host-specificity of certain phages.

In this study, putative structural and chaperone proteins of the newly sequenced Pantoea agglomerans-infecting phage vB_PagM_AAM22 were examined. It was done by engineering its recombinant proteins and analysing the self-assembled protein nanostructures with transmission electron microscope. In addition, two protein-cell interaction experiments were performed seeking to find out if these proteins may be responsible for host recognition.

The results show that the recombinant proteins of vB_PagM_AAM22 virions adopt characteristic structures resembling those of native phages. Therefore, this methodology is useful for confirmation of the predicted function of phage-encoded proteins. Moreover, the resulting nanostructures can be suitable as a basis for engineering of more complex devices that may be used depending on their physical and biochemical characteristics. According to the results of cell receptor blocking experiment, function of the fiber-assembly protein may be related to Pantoea agglomerans AUR receptor binding.

A2. THE ROLE OF HYPOXIA AND SPLICING FACTORS IN LEUKEMIA-ASSOCIATED mRNA FORMATION

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Leukemia is malignant blood disease which is characterized by an increase in the number of immature and functionally impaired types of their precursor cells in the blood or bone marrow. It is divided into several types according to the symptoms or treatment and prognosis of each of them differ. However, the available data suggest that current treatment strategies for leukemia are inadequate [1]. Altered expression of mRNA isoforms has been identified in a variety of tumors. The dysregulation of alternative splicing has emerged as a mechanism of diferent hematopoetic disease. The splicing pattern of specific isoforms of numerous genes is altered as cells move through the oncogenic process of gaining proliferative capacity while acquiring anti-apoptotic and survival properties [2]. It is clear that the pathological processes taking place in tumor cells depend not only on the genetic context but also on the effects of the cell microenvironment. Hypoxia is one of the main characteristics of the microenvironment of tumor cells. Hypoxia is a state which is described as a deficiency of oxygen supply in cells and tissues to maintain normal cellular functions. Moreover, hypoxic microenvironment in cells alters various genes pre-mRNA alternative splicing [3].

It is established that changes in the expression of splice-regulating proteins are one of the reasons for the changes in mRNA formation associated with oncological diseases [4]. One of the most important trans factors involved in the pre-mRNA splicing process are the SR and hnRNP family proteins, which act as positive or negative splice regulators by interacting with regulatory pre-mRNA sequences [5]. However, the effect of these changes on the formation of mRNA isoforms associated with blood oncology in a hypoxic environment is not yet known. For this reason, our objective is to investigate the influence of splicing factors on the formation of mRNA isoforms of leukemia-associated genes in K562 cells on reduced oxygen content in the environment (hypoxia). The obtained results show that the hypoxic microenvironment changes the expression of leukemia associated MAX, GFI1B and PUF60 genes mRNA isoforms in leukemia K562 cell line. It also reveals how hypoxia affects SRSF1, SRSF and U2AF splicing factors expression in the same cell line. These results provide new insights into the understanding of the influence of the hypoxic microenvironment of cells on the expression of tumor-associated mRNA formation.

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A3. Optimization of recombinant L-BC Gag protein synthesis in S. cerevisiae

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During the past decade, virus-like particles (VLPs) were widely employed as vaccine platforms and nanodelivery systems. These nanoparticles are formed of self-assembling viral proteins so they can be functionalized and used for various purposes. Since VLPs are free of replicating viral genetic material, they are considered as safe [1]. Main steps in production of VLPs are: a) viral gene cloning into vector, b) synthesis of VLPs in selected expression system, c) purification of VLPs, d) identification and characterization of the particles [2]. One of the most limiting factor in successful VLP production is solubility of synthesized viral protein. In order to overcome this limitation, it is important to select suitable expression system and optimize conditions for soluble recombinant protein synthesis. Also, renaturation of insoluble proteins can be applied to obtain proteins that would self-assemble into VLPs [3].

The aim of this study was to synthesize the soluble *S. cerevisiae* dsRNA virus L-BC Gag protein that would self-assemble into VLPs to be used for further experiments. L-BC virus *gag* gene was cloned into expression vector which contains galactose-inducible promoter. The synthesis of the recombinant L-BC Gag protein was launched by induction for 18 hours in different *S. cerevisiae* strains. Our initial studies showed that protein expression was succesful while most of the protein is in the insoluble form. Since for formation of VLPs it is essential to obtain the soluble protein, optimization of synthesis and protein purification conditions was performed. We tested different induction times of protein synthesis and cell disruption buffers with different concentrations of salt and detergents. Since all of the results indicated that the L-BC Gag protein is synthesized in rather insoluble form, we decided to perform additional refolding using urea, guanidine hydrochloride and arginine solutions. Unfortunately, the protein did not dissolve in any of the solutions, so further optimization for solubilization of L-BC Gag protein is required.

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A4. Plasmid profiles and replicon types among clinical Acinetobacter baumannii isolates

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Acinetobacter baumannii is a gram-negative, opportunistic pathogen that is known for being resistant to multiple antibiotics, most notably – carbapenems. A. baumannii capabilities to be multi- or extensively-drug resistant arise due to its genome plasticity that is inherent to the genus [1]. They use multiple different mobile genetic elements (MGEs) to shuffle genes within cells and among other bacteria of the same species, sometimes even between different species [1,2]. Plasmids are among the most important MGEs that are responsible for antimicrobial resistance genes transfer therefore it is important to access the diversity and resistance potential of these vehicles in A. baumannii.

This study focuses on plasmids found in clinical isolates of A. baumannii and other members of this genus, obtained from specimens of patients of National Cancer Institute during the period of 2010-2018. Of 48 isolates investigated more than 98% were A. baumannii, while one isolate was a member of a previously unidentified Acinetobacter species. Acinetobacter plasmids were typed by the PCR-based replicon-typing scheme (PBRT) according to Bertini et al. [3]. Additionally, pulsed-field gel electrophoresis (PFGE) was employed to identify plasmid profiles. Of 48 tested isolates, 5 had one plasmid, 30 had two plasmids, 6 had three plasmids and 5 had four or more plasmids. According to the plasmid profiles, approximately 52% of all plasmids were 40-300kb in size, whereas the rest 48% were 2-40kb. The most common A. baumannii replicon types identified were GR2 and GR6, detected in approximately 77% and 73% of isolates, respectively. Less common types were GR4 and GR13, both found in a single isolate, GR16 and GR24 found in another isolate, and GR28 found in the non-baumannii isolate. Additionally, GR24 was found in two different A. baumannii isolates. In silico analysis was done, in order to compare observed replicon profiles of clinical isolates with data from NCBI GenBank. The rep gene sequences, which were detected among the isolates, were used in NCBI Blastn (MegaBLAST) algorithm to find closest homologs [4]. It revealed that among the aforementioned seven identified GR groups (GR2, GR4, GR6, GR13, GR16, GR24, GR28), the most common were GR6 with 122 unique NCBI entries, closely followed by GR2 with 106 unique NCBI entries. These entries constituted approximately 63% of all entries that were gathered in this analysis. They were followed by GR24, GR13, GR4 with 54, 43 and 29 unique NCBI plasmid entries, respectively, while the replicon types GR28 and GR16 had only 4 and 2 unique entries, respectively.

In conclusion, in this study of A. baumannii plasmids, it was found that all isolates contained at least one plasmid and they were similarly distributed among large and small sizes. Majority of the identified plasmids were either GR2 or GR6 type with a very few being other GR types. In silico analysis of those types revealed, that GR2 and GR6 indeed comprised the majority of the replicons observed in A. baumannii.

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B1. S100A9 PROTEIN INTERACTION WITH BIOMIMETIC LIPID MEMBRANES

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Pro-inflammatory, calcium-binding protein S100A9 is localized in the cytoplasm of a wide range of cells and regulates several intracellular and extracellular processes [1]. One of them is the participation in the inflammation associated with the pathogenesis of Alzheimer's disease (AD). In Alzheimer's disease, S100A9 serves as a junction between amyloid and neuroinflammatory cascades. Due to its amyloidogenicity, together with β-amyloid S100A9 forms neurotoxic amyloid plaques [2], which results in neuronal death and memory impairment. The number of studies on the impact of S100A9 in co-aggregation processes with amyloid-like proteins is increasing. However, the interest in the interaction mechanism of protein S100A9 with biological membranes is still limited.

In this work various biomimetic membrane models, as lipid vesicles in solution and tethered lipid bilayers (tBLMs), were used to examine the binding and interaction between protein and the membrane surface. For this purpose we employed atomic force microscopy (AFM) and fluorescence spectroscopy techniques. Our results indicate that the initial binding and accumulation of S100A9 protein on the lipid membrane surface is lipid phase-sensitive. We demonstrate that monomeric form of S100A9 interacts with tethered bilayer lipid membranes in phase specific manner and cause bilayer disruption through liquid-ordered lipid domains (Fig. 1). The most significant loss of integrity is observed in lipid bilayers composed of lipid mixture (total brain lipid extract). Our findings were further supported by thioflavin T fluorescence data which indicates inhibition of S100A9 fibrillation by ordered lipid domain systems due to potential protein penetration into bilayer. These results might broader the understanding of S100A9 interactions with lipid membrane and potentially affect the development of new diagnostic and therapeutic approaches for AD or other related diseases.

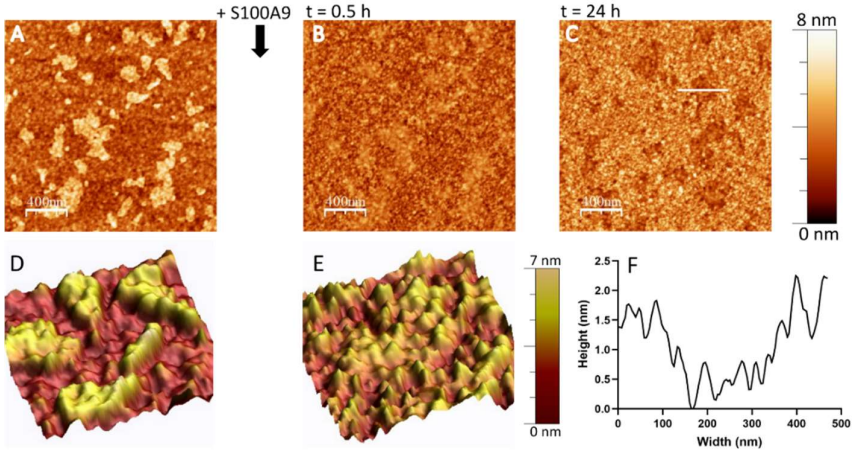

Figure 1. AFM observation of phase-specific S100A9 protein interaction with tBLM. (A) AFM topographic image of two-phase tBLM. S100A9 (5 uM) induced disruption of liquid-ordered phase after 0.5 (B) and 24 (C) hours. 3D images of non-disrupted (D) and S100A9 disrupted (E) tBLM. (F) Cross-section along the highlighted line (white line in the insert C).

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B2. PRODUCTION OF RECOMBINANT MOUSE CARBONIC ANHYDRASES PROTEINS

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Carbonic anhydrases (CA) catalyze reversible carbon dioxide hydration ($CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$); therefore, these enzymes participate in pH regulation, transport of CO_2 and bicarbonate, gluconeogenesis, lipogenesis, and other processes. Mammals have up to fifteen structurally similar CA isozymes widely expressed in different tissues and organs. Some of CA isozymes are drug targets for managing fluid secretion and pH change-linked diseases, such as glaucoma, edema, and even cancer [1].

Several clinically used CA inhibitors are available for systemic or topical use; however, non of them are selective towards a specific CA isozyme, resulting in frequently observed side effects. New and isozyme-specific small molecule inhibitors are designed, their affinity is tested in vitro and then subjected to preclinical testing in animals (most often mouse (Mus musculus)). While orthologous proteins have high sequence similarity, even minor structural alterations may have significant differences in small-molecule inhibitor affinity, and compound selectivity/affinity profile observed during in vitro testing using human CA isozymes may not correlate to preclinical testing in mice. Control experiments with recombinant murine CA proteins could help interpret the results of small molecule compound preclinical testing.

One of the methods in pharmaceutical industry used to measure drug candidate binding to target proteins of therapeutic interest is thermal shift assay. Because of its broad application method is applied for high-throughput screening of chemical compounds that could develop into therapeutic compounds. Most ligands stabilize proteins upon binding therefore increasing protein melting temperature thus with the assay dissociation constant can be determined. And in the past method has been used to measure ligand binding constants for carbonic anhydrases. Hence our main goal to produce all catalytically active mouse carbonic anhydrases that can be used in experiments with this method [2].

Here we are able to present purification, and small-molecule compound binding to several recombinant murine carbonic anhydrase isozymes (Figure 1).

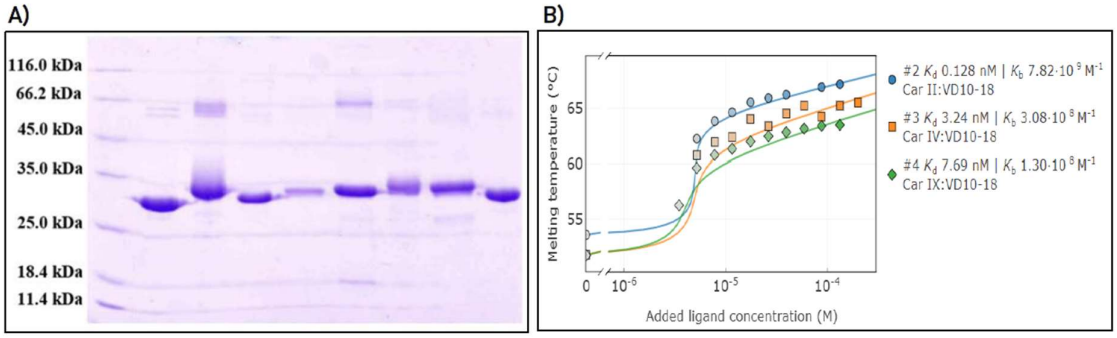

Figure 1. A) SDS-PAGE of carbonic anhydrases (from left to right protein marker Car I, Car II, Car III, Car IV, Car VA, Car VI, Car VII, Car XIII). B) Fluorescent thermal shift assay (FTSA) results of Car II, Car IV, Car IX binding to VD10-18 – symbols correspond to experimental data while line shows fitting using Kd determination model [2].

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B3. ENZYMATIC HYDROLYSIS OF PLANT-DERIVED PROTEINS

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As the world population is growing, the current demand for proteins in food and feed systems is also facing an increasing trend. Proteins are one of the key nutrients associated with human health, which are very important component of the human diet. They are essential for maintenance of body structural framework and for coordination of metabolic functions. Proteins are obtained from products containing plant or animal origins. Plant proteins provide high levels of proteins without the high content of fats that is often related to animal proteins. Furthermore, due to their lower cost compared to animal proteins, plant proteins have been well studied as a way of manufacturing cheaper food ingredient. Current policy directives and initiatives (EU Green Deal, EU Bioeconomy Strategy, etc.) are also directed to boost the use of plants for functional food. Plants are sustainable and environmentally feasible raw biomass and protein source [1].

The traditional chemical hydrolysis methods of plant proteins are usually performed under hazardous conditions, and it influences protein structure and other functional properties [2]. One of the alternative ways to hydrolyze plant-derived proteins is enzymatic hydrolysis. Enzymatic hydrolysis eliminates the use of antinutritional components, increases protein solubility and improves functional and nutritional properties of proteins. Hydrolysates, which are more readily absorbed by the body, can be applied in food industry as a functional food and in pharmaceutical industries because of formed bioactive peptides. The hydrolysates exhibit various beneficial features such as antioxidant activity, antihypertensive activity, and hypoglycemic activity, etc [3].

The aim of this study is to develop enzymatically based process for hydrolysis of plant isolated proteins. An investigation object is alfalfa plant, its flour and protein isolate. The obtained results in more detail will be presented during the poster session.

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B4. GENERATION OF CONDITIONAL TCF21 ALLELE BY OLIGONUCLEOTIDE-MEDIATED INTEGRATION OF LOXP SITES IN ZEBRAFISH

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Cardiovascular diseases are the leading cause of death worldwide. Zebrafish are becoming an increasingly powerful vertebrate model system in heart research due to their extensive regenerative capabilities. Transcription factor 21 (Tcf21) is expressed in the epicardium [1], which plays a crucial role in heart regeneration [2], yet the molecular mechanisms underlying this process are still unclear.

Gene knock-out is widely used to study gene function. However, many genes play pivotal roles in different developmental stages and cell types. Mutations in tcf21 gene lead to a lethal phenotype [3], which makes studying the function of this gene in adult organisms difficult. Conditional mutations allow to control gene expression in tissue-specific and temporal manner.

Site specific recombinases, such as Cre, are the most often used tool for conditional mutagenesis. They recognise specific target sites, in this case loxP, and recombine the sequence between them. Traditionally, two loxP sites are inserted into a gene to flank an exon, creating floxed allele. By using CRISPR/Cas9 and single stranded oligonucleotides (ssODN) we have successfully inserted two loxP sites into non-coding DNA regions of tcf21 gene. As tcf21 is composed of just two exons, first loxP site was inserted into 5' UTR region. Successful integration of this site has previously been reported [4]. Second loxP site was inserted into the intron to flank the first exon of the gene. Sequencing results indicated the integration of the full-length loxP sites. Presence of both loxP sites did not have any impact on the phenotype and viability of homozygous tcf21 floxed mutants. To validate the conditional tcf21 allele, a pair of F2 generation mutants, one heterozygous and one homozygous for the floxed tcf21 allele, were incrossed. Single cell stage embryos were injected with Cre mRNA. Phenotypical changes of injected embryos were analysed at 5 days post fertilisation (dpf). Uninjected control group and half of the injected embryos did not show any phenotypical abnormalities while the other half of injected embryos had severe defects in branchial arches formation. Genotyping results confirmed that individuals with severe abnormalities (the result of complete loss of Tcf21 function) were homozygous for tcf21 allele while phenotypically normal ones were heterozygous.

In summary, we have successfully used ssODN and CRISPR/Cas9 system to induce homology directed repair in zebrafish and integrate two loxP sites into tcf21 locus to create functional conditional tcf21 allele. These mutants will be further used to study the role of tcf21 gene in heart regeneration in zebrafish.

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C1: Characterization of Deaminases from Microbacterium sp. SINO2

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Canonical nucleic acids undergo many natural modifications some as uncomplicated as methylation, hydration of double bond, deamination, or more complex ones like the incorporation of amino acids or monosaccharides. Hypermodification systems found in bacteria and phages reveal the potential and diversity of yet unknown modifications [1,2]. Modified bases due to their importance in transcriptional regulation are associated with various diseases. 7-Cyano-7-deazaguanine ($PreQ_0$) is a biosynthetic precursor of the 7-deazaguanosine-modified tRNA nucleosides as well as a precursor to several natural products, such as toyocamycin and sangivamycin. Both of these pyrrolopyrimidine nucleosides and the $PreQ_0$ molecule itself are therapeutically interesting compounds known to have anti-cancer properties [3]. Although the synthesis of $PreQ_0$ has been extensively explored, the biodegradation pathway and the enzymes involved are still unknown.

Previously we isolated and identified soil bacteria Microbacterium sp. SINO2 that is capable of transforming $PreQ_0$ to 2,4-dioxo-2,3,4,7-tetrahydro-1H-pyrrolo[2,3-d]pyrimidine-5-carbonitrile (Fig. 1). In this study, we analyzed three hypothetical deaminases from Microbacterium sp. SINO2 that are likely to participate in the deamination of $PreQ_0$.

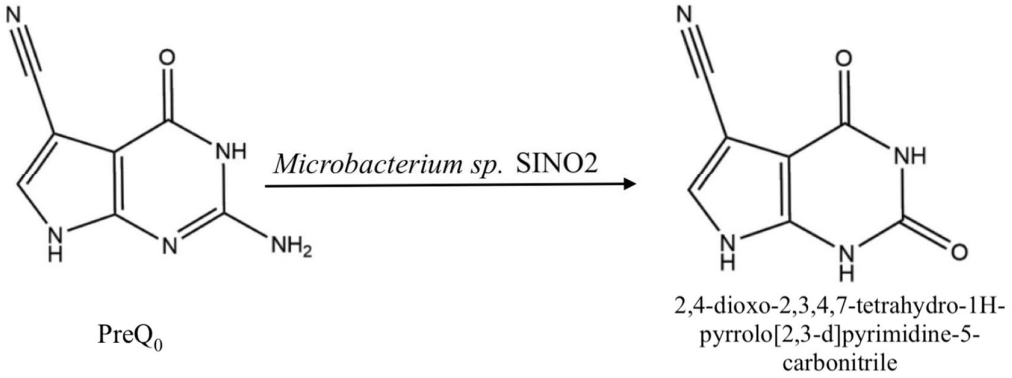

Figure 1. Deamination of $PreQ_0$ by Microbacterium sp. SINO2.

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C2. Improving sRNA binding selectivity using dimer MCP-GST protein

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Small RNAs (sRNAs) are considered as one of the major post-transcriptional regulators in bacteria. These molecules vary between 50 and 250 nt in length, they are highly structured and typically act by binding or being bound by other molecular factors. Usually, sRNAs base-pair other RNA molecules affecting their stability, function, and/or other characteristics. However, for the majority of currently known sRNAs, only a small subset of targets has been identified. In some cases, this can be caused by a relatively low abundances of sRNAs which require additional enrichment steps. One of the solutions to solve this problem is to utilise MS2 system, where RNA molecule is tagged by an MS2 aptamer, which is specifically bound by an MS2 coat protein (MCP). In case the latter is tagged with an affinity tag, then the *in vivo* assembled complexes from lysed cells can be purified using affinity chromatography. It has been determined that MS2 sequence is bound by an MCP dimer only, therefore in this work we tried to increase the specificity and selectivity of the system by merging an MCP dimer (2xMCP) instead of a monomer to a Glutathione S-transferase (GST) tag at the N-terminus in pGEX5 plasmid. In addition, we optimised induction and purification conditions to obtain a mainly soluble product. After utilising a two-step purification protocol, which included an immobilized glutathione sepharose purification followed by an affinity chromatography on heparin sepharose, we obtained a quite pure (~85%) GST-2xMCP protein with the total yield of 1.22 mg/L. Following that, we evaluated the performance of the purified protein to bind and specifically purify selected sRNAs from the whole bacterial cell lysates using glutathione sepharose beads. Subsequently, by employing reverse transcription (RT) and real-time, quantitative (q)PCR reactions we determined how well these RNAs can be extracted when compared to the untagged controls.

C3. EFFECT OF ORGANIC SOLVENTS ON NANOTUBE-FORMING CHIMERIC PROTEINS

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Biocatalysis is one of the most promising technologies, offering many benefits for the sustainable synthesis of various molecules for pharmaceuticals and biotechnological and industrial purposes [1]. Quite a few enzymes have been implemented in industrial biotechnology, however, some issues arise when enzymes exhibit poor stability under processing conditions. While in nature enzymes operate under aqueous conditions, the application of organic solvent systems might offer numerous advantages, such as increased solubility of hydrophobic substrates or water-dependent side reactions [2]. The downside is that organic solvents often inactivate enzymes. However, methods have been developed in order to enhance their stability in presence of organic substances – such as screening and isolation of biocatalysts that are robust under harsh conditions, chemical and genetic modifications and various immobilization strategies [2].

In this study we present two nanotube-encapsulated enzymes, constructed from bacteriophage structural proteins as scaffolds (bacteriophage vB_EcoS_NBD2 tail tube protein gp39 or bacteriophage vB_EcoM_FV3 tail sheath protein gp053) and amidohydrolase YqfB [3]. The encapsulation was performed by gene-fusion to obtain chimeric proteins. The chimeric proteins were synthesized in *E. coli* Bl21 (DE3) cells and self-assembled into nanotubes. Purified by sucrose density gradient centrifugation, the nanotubes were catalytically active and hydrolysed N^4-acetyl-2'-deoxycytidine to 2'-deoxycytidine. We investigated the catalytic activity of chimeric proteins in organic solvents: acetonitrile, 1,4-dioxane, methanol and dimethyl sulfoxide. Nanotubes displayed enhanced stability in methanol and dimethyl sulfoxide. We also studied the effect of trypsin on nanotube-encapsulated YqfB and found that stability against protease digestion increased. This immobilization approach appears to improve enzyme stability and could be applicable in functional biomaterials and biotechnology.

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C4. FUNCTIONAL ANALYSIS OF SMALL REGULATORY RNAS sLLM2- AND sLLM1042+ INVOLVED IN STRESS RESPONSE IN LACTIC ACID BACTERIA *LACTOCOCCUS LACTIS*

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Lactic acid bacteria *Lactococcus lactis* are commonly used in food and medicine industry, for fermentation and as probiotics, respectively. During these applications, *L. lactis* inevitably encounter a variety of stressors including acid, base, heating and cooling, oxidation and antibiotics [1]. Bacteria are able to cope with harsh conditions by changing the expression of various stress-response proteins. Meanwhile, the expression of these proteins is often itself regulated by 50–500 nt long small regulatory RNAs, sRNAs, as part of networks that respond to environmental changes [2]. Although numerous potential stress-response sRNAs have been identified in other bacteria, there are little to no data about sRNAs and their mRNA targets in *L. lactis* [3].

The aim of this work was to identify sRNAs-mediated target mRNAs associated with antibiotic-induced stress response in *Lactococcus lactis* subsp. *cremoris* MG1363. In this study two sRNAs – sLLM2- and sLLM1042+ involved in lysozyme and penicillin G-induced stress response – were analyzed in order to examine the phenotypic changes in bacteria caused by these stress conditions. Based on bioinformatic total RNA sequencing data analysis of bacteria overexpressing particular sRNAs, it was shown that sLLM2- modulates the expression of 177 genes, while sLLM1042+ regulates the expression of 45 genes, 11 of each were confirmed by real time quantitative PCR. Our analysis revealed that overexpression of sLLM2- inhibits the bacterial growth in medium containing galactose, whereas overexpression of sLLM1042+ supresses bacterial growth in medium containing glucose, galactose and cellobiose. After the bioinformatic identification of the most conserved and the strongest interactions between potential stress-response mRNAs and sRNAs' regions, 6 plasmids carrying different mutated sRNAs were constructed. It was shown that sLLM2- regions between 12-16 and 66-69 nt positions are responsible for increased bacteria resistance to lysozyme and sensitivity to penicillin G. While 57-60 nt region of sLLM1042+ confers the partial bacteria resistance to lysozyme, whereas both 30-33 nt and 57-60 nt regions appears to be partially responsible for *L. lactis* sensitivity to penicillin G. Thus, our results suggest that interactions between specific sLLM2- or sLLM1042+ regions and their target mRNAs are associated with specific changes in *L. lactis* phenotype under the overexpression of these sRNAs .

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D1. ANALYSIS OF 5'-NAD-RNA IN MODEL ORGANISM *ESCHERICHIA COLI* AND INDUSTRIALLY AS WELL AS MEDICALLY IMPORTANT LACTIC ACID BACTERIA

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It is known that eukaryotic mRNA has a 7-methylguanosine cap (m^7G) at the 5'-end that protects the transcript from degradation and regulates its maturation, localization and transcription. For a long time, it was thought that only eukaryotic RNAs can have a unique structure at their 5'-end, but in the year of 2009, it was shown that prokaryotic RNAs can also be modified with a nicotinamide adenine dinucleotide (NAD^+) cap [1]. Later this modification was found to be universal, as it was also observed in eukaryotes, including yeast, human and plant cells [2]. The main known function of NAD^+ cap in bacteria is the protection of RNA from degradation [3], however there is a lack of information on the modulation of capping depending on the bacterial growth phase, environmental conditions or intracellular NAD^+ level. Moreover, only few proteins have been found capable of regulating NAD^+ capping. One of them is *Escherichia coli* NudiX hydrolase NudC that can hydrolyse 5'-NAD-RNA [4]. As it is unclear how this protein interacts with RNA, we raised a hypothesis that this reaction could require additional proteins. Indeed, our group identified one of the potential partners, though the function of it remained unknown. In addition, until now the 5'-NAD cap and its hydrolases have not been studied in industrially and medically important lactic acid bacteria.

To fill the existing knowledge gap, in this study we (i) evaluated the dependence of RNA capping with NAD^+ in the model organism *Escherichia coli* on bacterial growth phase and environmental conditions or intracellular NAD^+ level, (ii) analysed the impact of *E. coli* NudiX hydrolase NudC and its associated protein on bacterial physiology and (iii) conducted a search for 5'-NAD^+-RNA in lactic acid bacteria *Lacticaseibacillus paracasei* and *Lactococcus lactis*. High performance liquid chromatography–mass spectrometry analysis revealed that in *E. coli* the level of 5'-NAD^+-RNA depends on bacteria growth stage and temperature but not on the fluctuations of intracellular amount of NAD^+. *Northern* hybridization experiments for the first time showed that both NudC and its associated protein affect the quantity of not only 5'-NAD-sRNA, but also the total amount of sRNA. We also discovered that RNA of lactic acid bacteria contains 5'-NAD^+ cap and that the quantity of 5'-NAD^+-RNA in *L. lactis* is growth-phase dependent while in *L. paracasei* – it is not. Additionally, using bioinformatics methods, we found 11 and 8 potential NudiX hydrolases in *L. lactis* and *L. paracasei,* respectively. These proteins could be involved in the metabolism of 5'-NAD-RNA, thus adding lactic acid bacteria to the list of organisms with elaborate 5'-NAD-RNA pathway. Together, the results of our study suggest that RNA is modified by 5'-NAD in a controlled manner across different bacterial strains, highlighting the significance of this modification in bacterial physiology and the need for even deeper research including identification and detailed analysis of proteins that regulate NAD^+ capping.

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D2. CHARACTERIZATION OF NOVEL YQFB-LIKE AMIDOHYDROLASES ACTIVE TOWARDS MODIFIED CYTOSINE AND CYTIDINE COMPOUNDS

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Human activating signal cointegrator homology (ASCH) domain is widely dispersed in all domains of life and some prokaryotic viruses. Based on the known properties and functions of homologous domain-containing proteins in RNA metabolism, the ASCH domain is thought to be able to interact with RNA molecules as a transcription co-regulator[1]. However, there is a lack of available experimental data highlighting the biological functions of this diverse protein superfamily.

In a previous study, a hypothetical ASCH domain-containing protein from *Zymomonas mobilis* was described as a monomeric ribonuclease, which binds nucleic acids and degrades single-stranded RNA molecules *in vitro*[2]. Recently, the *Escherichia coli* ASCH domain-containing protein YqfB has been characterized as the smallest known amidohydrolase of just 103 amino acids[3]. The primary substrate of the enzyme is the modified nucleoside N^4-acetylcytidine (ac4C), which is highly abundant in both eukaryotic and prokaryotic RNA molecules. The presence of ac4C is important for efficient protein synthesis and eukaryotic ribosome biogenesis. It is also suggested that cytidine acetylation and deacetylation might add another layer of regulation of the responses to environmental stressors[4]. ac4C writers are quite well studied in prokaryotes, however, to this day, no proteins, responsible for the removal of acetyl modification, were identified.

To gain more insight into the aforementioned protein superfamily, our study aimed to characterize hypothetical ASCH domain-containing proteins identified in mesophylic bacteria, such as *Buttiauxella agrestis*, *Cronobacter universalis*, *Klebsiella pneumoniae,* and *Shewanella loihica*. To achieve this goal, recombinant protein purification and activity assays with modified cytosine and cytidine derivatives were performed. To determine whether amidohydrolase YqfB and its' analogs can bind RNA molecules, the electrophoretic mobility shift assay (EMSA) using radioactive isotope-labeled *E. coli* tRNA was applied.

Our findings show that all target proteins exhibit amidohydrolase activity with a wide range of modified cytosine and cytidine compounds. Based on the kinetic properties, the ac4C might be the primary substrate of YqfB analogs in vivo. However, we did not detect any RNA-binding activities of the tested proteins. Thus, this work provides new data about proteins belonging to the widespread ASCH protein superfamily, the cellular role of which remains to be proven by further studies.

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D3. THE INVESTIGATION OF MOUSE METHIONINE ADENOSYLTRANSFERASE MAT2A MUTANTS

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Methionine adenosyltransferase MAT2A synthesizes SAM (S-adenosylmethionine) from L-methionine and ATP. SAM is used by DNA methyltransferases as a donor of methyl group for DNA, RNA and protein modification and thus plays important part in gene expression, enzymatic activity, DNA reparation and are necessary for normal development of an organism. Methyl group is not chemically reactive and hard to detect. Therefore, synthetic SAM analogues were created that are able to transfer more chemically reactive groups which can be linked with reporter group [1]. This method is known as methyltransferase-directed Transfer of Activated Groups (mTAG). SAM and it's analogues can not cross the cell membrane, thus this method is not applicable for *in vivo* experiments. Previously, it was shown, that MAT2A I117A and I322A mutants are able to synthesize SAM analogues, but only in the absence of L-methionine [2]. These conditions are not suitable for experiments *in vivo* as well, considering that the lack of L-methionine could disrupt drastically the epigenetic regulation and protein synthesis. Therefore, MAT2A mutants that are able to synthesize SAM analogues in presence of L-methionine are under investigation.

One of the possible solutions is to replace the sulfur atom with selenium and use L-seleniumhomocysteine with extended 6-aminohex-2-yn-1-yl functional group (NH_2-SeMet). This reaction would produce Se-adenosyl-Se- (6-aminohex-2-yn-1-yl) -L-selenohomocysteine (NH_2-SeSAM), which could be used as a substrate by DNA methyltransferases.

Therefore, the goal of our research is to identify *Mus musculus* MAT2A mutants that can synthesize SAM analog NH2-SeSAM from ATP and NH2-SeMet, in the presence of L-methionine. To achieve this goal, the following tasks were set:

1. Introduce specific mutations in MAT2A I117A gene potentially conferring selectivity for NH_2-SeSAM over L-Methionine.
2. Purify recombinant MAT2A proteins.
3. Measure the activity of purified proteins by performing MS-HPLC analysis to detect SAM analog NH_2-SeSAM, in the presence of L-methionine.

The following results were achieved:

1. Vectors of *Mus musculus* MAT2A gene containing the target mutations were genetically engineered.
2. Mutant variants of Mus musculus MAT2A with greater than 95% purity were purified from E. coli BL21 RIL (DE3) cells.
3. Two MAT2A mutant variants were identified to be able to synthesize SAM analog NH_2-SeSAM, in the presence of L-methionine.

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D4. STUDY OF THE INTERACTION BETWEEN BIOMIMETIC LIPID BILAYER AND AMYLOID-β PEPTIDE

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A common sign in neurodegenerative diseases is the misfolding, aggregation, and accumulation of proteins, leading to cellular dysfunction, loss of synaptic connections, and brain damage. Proteins can undergo misfolding from their native state to form intermolecular β-sheet rich structures, ranging from small oligomers to large fibrillar aggregates, in the diseased brain. Amyloid β that confers neurotoxicity has been central to the amyloid hypothesis of Alzheimer's disease pathology [1]. Intermediate structures of aggregation process are transient and heterogeneous and the mechanism by which amyloid β can be neurotoxic has not been fully elucidated. One prevailing hypothesis suggests that amyloid β can be toxic through a membrane disruption mechanism, which leads to loss of synaptic efficiency, neuronal dysfunction and degeneration [2].

Biomimetic lipid bilayer systems are a useful tool for modelling specific properties of cellular membranes in order to get more knowledge about their structure and functions. In this study, as simplified membrane model, we are using large unilamellar liposomes. The aim of our work is to find out whether composition of lipids is an important factor for amyloid β and membrane interaction. By incorporating lipids, such as sphingomyelin, we can mimic membrane microdomains, also known as lipid rafts. Lipid rafts appear to be involved in the development of Alzheimer's disease by affecting the aggregation of the amyloid β peptide at neuronal membranes thereby forming toxic oligomeric species [3]. Additionally, in this work we study what extent of damage is caused by amyloid β at different stages of the aggregation process (small oligomers and fibrils). To study interaction between amyloid β and unilamellar liposomes we are using fluorescence spectroscopy. Fluorescent dye Thioflavin T allows us to monitor fibrils formation and calcein leakage essay helps to evaluate liposomes integrity changes. Our results show that small oligomers of amyloid β disrupt the liposomes at much higher level, compared to large fibrils.

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E1. BIO-POTPOURRI OF SACCHAROMYCETALES KILLER YEASTS

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Many members of the flora tend to co-exist with various other organisms, some of which drive plants' survival and biological prosperity. It is hardly astonishing that a wide spectrum of those symbiotic organisms are yeasts, however, their properties can be rather peculiar – they significantly diverge from a family point of view and can possess biocidic properties, regardless of their species. Our ambition was to disclose this magnitude of different yeasts with double-stranded RNA (dsRNA) killer viruses and identify their position in the Saccharomycetales family tree. Comprehending such biodiversity with linkage to yeasts' virulent capabilities may be fundamental for novel discernments of life.

We have inspected 384 unique yeast strains from a vast range of natural niches in different geographical zones of Lithuania. More than a tenth of them had some sort of killer phenotype – a phenomenon which was verified by combining methods of RNA gel electrophoresis and growth on a selective medium. Out of the biocidic yeasts we selected 12 unique strains with most palpable morphological disparities and performed parallel analyses of restriction fragment length polymorphism (RFLP) and internal transcribed spacer (ITS) sequencing. The outcome suggests that most yeast strains are of Metschnikowia sp. origin, yet several other species were also identified. This may imply that killer activity is much more moderately dispersed throughout a variety of Saccharomycetales, and new types of viruses with their unique capabilities are yet to be perceived.

E2. ELECTROCHEMICAL BIOSENSOR FOR DEOXYRIBONUCLEASE DETECTION AND MEASUREMENT

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Deoxyribonucleases (DNases) are targets in biotechnology presenting both as a contaminant as well as a tool for fragmentation in DNA sequencing,[1] DNA manipulation and for the removal of unwanted biomolecules. Also notably, DNAses may prove to be an important biomarker in autoimmune or inflammatory disease.[2] Electrochemical biosensors provide a simple and inexpensive platform for biomolecule detection while maintaining a high degree of accuracy and sensitivity.

Our study was aimed at the development of an electrochemical biosensor for detecting the activity of DNase I as the model DNase. The sensor employs a labelled DNA target which is cleaved in the presence of DNases and is able to hybridize to an immobilized DNA probe to generate an electrochemical signal. The signal is related to the concentration of the intact DNA target and can be observed using cyclic voltammetry. Signal loss becomes apparent upon the target cleavage, thus the presence of DNAses can be detected by measuring the rate of hybridization of the intact DNA target. The DNA target was designed to have a both single- and double-stranded DNA regions to ensure the activity of various DNAses. To specifically detect the DNA target a self-assembled monolayer (SAM) of DNA probe was attached to the sensing gold electrode surface.[3] SAM formation is an attractive method to form a stable and accessible surface for DNA hybridization.

Our results indicate that the electrochemical signal increases over time as hybridization of the target occurs. The DNA target shows high rates of hybridization when incubated in nuclease-free DNase I working buffer solution while the hybridization rate decreases when the target is incubated with DNAse I. The detectable concentration range is up to 0.001 U mL^{-1}. Any liquid sample such as saliva can be added to the DNA target and DNase I was detectable from surface swabs (0.35U spread on a ceramic surface). The method was compared with a currently available fluorescence based kit (DNaseAlert™ QC System, Thermo Fisher Scientific) showing very similar results in the lowest DNase I concentration range. Finally, we have designed a model device consisting of a cell with gold sensing, Ag/AgCl reference and platinum counter electrodes, temperature and stirring control unit and signal analysis electronics, and custom built software.

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E3. SYNTHESIS OF 6-(3-METHOXY-1-PHENYL-1*H*-PYRAZOL-4-YL)-6*H*-CHROMENO[4,3-*b*]QUINOLINE DERIVATIVES

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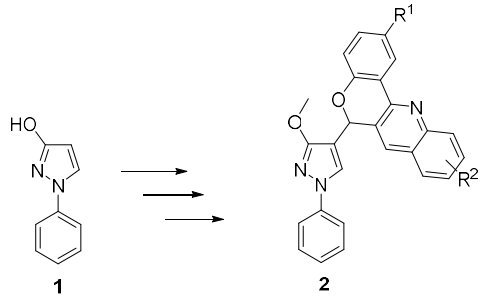
Pyrazole ring is an important structural unit within the structure of many synthetic and natural biologically active compounds. Derivatives containing pyrazole fragment exhibit significant biological activities, such as antimicrobial, anti-inflammatory, antibacterial, anticancer and other properties. Because of these biological activities, pyrazole derivatives are widely used in medicinal chemistry and many studies are devoted to the synthesis and biological evaluation of novel pyrazole moiety-bearing molecules [1].

Chromene and quinoline derivatives also stand out by their biological activity. The chromene derivatives show various pharmacological properties such as anticancer, anticoagulant, anti-HIV, diuretic, antimalarial, antitumor, antibacterial and other activities [2]. Quinoline derivatives also exhibit wide range of biological properties such as antibacterial, antifungal, antiviral, anti-protozoal, antimalarial, anticancer, cardiovascular, CNS effects, antioxidant, anticonvulsant, analgesic, anti-inflammatory, anthelmintic and others [3].

All three heterocycles, pyrazole, chromene and quinoline, belong to the group of priviledge structures for drug development, so in this work we developed new derivatives containing pyrazole, quinoline and chromene fragments and investigated their structure and properties. Herein we present an access to yet unknown 6-(3-methoxy-1-phenyl-1*H*-pyrazol-4-yl)-6*H*-chromeno[4,3-*b*]quinoline derivatives.

First, according to the already known methodology [4], synthesis of 3-methoxy-1-phenyl-1*H*-pyrazole-4-carbaldehyde was performed from 1-phenyl-3-hydroxy-1*H*-pyrazole. 3-Methoxy-1-phenyl-1*H*-pyrazole-4-carbaldehyde was further reacted with 2-hydroxyacetophenones affording appropriate flavanones. Then the Wilsmeier-Haack reaction was employed to form pyrazolyl-2*H*-chromene-3-carbaldehyde which was further used for the synthesis of target 6-(3-methoxy-1-phenyl-1*H*-pyrazol-4-yl)-6*H*-chromeno[4,3-*b*]quinoline system. (Scheme 1)



Scheme 1. Synthesis of 6-(3-methoxy-1-phenyl-1*H*-pyrazol-4-yl)-6*H*-chromeno[4,3-*b*]quinoline derivatives

The structures of products were confirmed by data of nuclear magnetic resonance spectroscopy, infrared spectroscopy, and mass spectrometry.

Acknowledgements:

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E4. STUDIES OF MIR-7, MIR-153 AND MIR-214 IN BLOOD OF PATIENTS WITH PARKINSON'S DISEASE

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Parkinson's Disease (PD) is the second most common progressive neurodegenerative disorder that is characterized by both motor and non-motor manifestations. Motor symptoms include resting tremor, bradykinesia (slow movement), rigidity, shuffling gait, and postural instability. Non-motor symptoms of PD are cognitive changes, behavioral/neuropsychiatric changes, autonomic nervous system failure, sensory and sleep disturbances [1]. Considering the accuracy of clinical diagnosis of PD is currently inadequate and there is no reliable quantitative diagnostic test for PD, molecular biomarkers could be potential clinical tools to ease early and accurate PD diagnosis [2,3]. For example, circulating plasma miRNAs could possibly be used as one of the non-invasive biomarkers, facilitating the early detection of PD and monitoring the progression of the pathology [2]. However, more research is needed to further evaluate the potential of miRNAs and other small molecules as candidate biomarkers before application in clinical practice [4]. The aim of the study was to investigate miR-7, miR-153, miR-214 expression in blood samples that were collected from patients with PD who received DBS or Gamma knife surgery, examine if there were any differences and try to link the results with clinical symptoms and other patient-related data.

First of all, blood samples were centrifuged, and serum was collected. Extracellular vesicle miRNA was then isolated from blood serums and miRNA cDNA was synthesized. The expression levels of miR-7, miR-153, miR-214 were evaluated by RT-PCR in 42 samples of patients, who underwent DBS surgery, 16 samples of patients, who underwent Gamma knife surgery, and 37 samples of PD control group patients.

After observation, the results showed that the difference between the expression levels of miR-7, miR-153 and miR-214 was statistically significant. The statistical analysis of the results showed that miR-7 expression is significantly different between the control group and the DBS group ($p \leq 0,05$). Furthermore, the results showed that miR-214 expression levels between patients with first and forth level of bradykinesia were statistically different ($p \leq 0,01$). Moreover, the results showed that miR-214 expression is positively correlated with the age of the patients ($p \leq 0,05$). Lastly, statistical analysis showed a positive correlation between expression of miR-7 and the duration of PD ($p \leq 0,01$).

In conclusion, the different miRNA expression levels in PD blood serum samples showed patient heterogeneity and indicate a potential role of miRNA in PD pathogenesis, however more research is still required.

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F1. 5-(BUTYLTHIO)-2-(1*H*-INDOLYL)-1,3,4-OXADIAZOLES LOW MOLECULAR WEIGHT COMPOUNDS WITH POTENTIAL PROTECTIVE ACTIVITY AGAINST OXIDATIVE STRESS

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Nitrogen containing compounds, such as oxadiazoles and indoles, are of great importance in medicine and industry. As 1,3,4-oxadiazole heterocycles are good bioisosters of esters and amides, which can substantially contribute to hydrogen-bonding interactions with receptors [1] combining it with indole into one molecule is expected to result in compounds with enhanced biological properties. Recently we reported synthesis of a small library of 2-((1H-indol-3-yl)methyl)-5-(alkylthio)-1,3,4-oxadiazoles with results from screened their protective effects *in vitro* and *in vivo* [2]. Three of the prepared compounds, protected FA-fibroblasts against glutathione depletion induced by *γ*-glutamylcysteine synthetase inhibitor BSO. Two of the active compounds increased the survival of *Caenorhabditis elegans* exposed to juglone-induced oxidative stress. During these experiments it was defined that 5-(butylthio)-1,3,4-oxadiazole structural unit might be the key part of molecule leading to desired biological activity.

Inspired by the obtained results, we intended to further elucidate protective potential of 1,3,4-oxadiazole-indole hybrids. Seeking to evaluate the influence of 5-(butylthio)-1,3,4-oxadiazole fragment connection position in the indole ring to their protective activity against oxidative stress the goal of this project was to prepare a number of 5-(butylthio)-2-(1*H*-indolyl)-1,3,4-oxadiazole regioisomers.

In a three-step synthesis, a series of compounds were prepared from various 1H-indole carboxylates regioisomers. The first step was formation of corresponding intermediate hydrazides. The immediate base-mediated treatment of crude hydrazides with carbon disulfide, followed by *in situ* acidification gave rise to corresponding regioisomer of 5-(1*H*-indolyl)-1,3,4-oxadiazole-2(3*H*)-thione. The final step was base catalized *S*-alkylation with 1-iodobutane. The structures of the obtained compounds were elucidated and confirmed by NMR, IR and mass spectrometry. All compounds were sent for biotesting to Czech Republic.

Short overview of recent biotesting results and detailed synthesis schemes will be presented in poster presentation.

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F2. EXTRACELLULAR miRNAs AS DIAGNOSTIC BIOMARKERS FOR PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease, that progresses with aging. PD is characterized by a progressive loss of dopaminergic neurons that leads to severe motor and non-motor symptoms. Although the initial diagnosis is usually based on clinical motor symptoms, such as tremor and bradykinesia, other non-motor symptoms also occur commonly [1]. Despite significant recent research progress of PD, that can afford symptomatic relief, clinicials still face a lot of difficulties in diagnosing and choosing the proper treatment for PD patients. A disease modifying agent capable of halting the progression is not yet available. The lack of a robust biomarker with high sensitivity and specificity has limited the progress towards the development of effective therapeutics for PD. Deep brain stimulation (DBS) surgery and Gamma knife (GK) radiosurgery have become widely used [2]. However, it raises concerns regarding infections and hardware complications. There is a need for molecular markers that could contribute to better treatment selection and outcome prediction. The possible alternative could be miRNA [3]. The purpose of this pilot study is to determine the expression of miRNA in blood samples from patients with Parkinson's disease who have received Deep brain stimulation implantation surgery and Gamma knife radiosurgery and examine whether there are any differences and try to relate to clinical symptoms and other patients-related data.

PD patients, who have participated in this research, blood samples were collected before and after DBS and GK surgeries. Firstly, blood samples were centrifuged and the serum part was collected. miRNA was isolated from the extracellular vesicles in the serum. Following the protocol, miRNA cDNA synthesis was performed. The expression levels of miR-29c, miR-30b and miR-181a were evaluated by RT-PCR in 42 PD patients, who underwent DBS implantation surgery, 16 PD patients, who underwent GK radiosurgery, samples, and 37 PD controls. Statistical analysis was performed to find correlations between expression levels of miRNA in serum from PD patients before DBS implantation surgery (DBS-1) and after (DBS-2), before Gamma knife radiosurgery (GK-1) and after (GK-2), and PD controls (CTL).

The results showed that miR-30b expression was significantly lower in DBS-1 and GK-1 than in CTL ($p<0.05$). The miR-29 expression among patients with second- and third-degree bradykinesia ($p<0.05$) and miR-30b between patients with no evidence of bradykinesia or with first-degree bradykinesia and third-degree bradykinesia were statistically significant ($p<0.01$, $p<0.5$, respectively). Futhermore, the miR181-a expression level between patients with no evidence of tremor or with first-degree tremor and second-degree tremor was statistically significant ($p<0.01$, $p<0.5$, respectively). Moreover, the results showed that miR-30b and miR-181a correlate negatively with the age of PD patients ($p<0.05$). However, only miR-181a was significantly related to the age when patients were diagnosed with PD ($p<0.01$).

In conclusion, the different miRNA expression levels in PD blood samples showed patients heterogeneity and indicate a potential role of miRNA in PD pathogenesis, but the correspondinng details require intensive research.

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F3. OXYFUNCTIONALIZATION STUDY OF AROMATIC COMPOUNDS BY BIOCATALYTIC AND CHEMICAL METHODS

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Oxiranes, also known as epoxides, are three-membered heterocycles with an oxygen heteroatom in their structure. Oxiranes prepared by the treatment of an alkene with peroxy acids are the smallest member of heterocyclic compounds and are important intermediates in organic chemistry. They also bear the capacity of wide–ranging ring–opening reactions, which usually occur with predictable regioselectivity and stereospecificity [1,2]. Chemical synthesis requires strong oxidants, reactions are not always stereo and regioselective. Biocatalytic methods, meanwhile, are environmentally friendly, characterized by stereo and regioselectivity. Previous studies have shown that soluble di-iron monooxygenase PmlABCDEF (PML) can oxidize various N-heteroaromatic compounds to their N-oxides [3]. It is also interesting whether PML monooxygenase can use other aromatic compounds as substrates: phenols, styrenes, allyl-substituted compounds. Phenol is widely used in many industries such as petroleum refining, pharmaceutical, and resin-manufacturing plants. Therefore phenol is the major pollutant found in wastewaters of these industries [4]. We also investigated how PML monooxygenase converts various phenols containing amino, aliphatic, methoxy substituents. By oxidizing various allyl-substituted aromatic compounds both chemically with mCPBA (meta-chloroperoxybenzoic acid) and using PML monooxygenase, the aim is to investigate the specificity of the substrates of this enzyme and whether the enzyme is inherently stereoselective.

The various allyl-substituted aromatics (o-eugenol, 4-allyl-1,2-dimethoxybenzene) and styrene were oxidized using mCPBA, synthesized compounds checked by color reaction with 4-(4-nitrobenzyl)pyridine and the resulting epoxides were purified by column chromatography using a column filled with silica gel and a mobile phase of hexane-ethyl acetate (1:1).

Chemically synthesized epoxides were compared with oxidized compounds of PML monooxygenase. All biocatalytic reactions were performed using Pseudomonas putida KT2440 bacterial strain harboring a recombinant plasmid containing the pmlABCDEF gene.

Compounds were analyzed using methods such as thin layer chromatography (TLC) or high-performance liquid chromatography – mass spectrometry (HPLC-MS).

Also, combining chemical and biocatalytic methods could be an attractive new platform to synthesize compounds that would be difficult to obtain by chemical synthesis alone.

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F4. Wood ticks (*Ixodes ricinus*) and the agent of Lyme borreliosis Lithuanian city parks

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Tick-borne diseases (TBDs) are an emerging health problem for humans and domestic animals. The most common and well-known tick-borne disease in Europe is Lyme borreliosis (LB), caused by spirochaetes of the *Borrelia burgdorferi* s. l. and transmitted by ticks mainly from the genus *Ixodes*. *Notably, Ixodes ticks presence and an* increasing number of tick bites *are commonly reported in urbanized areas such as suburban forests and city parks. Global warming, progressive environmental changes, and urbanization force plants and animals to leave their habitats or to adapt to the new environment.* Ticks are an example of species that adapt to new conditions. Unfortunately, in Lithuania, the risk posed by ticks in such areas is largely unknown. This study aimed to investigate the presence of Ixodes ticks and their infection with *Borrelia burgdorferi* s. l. in eleven urban parks in Lithuania. A total of 361 questing ixodid ticks (2 larvae, 161 nymphs, 107 males, and 91 females) were collected by flagging from nine different parks in Lithuania during the spring of 2021. All ticks were identified as *Ixodes ricinus* based on morphological criteria. After DNA extraction, ticks were further analysed for the presence of *Borrelia* spp. by real-time PCR amplification of the partial 16S rRNA gene. Overall, 26,5% (96/361) of ticks collected in the parks were infected with *Borrelia*. The prevalence of *Borrelia* sp. in different parks varied from 0% to 38,9%. *Borrelia*-positive ticks were found in eight city parks. In order to identify *Borrelia* species, both partial outer surface protein A (*ospA*) gene and 16S-23S rrs-rrlA intergenic spacer region were amplified and sequenced. Our results demonstrate the potentially high human risk of exposure to tick-borne infection with *Borrelia burgdorferi* s. l. in urban parks in Lithuania.

G1. CHEMOENZYMATIC SYNTHESIS OF ACETYLATED 2'-AMINO-2'-DEOXYURIDINES

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Different specificity of enzymes enables us to use biocatalytic reactions alongside chemical ones in biodegradation and synthesis of various chemical compounds. Specifically, the enzymes show an excellent regio- and stereoselectivity, hence more efficient processes can be developed using appropriate biocatalysts.

In this work, 21 metagenomic ester or amido-hydrolases exhibiting a differed by substrate specificity [1] were analyzed for regioselective deacetylation of nucleosides. For this purpose 2'-amino-2'-deoxyuridine was treated with acetic anhydride and sodium acetate [2] at 90°C for 1 h to produce 2',3',5'-triacetyl-2'-amino-2'-deoxyuridine. The reaction product was purified by silica gel column chromatography and further analyzed by HPLC-MS and nuclear magnetic resonance (NMR).

A hydrolytic activity of each enzyme was assayed in reaction mixture containing 50 mM potassium phosphate buffer, pH 7.5, 2 µl of the appropriate enzyme and 10 mM substrate - 2',3',5'-triacetyl-2'-amino-2'-deoxyuridine. Reactions were carried out in an overall volume of 20 µl and analyzed by thin-layer chromatography (TLC) under UV light after 1 hour and 20 hours. Results showed that mainly three types of the product can be observed (Fig. 1). After 20 hours, most enzymes hydrolyzed both of ester groups and left untouched 2' amide group. A full deacetylation was also observed in the case of the hydrolase M013, that was confirmed by staining TLC plates with ninhydrin solution.

For further analysis of products, a semi-preparative conversion was carried out. Hence, 2',3',5'-triacetyl-2'-amino-2'-deoxyuridine was subjected to the enzymes GRU1 and SVG1, and the reaction products were purified using silica gel chromatography. SVG1 mainly hydrolyzed one of acetyl groups. In contrast, GRU1 was active towards both ester groups. The reaction product in the case of SVG1 was identified as 2',3'-diacetyl-2'-amino-2'-deoxyuridine through NMR analysis.

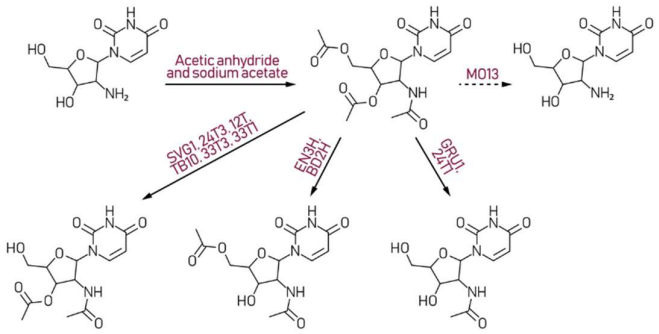

Figure 1. Chemoenzymatic synthesis of acetylated 2'-amino-2'-deoxyuridines

In conclusion, a chemoenzymatic synthesis of mono- and diacetylated 2'-amino-2'-deoxyuridines was established. The purified compounds will be further used as substrates for identification of novel regioselective hydrolases using a previously described screening method based on auxotrophic complementation [1].

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G2. FUNCTIONAL ANALYSIS OF CRISPR-CAS TYPE I-D EFFECTOR COMPLEX BY IN VIVO ASSAYS

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Many molecular tools now used in genetic engineering were discovered during the research of arms race between viruses and bacteria [1]. The CRISPR-Cas system is an adaptive immunity found in bacteria and archaea which lately receives remarkable attention because of its programmable RNA-guided targeting of DNA which can be used in gene expression regulation [2] or genome editing [3].

Class 1 type I CRISPR-Cas systems are the most abundant and consist of multiprotein crRNA-guided effector which binds dsDNA target distinguished by protospacer adjacent motif (PAM) – a short sequence next to the target and absent in the host's genome. After the formation of R-loop a Cas3 helicase-nuclease is recruited and cleaves the target DNA [4]. The subtype I-D is considered an evolutionary intermediate between type I and type III systems for the presence of type III like large subunit dCas10 which encodes a HD-nuclease domain. Therefore the complex itself might perform the target cleavage, with Cas3 later acting as a helicase. Recent studies of S. islandicus LAL14/1 CRISPR-Cas I-D system showed its ability to cleave both double-stranded and single-stranded DNA [5], though the exact mechanism of this cleavage remains elusive.

Our research focuses on I-D Cascade complex from A. flos-aquae, a mesophile whose Cas proteins could be suitable for genetic engineering. The mechanism of action of this particular system has not yet been characterized. Thereby, wild-type and mutated Cas genes were cloned into heterologous host E. coli, then different targets were designed: M13 bacteriophages and pUC plasmids with various PAM sequences or reduced target sequence lengths. In vivo assays with M13 bacteriophage targets were performed to elucidate which PAM sequence is recognized the best and therefore which phage's proliferation is inhibited the most by I-D Cascade complex. The aim for plasmid assays is to determine the minimum length of a target sequence that is still recognizable by the CRISPR-Cas I-D system. To elucidate the role of different Cas proteins for plasmid or phage interference, we cloned the mutant variants of each potential effector protein of type I-D CRISPR-Cas system. Furthermore, the I-D system from A. flos-aquae encodes toxin-antitoxin (TA) system in the same operon, though the functional significance of this remains unclear. Therefore, our study aims to analyse the potential co-action of TA system and CRISPR-Cas system applying phage or plasmid interference assays.

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G3. PRO-INFLAMMATORY S100A9 PROTEIN PROMOTES TAU PROTEIN AGGREGATION

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Neurodegenerative diseases are among the most common disorders in the world. Unfortunately, despite intensive research, the understanding of the mechanism of these diseases is limited, and almost all existing treatments are symptomatic [1]. Alzheimer's disease has attracted the most attention from scientists because it is the most common neurodegenerative disease, affecting about 50 million people worldwide. In addition to amyloid plaques composed of amyloid-β peptides, neurofibrillary tangles formed from the protein Tau are a hallmark of this disease as well as other tauopathies [2]. Therefore, it is essential to understand the mechanisms involved in process of protein amyloid aggregation and determine the best way to curb them.

One of tauopathies – Chronic traumatic encephalopathy – registers high levels of Tau aggregates, and the exact reasons for their formation are unknown. Researchers observed that this disease is quite prominent in contact sport players (e.g., American football) who experiences chronic head concussions [3]. There has been some speculation from the scientific community that neuroinflammation could induce Tau pathology; thus, it is feasible that S100A9 as a pro-inflammatory protein could be a culprit behind it. However, there is no information showing if the S100A9 protein or its aggregates could be involved in Tau aggregation [4]. Therefore, we examined the ability of the S100A9 protein and its aggregates to promote Tau aggregation.

We used recombinant S100A9 and Tau (2N4R isoform) proteins. All experiments were performed in pH 7,4 (50mM HEPES, 10mM phosphate) buffers complemented with 2mM DTT, at 37^{0}C temperature. For Tau aggregation 15μM of protein were used and S100A9 in range of concentrations from 5μM to 90μM. For Tau aggregation experiments using S100A9 fibrils, aggregates were pre-formed by incubating protein for 72h at 37^{0}C. Aggregation kinetics were followed using the amyloidophilic dye thioflavin T fluorescence assay. Atomic force microscopy was performed to analyze the morphology of the formed aggregates.

We observed that Tau aggregation is dependent on S100A9 aggregate formation as S100A9 monomers alone do not induce Tau aggregation, while S100A9 aggregates induce notable fluorescence changes in the reaction mixture with Tau protein.

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G4. CYTOGENETIC EFFECTS OF EXPOSURE TO MICROPLASTICS ON LARVAL-STAGE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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A growing amount of microplastics (MPs) is released into the aquatic ecosystems due to large-scale use and improper waste management. MPs are widespread plastic particles smaller than 5 mm in size and pose a serious threat to fish and other aquatic organisms. With the increasing amount of information about the adverse effects of MPs, researchers are encouraged to better understand the toxicity of this anthropogenic pollutant. Therefore a study was conducted with the following objective: to examine the effects of microplastics on genotoxicity and cytotoxicity endpoints in rainbow trout (*Oncorhynchus mykiss*) larvae during the endogenous feeding phase. *O. mykiss* were exposed to high density and low density polyethylene (HDPE and LDPE respectively), polypropylene (PP) and polystyrene (PS) microplastics. Cytogenetic damage was assessed using erythrocytic nuclear abnormalities assay with criteria described by Baršienė *et al.* (2012) [1], Fenech *et al.* (2003) [2] and Heddle *et al.* (1991) [3]. The formation of micronuclei (MN), nuclear buds (NB), nuclear buds on filament (NBf), blebbed nuclei (BL) cells were assessed as genotoxicity endpoints, and induction of 8-shaped and bean-shaped nuclei, fragmented apoptotic (FA) and bi-nucleated (BN) cells as cytotoxicity endpoints. Data analysis was performed using R [4] software (version 4.1.1).

This study revealed a significant increase in genotoxicity but not cytotoxicity endpoints. Percentage change was calculated using formula (1).

$$\%\,Change = \frac{Treatment\,‰ - Control\,‰}{Control\,‰} \cdot 100\% \qquad (1)$$

Compared to the control, significantly increased genotoxicity (MN + NB + NBf + BL) was observed in HDPE (by 142%), PP (by 318%) and PS (by 138%) groups. Significantly increased MN were observed in PP (by 358%) and PS (by 218%) groups. Elevated levels of sum of NB (NB + NBf + BL) were detected in HDPE (by 182%) and PP (by 270%) groups. Different polymer types showed different toxicity levels (PP was the most genotoxic while LDPE genotoxicity was not even significant). HDPE, PP and PS displayed twofold and even threefold increase in genotoxicity endpoints. The findings indicate that MPs have genotoxic effects on fish, implying that more studies are required to determine the mechanisms of genotoxicity and the environmental implications.

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H1. KAZOKIŠKĖS LANDFILL LEACHATE: CONTAMINATION AND TOXICITY

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Around 95% of all municipal solid wastes collected worldwide are deposited in a landfills. The increasing generation of municipal solid waste (MSW) has become a major burden on our society with serious environmental and economic problems [1]. Physical, chemical and biological interactions in landfill result in formation of landfill gas and harmful leachate. The leachate which is formed in the landfill is a particularly toxic and hazardous type of wastewaters that has a strong impact on the biota due to its complex components such as ammonia, metals, and organic compounds [2].

The purpose of the current study was to investigate the physical and chemical characteristics of leachate from the Kazokiškės landfill and to assess the toxicity of leachate to model organisms: lettuce (Lactuca sativa L.), garden cress (Lepidium sativum L.) and bioluminescent bacteria Aliivibrio fischeri (Beijerinck). Three samples of landfill leachate (S1 - after Reverse Osmosis wastewater treatment, S2 and S3 without treatment) were collected from different sites on October of 2021. Concentration of heavy metals ions (Cu, Zn, Ni, Pb, Cd, Hg, Crtotal) were determined using standard methods (ISO). Comparison of determined metal concentrations with maximum permissible concentrations (MPC) showed that concentration of all analyzed metals didn't exceed MPC in treated sample. However, significant increase in chromium was detected in untreated landfill leachate samples.

Data of ecotoxicological bioassays showed that sample of the treated leachate was toxic to bacteria and garden cress, and samples of untreated leachate were found to be highly toxic to all exposed model organisms. Bacteria A. fischeri were the most sensitive bioassay organism for all landfill leachate samples, garden cress was more sensitive than lettuce in assessing the phytotoxic effects of all leachate samples. According to the sensitivity to all tested leachate samples, the bioassay organisms are arranged in the following order: Aliivibrio fischeri > Lepidium sativum > Lactuca sativa. Based on the results of the studies, it is concluded that although the chemical analysis of the treated leachate does not show contamination, the ecotoxicological assessment indicates that the treated leachate may pose a risk to the microorganism and plant communities.

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H2. ALLELOPATHIC ACTIVITY OF NATIVE AND INVASIVE SOLIDAGO L. SPECIES

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Various invasive species are considered to be one of the greatest threats to biodiversity and ecosystems, along with factors such as habitat degradation, pollution, exploitation and climate change. Widespread alien species not only change the landscape, overgrow the populations of native species, but also damage the economy, industry and cause huge losses.

The success of alien species in new territories depends on several reasons: invasive plants tend to have small seeds, great tolerance for climate and soil conditions, phenotypic plasticity and allelopathic properties. Allelopathy is defined as any direct or indirect harmful effect of one plant on another through production and release of chemical compounds [1]. Plant secondary metabolites have allelochemical effects and are important for the initial establishment and spread of invasive species. It is known, that allelochemicals can be obtained from different parts of the plant: leaves, stems, flowers, buds, fruits, roots and can affect seed germination and root formation.

Most of Solidago species are herbaceous flowering plants, which occur in the spontaneous flora or are cultivated as decorative plants [2]. S. canadensis belongs to highly invasive species in Europe. In 2004 S. canadensis among other 17 plant species was listed as invasive to Lithuania. Quickly establishing itself via seeds and underground rhizomes, it began to compete with S. virgaurea, which is native plant to Europe. Under unusual conditions, alien species may form hybrids with both closely related native species and other alien plants that settle in the same territory [3]. Hybrids between invasive and native species have been suggested to be treated as invasive species, because they occur in areas where geographical and ecological barriers have been removed by human anthropogenic activities [4]. Distribution of S. × niederederi, a natural hybrid between S. canadensis and S. virgaurea, is related to the overlapping territories of the parental species, but the number of sites, currently recorded, reveals a wide range of research opportunities.

The aim of the present study was to determine the allelopathic activity of aqueous extracts from three Solidago morphological parts: roots, leaves with stems, flowers and buds. Under laboratory conditions allelopathic properties of different aqueous extracts (concentrations 0.1; 0.5; 1.0 M) were tested on Lettuce (Lactuca sativa L.) and Garden pepper cress (Lepidium sativum L.) seeds. Bioassay results have shown that Solidago extracts significantly affected tested seeds germination and growth. However, Solidago inhibitory effect differs upon species. In case of S. virgaurea root and flower extracts indicated the highest allelopathic activity. While S. canadensis and S. × niederederi leaves with stems and as well as flower extracts have demonstrated similar inhibitory effects on model plants. Overall, in all tested Solidago species the strongest inhibitory effect was caused by aqueous extract of flowers and buds: at the 0.5 M and 1.0 M concentration, L. sativa seed germination and growth was reduced from 81.2 % to 94.8 %, L. sativum - 100%. This study revealed that it is important to investigate allelopathic mechanism and its role in the spread of invasive species.

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H3. RESPONSES OF BIOCHEMICAL BIOMARKERS IN *UNIONIDAE* MOLLUSCS FROM NERIS RIVER (LITHUANIA)

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Contamination by hazardous substances poses potential threat to aquatic ecosystems. In recent decades performed comprehensive pollution analysis of the second largest river in Lithuania, the Neris, has caused concern about the levels of polycyclic aromatic hydrocarbons (PAHs), heavy metals and other pollutants which enter the river from urban and industrial effluents, road and agricultural runoffs [1].

Bivalve molluscs are widely applied bioindicator organisms for evaluation of impact of the environmental pollution, because they are broadly distributed, have sessile life style and by intensive filter feeding are able to accumulate high concentrations of pollutants [2]. In order to determine the biological effects of environmental contamination different biomarkers are assessed. Evaluation of biomarker responses could provide detailed and accurate information about the ecological status of aquatic ecosystems [1]. The aim of this study was to analyze the responses of biochemical biomarkers in bivalve molluscs Anadonta anatina and Unio pictorum collected at different sites of the Neris river.

Bioindicator organims were collected in 2020 in summer and autumn at four stations of the Neris river. At the first station (N1), which is near the border with Belarus Republic, effluents from small households and pollutants of agricultural origin are possible. Second station (N2) is comparatively distant from major sources of pollution, however pollutants due to long-range transport are possible. Municipal and industrial wastewaters from Vilnius city and suburbs pose threat at downstream stations (N3, N4). Biomarker responses in molluscs hemolymph were analyzed by a set of various methods. Acetylcholine esterase activity was determined using a modified Ellman (1961) method [3] adapted for microplates, antioxidant capacity was determined using the ferric reducing antioxidant power (FRAP) assay [4], the semi-quantitative determination of polycyclic aromatic hydrocarbons (PAHs) was performed by fixed wavelength fluorescence method [5].

Based on the results, the activity of AChE in molluscs A. anatina and U. pictorum were lower in the summer compared to the autumn at the stations N1, N2 and N3. The lowest values of AChE activity were found in both mollusc species from the station N2 collected in the summer. The results of FRAP assay, have indicated that in the summer, molluscs were exposed to oxidative stress at higher degree at stations N1 and N2, compare to other two stations in both species. The lowest levels of AChE activity found at the N2 station in the summer in A. anatina molluscs may be related to the increased contamination with polycyclic aromatic hydrocarbons, as the highest fluorescence values of PAH metabolites were found at this station. Results of AChE and FRAP analysis performed with U. pictorum collected in summer indicate, that these molluscs were mostly affected at N2 station in comparison with other stations, however analysis of PAH's fluorescence haven't showed reliable differences between stations and doesn't explain the causes of the observed effects. Based on study results we can conclude that applied set of biomarkers are suitable for monitoring the status of river ecosystem, however responses of biomarkers cannot be explained without detailed chemical analysis.

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H4. EVALUATION OF METALLOTHIONEIN LEVELS IN *ONCORHYNCHUS MYKISS* EMBRYOS AFTER EXPOSURE TO POLYSTYRENE NANOPLASTICS

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Polystyrene is a highly valued plastic material. The commercial use of polystyrene began in 1941 when Styrofoam™ – a closed-cell extruded polystyrene foam (XPS) was invented. Since then, it is widely used for thermal insulation purposes. Nowadays, polystyrene is commonly used to make disposable items such as cutlery, plates, caps for takeaway cups, as well as food storage containers. An extensive use of polystyrene plastic contributes to the rapid growth of its waste. Common practice shows that not all plastic, including polystyrene, is handled in a way that is better for the environment. A simple example would be plastic waste in the oceans and coastlands, which shows a lack of participation in proper disposal of plastic waste from its users. It is well-known that plastic cannot biodegrade in the environment. However, in the last decade a new phenomenon, called plastic fragmentation, was witnessed. It is now suspected to be the consequence of biotic and abiotic influence, which is giving rise to nanosized plastic particles – nanoplastics[1]. Research shows that polystyrene nanoplastics (PS-NPs) hold similar physicochemical properties as engineered nanoparticles, therefore pass-through biological barriers and accumulate in living organisms[2]. Aquatic species, such as fish, where found to experience physiological disturbances and redox state imbalance as early as the embryo stage after exposure to PS-NPs[3,4].

The aim of this study was to evaluate the level of metallothioneins (MTs) in rainbow trout (Oncorhynchus mykiss) embryos exposed to 25 nm sized PS-NPs. Fish embryos were exposed to 10 µg/L and 1000 µg/L PS-NPs concentrations for 4- and 12 days. As aforementioned, redox state imbalance may occur during exposure to PS-NPs, therefore, for the first time, a ROS scavenging protein, metallothionein, was chosen as biomarker.

Results obtained during our experiments showed non-significant changes in MT levels after exposure to 10 µg/L and 1000 µg/L PS-NPs concentrations in both 4- and 12 day embryos. However, it was noticed that MT levels change depending on the exposure time and concentrations of PS-NPs. Embryos exposed to 1000 µg/L PS-NPs concentration for 12 days had the highest level of MTs compared to control group. Nevertheless, further experiments should explore a wider spectrum of PS-NPs concentrations and exposure times for a more accurate profile of MTs induction in fish embryos.

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I1. PRELIMINARY RESULTS ON MOLECULAR INVESTIGATIONS OF *TRICHINELLA* SPP. IN PREDATORS FROM LITHUANIA

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Nematode parasites of the genus Trichinella can be transmitted through the foodborne route in carnivores, omnivores, and scavengers. These pathogens are dangerous cosmopolitan parasites that cause a zoonotic disease, trichinellosis. The genus of Trichinella has a broad host spectrum encompassing mammals, birds, and reptiles. Trichinella parasites are circulated in two cycles maintained in nature, the domestic and the sylvatic. High infection rates have been detected in the Canis lupus, Vulpes vulpes, Sus scrofa, Meles meles and Nyctereutes procyonoides. The previous detailed research on Trichinella parasites in wild predators from Lithuania was performed 15 years ago. In recent years, significant changes on the quality, care and inspection of the consumed meat have been conducted in Lithuania. The consumed meat regulation efforts may have affected the circulation rates of trichinellosis in wildlife. Therefore, objectives of the present study were to establish infection prevalence and intensity, to isolate larvae for molecular species identification and to evaluate the suitability of the internal transcribed spacer (ITS1) region for intraspecific genetic variability.

In 2020-2022, 55 wild predators, 24 Vulpes vulpes, 6 Canis lupus (Canidae), 7 Martes martes, 5 Meles meles, 4 Mustela putorius, 3 Neovison vison (Mustelidae), 4 Procyon lotor (Procyonidae), 2 Lynx lynx (Felidae) were collected in various regions of Lithuania. The active method of artificial digestion has been used to study the prevalence and intensity of infection in wild animals. This method is recognized by the European Food Safety Authority (EFSA) as the most effective method for detecting Trichinella spp. The infection intensity was estimated by counting lpg (number of larvae per gram of sample). Trichinella species were identified using multiplex-PCR. Overall, 12 larvae isolated from 6 canids and 6 mustelids were genetically characterized at the ITS1 region.

Of the 55 animals tested, 17 (30.9 %) were positive for Trichinella spp. The highest infection intensity was calculated in the Canis lupus (16.0–46.0 lpg). Whereas in Vulpes vulpes the determined intensity was from 0.07 to 9.0 lpg. Overall, 170 larvae were isolated for molecular confirmation of Trichinella species. The multiplex-PCR conditions were optimized to increase product yield and to avoid the multiplication of non-specific bands. The forty larvae isolated from 2 Vulpes vulpes and 2 Meles meles were identified as T. britovi which is the dominant Trichinella species in wild animals in Lithuania. In the future other isolated larvae will be attributed to Trichinella species as well as a larger sample of wild predators will be investigated. The 12 obtained 775 bp-long ITS1 sequences were identified as T. britovi. Altogether 6 segregating sites and 6 different haplotypes were observed. The primary results show higher haplotype diversity of T. britovi isolated from mustelids rather than canids. In conclusion, the ITS1 is appropriate for the evaluation of intraspecific genetic variability of T. britovi.

I2. PREVALENCE OF DOMESTIC ANIMALS INFECTING *SARCOCYSTIS* PARASITES IN SAMPLES FROM DIFFERENT TYPES OF WATER IN LITHUANIA

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There are currently about 15,000 species of parasitic protozoa known in the world. The most studied parasitic protozoans transmitted through water and causing human, or animal infections are *Toxoplasma gondii*, *Entamoeba histolytica*, *Cyclospora cayetanensis*, *Isospora belli*, *Acanthamoeba* spp., *Sarcocystis* spp. and *Naegleria* spp. [1]. However, to date there are no suitable methods for accurate identification of various parasitic protozoa in water bodies. Therefore, during drinking-water quality studies usually only the prevalence of *Giardia* and *Cryptosporidium* spp. is being tested. The aim of this study was to determine the prevalence of *Sarcocystis* parasites infecting domestic animals (bovine, ovine, caprine and porcine) in different types of water in Lithuania using molecular-based method.

Ninety samples were collected from freshwater (lakes, rivers, ponds, canals and wetlands) and marine water (Baltic Sea) during the summer of 2021. Filtration was used to collect sporocysts of *Sarcocystis* parasites from samples. Primer pairs that were specific for identification of *S. cruzi* infecting cattle, *S. arieticanis* infecting sheep, *S. capracanis* infecting goats and *S. miescheriana* infecting pigs were selected for cox1 gene PCR targeting. To assess the prevalence of *Sarcocystis* parasites water bodies were divided into three groups: neutral pH water (lakes, rivers, ponds and canals), acidic pH water (wetlands), and saline water (Baltic Sea). Based on molecular methods, two (37%) or three (33-40%) different species of *Sarcocystis* were usually identified in samples from freshwater sources, but two (50%) or one (33%) in samples from the Baltic Sea. The highest contamination by parasites was detected in Strėvininkai lake and canal near the reserve in Uostadvaris, they were found to contain four species of *Sarcocystis* in the sample. Meanwhile, tested *Sarcocystis* species were not found in wetland located in the forest in Švenčionys district.

The most common species identified in the samples was *S. cruzi* (98%), less frequently *S. arieticanis* (60%) and *S. capracanis* (43%) were detected, and minor observed species was *S. miescheriana* (8%). *S. arieticanis* was most commonly found in neutral pH water samples (73%), less frequently in samples from wetlands (57%) or Baltic Sea (50%). Meanwhile, *S. capracanis* was mostly identified in acidic pH water samples from wetlands (57%) and less frequently in lakes, rivers, ponds and canals (40%) or samples from the Baltic Sea (33%). *S. miescheriana* has been found in lakes, rivers, ponds and canals (17%) and wetlands (7%).

This study provides first insight into contamination of different types of water with sporocysts of *Sarcocystis* species infecting domestic animals. From the results obtained, it can be concluded that parasites of the genus *Sarcocystis* are significantly common in fresh, and marine water sources; high salt concentration or low pH of water does not affect survival of *Sarcocystis* parasites.

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I3. MOLECULAR IDENTIFICATION OF SARCOCYSTIS INFECTION IN THE NORTHERN GOSHAWK (*ACCIPITER GENTILIS*) FROM LITHUANIA

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Representatives of the genus *Sarcocystis* (Apicomplexa: *Sarcocystidae*) are widespread protozoan parasites. They are characterized by two-host prey-predatory life cycle. Sporocyst develop in the small intestine of definitive host, while sarcocysts are formed in muscles and CNS of intermediate host. Definitive host (predator, omnivore, or scavenger) become infected by eating meat contaminated with parasite sarcocysts and intermediate host become infected by ingesting sporocyst-contaminated food or water. Currently about 200 species of Sarcocystis are known to infect mammals, birds, and reptiles. Three *Sarcocystis* spp. are zoonotic, also some species are pathogenic for domestic and wildlife animals. It was considered for a long time that *Sarcocystis* parasites are not harmful for European bird populations. However, recent data showed *S. calchasi* and *S. halieti* to cause meningoencephalitis and hepatitis in various bird orders [1]. In Lithuania *S. halieti* was previously detected in muscles of birds of families *Phalacrocoracidae*, *Corvidae* and *Laridae*, while *S. calchasi* was not reported [2]. The aim of the present study was to identify *Sarcocystis* infection in muscle and intestine samples of the northern goshawk (*Accipiter gentilis*) by molecular methods.

In the period of 2019-2020 samples of 11 northern goshawks (*Accipiter gentilis*) obtained from taxidermist were investigated for the presence of *Sarcocystis* spp. Birds were examined as possible definitive and intermediate hosts of *Sarcocystis* parasites. Clippings of legs, chest and neck muscles were examined in fresh preparations by squashed method. Small and large intestines scrapings were homogenized, centrifuged with Hanks' Balanced Salt Solution and sodium hypochlorite 5.25% solution. Prepared intestinal sediments were inspected under a light microscope at ×200 magnification. The 400 µL of sporocysts sediments were taken from each sample and used for DNA extraction. The highly variable the internal transcribed spacer 1 (ITS) was chosen for the confirmation of *Sarcocystis* infection. Ten *Sarcocystis* species employing birds as intermediate hosts were tested in samples.

Sarcocysts were not found in muscles samples examined. Intestinal samples microscopy detected sporocysts, oocysts and sporulating oocysts in all examined northern goshawks. Using species-specific PCR of ITS1 region *S. cornixi* 72.7% (8/11), *S. halieti* 72.7% (8/11), *S. wobeseri* 54.5% (6/11), *S. turdusi* 54.5% (6/11), *S. kutkienae* 45.5% (5/11), *S. columbe* 45.5% (5/11), *S. calchasi* 45.5% (5/11) and *S. lari* 9.1% (1/11) were confirmed. The number of *Sarcocystis* species per one bird varied from one to seven. *Sarcocystis* species co-infections were identified in ten birds. In the present study it was for first time determined that the northern goshawk can be definitive hosts of *S. kutkienae*, *S. lari* and *S. wobeseri*. Furthermore, the highly pathogenic *S. calchasi* was identified in birds from Lithuania for the first time. Further investigation should be focused on the possible intermediate hosts of *S. calchasi* in Lithuania.

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I4. DYNAMICS OF GLOBAL BIODIVERSITY INVENTORY: SPECIES DESCRIPTIONS OF THREE MODEL, PLANT-MINING LEPIDOPTERAN FAMILIES

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The task to inventory and monitor global biodiversity is huge [1]. Closing this knowledge gap will require a strong, renewed effort in exploration and taxonomy, as well as the continuing effort to catalogue existing biodiversity data. We also need a clearer understanding of the total number of species to reveal the structure and function of ecosystems [1, 2, 3].

The goal of our research (conducted by us with co-authors; [3]), was to apply quantitative criteria for the study of species diversity with three model families of mining Lepidoptera to assess and compare the abundance and current knowledge about these taxa.

We inventoried, counted, summarized, and provided precise data on the documentation history of Nepticulidae and Opostegidae (Nepticuloidea), and Tischeriidae (Tischerioidea) for the first time. We checked and analyzed many original descriptions of species of the above-mentioned families, but the actual data were retrieved from our unpublished electronic "Global Species Database 1995–2021" maintained by Arūnas Diškus [3]. All doubtful synonymies were checked and confirmed and, if the synonymy proved true, the synonymic names were excluded from our analysis. Species which had been previously documented (described or illustrated), but published unnamed were excluded.

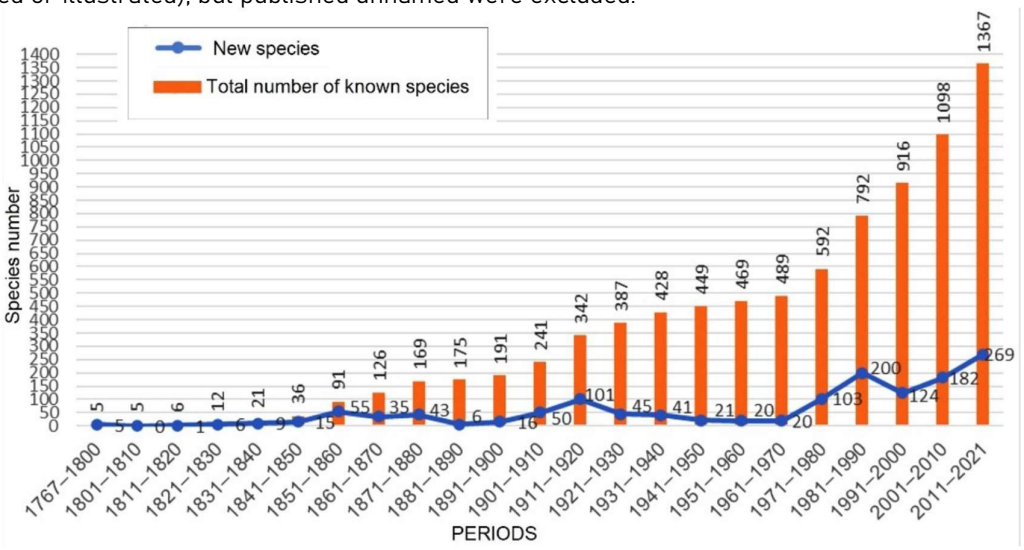

Figure 1. Species description dynamics of Nepticulidae, Opostegidae, and Tischeriidae (after [3]).

At the end of 2021, we estimated that a total of 1000 Nepticulidae species, 197 Opostegidae species, and 170 Tischeriidae species have been described since the taxonomic practice of describing species began in the 18th century (Fig. 1.) [3]. We examined, discussed, and published the history of descriptions and authorship of species worldwide for each of the three families. We found that the total (accumulative) number of species described increased with each time period delineated. About five new species were described per year on average, or about 22 new species were described per year in the 21st century [3]. We also recognized researchers with the most number of described species in these three taxa.

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MARCH 2nd

Microbiology and Biotechnology:

A1. ENZYMATIC BIOFUEL CELL BASED ON GLUCOSE OXIDASE MODIFIED ANODE AND CATHODE

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The biological origin of fuel cells has attracted scientists' interest for many years, due to the cell ability to generate environmentally friendly energy under mild conditions, good biological compatibility and a high potential for implantation in human organisms in order to supply the energy to implanted electrical devices such as biological sensors, pacemakers and others [1,2]. Biological fuel cells (biofuel cells, BFCs) are devices that can directly convert chemical energy into electrical energy via electrochemical reactions involving biochemical pathways. One of the most promising BFCs is based on purified redox enzymes and is called enzymatic BFCs (EBFCs). Due to the high selectivity of the enzymes, it is not necessary to separate the anodic part from the cathodic part, so EBFCs open the possibility to develop membrane-less miniature EBFCs [3]. In order to apply EBFCs for practical use, they must produce enough energy and have a long lifetime, which is still an unresolved problem.

The aim of this work was to designe and investigate the performance of an EBFC, which anode and cathode are modified by the same enzyme. The anode surface was modified with poly(1,10-phenanthroline-5,6-dione) and a poly(pyrrole-2-carboxylic acid) (PPCA) layer with encapsulated gold nanoparticles by cyclic voltammetry (CV) and covalently immobilised glucose oxidase (GOx). The immobilized enzyme acted as a catalyst, oxidizing glucose by molecular oxygen and converting the chemical energy of this chemical reaction to electrical energy.

Similarly, Prussian blue nanoparticles (PB) were synthesized in conductive PPCA polymeric layers, also by CV, and then GOx enzyme was covalently linked to the cathode surface. H_2O_2, which was produced during the enzymatic glucose oxidation reaction, was reduced on the biocathode surface by PB, thus creating an electron flow from anode to cathode in the constructed EBFC.

The power, stability, impact of glucose concentration and pH on the EBFC performance was also studied. The obtained data indicates that GOx immobilized on both anode and cathode increases the power output and constructed EBFC can generate electricity using glucose as the main fuel source.

Acknowledgment
This project was funded by the European Union (project No 09.3.3-LMT-K712-25-0013) under the agreement with the Research Council of Lithuania (LMTLT).

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A2. SCREENING AND CHARACTERIZATION OF AMINOTRANSFERASES FOR CHIRAL AMINE SYNTHESIS

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Chiral amine compounds are widely used as active pharmaceutical ingredients, agricultural chemicals, and other biologically active compounds [1]. Aminotransferases (AT) are enzymes that mediate the transfer of an amino group to a ketone acceptor. ATs have been already proven to be a promising catalyst in the synthesis of chiral amine compounds. The catalysis by ATs is performed under mild conditions, without the use of toxic metals and solvents, and has a higher stereo- and regio-selectivity compared to the organic synthesis [2]. However, the broad application of such enzymes is restricted by a limited number of the identified ATs.

In this study, we searched for aminotransferases in the metagenomic DNA libraries using indol-3-ylmethylamine as a prochromogenic amino donor. We found and recombinantly expressed 18 different ATs. The experimental analysis revealed that the tested aminotransferases were active towards a wide variety of aromatic and aliphatic keto compounds and were capable of using isopropylamine as an amino donor (Fig. 1). In small-scale amination experiments, we were able to achieve a conversion of 95% for 2-acetylpyridine and 2-indanone using AT as a biocatalyst. Furthermore, some ATs showed activity towards monosaccharides and, therefore, could be employed for the synthesis of different aminopolyols – a class of products that are of particular interest as carbohydrate mimetics.

In summary, we successfully identified ATs active towards a wide scope of keto compounds. We showed ATs to be promising biocatalysts for chiral amine production.

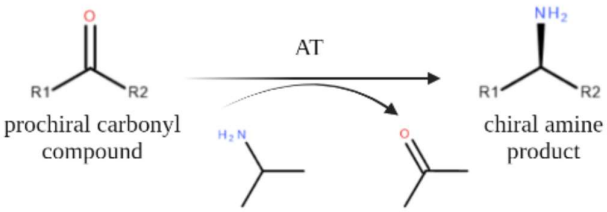

Figure 1. Asymmetric synthesis using ATs.

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A3. ANTIBACTERIAL ACTIVITY OF MERCAPTO-SUBSTITUTED 5-(2-((4-ETHOXYPHENYL)AMINO)ETHYL)-4-PHENYL-2,4-DIHYDRO-3H-1,2,4-TRIAZOLE DERIVATIVES

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Antimicrobial resistance (AMR) has been identified as one of the main public health problems, which limits the effective prevention and treatment of infections. The problem of AMR is especially urgent regarding antibiotic resistance in bacteria. The World Health Organization has long recognised the need for an improved global effort to contain AMR and one of the goals set is development of new antibacterial agents [1].

1,2,4-Triazole scaffold is an important heterocyclic moiety widely investigated due to versatile medicinal and pharmaceutical properties. It has been incorporated into a wide variety of therapeutically important agents available in clinical therapy, such as itraconazole, posaconazole, voriconazole (antifungal), ribavirin (antiviral), letrozole and anastrozole (antitumoral). Compounds, which triazole ring incorporates sulphur in a form of mercapto and thione substitution, show more potency compared to parent derivatives. Triazolethione-thiols are attractive scaffolds in medicinal chemistry owing to their potential antimicrobial, antioxidant, anti-tuberculosis, antifungal, antitumor, anticonvulsant, antiepileptic, and anti-inflammatory activities [2,3].

The screened 1,2,4-triazole derivatives 1-6 were synthesized in the reaction of 5-(2-((4-ethoxyphenyl)amino)ethyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione, which was prepared from 3-[(4-ethoxyphenyl)amino]propanehydrazide treated with phenyl isothiocyanate in methanol to provide semithiocarbazide and subsequent cyclization reaction of the latter under alkaline condition, and various acetophenone derivatives [4].

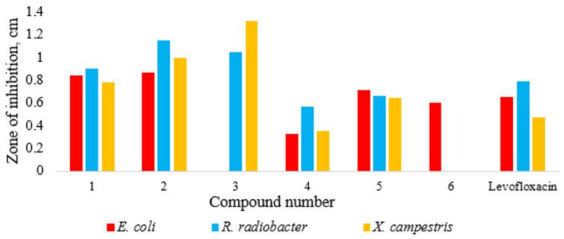

Figure 1. Antibacterial activity of 1,2,4-triazole-3-thiol derivatives 1-6 against Escherichia coli, Rhizobium radiobacter, and Xanthomonas campestris bacteria by agar diffusion method.

Antibacterial activity of compounds 1-6 was screened by agar diffusion method against Escherichia coli, Rhizobium radiobacter and Xanthomonas campestris bacteria. 1 mg/mL solutions of the test compounds and control antibiotic in DMSO were used in the experiments. Inhibition zones (IZs) were estimated by measuring the diameter of the microbial growth inhibition zone after 24 h of incubation at 37 °C.

Antibacterial activity of the compounds 1 and 2 was higher than that of control antibiotic levofloxacin against all three bacterial strains. Compound 3 (IZ = 1.32 cm) was the most active against X. campestris. The growth of E. coli bacteria was best inhibited by 1 and 2 (IZ = 0.84 cm and 0.87 cm, respectively). Compounds 2 and 3 were the most active against R. Radiobacter (IZ = 1.15 cm and 1.05 cm, respectively).

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A4. GREEN EXTRACTION OF ISOFLAVONES FROM *TRIFOLIUM PRATENSE* L. FLOWERS USING γ-CYCLODEXTRIN

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Trifolium pratense L. from the Fabaceae family, commonly known as red clover, is both a forage plant and a medicinal herb used in dietary products and for the alleviation of menopausal symptoms as an alternative to hormone replacement therapy, for hyperlipidemia, and to prevent osteoporosis, as well as for benign prostatic hypertrophy and possibly prostate cancer [1]. Isoflavonoids aglycones in leguminous plants occur at low concentrations. However, the yield of isoflavonoid aglycones such as genistein and daidzein in an extract will dramatically increase when removing the sugar moieties from their corresponding isoflavonoid genistin and daidzin [2].

Thermal hydrolysis with additional materials, such as excipients can be used to extract isoflavone aglycones in water or another solvent and thus increase their yield in extract. Cyclodextrins (CDs) can be suitable compounds for increasing the solubility of isoflavones [3]. Therefore, the main aim of this study was to evaluate the eco-friendly method of using the effects of γ-CD to obtain higher yields of isoflavones from red clover extracts in comparison to a standard extract without excipients.

Plant aerial parts were harvested and dried, and then aqueous or ethanolic extracts were prepared. Ultrasound assisted extraction was performed using 1.0 ± 0.001 g of dried and milled flower head, 0.3 ± 0.001 g γ-CD and 30 mL of distilled water or 50 % of ethanol. Temperature during ultrasound processing was 40 °C. Sonication time was 10 or 30 min. Part of the samples were heated for 1 hour under reflux after the sonication. Heat reflux extraction was done using 1.0 ± 0.001 g of dried and milled flower heads, 0.3 ± 0.001 g γ-CD and 30 mL of water or 50 % of ethanol mixed in a 250 mL round bottom flask. Control samples were prepared using the same conditions without excipient. Extraction samples for identification and quantification of isoflavones glycosides and aglycones were investigated using HPLC.

Samples prepared with water and excipient showed statistically significantly higher isoflavones aglycones yields compared with control samples. Glycosides (genistin and daidzin) ware not detected in any samples prepared with water. Highest isoflavones aglycones yield was detected in the sample prepared using heat reflux – 115.40 ± 5.77 genistein and 227.83 ± 11.39 µg/g daidzein. Using ultrasound processing and increasing processing time from 10 to 30 minutes isoflavones aglycones yield decreased.

Using 50% ethanol as a solvent and γ-CD, samples yielded higher isoflavones results than those prepared with water. The highest yield of isoflavones (glycosides and aglycones) were determined in the sample prepared using ultrasound for 30 minutes and then refluxed for 1 hour – 226.96 ± 11.30 µg/g genistein, 682.90 ± 34.15 µg/g daidzein, 93.40 ± 4.67 µg/g genistin and 10.30 ± 0.52 µg/g daidzin. Although, highest glycosides yields were observed in saample prepared using only heat reflux – 136.20 ± 6.81 genistin and 27.67 ± 1.38 µg/g daidzin.

Using water as solvent and increasing ultrasound processing from 10 to 30 minutes isoflavones aglycones yields decreased, however, using 50 % ethanol and increasing processing time isoflavones yields increases. Using γ-CD as excipient in the extraction media increases isoflavones yield significantly using both water and ethanol compared with control samples.

This work was supported by the Research council of Lithuania grant no. 09.3.3 ESFA V 711 01 0001.

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B1. TAXONOMIC CHARACTERIZATION OF BACTERIAL STRAINS FROM KRUBERA-VORONJA CAVE

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Caves are an interesting study object since their environmental conditions are considered to be extreme as the result of low temperatures, high humidity levels and little to no light. Microorganisms found in caves are well adjusted to these harsh conditions, therefore, their metabolic diversity is being explored with hopes of finding new biotechnological products [1]. Krubera Voronja cave with a depth of 2212 m. is the deepest known cave in the world. However, there are not a lot of studies done on the caves microbial diversity [2]. The analysis of bacterial extinction rates has estimated that most of bacterial lines that ever existed are now extinct, therefore, we cannot analize their genomes or study them for possible applications in biotechnology [3]. Due to this reason, while researching microorganisms from such deep caves, it is important to properly preserve them as well as to collect information for future usage.

The aim of this study was to characterize and identify bacterial strains from Krubera-Voronja Cave using phenotypic and molecular analysis.

Taxonomic characterization of 16 bacterial isolates was carried out. Most (13 out of 16) cultures were Gram-positive while the remaining 3 cultures were Gram-negative. Most isolates (12 out of 16) were non-motile. The main focus of physiological analysis was an ability to degrade polymeric compounds – polysaccharides and proteins. A few substrates were used for this analysis. Our results showed that all 16 cultures were highly active against polysaccharides - they produced enzymes that break down birchwood xylan, carboxymethyl cellulose, and apple pectin. Polygalacturonic acid was degraded by 12 cultures. Some isolates also produced proteases that hydrolysed casein (6 isolates) and gelatin (7 isolates).

In order to determine the exact number of the strains represented by the studied isolates, both BOX-PCR and (GTG)5-PCR genotyping techniques were used. Combined results of both genotyping experiments let us to conclude that 16 bacterial isolates belong to 13 different strains. To identify these strains, 16S rRNA genes were amplified, cloned, and sequenced. Phylogenetic analysis of 16S rRNA gene was also performed. Our results showed that the strains from Krubera Voronja Cave can be assigned to three bacterial phyla: Firmicutes, Actinobacteria, and Proteobacteria. The strains have been found to be members of the following genera: Bacillus, Brevibacterium, Nocardia, Paeniglutamicibacter, Pseudarthrobacter, and Pseudomonas. The closest phylogenetic neigbours of the examined strains were established, and 16S rRNA gene sequence similarity with their sequences was determined.

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B2. ANALYSIS OF *STREPTOMYCES SCABIEI* 87.22 CUTINASE BY USING ENZYME FUSION STRATEGY

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Cutinases are hydrolytic enzymes that catalyze hydrolysis, synthesis, or transesterification reactions of ester bonds, which makes them attractive to the industrial sector. One of the most promising applications of such enzymes is degradation of polymers (plastics), such as PCL (polycaprolactones) and PET (polyethylene terephthalate). Nevertheless, the amount of research related to the analysis of the structure-function relationship of microbial cutinases and the perspectives of their application is limited. There are only a few publications about the purification and characterization of these enzymes [1]. Moreover, cutinases themselves are often classified as lipases, esterases, or other hydrolytic enzymes. Thus, there is a lack of fundamental knowledge about these bacterial enzymes.

During earlier studies, a synthetic *Streptomyces scabiei* 87.22 cutinase was created. It was shown that the removal of the N-terminal signal peptide significantly reduced protein synthesis in *E. coli* BL21 (DE3) cells. Based on other researchers, some microbial cutinases can possess phospholipase activity [2]. It is possible that *S. scabiei* mature cutinase also acts as a phospholipase and is released into the environment or is toxic to *E. coli* cells. One possible solution to limit the phospholipase/toxic activity of this enzyme is to construct fused enzymes.

GD-95 lipase and GDEst-95 esterase can be excellent fusion domains, that can help modify and modulate the properties of other enzymes: yield, activity and thermostability [3]. It was hypothesized that in the presence of a lipase/esterase domain at the N-terminus of the fusion protein, synthesis in *E. coli* cells should be more efficient. Thus, four new chimeric enzymes were created: 1) Cutinase-Lipase (Cut-Lip); 2) Cutinase-Esterase (Cut-Est); 3) Lipase-Cutinase (Lip-Cut); 4) Esterase-Cutinase (Est-Cut) (Fig. 1). Different restriction sites, which were used for the fusion of enzymes, were inserted during PCR. Furthermore, synthesis of newly constructed proteins was evaluated by SDS-PAGE.

The results obtained during this study showed that Lip-Cut and Est-Cut fused enzymes were efficiently synthesized. However, Lip-Cut enzyme was proteolytically cleaved into two separate enzymes, thus GDEst-95 esterase is a more suitable fusion partner.

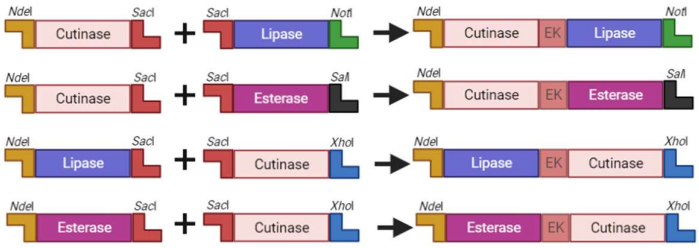

Figure 1. A scheme for the construction of fused Cut-Lip, Cut-Est, Lip-Cut and Est-Cut enzymes. EK (E - glutamate, K - lysine) - a linker occurring at the cleavage site of the *Sac*I restriction endonuclease.

Acknowledgment. This research was funded by the European Social Fund under the No 09.3.3-LMT-K-712 "Development of Competences of Scientists, other Researchers and Students through Practical Research Activities" measure, Grant No. 09.3.3.-LMT-K-712-25-0030.

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B3. INVESTIGATION OF TAIL FIBER PROTEIN GP24 FROM PANTOEA AGGLOMERANS-INFECTING PHAGE VB_PAGS_AAS23

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In recent years, antimicrobial drug resistant microorganisms became a global concern affecting various spheres. One of such happens to be agriculture that suffers immensely from pathogens from closely related Erwinia and Pantoea genera, that not only cause plant diseases, but also frequently happen to be resistant to antibiotics. Although it is known that bacteria which resists antimicrobial drugs can be eliminated by the use of bacteriophages and their proteins depolymerases, which degrade bacterial surface polysaccharides, Pantoea-infecting viruses remain underexplored to date. In order to at least partially change the described situation, we aimed to study Pantoea agglomerans-infecting phage vB_PagS_AAS23 (AAS23) by investigating activity of its tail fiber protein gp24 in vitro.

Tail fiber protein gp24 of bacteriophage vB_PagS_AAS23 was chosen to be the object of our study after its bioinformatics and phylogenetic analysis. These analyses revealed that gp24 was not reliably homologous to any phage proteins deposited in publicly available databases, but it is closest to Stf protein of Salmonella virus SP126 and tail fiber protein of Shigella-infecting phage SH6 (with aa percent identity of 40.78% and 30.98% respectively). Furthermore, tail fiber protein of Shigella phage SH6 carries a C-terminal Peptidase_S74 domain, a conserved chaperone domain that is frequently found in a type of depolymerases - endosialidases. These results showed that gp24 is a unique protein that potentially has properties associated with depolymerases, which is why a more detailed analysis of this protein is therefore necessary.

To study the potential activity of gp24 in vitro, the g24 of AAS23 was cloned into two inducible vectors (with a His-Tag attached to their N- or C-terminus) with the goal of them to be expressed in E. coli expression strain BL21 (DE-3). The recombinant proteins were purified and analysed by SDS-PAGE, which demonstrated that recombinant proteins are soluble and are the size of approximately 50 kDa. It is worth mentioning that gp24C-his was purified alongside with a protein from E. coli which allows us to speculate that gp24 might interact with the unknown protein via its free N-terminus. To test the activity of recombinant gp24 the spot test on a double-layer agar using Pantoea agglomerans strain AUR was used. The results showed that only gp24 with His-Tag in N-terminus forms a turbid zone, known to be a hallmark of a depolymerase activity, which not only proves that gp24 potentially demonstrates a depolymerase activity, but also lets us speculate that C-terminus of the protein is responsible of its functional activity.

Results of this study not only extend our knowledge about Pantoea-infecting viruses, but also imply that some of them potentially might be used as biocontrol agents due to their proteins depolymerases that are able to degrade essential bacterial polysaccharides.

This research was funded by Research Council of Lithuania (Grant No. S-MIP-20-38).

B4. Characterization of Bacteriophages KLER1-1 and KLER1-2 Isolated from Gypsum Karst Lakes

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Gypsum karst lakes with sulfate type water can be found in the northern part of Lithuania, however, information about their biotic components such as bacteria and their viruses is scarce. We isolated two novel bacteriophages vB_PsoM_KLER1-1 (KLER1-1) and vB_PsoM_KLER1-2 (KLER1-2) that *infect Pararheinheimera soli* strain KR2-1 derived from sulfate-type gypsum karst lake Ramunėlis located near Biržai, Lithuania.

Pararheinheimera is a purple sulfur bacteria from the family *Chromatiaceae* that were only distinguished as a separate genus in 20171. It is supposed that bacteria from the family *Chromatiaceae* are of great importance to aquatic ecosystem sulfur and carbon biogeochemical cycling regulation[2]. Some species of the genus *Pararheinheimera* exhibit antimicrobial properties[3] and are utilized while exploring the origin of a wide spectrum of beta-lactamases that are responsible for antibiotic resistance[4]. Until now there have not been any publications in databases of bacteriophages infecting *Pararheinheimera* genus bacteria.

In this study we present characterization of two novel bacteriophages vB_PsoM_KLER1-1 (KLER1-1) and vB_PsoM_KLER1-2 (KLER1-2) that have been isolated from a gypsum karst lake Ramunėlis in Biržai, Lithuania. Based on TEM analysis both phages are myoviruses, that have an isometric head of about 62 nm in diameter and a contractile, non-flexible tail about 125 nm long. The host range determination tests revealed that out of 26 bacterial strains tested, only *Pararheinheimera soli* isolate KR2-1 was sensitive to KLER1-1 and KLER1-2 in the temperature range of 4 to 28°C, 15°C being the most optimal temperature for infection. While KLER1-1 forms clear plaques of about 1.2 mm in diameter, KLER1-2 forms opaque and smaller ones of about 1 mm. Adsorbtion experiments demonstrated that in selected conditions adsorption of viruses to bacterial cells is efficient. Also it was showed that both phages are not sensitive to chloroform under investigated conditions. Furthermore, restriction analysis suggests that these phages are genetically closely related and that DNA of both phages is potentialy modified, with similar modification profiles.

Results of this study not only extend our knowledge about *Pararheinheimera* infecting viruses but can also contribute to future studies of bacteriophage impact to sulfur biogeochemical cycling.

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C1. ANALYSIS OF STAPHYLOCCOCUS SP. H6 MICROBIALLY INDUCED CALCIUM CARBONATE PRECIPITATION DYNAMICS USING ATOMIC FORCE MICROSCOPY

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Biomineralization or the formation of minerals through biological activity is known to form over 60 different types minerals – chlorides, phosphates, and carbonates are some examples of those minerals [2]. Biomineralization can be classified into three types: 1) biologically controlled mineralization, 2) biologically influenced mineralization and 3) biologically induced mineralization [1]. Of all the types of biomineralization, microorganism induced calcium carbonate precipitation (MICCP) is the most studied and has the greatest potential for application. MICCP is the formation of calcium carbonate from supersaturated solution through the biochemical activity of bacteria - during this process, organisms release one or more metabolic products that react with ions present in the environment, resulting in the precipitation of minerals [1]. Many metabolic processes can promote biomineralization, however, precipitation of calcium carbonate by urea hydrolysis is the most widely used and the simplest method of calcium carbonate precipitation. It is also important to note that calcium carbonate is polymorphic - in the solid state, it can adopt several different crystal structures - polymorphisms [3].

This study presents the analysis of Staphyloccocus sp. H6 microbially induced calcium carbonate precipitation dynamics atomic force microscopy (AFM).

Using AFM, we examined the changes in surface topography of Staphylococcus sp. H6 after treatment with cementation mixture. The results showed that after only 30 minutes of incubation with the corresponding solutions of urea, CaCl2, and $NaHCO_3$, the topology of Staphylococcus sp. H6 culture starts to change and crystal-like structures start to become detectable (Fig. 1a). Topographical changes were observed to form from one pole of the cells; however, this could be due to cell immobilization in the monolayer. It was hypothesized that the cells were forming amorphous CaCO3 precipitates.

To analyze the dynamics of developing crystals on the Staphyloccocus sp. H6 cells, different incubation time intervals were chosen, from 15 minutes to 1 hour and 30 minutes with gaps between different measurements being 15 minutes. The obtained results indicated that Staphylococcus sp. H6 cells exposed to the cementation mixture start to rapidly form uneven structures, which grow in size exponentially according to the incubation period. After a prolonged period, the crystals grow too big and coat the entirety of the cell. Analysis of the change in height shows that, in comparison, the following surface elements are roughened, and protrude ~50% more than the unaffected part of the cell (Fig. 1b.)

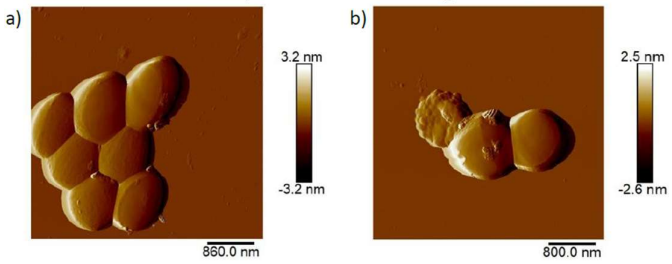

Fig. 1. Changes in bacterial topography observed after different incubation time intervals using AFM. a) after 30 minutes of incubation with cementation solution. b) After 1 hour of incubation with cementation solution. Scan area size 4:4 µm.

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C2: THE RESEARCH OF NEW BACTERIOCINS ACTIVE AGAINST PHYTOPATHOGENIC BACTERIA

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An increasing antimicrobial resistance among phytopathogenic bacteria has shown significant problems in the soil ecosystem, since these pathogens can cause variety of plant diseases as well as economical damage in various plant crops. Current methods used to control these plant pathogens are chemicals, which could be harmful for the environment and threatening to human health [1]. Therefore, bacteriocins, a heterogeneous group of ribosomally synthesized antimicrobial peptides with the ability to kill closely related or a diverse range of bacteria species, could be used to overcome this problem [2]. Bacteriocins are much safer to use than some of the chemicals, moreover, due to their specificity towards purposive targets it comes off with no side effects.

The aim of this study is to find bacteriocins, secreted by the microorganisms isolated from the soil,which could affect phytopathogenic bacteria. Sixty-three isolates were isolated from the soil and they were screened in order to evaluate their antibacterial activity against ten phytopathogens, such as , , etc. We successfully found two bacteria strains that produce antibacterial substances. One of these strains DM1-10 has had a relatively wide antibacterial activity against most of the tested phytopathogens. The results of the 16s rRNA gene analysis has showed that this bacterium can be designated as . With all the data about gene clusters and genes encoding the secondary metabolites, we are led to believe that the substances that we found could be bacteriocins [3].

In the future, by using protein extraction and chromatography methods we will determine the natureof produced antimicrobials. Furthermore, the rest of the bacteria isolates will be tested in order to find more molecules, which are biologically active against plant pathogens. Thus, the data collected carrying out this research will be helpful in the future developing new methods for phytopathogens control.

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C3. CHARACTERIZATION OF POLYSACCHARIDE LYASES FROM *PAENIBACILLUS* SP. 23TSA30-6

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Polysaccharide lyases are widespread enzymes that catalyse polysaccharide decomposition reactions by beta-elimination mechanism. These enzymes are extensively applied in many industry fields, such as food industry, wastewater treatment, textile processing etc. As a result, polysaccharide lyases have attracted great interest of recent studies, analyzing variuos properties of these enzymes. Endospore-forming bacteria of the genus *Paenibacillus* are known to be potent producers of polysaccharide-degrading enzymes including polysaccharide lyases. [1] [2]

The aim of this study was to characterize polysaccharide lyases from *Paenibacillus* sp. 23TSA30-6. This strain was isolated from Krubera-Voronja Cave, and its genome was previously sequenced in our laboratory.

In order to reach the aim of this study, a few tasks were established: to carry out characterization of pectin and polygalacturonic acid degrading enzymes in secretome of the native bacterial strain as well as to analyse *Paenibacillus* sp. 23TSA30-6 polysaccharide lyases genes *in silico*,

It was determined that *Paenibacillus* sp. 23TSA30-6 polysaccharide lyases are constitutively secreted enzymes since their secretion did not require induction with substrate. Further research using zymography revealed 17 polysaccharide lyases, secreted by *Paenibacillus* sp. 23TSA30-6. Majority of polygalacturonic acid degrading enzymes were active in pH 8.0 – 9.0 range, two polysaccharide lyases were active in pH 7.0 – 9.0 conditions and one enzyme could function in pH 6.0 – 10.0. The optimal conditions for these enzymes were pH 9.0 and 23 – 30 °C, although enzymatic activity was detected in 23 – 37 °C temperature. Using apple pectin as a substrate, polysaccharide lyases showed greater activity when incubated with calcium, whereas polygalacturonic acid degradation was inhibited by Ca^{2+} ions. Moreover we assessed that these enzymes are thermostable, which means they could be applicable in industry.

In silico analysis was performed using CAZy database, MEGA7 software, and the BLAST tool in order to compare genomic potential of *Paenibacillus* sp. 23TSA30-6 for polysaccharide degradation with that of the reference organism *Paenibacillus* sp. IHB B 3084. As a result, 9 different polysaccharide lyase genes were indentified in the genomes of both *Paenibacillus* sp. IHB B 3084 and *Paenibacillus* sp. 23TSA30-6. Majority of these genes coded pectate lyases with a few rhamnogalacturonate lyases and hypothetical proteins. Predicted substrates of these hypothetical enzymes were pectin, chondroitin, heparin and rhamnogalacturonan I. SignalP 5.0 server was used for the prediction of signal peptides in 7 enzymes - 5 pectate lyases and 2 hypothetical proteins. Results of this analysis let us to suppose that all these enzymes are extracellular enzymes. Majority of these enzymes (6) most likely have Sec signal peptide while one hypotethical protein was presumed to be secreted through TAT pathway.

During further research we are going to clone polysaccharide lyase genes of *Paenibacillus* sp. 23TSA30-6 and characterize recombinant polysaccharide lyases more extensively.

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C4. SURFACE DISPLAY OF MALTOSE-BINDING AND GREEN FLUORESCENT PROTEINS USING THE YEAST *Saccharomyces cerevisiae*

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Yeast surface display (YSD) is a powerful protein engineering tool used for the expression of target peptides or proteins on the cell surface of yeast. The protein of interest is immobilized on cell surface by fusing with anchoring protein maintaining its relatively independent spatial structure and biological activity [1]. Over the past decades this technology has become one of the most widely used platforms for protein display due to several unique advantages, which include a eukaryotic expression system that can incorporate post-translational modifications and compatibility with flow cytometric analysis, which allows to analyze and sort cells that display proteins with desired properties, such as increased stability or specificity [2]. One of the most frequently used YSD system is based on the mating protein a-agglutinin of *Saccharomyces cerevisiae*. This system consists of two subunits, Aga1 and Aga2. Aga1 is secreted from the cell and covalently bounds to the cell wall by a glycosylphosphatidylinositol (GPI) anchor. It interacts by two disulfide bonds to the secreted Aga2 subunit, to which the protein of interest is fused [3]. So far, a-agglutinin system has been applied to various biotechnological purposes, such as biosensor design, whole-cell biocatalysts and library screening. In addition, this system has significant potential in oral vaccine development against infectious diseases [4].

The a-agglutinin-based display system is a new platform in our research and little is known about protein display efficiency. The aim of this study was to test this system and immobilize a maltose-binding protein (MBP) and green fluorescent protein (GFP) on the surface of *Saccharomyces cerevisiae*. In this work, the genes encoding MBP and GFP proteins were fused to the gene encoding the Aga2 subunit. Display of maltose-binding and green fluorescent proteins were investigated and confirmed by western blot and immunofluorescence microscopy analysis. This study laid out the foundation for the application of the a-agglutinin system to further studies, as it provided knowledge for determining the optimal conditions for protein display and proper detection of immobilized proteins.

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D1. DETECTION OF ANTIBIOTIC RESISTANCE GENES IN THE SEDIMENTS OF FISH FARMING PONDS AND ASSOCIATED WATER BODIES

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Aquaculture is the rearing of aquatic animals and plants in a variety of aquatic environments. In recent decades, aquaculture has become one of the fastest growing food industry sectors. One of the main advantages of this industry is the high nutritional value of the products [1]. Farmed fish is a rich source of protein, vitamins and minerals, which are important for feeding a rapidly growing human population. Considering global challenges such as global warming or air pollution, it is becoming increasingly important to choose industrial sectors of lesser environmental impact. Differently from other types of farming, fish farming has lower greenhouse gas emissions [2]. However, despite the benefits of aquaculture, it is important to take into account its shortcomings. As aquaculture farms develop, efforts are made to maximize the production, which is why fish are farmed in high densities in limited reservoirs. Such cultivation conditions often lead to outbreaks of infectious diseases, which result in a significant loss of resources [3]. Various antimicrobials, most commonly antibiotics, are used in farms to fight bacterial infections. Unconsumed antibiotics residues may remain in the sediments as well as partially metabolised antibiotics can remain in the intestines of the fish and may be released into the environment through the faeces. Such conditions increase the selective pressure and facilitate the emergence and the spread of resistant pathogens, thus contributing to a major antibiotic resistance problem in the world [4].

The aim of this study was to evaluate the impact of intensive fisheries on the environment by investigating the prevalence of antibiotic resistance genes and their possible spread to the environment. Sediment samples were collected from three water bodies: Simnas fishery ponds and adjoining Simnas and Dusia lakes, and antibiotic resistance genes were detected. Tetracycline resistance genes were the most commonly detected in the sediments of the Simnas fish farming ponds. Meanwhile aminoglycoside resistance genes were the most prevalent in the sediments of Dusia and Simnas lakes. The detection of resistance in the unaffected lake Dusia indicated that these genes are a part of natural environmental microbiota and are likely not related to the use of antibiotics in the farm. The genes found in the sediments of the fishery ponds and lake Simnas indicate a possible spread of resistance genes and raise concerns about their transmission to humans.

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D2. INVESTIGATION OF TAIL-FORMING PROTEINS OF BACILLUS MYOVIRUS vB_BauM_KLEB27-3

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Bacteriophages (phages) are the most abundant entities on Earth. Despite their genetic diversity, the vast majority of bacteriophages described to date have a tailed morphology and belong to the order Caudovirales [1]. Tailed phages share the structure of three morphotypes: sipvoviruses (phages with long noncontractile tails), myoviruses (phages with long contractile tails) and podoviruses (phages with short noncontractile tails). The tail of myoviruses consists of a contractile sheath enveloping a rigid tube that is sharpened by a spike-shaped protein complex at its tip [2]. Usually, one copy of tail sheath and tail tube proteins are coded in the genome of bacteriophages. Some phages have duplications of these proteins in their genome. For example, giant Pseudomonas aeruginosa phage PaBG is notable for its possession of two tail sheath proteins [3]. Bacillus subtilis bacteriophage SPP1 tail tube is composed of two major tail proteins, gp17.1 and gp17.1* [4]. To our knowledge, none of three tail tube protein-encoding genes were identified in the genomes of bacteriophages to date.

In this study, tail-forming proteins of newly isolated Bacillus phage vB_BauM_KLEB27-3 (KLEB27-3) were examined. TEM analysis revealed that KLEB27-3 is a jumbo phage characterized by an isometric head (~102 nm in diameter)) and a contractile tail (~252 nm in length, and ~19 nm in width). Based on results of bioinformatic analysis, two tail sheath proteins (gp23 and gp25) and three tail tube proteins (gp26, gp27, and gp28) encoding genes are present in the genome of KLEB27-3. Bioinformatic and phylogenetic analysis revealed that none of the aforementioned KLEB27-3 proteins have close homologs in publicly available databases. In order to confirm the function and to determine the potential structures formed of theses proteins, investigation of biosynthesis of recombinant gp23, gp25, gp26, gp27, and gp28 was performed. E. coli cells were designed to express these proteins individually using pET16b and pET21a vectors. The recombinant, His-tagged and non-tagged proteins were expressed in E. coli BL21(DE3) and E. coli Rosetta (DE3) cells. SDS-PAGE analysis revealed that all of tested proteins were detected in soluble fraction under investigated conditions. The cell-free extracts were analysed by TEM, however no formation of regular structures was observed.

The results of this study not either provide new knowledge about non-canonical tail-forming proteins of myoviruses but also give new insights for further investigation of such contractile structures.

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D3. ISOLATION AND VIRULENCE ASSESMENT OF ENVIRONMENTAL STENOTROPHOMONAS MALTOPHILIA STRAINS

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Stenotrophomonas maltophilia is a gram-negative opportunistic bacterium that causes nosocomial infections characterized by high mortality rates. Multidrug resistance, motility and biofilm formation on various clinical surfaces are the main traits characterizing *S. maltophilia* as a pathogen of high concern. Besides clinical settings *S. maltophilia* is also ubiquitously present in the environment [2]. Due to the presence of this microorganism in various environmental sources it is important to asses the potential of these strains to infect humans. Therefore, the aim of this study was to isolate *S. maltophilia* from a diverse spectrum of natural sources and compare virulence traits of isolated bacteria and the clinical strains.

Environmental samples from gardens, forests, plains, various water sources were incubated on selective medium with mannitol/bromothymol-blue system containing antibiotics vancomycin, imipenem and fungicide amphotericin B [1]. After incubation positive colonies were screened using genus Stenotrophomonas specific primers. Random amplified polymorphic DNA PCR was used for selection of distinct genetic profiles. The species confirmation of selected colonies was done by performing 16S rRNA gene sequencing analysis. After species identification analysis, biofilm formation ability, swarming and twitching motility assays of environmental and clinical isolates in 28°C and 37°C temperatures were performed.

38 samples from various environmental sources were collected. The selective medium system was sufficient to isolate 190 colonies out of which 138 belonged to the genus Stenotrophomonas. 42 isolates with distinct genetic profiles were selected - of which 26 isolates were identified as S. maltophilia. Different isolates displayed variable biofilm formation, growth and motility in 28°C and 37°C temperatures which may indicate different genes involved in virulence phenotype formation or differences in virulence genes expression levels.

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D4. EXPLORING DNA MODIFICATIONS OF BACTERIOPHAGES ISOLATED FROM GYPSUM KARST LAKES

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Bacteriophages have the largest variety of modified nucleobases in nature to protect their DNA from the bacterial restriction–modification systems. DNA-modifying enzymes of bacteriophages that exist in unique environments, such as gypsum karst lakes, have not been sufficiently studied, but they are considered to have an enormous potential to be applied in medicine and biotechnology due to their potentially unique properties. In order to explore DNA modifications and DNA-modifying proteins of bacteriophages isolated from gypsum karst lakes, we performed the genomic DNA (gDNA) restriction analysis of phages vB_BauM_KLEB27-3 (KLEB27-3) and vB_KaeM_F14 (F14), and also used bioinformatics methods to search for homologues of DNA-modifying proteins of KLEB27-3 phage.

Restriction analysis of KLEB27-3 gDNA showed, that phage does not have the most common modifications that are methylation and glycosylation, because the digestion results correspond with the profiles generated *in silico*. In the case of phage F14, restriction analysis showed that cytosine nucleobases (C) are potentially methylated or hydroxymethylated, also deoxyarcheosine can be presented in the genome of bacteriophage. Bioinformatics analysis revealed that bacteriophage KLEB27-3 genome is encoding a potential uracil-DNA glycosylase (*g128*). Uracil-DNA glycosylase is a repair enzyme that removes uracil from DNA. This enzyme can be used to develop uracil recognition strategies, for example, using inactive uracil-DNA glycosylase that binds to uracil but is not cleaved from DNA. Bioinformatics and phylogenetic analysis revealed that gp128 has no close homologues to the phage proteins presented in publicly available databases (the closest homologue (35% aa identity) is uracil-DNA glycosylase of *Bacillus* bacteriophage SP-15). Analysis of the tertiary structure revealed that the gp128 protein corresponds to the folding of the bacterium *Thermotoga maritima* uracil-DNA glycosylase. Because gp128 does not have close homologues, it is a potentially unique protein with interesting properties, and it is therefore useful to study it in more detail. The gene of potential uracil-DNA glycosylase was cloned into an expression vectors containing a histidine affinity tag, that the resulting products have either an N- or C- end His-tag. These plasmids will be used for further protein production, purification experiments and *in vitro* assays for protein activity.

Results of this study not only extend our knowledge about (hyper)modified nucleobases and DNA modifying enzymes in bacteriophages but can also contribute to future studies of phage DNA modifying enzymes applicability in medicine, biotechnology, ecology and more.

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E1. SEARCH FOR PYRETHROIDS-DEGRADING MICROORGANISMS

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Pyrethroids are pesticides used worldwide to control pests in agricultural, public and commercial buildings, livestock, greenhouses and veterinary facilities. Different pyrethroids have high insecticidal potential, however, they do not degrade immediately after use and their persistent use can affect non-target organisms and contaminate environment [1]. The biggest pollution is caused by the agricultural sector since it uses pyrethroids on a regular basis and it in turn pollutes terrestrial and aquatic environments and adversely affects living organisms [2]. As has been mentioned before, pyrethroids are not decomposed immediately after spraying and their residues such as permethrin, cypermethrin, deltamethrin and allethrin are detected in the soil, so the pyrethroid-contaminated environment needs to be remedied urgently. One of the potential solutions to the problem of pyrethroid decontamination could be the use of microorganisms for the bioremediation, which is cost-effective and ecological method of cleaning pesticide-contaminated soil. Microorganisms such as Bacillus sp., Catellibacterium sp., Serratia marcescens and Acidomonas sp. are reported to have the ability to break down ester-bond having pyrethroid pesticides residues such as cypermethrin, deltamethrin and allethrin [1,3], however, to date there is still a lack of research made in the area of effective microbiological decontamination of our environment from pyrethroids and their residues.

Therefore, in this work we aimed to perform primary search, identification and analysis of microorganism and their enzymes that could have a potential to break down pyrethroids. To achieve the set goal, we performed screening of microorganisms isolated from the substrate used for the cultivation of agricultural products in some Lithuanian farms. Primary screening of microorganisms was performed on agar plates enriched with fatty substrates having ester-bond (tributyrin and rhodamine B with olive oil agar plates) (Fig. 1). Microbial isolates (bacteria, yeast and fungi) having high activity towards mentioned fatty substrates were subsequently selected to be identified by 16S rDNA analysis and for further analysis of the ability to break-down some pyrethroids.

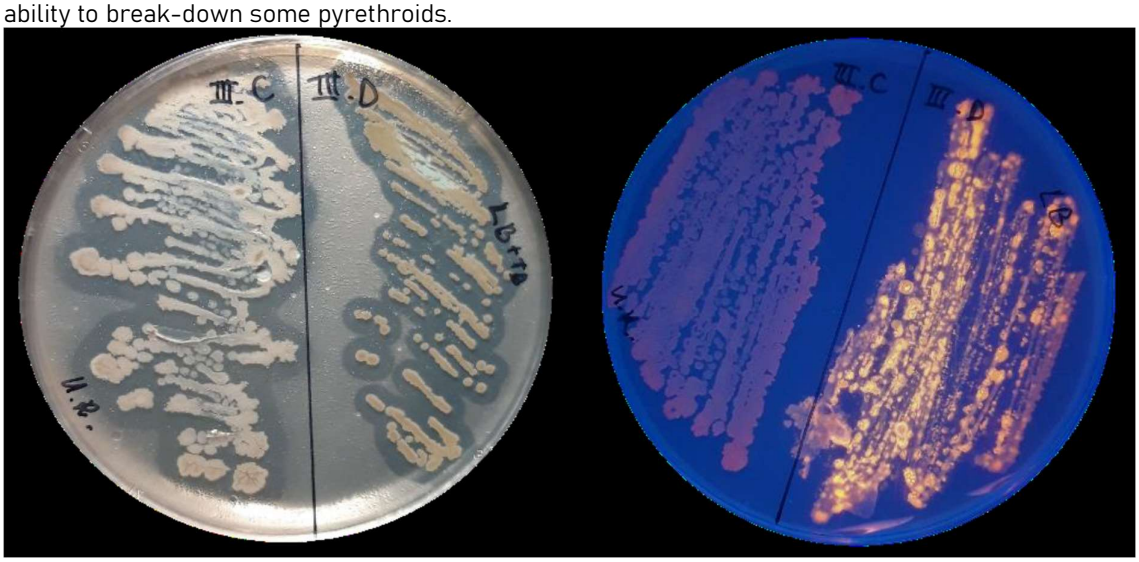

Figure 1. Example of potentially pyrethroid-degrading microorganisms on trybutirin and rhodamine B and olive oil agar plates.

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E2. MOLECULAR CHARACTERIZATION OF PSEUDOMONAS-INFECTING BACTERIOPHAGE VB_PCUM_KLEP17-4

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Plants are usually populated by a complex microbial communities composed of algae, fungi, bacteria, and their viruses. Plant-associated Pseudomonas sp. live as saprophytes and parasites on plant surfaces and inside plant tissues. Therefore, broad-temperature range bacteriophages could be useful for the prevention and therapy in both plants and humans or for food preservation.

In this study, we present a characterization of a novel, ambient-temperature adapted, Pseudomonas-infecting phage vB_PcuM_ KLEP17-4 (KLEP17-4) that has been isolated from a gypsum karst lake Kirkilai from North Lithuania. Based on the results of TEM analysis, phage KLEP17-4 is myovirus and has an isometric head of about 64 nm in diameter and a contractile non-flexible tail about 87 nm in length. The host range determination tests revealed that out of 29 bacterial strains tested, only Pseudomonas cuatrocienegasensis isolate KR2-17 was sensitive to KLEP17-4. Plating tests revealed that phage can form clear plaques of about 1.1 mm in diameter in the temperature range of 4 to 28°C, 15°C being the most optimal temperature for infection.

The 43.724 bp genome of KLEP17-4 has a G+C content of 55.5 % and contains 80 probable protein encoding genes and no genes for tRNA. Comparative sequence analysis revealed that 14 out of 80 KLEP17-4 ORFs encode unique proteins that have no reliable identity to database entries. Of the remaining 66 proteins, based on search of protein homologs in databases, 33 ORFs of KLEP17-4 have been given a putative functional annotation, including genes coding for structural proteins as well as those associated with phage-host interactions, DNA metabolism and morphogenesis. A proteomic analysis led to the experimental identification of 18 virion proteins, including 15 that were predicted by bioinformatics approaches.

Results of this study not only extend our knowledge about Pseudomonas infecting viruses but also imply that aforementioned low-temperature adapted phage potentially could be used as phage-based biocontrol agent to regulate plant, animal or human pathogens.

This research was funded by Research Council of Lithuania (Grant No. S-MIP- 20-38).

E3. MEASURING DETERGENT EFFECT ON RNA MOLECULE CAPTURE IN DROPLET BASED SINGLE CELL TRANSCRIPTOMICS

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In recent years single-cell RNA sequencing (scRNA-seq) has been established as the leading technique for characterizing the transcriptomes of individual cells, mapping cellular heterogeneity in diseased and healthy tissues and tracking cell development[1]. A large variety of different scRNA-seq methods have been developed to-date. Almost all of them can be categorized in to three broad categories based on cell capture and mRNA molecule indexing: valve-, plate- or droplet-based methods. While valve-based methods allow to capture and index cells nanoliter-size reaction chambers and plate-based methods can employ liquid handling robots none of these methods can achieve the scale and throughput at which droplet based sc-RNAseq methods can operate. Microfluidic droplets serve as independent nanoliter-sized reactors that are equivalent to tubes or wells, and can be generated at rates reaching thousands of droplets per second and can be used to barcode more than 12 000 cells per hour[2]. Inside the droplets the cells are lysed, and the released RNA is barcoded using oligonucleotide primers during RT reaction. As cell lysis, RNA molecule capture and cDNA synthesis occurs in the same reaction mix, it is crucial to select a suitable lysis reagent, a detergent that would ensure both efficient cell lysis and efficient RT reaction. Therefore, in this study we chose to investigate ionic, non-ionic and zwitterionic different detergents and their effect on cell lysis and cDNA synthesis using InDrops platform. First, we tested the detergent compatibility with RT enzyme in bulk, using conditions closely resembling those in droplets. After testing more than 100 conditions using various detergents and their combinations, we selected 5 most promising detergents for further testing using InDrops platform. Sequencing data analysis revealed that Brij58, Tween20, Tergitol 15S9 and dodecyl-β-maltoside non-ionic detergents improve mRNA molecule capture with increased UMI counts, gene capture and lower mitochondrial gene counts in cultre cells compared to IgepalCA-630.

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E4. Synthesis and Properties Investigation of Bipyrazole Derivatives

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Pyrazole and hydrazone are two very important moieties that can be found in many pharmacological drugs [1]. They possess broad spectra of biological characteristics ranging from antioxidant [1], anti-inflammatory [2], antiviral effects [2] to more specific qualities such as an antiplatelet activity that can help to fight diseases like thrombocytopenia [1] and even heart failure [1]. While demonstrating diverse biological effects, these two fragments can further be used to synthesize new scaffolds which incorporate not one but two pyrazole rings. Bipyrazole system could not only improve biological effects of compounds already possessing a pyrazole ring but also provide with a new asset of pharmacologically desired properties [3].

The aim of this work is to investigate the synthesis of bipyrazole compounds using condensation reactions of pyrazole-based hydrazone derivatives and optimize target reaction conditions.

Hydrazone derivatives containing 1-phenyl-1*H*-pyrazole moiety were obtained in a three-stage synthesis. In the first step, the alkylation of 3-hydroxypyrazole with iodmethane was performed using sodium hydride as a catalyst. Following the previous step, via *Vilsmeier-Hack* reaction the desired carbaldehyde was obtained which further reacted with different hydrazine derivatives to give a variety of functionalized hydrazones. Yields of pyrazole-based hydrazones ranged between 10% and 95%.

In search of a condensation reaction for the bipyrazole system various methods such as iron-based catalysis, hydrazone heating in acetic acid or *Vilsmeier-Haack* reaction were tested. However, sodium nitrite catalyzed cyclization of pyrazole-hydrazones proved to provide the highest yield of the target bipyrazole thus it was chosen for further development.

Influence of the reaction time, amount of sodium nitrite, temperature and heating method were investigated in order to optimize reaction conditions found in the literature. The yield of the bipyrazole derivative was increased from 20% to 55%.

There are no approved drugs that incorporate this important heterocyclic system, but the current studies with bipyrazole derivatives show that their biological attributes could help to tackle such neuropathological disorders as Parkinson disease. [4].

Acknowledgements. Part of this work has received funding from the Research Council of Lithuania (LMTLT), agreement No [09.3.3-LMT-K-712-25-0022].

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F1. Analysis of the Antifungal Effect of Silver Nanoparticles on *Candida* Yeast

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Today, due to the aging of populations in developed countries and the growing number of people with immune disorders, it is necessary to pay special attention to patients with comorbidities. The incidence of infections caused by the genus Candida is extremely high in the population of immunocompromised individuals. What's more, isolates of C. lusitaniae and C. guilliermondii are well known for their ability to rapidly acquire resistance to antifungal drugs [1].

One of the tools to solve this problem is silver nanoparticles (AgNPs). Due to their high penetration, the fungicidal efficacy of AgNPs is stronger than that of conventional antifungal drugs. Because AgNPs can affect different cellular targets simultaneously, they successfully inhibit the growth of not only susceptible but also antifungal-resistant yeast [2]. Smaller NPs are more permeable, so chemical synthesis that results in small AgNPs with a narrow size distribution is excellent for their production [3]. However, the application of AgNPs in medicine would require reducing the toxicity of AgNPs to humans by modifying them or combining them synergistically with other antifungal therapies.

One of the ways to reduce toxicity is the application of electroporation synergistically with AgNPs. The method results in an increase in the permeability of the cell membrane [4]. This way effective antifungal concentration of AgNPs can be reduced as well as toxicity.

In this work, a droplet test method was used to determine the minimum inhibitory concentration of AgNPs. It was found that AgNPs inhibit the growth of C. guilliermondii more than C. lusitaniae yeasts or C. lusitaniae pseudohyphae. Evaluation of yeast growth over time showed that AgNPs inhibited the growth of both yeasts statistically reliably over 24 hours. The strongest synergistic effect was demonstrated, when yeasts' cells or pseudohyphae were treated by both – electroporation (100 µs impulse, 2,5; 5; 7,5; 10; 12,5; 15 kV/cm) and AgNPs (0,11 µg/ml, 0,2 µg/ml or 0,5 µg/ml). The overall synergistic antimicrobial effect was significantly higher than after treatment with an electric field or AgNPs alone.

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F2. NOVEL METHODS FOR HIGH-THROUGHPUT SINGLE BACTERIA GENOMICS

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For all living entities, such as humans and other animals, it is crucial to understand the function of cells and the relations with other cells including microbes to an extent from human health to evolutionary biology. However, the majority of the microbes collected from the environmental samples cannot be isolated or cultivated in the laboratory. Modern shotgun metagenomics provides analysis for microbial ratios and genetic makeup of the sample but is limited to species resolution rather than single bacterial cells. Current single-cell genomics methods are expensive and low throughput, while typically involving individual cell processing in tubes and microplates [1].

Single-cell genomics (SCG), is an emerging approach examining the information about the genetic makeup of individual cells by utilizing next-generation sequencing (NGS) technologies. Heterogeneity within the samples demonstrates the particular importance of SCG, due to its ability to capture the single genome sequences [2]. Challenges in the microbial culture of variable samples and library preparation for sequencing govern the researchers for use of new techniques for their workflow. Droplet microfluidic technology enables scientists to generate millions of individual compartments, where unculturable cells can be trapped and massively processed for single-cell analysis. This technology is emulsion-based and requires a set of reactions, starting from encapsulation, continuing with the lysis, amplification, barcoding, and sequencing. In some cases, complete bacterial cell lysis can be difficult to achieve and might require harsh lysis conditions, which are incompatible with downstream genome amplification [3]. Here, we developed a novel single-cell genomics workflow for the next-generation sequencing platform by utilizing Semi-Permeable Capsule (SPC) technology developed by Droplet Genomics, to eliminate the aforementioned challenges. The core advantage of SPC technology is the exchange of small-molecular weight components through the hydrogel shell of the capsules while retaining the single-amplified genomes (SAGs) inside. In this project, individual cells were trapped, lysed and their genomes were amplified in SPCs, followed by barcoding and NGS sequencing. Overall we managed to sequence several hundred high-quality SAGs, with 20-80% genome coverage and low contamination rates.

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F3. BACTERIAL AMIDOHYDROLASES YQFB AND RL_D8: A NOVEL TOOL FOR GENE-DIRECTED ENZYME PRODRUG THERAPY

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The prodrug is a modified form of the active drug, specifically designed to improve its pharmacokinetic profile: the activity of the drug is "locked" and can only be restored by bioconversion, which takes place in the human body [1]. Although promising, the development of enzyme-activated drugs faces several limitations, such as the lack of suitable enzyme variants and the limited choice of chemical bonds that could be activated [2]. Developing prodrugs that cannot be activated by native human or gut microbiota enzymes, i.e., such prodrugs that can only be activated by specifically designed variants of bacterial enzymes, is one of the solutions to the problems that the gene-directed enzyme prodrug therapy faces.

The aim of this study was to determine the ability of bacterial amidohydrolases YqfB [3] and RL_D8 [4] to activate prodrugs that would affect the viability of eukaryotic cancer cells. These enzymes, when combined with the eukaryotic cytidine deaminase, have the potential to convert cytidine-based prodrugs to the therapeutic drug 5-fluorouridine (5-FUR) (Fig. 1). First, the HCT116 human colorectal carcinoma cell lines, which stably express the genes encoding the YqfB or the RL_D8 enzymes, were generated by retroviral transduction. In parallel, a selection of potential prodrugs were tested *in vitro* as possible substrates of the YqfB and RL_D8 enzymes. These cytidine derivatives were also tested *in vivo* for their possible toxicity before the activation. Four suitable prodrugs – 5-fluoro-*N*^{4}-acetylcytidine, 5-fluoro-*N*^{4}-benzoylcytidine, 5-fluoro-*N*^{4}-pivaloylcytidine, and 5-fluoro-*N*^{4}-[3-indolepropionyl]cytidine – were selected for further analysis. Next, the transduced cells expressing the bacterial amidohydrolases were exposed to several concentrations of the new prodrugs (in the range of 1 to 100 µM) and their viability was assessed using the MTT assay 24h or 48h after the exposure. Finally, the data obtained was processed and statistical analysis was performed. The results show significant decrease in the viability of both cell lines expressing either the YqfB or the RL_D8 amidohydrolase, compared to the control HCT116 cell line transduced with a vector without a gene insert. These results imply that the bacterial enzymes used in this study, together with the cellular cytidine deaminase, can convert the nontoxic prodrugs to a well-known chemotherapeutic drug 5-FUR in eukaryotic cancer cell lines.

In conclusion, our results suggest that bacterial YqfB and RL_D8 amidohydrolases, together with the modified cytidine-based prodrugs, may serve as future enzyme-prodrug systems for gene-directed enzyme prodrug therapy.

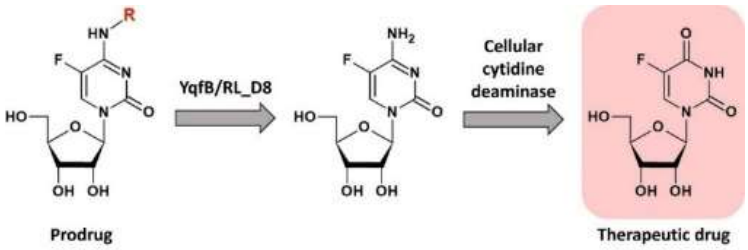

Figure 1. YqfB/RL_D8 enzyme-mediated activation of anticancer prodrug.

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F4. ISOLATION, IDENTIFICATION, AND ANTIMICROBIAL PHOTODYNAMIC INACTIVATION OF SPORES OF PATHOGENIC AND DETERIORATION MOLDS ASSOCIATED WITH STRAWBERRIES

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Diseases of strawberry (and other fruits) such as gray mold (Botrytis cinerea), anthracnose (Colletotrichum acutatum), powdery mildew (Podosphaera aphanis), Cladosporium sp., Alternaria sp., and others can cause severe losses by reducing yield and causing fruit decay during production and after harvest, if not controlled beginning early on in the production cycle [1]. With the current worldwide challenge to reduce food waste, while ensuring consumer safety, it is important to preserve fresh fruits from any molds. Photodynamic therapy (PDT) was discovered over one hundred years ago by the observation that microorganisms (Paramecia) were killed when exposed to both a photosensitizing dye and sunlight at the same time [2]. Since then, PDT has been studied and developed more as an anti-cancer therapy, and less as antimicrobial therapy [3].

This study aimed to evaluate the efficacy of PDT or antimicrobial photoinactivation (API) using natural photosensitizers (PS) in combination with 405 and 450 nm light against mold spores. Natural PS such as riboflavin (RF) and chlorophyllin (Chl) that are commonly used as vitamin B-2 and food additives, respectively, and do not cause any harm to human health were used in this work. For the analysis, strawberries from the greenhouse of "Audriaus Juškos ūkis" farm (Anykščiai, Lithuania) were used for the isolation of the fungi. 20 morphologically different isolates were obtained using PDA (Potato Dextrose Agar) and MEA (Malt Extract Agar) media. Macromorphological and microscopic structures revealed that isolated fungi could belong to different Cladosporium, Penicillium, Rhizopus, and Aspergillus species. Molecular barcoding of isolated fungi and more accurate identification of the fungi using PCR amplification and sequencing of the internal transcribed region (ITS) region, actin (ACT), elongation factor-α, β-tubulin (benA), Calmodulin (CaM), RNA polymerase II second largest subunit (RPB2-1) and other, was done. Further, regarding API, this study focused on Cladosporium spp. since it is reported as one of the most prevalent pathogens of the strawberries and, yet only a little or no research on Cladosporium sp. inactivation has been done. Some primary results of RF and Chl-based API of Cladosporium sp. are presented in this work.

Future studies and results obtained in this work may have the potential for use in the intensive field and the production of other fresh fruits and vegetables. More research is needed to make this system more profitable.

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G1. DISTRIBUTION OF DIFFERENT EFFLUX PUMP GENES IN CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII

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Acinetobacter baumannii is a pathogen that is acquiring resistance to antibiotics with a concerning speed due to the rise of antibiotic intake, and thus requires urgent attention. World Health Organization has noted A. baumannii as a 'Critical' pathogen that demands more studying in order to prevent the multidrug-resistant (MDR) phenotype from spreading, and to discover new ways to treat A. baumannii outbreaks.

Efflux pumps are one of the major mechanisms of antimicrobial resistance in A. baumannii [1]. This study aimed to understand the distribution of different types of pump genes and their relationship to the the antibiotic resistence phenotype in 61 clinical isolates of A. baumannii collected in 2013-2019 from the hospital of Lithuanian National Cancer Institute.

The isolates were identified as A. baumannii using species-specific PCR for the blaOXA-51-like and rRNR genes. After confirming the species, resistance to some antibiotics was determined using minimum inhibitory concentrations (MICs) for meropenem (carbapenem), gentamicin (aminoglycoside), ciprofloxacin (fluoroquinolone), and timetoprim-sulfametoxazole in accordance with EUCAST guidelines [2]. According to our preliminary results 8,7% of isolates are extensively-drug resistant (XDR) and 78,3% are multidrug-resistant: 88% of A. baumannii clinical isolates are resistant to gentamicin (MIC>4 mg/L), 83% - to meropenem (MIC>8 mg/L), 88% - to ciprofloxacin (MIC>1 mg/L). We found that 39% of A. baumannii isolates are resistant to trimethoprim-sulfamethoxazole treatment (MIC>4 mg/L).

The presence of efflux pump genes were tested by performing PCR. The genes tested were adeA, adeB, adeJ. These three genes code parts of efflux systems, the first two being parts of AdeABC efflux system, and the lather coding for a part of AdeIJK pump. Both of these pumps are known to contribute to the formation of the MDR phenotype. AdeA has been found in 90% of isolates, adeB in 84%, and adeJ in 98%. A correlation between particular efflux pump expression and resistance to certain antibiotics has not yet been established.

As mentioned above A. baumannii became one of the most hazardous pathogen in hospitals during the last decades. In Lithuania, 78,5% of A. baumannii isolates have shown the MDR phenotype [3]. Therefore it is important to identify clinical features, risks and virulence factors related to the pathogen. Knowledge about resistance mechanisms of A. baumannii can reveal a strategy on how the spread of these pathogens can be stopped.

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G2. POLYURETHANE DEGRADING ENZYMES FROM GENOMIC LIBRARIES

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Polyurethane (PU) is a synthetic polymer with a wide range of applications. Exceptional resistance to tearing and abrasion even in harsh environments, elasticity, impact resistance and other properties make polyurethane the 6th most used synthetic polymer in the world. More than 20 million metric tons of PU is produced annualy and over 30 % of it end up in landfills or oceans [1, 2]. PU waste can also enter food chains in a form of microplastic and nanoplastic which can have toxic effect to wild-life and natural ecosystems. Any method of increasing the value of PU waste can have a positive effect on PU recycling and waste management. Some microorganisms are capable of degrading PU. Analysis of these microorganisms could help to understand molecular mechanisms involved in biodegradation of PU.

The aim of this research is to construct a genomic library and identify the enzyme responsible for degradation of polyurethane by functional analysis. Genomic library is being constructed using genomic DNA of a microoganism that is capable of degrading polyurethane. This degradation can be observed by a formation of translucent halos around the microorganism colonies grown on PU containing media. With the help of 16S rRNA gene sequencing, it was elucidated that this microorganism belongs to the genus Glutamicibacter. Library is being prepared using a plasmid vector, various restriction enzymes and cloning methods. It is anticipated that a library of 3300 clones should be statistically sufficient to cover the whole genome of the microorganism of interest. Obtained results will be presented in more detail during poster session.

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G3. CIRCULAR PERMUTATION OF BACTERIOPHAGE vB_KleM-RaK2 SELF-ASSEMBLING PROTEIN 041

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In a circular permutation approach, native protein termini are connected via a covalent linker and new ends are introduced through the cleavage of the existing peptide bond. Such polypeptide chain reorganization can perturb local tertiary structure, change the dynamics, and properties of the protein. Circular permutations also occur naturally, albeit rarely. For example, bacteriophage AP205 coat protein is a natural circular permutant whose surface-exposed features (AB loops) are a perfect choice in vaccine development and peptide display applications. Currently, there are limited data on artificially engineered circularly permuted structural viral proteins. Such topological rearrangement could be a tool to manipulate the viral scaffolds aiming to investigate spatial structures of self-assembling proteins and improve their properties [1,2,3].

We focused on the research of a novel nanostructured material based on a self-assembling tail sheath protein 041 from the *Klebsiella*-infecting bacteriophage vB_KleM-RaK2 [4]. The spatial structure of the vB_KleM-RaK2 tail sheath is unknown. Therefore, the sites for circular permutations were designed between domains specified by FUpred, ThreaDom, ThreaDomEx, and Pfam servers. Six circular permuted proteins were constructed by joining native N- and C-termini via a polypeptide linker (GSGGTG) and new termini were introduced at positions N481/C480, N493/C492, N516/C515, N580/C579, N662/C661, and N783/C782. Constructed circular permutants were successfully synthesized in *E. coli* BL21(DE3) cells. The transmission electron microscopy analysis indicated different oligomerization states of the modified proteins 041, hence, two circular permuted proteins (041N493/C492 and 041N662/C661) self-assembled into tubular nanostructures, one (041N783/C782) formed thin tube-like structures, and three (041N481/C480, 041N516/C515, 041N580/C579) formed structures of irregular shapes. Moreover, the efficiency of recombinant protein synthesis, purification efficiency by affinity chromatography, and stability of tubular nanostructures (in 10 mM pH 7.5 potassium phosphate buffer, at 4 °C) differed between the constructed proteins. In conclusion, the results indicated that of all circular permutants the protein 041N493/C492 demonstrated the best properties: *in vivo* self-assembled into tubular nanostructures, and had the highest stability over time.

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G4: PHYSICOCHEMICAL PROPERTIES OF NISIN LOADED FUCOIDAN PARTICLES

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Microbial pathogens are one of the causes of foodborne diseases. Several methods (chemical or thermal ones) are used to extend the shelf life, assure microbial food safety. However, these methods could have undesired effects on the nutritional value or organoleptic aspects of food. Synthetic preservatives could cause allergic reactions. The use of antimicrobial peptides could be a natural alternative for these chemical and thermal methods [1].

Nisin is a cationic 3510 Da peptide produced by Lactococcus lactis which has been used in the food industry against Gram-positive bacteria. Sensitivity to environmental stresses, susceptibility to proteolysis, and undesirable interactions with food components can decrease the biological activity of nisin in food products. Nisin could be encapsulated using lipids, proteins, or carbohydrates to ensure antimicrobial activity [2]. In this study, for the synthesis of particles fucoidan was used. Fucoidan is an anionic, sulfated polysaccharide found in brown seaweed (e.g., Fucus vesiculosus, Macrocystis pyrifera, Laminaria japonica). This polysaccharide also has antivirus, antitumor, antithrombotic, anticoagulant, anti-inflammatory, and antioxidant activity. Also, fucoidan itself could eliminate cancer cells by inducing apoptosis [3].

Nisin-fucoidan particles were synthesized by a simple and cost-efficient complexation method at different pH values ranging from 4.0 to 7.0. Nisin concentration in a sample ranged from 0 to 1 mg/mL and a fucoidan concentration was 0.4 mg/mL. The size and zeta potential were measured at the initial moment and 4 weeks after complexation using Zetasizer NanoZS device. Nano-range size of the particles provides a high surface area, which allows them to interact more with the biological substrates and may lead to higher encapsulation efficiency, solubility, increased antimicrobial activity [3]. The size of nisin-fucoidan particles was found to be similar at all pH values, and with increasing nisin concentration from 0 to 0.6 mg/mL, the hydrodynamic diameter of particles varied in the range of about 200–500 nm. When the concentration of nisin approaches 1 mg/mL, particles growth and precipitation are observed. Also, there was no significant change in the particle size over time. The zeta potential measured at the initial moment of complexation had the highest absolute value at pH=7.0

compared to lower pH values. It was found that the zeta potential of the particles was always negative with nisin concentration from 0 to 0.6 mg/mL. Zeta potential increased in value and at times became positive when nisin concentration in the sample was 1 mg/mL. The biggest difference between zeta values measured at the initial moment and after 4 weeks after complexation was found to be when the nisin concentration was 0.1 and 0.2 mg/mL at pH=7.0 and 0.1 mg/mL at pH=6.0. Zeta potential does not change significantly in other samples.

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H1. SUBSTITUTED 1,3,4-OXADIAZOLE AND 1,3,4-THIADIAZOLE DERIVATIVES AS ANTIOXIDANT AND ANTIBACTERIAL AGENTS

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Oxidative stress induced by the reactive oxygen and nitrogen species is one of the major factors causing many contemporary diseases, such as cardiovascular diseases, cancer, neurodegenerative diseases, and various digestive disorders. Antioxidants are involved in the defense mechanism of the organism against pathologies associated with the attack of free radicals by slowing down or inhibiting completely the oxidation processes caused by reactive radicals. Several classes of organic compounds have attracted attention as potential scaffolds for the synthesis of antioxidant agents [1].

Furthermore, during recent decades, new infectious diseases have appeared and old ones have re-emerged. Many strains of bacteria and fungi have developed resistance to currently used drugs. Therefore, identification of novel structures leading to new potent and broad-spectrum antimicrobial agents or modification of already used drugs is of crucial importance, thus, the growth of microbial activity could be controlled.

Substituted 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives are among the most significant heterocyclic cores owing to their vast biological activities. The wide range of biological activities associated with 1,3,4-oxadiazoles include antimicrobial, antioxidant, antifungal, antiviral, antineoplastic acitivity. 1,3,4-Oxadiazole heterocycles can significantly contribute towards increasing the pharmacological activity by participating in hydrogen bonding interactions with the receptors. Due to the presence of (S-C=N) toxophoric units in substituted thiadiazoles, they show wide range of pharmacological activities. Substituted 1,3,4-thiadiazoles have been reported to possess such biological activities as antioxidant, antimicrobial, antitubercular, anti-inflammatory, anticancer, insecticidal, fungicidal, herbicidal, etc. [2].

The main goal of the study was to investigate antioxidant and antibacterial activity of azole derivatives synthesized from N-(4-methylphenyl)-β-alanine hydrazide [3].

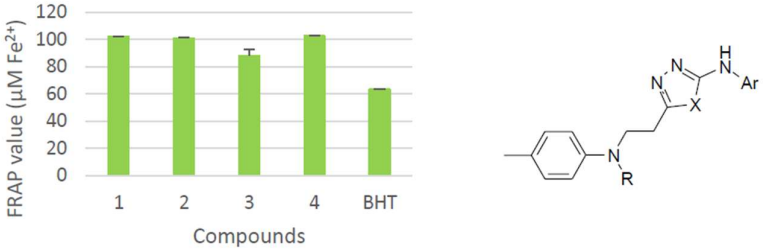

Figure 1. Antioxidant activity of compounds 1-4 tested by Ferric reducing antioxidant power assay.

The screening results of the antioxidant properties of the investigated compounds have revealed, that N-(3,5-dimethylphenyl)-5-{2-[(4-methylphenyl)amino]ethyl}-1,3,4-oxadiazole-2-amine (1) and 3-(4-methylphenyl)-1-phenyl-3-{2-[5-(phenylamino)-1,3,4-thiadiazol-2-yl]ethyl}thiourea (4) possess the highest antioxidant activity according to ferric reducing antioxidant power assay (FRAP) in comparison with the synthetic antioxidant 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene, BHT).

Antibacterial activity of oxadiazole and thiadiazole derivatives was screened by agar diffusion method against Escherichia coli, Bacillus subtilis, Rhizobium radiobacter, and Xanthomonas campestris bacteria. N-(4-Methylphenyl)-5-{2-[(4-methylphenyl)amino]ethyl}-1,3,4-thiadiazole-2-amine (3) has been identified as possessing the highest antibacterial activity against the tested bacteria strains.

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H2. COMPARISON OF LUTING CEMENTS ANTIBACTERIAL EFFECT ON *STREPTOCOCCUS MUTANS* AND SOLUBILITY *IN VITRO* FOR THE COINS 2022 POSTER SESSION

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The aim of the study: The objective of this study is to determine and compare antibacterial and solubility properties of different luting cements in different pH artificial saliva solutions *in vitro*, also to perform an analysis of cements' chemical composition and evaluate their chemical compounds' relationship with antibacterial properties and solubility. **Methods**: Bioceramic (C1), two resin-modified glass ionomers (RM-GIC) (C2) and (C3), zinc phosphate (C4) cements were used in this study. For measuring antibacterial properties were prepared 39 discs (8 x 1,5 mm) of each cement. Cement samples were stored on the *S. mitis* media with *S. mutans* and artificial saliva (pH 4,6 and 6,5). The control group was stored without artificial saliva. The antibacterial effect after 48 and 120 hours was evaluated with the disk diffusion method. In the solubility test: 54 samples of each cement (0,205 g) were prepared. Cement discs were stored in artificial saliva (pH 4,6 and 6,5) and distilled water (pH=7). After 48 and 120 hours solubility of the cement specimens was calculated. Chemical analysis of cements was performed using the X-ray fluorescence method.

Results: The growth inhibition zones of *S. mutans* induced by zinc phosphate cement (C4) were significantly larger than other cements at different pH media ($p<0,05$). Inhibition zones of RM-GIC (C2) were the smallest, however, not statistically significant. Different pH had a significant effect on solubility after 48 and 120 hours ($p<0,05$). The solubility of zinc phosphate cement (C4) was significantly higher than other cements. RM-GIC (C2) was the least soluble in all media during the observation period, however, there were no statistically significant differences. After 48 hours the solubility of zinc phosphate cement (C4) was significantly higher at pH=4,6 comparing with control. The solubility of cements excluding RM-GIC (C2) was significantly higher after 120 hours at pH=6,5. A significant association was found between a higher amount of zinc oxide in the cement and the larger zone of inhibition. Higher solubility was associated with higher amounts of aluminum and silicon oxides in the cement.

Conclusion: All cements demonstrated antibacterial activity against *S. mutans*. Zinc phosphate cement (C4) had the highest antibacterial effect, also it was the most soluble. The lowest solubility and antibacterial activity of RM-GIC (C2) were observed. Zinc phosphate (C4) and bioceramic (C1) cements were more soluble in acidic than in neutral media. Higher amounts of zinc oxide are associated with higher solubility and antibacterial activity, while higher amounts of aluminum and silicon oxides in cement were linked to lower solubility.

Keywords: antibacterial properties, solubility, luting cements, *Streptococcus mutans*, secondary caries.

H3. INVESTIGATION OF GENES AND ENZYMES INVOLVED IN THE METABOLISM OF PYRAZINE DERIVATIVES

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Pyrazines are volatile compounds with a six-membered aromatic ring containing two nitrogen atoms [1]. A wide variety of different pyrazine derivatives with different substituents are found in nature [2]. The most widespread are various alkylated pyrazines with simple structures [3]. These are found in most living organisms: bacteria, fungi, and animals. Pyrazines have a wide range of applications in the food industry, also are used as pharmaceuticals, insecticides, and pesticides [1]. Pyrazine-N-oxides are no exception. They are active biological compounds with applications in the aforementioned fields [4]. To this day, the chemical synthesis of these compounds is the main source of their production. Such synthesis methods are often based on the use of strong catalysts, organic solvents, and high temperatures. However, today more than ever, environmentally friendly methods with mild reaction conditions are becoming more and more popular.

The aim of this work is to develop an alternative and environmentally friendly platform for the synthesis of pyrazine N-oxides. We have shown that using whole cells of Escherichia coli HMS174 producing recombinant L-threonine-3-dehydrogenase and Pseudomonas putida KT2440 cells synthesizing PmlABCDEF monooxygenase, it is possible to produce 2,5-dimethylpyrazine-N-oxides (2,5-DMP-N-oxides) from L-threonine via a two-step reaction. Thus, attempts are being made to develop a more efficient and simpler platform for the synthesis of 2,5-DMP-N-oxides in order to simplify and speed up the synthesis process. The aim is to synthesize 2,5-DMP-N-oxides directly from renewable carbon sources (glucose, glycerol, etc.) employing appropriately modified cells of Pseudomonas putida KT2440. To achieve this, the first goal is to construct a genetically modified Pseudomonas putida KT2440 strain that would be capable of realizing elevated levels of L-threonine in the cells. Thus, based on the metabolic pathways of the organism, certain reactions have been identified for which the responsible genes should be removed, (markerless deletions) in order to reduce the number of possible side reactions in L-threonine biosynthetic pathway. Furthermore, it is important to optimize the synthesis of 2,5-DMP from L-threonine. To enhance the expression of certain genes involved in the biosynthetic pathway, responsible genes are amplified from the genome of the Pseudomonas putida KT2440 itself by PCR, integrated into plasmids, and introduced to the cells. Such a genetically modified P. putida KT2440 strain should ensure successful synthesis of 2,5-DMP. The last step should be the separation of mono and dioxide synthesis. This will be done by selecting a mutant PmlABCDEF enzyme that will carry out the synthesis of 2,5-DMP-N-oxide, but not 2,5-DMP-1,4-dioxide.

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H4. BIOMIMETIC CHONDROITIN SULFATE-TYRAMINE HYDROGELS FOR CARTILAGE TISSUE REPAIR IN VITRO

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Biomimetic hydrogels are widely used in tissue engineering as a promising strategy to restore damaged tissues [1]. Articular cartilage is an avascular tissue with a poor ability to restore its extracellular matrix (ECM) after trauma or disease [2]. Therefore, the application of cartilage ECM-based hydrogels is a potential tool for fulfilling and improving the integrity of cartilage tissue. Human mesenchymal stem cells (MSCs) have a strong ability to differentiate into the chondrogenic lineage and have been extensively used in hydrogel systems [3]. However, to date, no biomimetic cartilage ECM restoring technique has been developed.

The aim of this study is to investigate the potential of chondroitin sulfate-tyramine hydrogel for stimulating chondrogenic differentiation of BMMSCs and its biointegration into cartilage tissue *in vitro*.

BMMSCs were isolated from healthy human bone marrow samples and chondrocytes were isolated from human articular cartilage samples. Both cell types were incorporated into CS-Tyr hydrogels for viability and chondrogenesis studies. The viability of the cells was evaluated according to cell metabolic activity (CCK-8 and LDH kits) and the presence of live/dead cells (Live/Dead kit) using spectrophotometry and fluorescence microscopy, respectively. Chondrogenic differentiation was analyzed after 21 day of induction, according to the production of cartilage ECM proteins (collagen type II, glycosaminoglycans, proteoglycans) determined by histology, immunohistochemistry, ELISA and gene expression analysis. In addition, human cartilage explants were isolated for CS-Tyr/BMMSCs hydrogel biointegration test. Cartilage degradation biomarkers, including cartilage oligomeric matrix protein (COMP), glycosaminoglycans were analyzed after 3, 9 and 21 days of culturing hydrogels loaded on explants.

The results showed that CS-Tyr hydrogels are biocompatible to cells, evenly coat and adhere to cartilage explants, and stimulate a chondrogenic response in BMMSCs and chondrocytes, as demonstrated by collagen type II production, and expression of collagen type II and aggrecan genes. The release of COMP and glycosaminoglycans was decreased in cartilage explants coated with CS-Tyr/BMMSCs incubated in a chondrogenic medium for 21 day, while a significant increase in ECM components in the medium was observed in explants without CS-Tyr/BMMSCs. These results demonstrate integral coating and protective properties of cartilage BMMSCs-loaded CS-Tyr hydrogels on cartilage explants *in vitro*.

In conclusion, CS-Tyr-based hydrogels are suitable for stimulating chondrogenic differentiation of BMMSCs and chondrocytes *in vitro* by mimicking cartilage ECM and seem promising for cartilage tissue engineering studies.

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I1. THE IMPACT OF ALGAE BIOMASS ON THE GROWTH AND DEVELOPMENT PROCESS OF RAPESEED PLANT

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The area of organic farms increased rapidly (by 46%) in the European Union from 2012 to 2019. Meanwhile, Lithuania cannot boast an increased area of organic farms; moreover, the country is not among the leading EU countries in this area [1]. Due to this fact, it is very important to expand organic agriculture in Lithuania. Phytoplankton biomass-based fertilizers can help meet the nutritional needs of the entire population and are safe for the environment [2]. Phytoplankton, which causes water blooms, is widely used worldwide as an alternative extraction source (in the production of biodiesel, hydrogen, ethanol, and biogas); however, the agrotechnical application of phytoplankton biomass as an alternative to synthetic fertilizers is less studied [3].

The aim of the study is to asses experimentally the impact of phytoplankton biomass collected from the Curonian Lagoon on rapeseed plant (*Brassica napus* L.). Different amounts of freeze-dried phytoplankton biomass were added to a plant growth substrate: the inserted phytoplankton content corresponded to nitrogen (N) content: 30; 60; 90; and 120 kg / ha. An aqueous solution of ProbioHumus (1: 100 v/v) was added to the soil 14 days before sowing in order to improve the degradation of phytoplankton biomass and to release biologically active substances required for the mineral nutrition of plants. In order to evaluate the effect of phytoplankton as an alternative to synthetic fertilizers on the growth and development processes of the tested plants, we made analysis of their biometric parameters: aboveground height and fresh and dry weight; as well as of the content and ratio of photosynthetic pigments chl *a* and chl *b* and of the content of carotenoids, malondialdehyde (MDA), H_2O_2, and proline amount.

The study showed that the insertion of phytoplankton biomass into the plant growth substrate affected the growth and development of rapeseed. Phytoplankton biomass amounts corresponding to N 90 kg / ha and N 120 kg / ha had the greatest positive effect on the increase of photosynthetic pigment content in rapeseed and on the increase of proline content and hydrogen peroxide concentration. The aboveground height and dry and fresh weight of plants increased as well. Meanwhile, MDA levels decreased at these phytoplankton biomass concentrations. This experimental research showed that phytoplankton biomass can be used as an alternative to synthetic fertilizers.

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J1. THE INHIBITORY EFFECT OF BROMHEXINE ON LIPASE ACTIVITY OF Pseudomonas aeruginosa PA01

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Pseudomonas aeruginosa is a Gram-negative, rod shaped and aerobic bacterium. It is an opportunistic pathogen mainly affecting patients with impaired defense system. It can be important because of infection, particularly in the patients with cystic fibrosis, burn patients and hospitalized in intensive care unit [1] .The bacterium is capable of producing several exoenzymes such as lipases and exotoxins [2],which can be separated from its culture medium. Lipases are water-soluble enzymes produced by many organisms, that can hydrolyse the ester bond in lipid substrates [3]. Inhibiting the lipase from P. aeruginosa through the use of selective drugs can be the promising solution to decrease the growth rate of the bacterium in human wounds.

In this study, Bromhexine was for the first time introduced as an inhibitor against the lipase of Pseudomonas aeruginosa PA01. Structure analysis of the lipase in complex with octyl-phosphinic acid 1,2-bis-octylcarbamoyloxy-ethyl ester (PDB ID: 1EX9) revealed a large cavity unable to hold bromhexine bound. MD simulations of the holoenzyme revealed the bending of a key alpha-helix, largely closing the active site. Bromhexine docked strongly to the holo form of the enzyme with docking score -8.66 kcal/mol (Glide, Schrodinger 2021-2). In order to check the stability of the docked protein–ligand complexes, 200 ns MD simulations, and metadynamics were performed, revealing strong and stable interaction between LipA and the inhibitor.

The kinetic parameters of the enzyme were investigated using Lineweaver-Burk curve. The results showed that in the presence of Bromhexine, the maximum velocity of the enzyme did not change (Vmax = 2.85mM / min / mg protein) while the Km of the enzyme was significantly increased which verified that the drug inhibited the enzyme in a competitive manner. The IC50 values and Ki was determined to be 0.045 and 0.02 mM, respectively. The results show that bromhexine inhibits lipase and opens for the development of efficient drugs combating Pseudomonas aeruginosa.

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I2. DISCORHYTHM: RHYTHMIC DATA ANALYSIS MADE EASY

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Biological rhythms are a fundamental part of all life forms living on Earth. Clinical and experimental evidence show a direct connection between malfunctioning time keeping machinery and a wide range of morbidities, including increased cancer risk and psychiatric disorders. The advent of personalized medicine has given biological rhythm studies much needed attention and the concept of chronotherapy (time-of-day adapted therapy) is gaining traction in many medical areas. However, due to the complex nature of rhythmic data, pre-processing and analysis requires specialised statistical knowledge. Additionally, most common algorithms used in such analysis (e.g., JTK Cycle, ARSER and Lomb-Scargle) tend to be slow and require advanced computational expertise to use. Therefore, we created DiscoRhythm [1] as a tool aimed at addressing both of these issues and providing researchers with an easy-to-use R package coupled with a user-friendly web interface.

DiscoRhythm is implemented as a package in the R programming language (ver. 3.6+) with the web interface based on the R Shiny platform. While initially developed for the purpose of epigenomic data analysis, due to its general analysis procedures it can be used with any kind of temporal dataset, ranging from transcriptomics to applications in physics (Fig. 1). DiscoRhythm covers all the essential steps of data analysis: 1) outlier detection using either inter-sample correlation or principal component analysis (PCA), 2) feature selection by determining the signal-to-noise ratio for each feature using technical replicates (if available), 3) identification of dominant period of rhythmicity using cosinor regression or PCA, 4) estimation and visualization of feature-wise rhythmic characteristics. All these steps can be executed via an interactive web interface with links to additional resources and guidelines making DiscoRhythm perfect for non-computational researchers. Command line version of DiscoRhythm is also available for advanced users, making it easy to integrate it into their own research or scale it up for -omic-scale and real-time analysis. Lastly, runtime improvements of up to 30-fold were achieved for rhythm detection algorithms integrated in the MetaCycle [2] R package by developing parallelized versions of ARSER, JTK Cycle and Lomb-Scargle approaches.

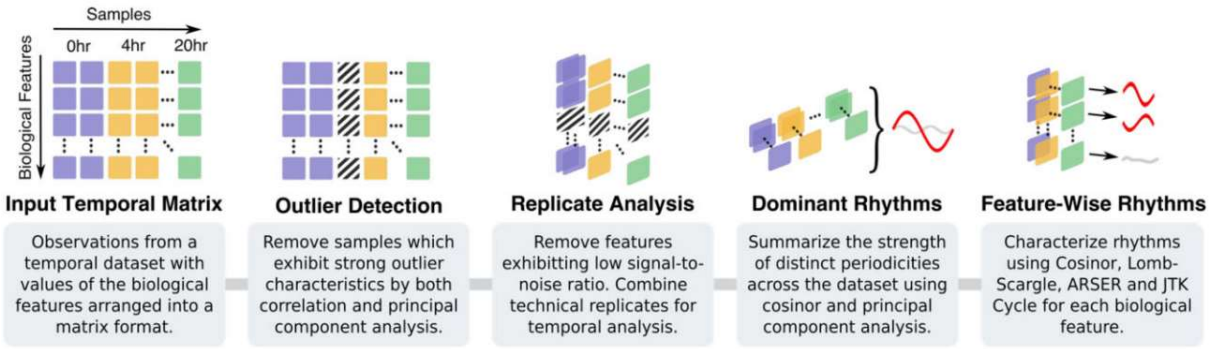

Figure 1. Stepwise overview of the analysis procedures performed by DiscoRhythm.

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I3. PROTEIN THERMOSTABILITY PREDICTION USING SEQUENCE REPRESENTATIONS FROM A PROTEIN LANGUAGE MODEL

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The variety of proteins is no less diverse than the variety of organisms. Just as the latter set is divided into domains, there are different attempts to classify proteins into distinct subsets. One way is to consider the heat-resistance property of biological macromolecules, which is an important trait for practical applications, for example, PCR.

Earlier studies show that protein's sequence and structural properties influence the thermostability of the macromolecule [1]. Furthermore, one of the most recent achievements in the field of deep learning are transformer architecture-based language models or, particularly, protein language models that have not yet been used to classify proteins based on their thermostability. Therefore, it was decided to apply protein representations from the protein language model to make inferences about thermostability of the biological macromolecules.

There are transformer architecture-based language models trained in an unsupervised fashion to predict probabilities of elements in sequences [2]. Simultaneously, the process of training creates sequences' embeddings – the real numbered vectors that represent semantic connections of language components. These representations can be transferred as input to specific application models trained using a supervised learning method to complete the defined task, such as the classification problem. Unsurprisingly, the transition between two types of learning has a name of 'transfer learning'. This separation is practically useful because the computationally-heavy task to train the language model can be excluded from the development of the application model.

Since proteins can be represented in aminoacid sequences assumed as a particular language, there are protein language models – models trained on protein sequences – that provide embeddings as output. The multi-dimensional vectors are transferred for the application neural network as its input to observe the results and decide whether the computed representations are suitable to solve the specific biological task.

This work presents a novel way to predict thermal stability of proteins. The solution is a feedforward neural network (FNN). To train the FNN, the evolutionary scale model 1b (ESM-1b) [3] is used to generate embeddings for proteins of organisms with annotated growth temperatures [4]. The model takes the generated embedding to predict the thermostability class of the input protein.

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I4. Characterization of *E. coli* phage VpaE1 gp87 and gp86 proteins using computational methods

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One way bacteria fight and resist phage infections is through the use of dedicated anti-viral systems such as CRISPR-Cas. This system provides bacteria and archaea with a way to detect, intercept and destroy nucleic acids from foreign organisms such as phages. Not only that, CRISPR-Cas also has mechanisms through which information about foreign nucleic acids can be collected, stored and reused during subsequent infection events as a way to enhance this defensive response. With this in mind, cases where Cas protein homologues are encoded as part of a phage genome are puzzling.

E. coli phage VpaE1 represents such an example. Using sequence homology methods, the VpaE1 protein, named gp87, was previously identified as a possible homologue of type III CRISPR-Cas system Cas7 protein. Another phage protein, gp86, was identified as a conserved and genomically adjacent to gp87 companion within related Felixounavirus taxa. The goal of this work was to investigate structure and possible function of these proteins using computational methods.

In the case of gp87, structure modeling revealed possible nucleic acid binding surfaces and a lack of catalytically important residues. Modeling of gp87 oligomeric states produced oligomerization patterns, similar to those of Cas7 within a CRISPR-Cas effector complex. Large-scale heterocomplex modeling experiments against target *E. coli* and VpaE1 proteomes were also conducted in an attempt to identify possible gp87 interactors. Unfortunately, no strong candidate could be identified.

Protein gp86, a companion to gp87, was also analyzed. Structure modeling suggested a homodimeric organization and the presence of a possible DNA-binding domain (wHTH) with a surface, suitable for electrostatic interaction.

Additionally, genomic contexts of gp87 homologs were collected and analyzed. This revealed that gp87 very often contains a downstream companion protein homologous to either gp86 or to another uncharacterized group of proteins. Analysis of this new group of proteins revealed that at the sequence level they show little homology to any other group of proteins including gp86. Monomeric models also provided little insight. However, homodimeric structure models revealed structural similarity to gp86 homodimeric models suggesting similar, albeit unclear, function.

Oral Presentations

Development of Recombinant C2 Domain Fused with SNAP or mKate Tags for Phosphatidylserine Labelling

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Synaptic pruning is a process when microglia eliminate redundant synapses during brain development to optimize neural networks. Inappropriate synapse remodeling can influence the development of nerve system disorders like epilepsy, schizophrenia or autism spectrum disorder [1]. For that reason, special signals are essential to ensure a strict control of this process. Recent research show that externalized phosphatidylserine (PS) is one of the synaptic markers for pruning. PS recognition occurs via microglial secreted milk fat globule-epidermal growth factor-factor 8 (MFG-E8). MFG-E8 protein is an opsonin that is able to bind to both exposed PS with its C2 domain and microglial integrin receptors. Binding to externalized synaptic PS initiates phagocytic processes in microglia [2]. Therefore, in order to investigate synaptic pruning process in the brain an effective and specific PS label is needed. However, all existing commercial PS labelling tools have drawbacks. C2 domain of MFG-E8 may provide a more sensitive probe of exposed phosphatidylserine on cell surfaces than existing PS markers like annexin V, because it binds membranes with lower PS percentage [3].

In this study we aimed to develop small genetically encoded and modifiable protein-based PS labelling tools suitable for synaptic pruning visualization *in vivo* and *ex vivo*. C2 domain proteins with or without fusion tags (mKate or SNAP-tag) were expressed in soluble fraction of *E. coli* ArcticExpress(DE3) strain and purified. Functionality of purified proteins was described by native and reducing protein electrophoresis methods. Protein interaction with PS was analyzed using staurosporine-induced apoptotic HEK293T cells. Specific apoptotic cells membrane labelling with C2-mKate and C2-SNAP proteins was achieved while mutated C2m2-mKate, C2m2-SNAP proteins did not bind to cells exposing PS. We compared C2-mKate and C2-SNAP proteins with commercial non-protein PS marker PSVue. Our results showed improved effectiveness of our protein-based PS markers over current labelling tools in neuroscience. These new PS labelling tools could potentially be used for synaptic pruning analysis in brain tissue. Further experiments with tethered lipid bilayer model and organotypic mouse brain slices will provide more information about protein affinity to PS and its potential as a tool to study synaptic pruning.

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ULTRA-HIGH THROUGHPUT EVOLUTION OF A BACTERIAL AUTOLYSIN AS ANTIMICROBIAL AGENT

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Peptidoglycan consists of N-acetylglucosamine and N-acetylmuramic acid chains interlinked by short peptides. These peptides – specific by class of bacteria – are targeted by autolysins, enzymes with an important role in bacterial cell growth and duplication that act by remodeling and relaxing the peptidoglycan structure.

Given the increasing threat posed by antibiotic resistance and the demand for new antimicrobial agents, we decided to explore whether it is possible to improve the proteolytic properties of an autolysin.

With our project, we aim to perform rounds of directed evolution on the autolysin SagA (Fig. 1) to make it suitable to act as an endolysin against the well-known pathogen *Staphylococcus aureus*. Using the aid of the powerful tool of droplet microfluidic (Fig. 2), libraries of SagA will be screened for increased activity against a model fluorogenic peptide, and the final product of evolution will be then used to perform *in vivo* assays to assess the new activity.

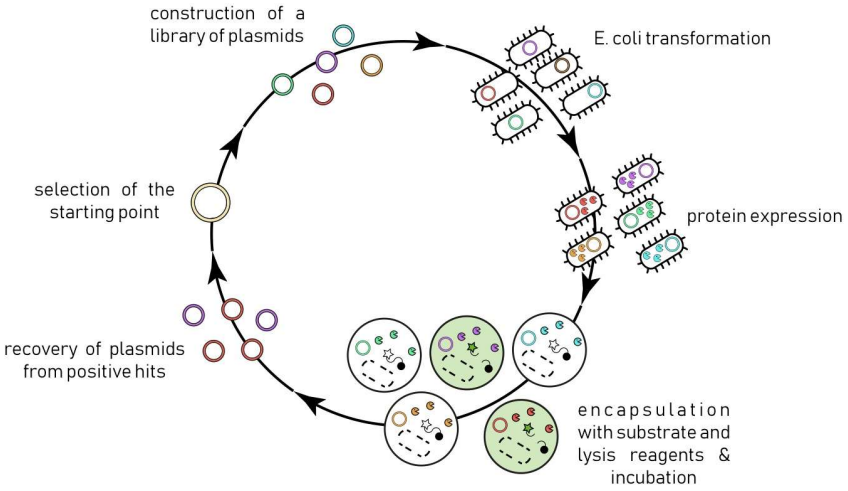

Figure 1. Scheme of iterative directed evolution workflow.

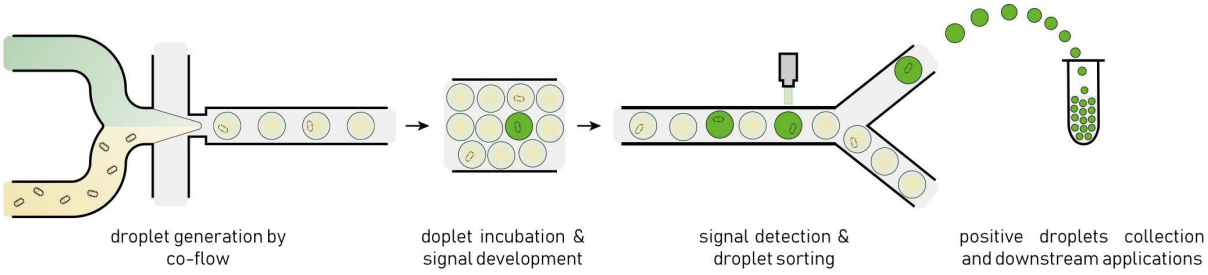

Figure 2. Scheme of the droplet microfluidics workflow.

MARCH 3rd

Cell Biology

A1. SERPIN B5 LOCALIZATION IN COLORECTAL CARCINOMA CELLS

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According to the WHO, colorectal cancer is second by highest morbidity and third by mortality rate worldwide [1]. At the first disease stage it is removed surgically. However, in a case of more developed colorectal cancer – chemotherapy is needed. Chemotherapy is usually composed of 5-fluoruracil (5-FU) and oxaliplatin (OxaPt). These compounds cause DNA damages and lead to a cell cycle arrest and apoptosis. Unfortunately, the treatment often becomes complicated because of the intrinsic or during treatment acquired chemoresistance. Despite already described molecular mechanisms of acquired chemoresistance, there are other important biochemical traits, which are vital to understand in order to develop efficient therapy.

One of possible chemoresistance mechanisms – altered gene expression of serine proteases inhibitors (serpins). Particularly, serpin B5 might act as tumour suppressor or oncogene depending on tissue and subcellular localization. Serpin B5 might be localized at cytoplasm, nucleus or secreted to the extracellular matrix. In colorectal cancer, nuclear localization of serpin B5 is related to microsatellites instability and metastasis formation, while cytoplasmic – tumour regression. From literature it is known that cellular stress might induce serpin B5 transportation to the permeabilized mitochondria, where serpin B5 competes with cytochrome C for binding to cardiolipin and releases cytochrome C into the cytosol, where it induces apoptosis [2]. Furthermore, 5-FU and OxaPt might induce stress granules formation in the cytosol. Stress granules are composed of RNA and proteins. These RNA-protein complexes help to overcome cellular stress and specialise the translation for proteins necessary for cell surveillance [3]. On the other hand, serpin B5 might be localized in the nucleus. Transportation to this organelle is at least partially controlled by epidermis growth factor (EGF) activated EGF receptor (EGFR) signalling [4]. Nuclear serpin B5 binds to the chromatin and acts as transcription factor altering several genes expression. In addition, the well-known tumour suppressor and the main factor inducing apoptosis – transcription factor p53 induces serpin B5 expression. In our laboratory it has been found that programmed cell death apoptosis is impaired in OxaPt resistant colorectal carcinoma cells HCT116/OXA. Moreover, in previous experiments we have found the enhanced serpin B5 expression in HCT116/FU and HCT116/OXA cells compared to HCT116 cells. Furthermore, serpin B5 expression is enhanced after 5-FU or OxaPt treatment in HCT116 and HCT116/FU cells and slightly enhanced in HCT116/OXA after 5-FU treatment.

In this study, we have examined serpin B5 subcellular localization and colocalization with mitochondria and stress granules after treatment by 5-FU or OxaPt in HCT116 and SW620 cells and their 5-FU and OxaPt resistant sublines.

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A2. APPLICATION OF CRISPR-CAS13 SYSTEM TO KNOCKDOWN RIPK3 TRANSCRIPT

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Colorectal cancer (CRC) is one of the most frequent malignancies worldwide, being the second most common cause of cancer death in Europe. Depending on the cancer progression, a surgical removal of the tumour can be performed, other times, a first-line chemotherapy such as FOLFOX – a combination of 5-fluorouracil (5-FU), leucovorin and oxaliplatin (OxaPt), is used to battle the disease. Unfortunately, intrinsic or acquired chemoresistance is understood as a major reason for therapy failure, with consequent tumour growth and spreading eventually leading to the patient's premature death. The ultimate function of chemoresistance is to ensure the survival of malignant cells through continuous adaptation within an organism, therefore, the nature and spectrum of cell-survival strategies in CRC represent a highly significant target of scientific inquiry.

Cas13 is a Class II Type VI CRISPR-Cas system, Cas13 protein family consists of four proteins - Cas13a, Cas13b, Cas13c and Cas13d. All Cas13 proteins are found in bacteria, where they elicit a robust response against RNA bacteriophages. Cas13 enzyme works by recognising, binding, and cleaving a target RNA molecule, allowing to utilise this system in modifying target protein expression levels by mRNA knockdown. Cas13d system consists of Cas13d protein responsible for RNA cleavage and a guide-RNA (gRNA) responsible for recognition of the target RNA. CRISPR-Cas13d system is highly specific and has an enhanced knockdown efficiency due to WYL domain, making it ideal for mRNA silencing.

The aim of this study was to determine the effects of CRIPSR-Cas13d knockdown on necroptosis inducing protein RIPK3. Firstly, we constructed two different gRNAs, both of which are able to recognise RIPK3 protein mRNA. We then transfected both of these systems into HCT116 cells and evaluated the efficiency of RIPK3 knockdown using RT-PCR analysis. We also analysed how RIPK3 silencing affected cytotoxicity of 5-FU and OxaPt. Lastly, we sought to determine CRISPR-Cas13d systems mediated RIPK3 mRNA knockdown dependency on time, by collecting transfected HCT116 cells at different time periods and measuring silencing efficiency.

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A3. CYTOTOXIC EFFECT OF BAICALEIN ON 2D AND 3D OVARIAN CANCER CELL LINES *IN VITRO*

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Ovarian carcinoma is one of the most lethal female reproductive system malignancies. It has been shown that flavonoids, secondary metabolites of plants, frequently possess anti-cancer effects. Flavonoids, such as baicalein, were recently studied for a cytotoxic effect on multiple cancer types, including ovarian [1].

To study the response of the cells to this flavonoid, we selected two ovarian carcinoma cell lines – A2780 and SKOV3. Both A2780 and SKOV3 are human ovarian cancer cell lines with epithelial-like morphology. However, SKOV3 cells are resistant to tumor necrosis factors and other cytotoxic drugs such as diphtheria toxin, cisplatin, and adriamycin, while A2780 cells carry no resistance potential [2]. Although the literature on differentiation of these cell lines is contradictory, A2780 cells are attributed to more invasive and migratory high-grade tumor differentiation type. While SKOV3 cells are associated with low-grade tumor differentiation due to being derived from well-differentiated clear cell ovarian adenocarcinoma. We selected these two cancer cell lines as representatives of differentiation grade variation and overall heterogeneity of ovarian cancer.

Our study was designed to evaluate the effect of baicalein on cytotoxicity, growth, and morphological changes of human ovarian carcinoma cell lines by cultivating them in 2D (two-dimensional) cell cultures and 3D (three-dimensional) multicellular tumor spheroid cultures (Fig. 1). The results of 2D cell cultures demonstrated a significant baicalein cytotoxic effect on A2780 and SKOV3 cell lines in a dose-dependent manner (from 40 to 60 μM) along with morphological changes. Furthermore, we decided to examine its effect on spheroid models by evaluating their growth kinetics and integrity. Exposure of 3D cultures to baicalein (concentrations from 40 to 60 μM) resulted in inhibited growth and disintegration of multicellular tumor spheroids.

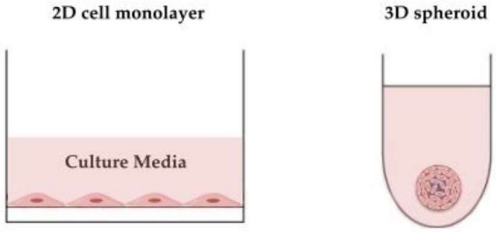

Figure 1. Schematic representation of 2D cell and 3D spheroid cultures.

This study contributes to the existing literature on the anti-cancer effect of baicalein on 2D cell cultures and for the first time examines this compound's influence on 3D spheroid cultures, which are typically more resistant and less sensitive to treatments than a monolayer culture of cells [3]. After assessing morphological, growth, and viability changes on high- and low-grade ovarian cancer cell lines in the presence of baicalein, it is vital for future studies to look for changes on a genetic and transcriptomic level that would give deeper insight into this flavonoid's mechanisms of action in 2D and 3D cell cultures.

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A4. THE EFFECT OF NOVEL TYROSINE KINASE INHIBITORS ON HUMAN PANCREATIC CANCER CELL LINES UNDER NORMOXIA AND HYPOXIA

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Pancreatic cancer remains one of the deadliest cancer types. Adenocarcinoma accounts for more than 90% of all pancreatic malignancies [1]. Targeted drugs have made significant progress in the treatment of cancer. Therefore, tyrosine kinase inhibitor sunitinib and its derivatives are expected to contribute to the efficacy of treatment. The aim of our research was to evaluate the anticancer activity of sunitinib analogues synthesized at Cagliari University (Italy) in human pancreatic cancer cell cultures under normoxia and hypoxia conditions.

In our study we investigated the activity of sunitinib and its seventeen analogues on human pancreatic adenocarcinoma cell lines MIA PaCa-2 and PANC-1 under normoxia and hypoxia. The effect on cell viability was determined by the MTT assay after 72 hours of incubation and the EC50 values were calculated. The impact of the compounds on cell clonogenicity was assessed by the ability of cells to form colonies; the number of colonies and area of colonies were calculated. Compounds were used at 10 and 90% of their established EC50 values. Results were compared to the clinically used drug sunitinib. Hypoxia was induced using $CoCl_2$ solution.

Compounds 1, 6, 7, 8, 9 and 17 reduced the viability of at least one cell line by 90% and statistically significantly stronger reduced the viability of both cancer cell lines compared to sunitinib. Evaluation of the selectivity of the compounds for cancer cells showed that not all compounds inhibited cancer cells more strongly compared to than non-cancer cells [2]. The most promising compounds 1, 6, 8 and 17 were selected for further research. Based on estimated EC50 values the most active was compound 1. It was more active than sunitinib from 24 and 35 times against MIA PaCa-2 cells in normoxia and hypoxia and to 36 and 47 times against PANC-1 cell line in normoxia and hypoxia. Compound 1 at 90% of its EC50 concentration showed the highest ability to inhibit the colony formation of MIA PaCa-2 and PANC-1 cells under normoxia and hypoxia. Compound 6 at 90% of its EC50 concentration selectively and more than sunitinib inhibited PANC-1 cell line to form colonies.

In conclusion, sunitinib derivative 1 is more effective than sunitinib in vitro in normoxia and hypoxia conditions and could be developed further as anticancer agent against pancreatic cancer.

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B1. ANTICANCER ACTIVITY OF SUNITINIB ANALOGUES IN BRAIN CANCER MODELS

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Glioblastoma (GBM) is the most aggressive type of brain cancer. Median survival time for GBM patients is about 12.1 to 14.6 month [1], and there are still extremely limited opportunities of conventional therapy. Tyrosine kinase inhibitors currently are considered as perspective targeted therapy for GBM [2]. One of them is sunitinib, approved for the treatment of renal and gastrointestinal stromal tumours [3]. Moreover, the new chemical compounds are suggested as more effective sunitinib analogues [4]. The aim of our study was to evaluate the effect of new sunitinib analogues on 2D and 3D human brain cancer cell culture models.

The two human GBM cell lines, U87-MG and A172 were used to investigate cytotoxic effect of 16 sunitinib analogues in vitro. The effect of kinase inhibitors on cell viability was evaluated by MTT assay after 72 hours of incubation. The EC50 values of the most active compounds were established and compared to the activity of sunitinib. Single cell migration assay was performed to examine the effect of selected compounds on U87-MG and A172 cell migration. Cells were seeded in the dishes with numbered grids. Photos were taken of cells every hour up to 8 hours. Then coordinates of each cell were calculated. The single cell distance travelled per hour was determined and defined as cell velocity. The data are presented as average cell velocity ± standard deviation. The activity of compounds in 3D cell cultures was examined by measuring the size change of spheroids. Tumor spheroids were formed from cancer cell lines using Hanging drop method. Spheroids were allowed to grow for 72 hours. Then individual spheroids were embedded in wells of a 96-well plate containing solidified 2% agarose and treated with 100 nM and 500 nM concentrations of tested compounds. Spheroid's size was determined using the ImageJ software.

The results of MTT assay have shown that compounds 1, 6 and 7 were the most active ones. Compound 1 had the greatest effect on cell viability (EC50 values on U87-MG, A172 and HF were 82 ± 6 nM, 72 ± 6 nM and 82 ± 6 nM, respectively). All three tested sunitinib analogues were more active than sunitinib. Regarding migration assay in U87-MG cell line, compounds 1 and 6 were more effective than sunitinib and decreased average cell velocity by 2.5 times compared to the control. In A172 cell line, compound 7 had the greatest effect on cell migration. Compounds 6 and 7 had statistically significant effect on spheroid growth at 500 nM concentration in U87-MG cell line and compound 7 had statistically significant effect on spheroid growth of A172 cell line at lower and higher concentrations similarly to sunitinib.

To conclude, new sunitinib analogues had stronger effect on brain cancer cell viability and migration based on the cell line, however none of new tested compounds were more active in 3D cell cultures than sunitinib.

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B2. STARVATION-INDUCED CHANGES IN MESENCHYMAL STATE MEDIATE PANCREATIC CANCER CELL RESISTANCE TO FERROPTOSIS

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Pancreatic cancer is one of the most aggressive cancers in the world. Due to a poor prognosis and the lack of specific biomarkers, 94% of pancreatic cancer cases lead to patient death [1]. Most of the time pancreatic cancer is diagnosed in an advanced stage. As surgical resection is then impossible, chemotherapy becomes the only hope for the patient. Gemcitabine is a classical pancreatic cancer chemotherapeutic, however, development of resistance is almost inevitable. For this reason, new combination therapies are constantly under development.

In 2012, scientists discovered a new iron-dependent cell death form, ferroptosis [2], which is caused by an excessive membrane lipid peroxidation. Iron mediated Fenton reactions constantly generate lipid peroxides. Normally, to prevent oxidative damage, cells activate lipid antioxidant glutathione peroxidase 4 (GPX4). Ferroptosis inducers inhibit activity or expression of GPX4, which leads to accumulation of harmful lipid peroxides and eventual cell death. It was shown that cancer cells are especially sensitive to ferroptosis inducers, as they have more cytosolic iron in comparison to their normal counterparts. Thus, ferroptosis can be successfully targeted to different types of cancer cells, including pancreatic. Another emerging anticancer strategy is starvation based therapy. It includes various methods: blocking angiogenesis, enhancing intratumoral nutrient catabolism and treatment with compounds that mimic starvation, such as inhibitors of growth factor receptors and mammalian target of rapamycin (mTOR) pathway.

In this study, we explored a novel anticancer approach: ferroptosis induction combined with cell starvation. In our experiments, we used pancreatic cancer cell line Miapaca-2 and a unique cell line Capan-26, previously established in our laboratory [3]. We showed that growth factor starvation made cells alternate between epithelial and mesenchymal states. Serum withdrawal induced cell morphology changes, elevated epithelial or mesenchymal marker expression, which was confirmed by fluorescence microscopy and Western blot; wound healing assay highlighted changes in Miapaca-2 cell motility. In addition, we found out that mesenchymal cells tend to be more sensitive to ferroptosis inducer erastin, whereas epithelial-like cells are resistant. Finally, we showed that compounds targeting epithelial-to-mesenchymal transition (EMT)-related signaling pathways enhance ferroptotic cell death. In this way, our study elucidates new prospects of combination therapy using ferroptosis inducers and kinase inhibitors.

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B3. ANTICANCER ACTIVITY OF SUNITINIB ANALOGUES IN 2D AND 3D BREAST CANCER CELL CULTURES

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Protein kinases are responsible for regulating different cellular functions, such as proliferation, cell cycle, apoptosis, motility, differentiation. Moreover, recent studies have shown that protein kinase dysregulations or mutations may play a role in different human diseases, including cancer [1]. Kinase inhibitors have been widely investigated for their potential anticancer activity. Over 25 kinase inhibitors are approved as anticancer drugs [2]. One of them is sunitinib, which is used to treat kidney cancer and gastrointestinal stromal tumors. However, despite the innovations in the treatment of cancer, cancer is still the second leading cause of death globally, so there is a need to search for new, more effective agents for the treatment of cancer.

The aim of our study was to evaluate the anticancer activity of new sunitinib analogues on triple-negative breast cancer cell line MDA-MB-231.

Materials and methods. Experiments were performed on human breast cancer cell line MDA-MB-231. Kinase inhibitors, which are sunitinib analogues, have been synthesized at the University of Cagliari, Italy, and their synthesis and characterization has been published [3]. Compound effect on breast cancer cell ability to migrate was examined using wound healing assay. The wound was created in a cell monolayer. Images were taken at the beginning and at regular 24 hours intervals. All compounds were used at 50% of their established EC_{50} values. 3D cell cultures were formed using magnetic *3D Bioprinting* method and the activity of kinase inhibitors was examined by measuring the size change of spheroids. All compounds were used at their established EC_{50} values.

Results. Wound healing assay results showed that kinase inhibitors 1, 6 and 17 had the greatest effect on the migration of the MDA-MB-231 cells. Among tested compounds, compound 1 was the most potent one and reduced the migration of MDA-MB-231 cells after 72 hours after incubation about 2 times compared to the control. Results from experiment in spheroid showed that kinase inhibitors 1 and 8 had the strongest effect on spheroid growth and reduced the diameter of spheroids after 10 days of incubation compared to the control. The diameter of spheroids decreased by 6.2% and 11.9% after their treatment with compounds 1 and 8, respectively.

Conclusions. Tested kinase inhibitors possess anticancer activity on triple-negative breast cancer cell line. They inhibit migration of cells and growth of spheroids, and may be worthy for further studies.

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B4. REGENERATIVE PROPERTIES OF HUMAN DILATED ATRIUM-DERIVED MESENCHYMAL STEM/STROMAL CELLS

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One of the main causes of heart failure and sudden death in children and adults is dilated cardiomyopathy (DCM), which starts mainly in left ventricle and is characterized by myocardium dilation and systolic dysfunction [1]. Later on, the myocardium dilation also spreads into atriums impairing rhythm generation. Despite intensive research and advanced medicine to manage the disease, the morbidity and mortality rates of DCM remains high [2]. Current DCM therapy is mainly symptomatic, based on drug treatment, neurohumoral blockade, pace-making devices and at the end-stage - by heart transplantation [3]. In this study, a response of human healthy and dilated atrial appendage-derived mesenchymal stem/stromal cells to histone deacetylase (HDAC) inhibitor suberoylanilide hydroxamic acid (SAHA) have been investigated. Cells were isolated from the human heart atrial appendage biopsies, cultivated under appropriate conditions and the effect of SAHA on cell proliferation, intracellular energy-dependent processes and differentiation have been investigated using spectrophotometric, flow cytometer and Western blotting.

Data of this study showed that SAHA have a positive effect on cell proliferation, overall NAD^+/NADH level, increased differentiation-based plasma membrane hyperpolarization and activated mitochondria and intracellular calcium regulating processes. In addition, SAHA reduced levels of prolonged intracellular stress-related chaperones and activated cell surviving signaling systems such as EKR1.2. Thus, HDAC inhibitor SAHA has a positive effect on the energetic and cell protecting functions that could improve regenerative properties of dilated atrium and further broaden stem/stromal cell-based therapeutic applications in the future.

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C1. DIFFERENT RESPONSE OF CHONDROCYTES AND MESENCHYMAL STEM CELLS TO BETAINE TREATMENT

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Osteoarthritis (OA) is a long-term joint disease and it is a leading cause of disability across the world [1]. Betaine is a modified amino acid acting as an osmoprotectant and a methyl-group donor with hepatoprotective, neuroprotective and cardioprotective role [2]. Its anti-inflammatory, antifibrotic and antiangiogenic properties might lead to attenuation of osteoarthritic lesion progression in cartilage and subchondral bone [3]. However there is still limited knowledge about the effects of betaine on chondrocytes and mesenchymal stem cells. The aim of our study was to compare the responses of human chondrocytes and bone marrow mesenchymal stem cells (BMMSCs) to betaine treatment under normal and inflammatory conditions.

Human chondrocytes were isolated from knee cartilage of OA patients undergoing joint replacement and mesenchymal stem cells were derived from bone marrow. Cell proliferation was measured by alamarBlue™, (fluorescence was measured at 560/590 nm), with/without betaine (10 mM) pretreatment for 24 hours under normal and inflammatory (with IL-1β) conditions. Intracellular calcium (iCa2+) levels were evaluated in both cell types as determined by the iCa2+ specific fluorescent dye Cal-520, mean fluorescence was counted using ImageJ. 2 different doses of betaine (10 mM and 40 mM) were used for morphological analysis by HoloMonitor® Live Cell Imaging System.

Betaine reduced proliferation of both chondrocytes and BMMSCs, but only in chondrocytes the difference was significant. Under stimulation with with IL-1β, the proliferation of both cell types was increased while the response to betaine pretreatment was different. Reduced proliferation of BMMSCs and increased proliferation of chondrocytes was observed on the 5th day of IL-1β conditioning . ICa2+ analysis showed similar tendency in both cell groups as betaine increased iCa2+ level, but the difference was significant only in BMMSCs group. Migration of BMMSCs was reduced by 10 mM betaine while upregulated by 40 mM betaine and comparing to control group, whereas no effects of betaine on chondrocyte migration were observed.

In concusion, betaine differently modulates responses in chondrocytes and BMMSCs and under normal and inflammatory conditions. Although similar effects of betaine on proliferation and iCa2+ level of human chondrocytes and BMMSCs under normal conditions were observed, it differently affected cell migration and modulated cell proliferation under inflammatory conditions

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C2. PARACRINE EFFECTS OF HUMAN MENSTRUAL BLOOD MESENCHYMAL STEM CELLS ON CARTILAGE TISSUE REGENERATION IN VITRO

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Mesenchymal stem cells (MSCs) have been widely used in regenerative medicine due to their capability of differentiating and self-renewing [1]. These cells are being isolated from different tissues, including bone marrow, dental pulp, adipose tissue, etc.. Menstrual blood MSCs (MenSCs) are much less characterized, however, they are known to possess more pronounced stem cell properties, as compared to classical bone marrow MSCs (BMMSCs). MenSCs exhibit a higher proliferation rate, a different profile of surface markers and a broader differentiation capacity [2][3]. Although MenSCs is an attractive alternative to BMMSCs, their use for cartilage tissue regeneration is still under consideration and research. This study was focused on potential of MenSC to stimulate chondrogenic differentiation and to protect cartilage from degradation under inflammatory conditions in vitro.

The aim of the study is to evaluate paracrine effects of MenSCs on BMMSCs chondrogenic differentiation capacity and cartilage explants in co-culture conditions.

Cells were isolated from healthy human menstrual blood (n=3) and bone marrow (n=3) samples, while cartilage explants obtained from OA patients after joint replacement surgeries. The study was performed in compliance with all bioethical requirements. MenSCs and BMMSCs were characterized according to stem cell surface marker expression (flow cytometry) and adipogenic/osteogenic differentiation capacity (Oil-Red/Alizarin). For co-culture conditions, MenSCs were seeded in 24 well plates, while BMMSCs were seeded in 0.4 µm membrane inserts placed into wells with seeded MenSCs. Chondrogenic differentiation of co-cultured cells was additionally stimulated for 21 day with transforming growth factor-beta (TGF-β3). Chondrogenic differentiation of BMMSCs was evaluated according to cartilage extracellular matrix protein formation (histology) and secretion of TGF-β1 (ELISA). The results demonstrated that chondrogenic differentiation of BMMSCs was more pronounced in co-culture with MenSCs, as compared to single BMMSCs cultures. Furthermore, secretion of TGF-β1 was increased in cell co-cultures, as compared to single MenSCs/BMMSCs populations. In addition, human cartilage explants co-cultured in 0.4 µm membrane inserts with MenSCs seeded on the bottom of 6-well plates for 21 day in chondrogenic medium without growth factors. An inflammatory cytokine Interleukin-1β (IL-1β) was additionally added in order to stimulate cartilage degradation in vitro. Cartilage degradation was evaluated by measuring glycosaminoglycan concentration in the medium, using Blyscan Sulfated Glycosaminoglycan (GAG) colorimetric assay. Results revealed that levels of released glycosaminoglycans were reduced in co-culture conditions with MenSCs, in both presence or absence of IL-1β, as compared to single explant cultures.

In conclusion, MenSCs may turn out to be a promising population of stem cells for the development of cell-based therapies with the capacity to stimulate BMMSCs chondrogenic differentiation and preventing cartilage tissue from degradation.

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C3. The Discovery of Drugs for Pancreatic Cancer via Multi-proteomic Analysis

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Poor long-term outcomes of pancreatic cancer are usually attributed to several factors, including late stage at the time of diagnosis and multi-drug resistance. Therefore, pancreatic ductal adenocarcinoma (PDAC) remains one of the most lethal cancers worldwide. In this study we present a novel integrative approach for anticancer drug discovery and evaluation.

Here we performed a global differential proteomic and phosphoproteomic analysis combined with kinome study based on multiplexed kinase inhibitory beads mass spectrometry on PDAC surgical specimens and patient-derived cancer cell lines.

First, dataset of PDAC-specific differentially expressed proteins was analyzed for enrichment using gene expression perturbation with small compounds libraries, and a set of potential anti-PDAC compounds was predicted in our analysis. We have chosen the most promising drugs in the set for the validation in patient-derived and established PDAC tumor cells. A number of these drugs induced cell death in the patient-derived as well as in established PDAC cell lines. Combinatorial drug application led to efficient cell death induction even in the cell line partially resistant to the traditional PDAC drugs. In addition, kinomic and phoshoproteomic analysis identified changes in the activity of a number of the same protein kinases in PDAC tumors as well as in patient-derived tumor cells. This allowed us to use these kinases as small molecular inhibitor targets for successful in vitro PDAC cell eradication.

The successful prediction and validation in vitro of anticancer drugs in our study demonstrates the benefits of combining multiple proteomic and bioinformatic approaches for the selection of effective compounds for tumor treatment.

C4. INVESTIGATIONS OF STRESS KINASE JNK AS A MOLECULAR TARGET IN THE TREATMENT OF LUNG CANCER CELLS

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Lung cancer is one of the leading causes of cancer-related deaths. Despite significant advances in the treatment of lung cancer, resistance almost always occurs. Combination of conventional anticancer drugs with signal molecule-targeted inhibitors is one of the most promising treatment strategies to overcome resistance. Here, by combining the chemotherapeutic drug cisplatin with inhibitors of JNK (c-Jun N-terminal Kinase) signaling pathway, we investigate the role JNK in lung cancer cell chemoresistance and death.

JNKs belong to the mitogen-activated protein kinase (MAPK) family and are involved in regulation of major cellular functions. JNK activation was reported upon genotoxic stresses and in response to anticancer treatment, including chemotherapy. Reactive oxygen species are potent activators of JNK as well. Recent evidence has shown that JNK is involved in a variety of cancers, including the lung. JNK can promote drug resistance.

Cisplatin action involves activation of multiple signaling pathways leading cells to death. Here we show that cisplatin induced expression and Ser6 phosphorylation of P53 (tumor suppressor), activation of AKT (prosurvival protein kinase), accumulation of BAX (proapoptotic mitochondrial protein) and simultaneous reduction of BCL-2 (antiapoptotic protein) in A549 adenocarcinoma cells. Our studies reveal different baseline levels of JNK phosphorylation in non-small cell lung cancer (NSCLC) cell lines. Furthermore, gradual and prolonged increase of JNK and its target transcription factor C-JUN phosphorylation was observed in A549 and other lung cancer-derived cell lines after cisplatin treatment, in contrast to H2O2. Meanwhile, expression of these signaling molecules differed: JNK expression was constant while C-JUN expression increased during cisplatin treatment.

Therefore, we applied genetic and pharmacologic approaches to modulate JNK signaling pathway. To elucidate the role of C-JUN, we overexpressed exogenous c-jun in A549 cells. ATP-competing JNK inhibitors (SP600125 and AS601245) have been used to decrease JNK activity and to ascertain its role in cisplatin-induced cell death. We found that in A549 cells, the role of JNK in cell fate determination was cisplatin concentration-dependent. At concentrations consistent with their levels in patient blood during conventional chemotherapy (10-50 μM), JNK played a cell-protective role as its inhibitors effectively increased cell death. In such conditions, inhibition of JNK had no influence on P53 expression nor on AKT phosphorylation, however, it augmented expression of proapoptotic BAX. Subcellular localization of JNK was cytoplasmic, as determined by immunocytochemistry and Western blot analysis, confirming the attitude that nuclear localization was required for the opposite – proapoptotic - activity of JNK. On the other hand, cells overexpressing c-jun were more resistant to cisplatin-induced cell death, suggesting antiapoptotic activity of C-JUN in A549 cells.

Therefore, JNK signaling pathway may be a promising target for anticancer therapy to improve the efficacy of targeted and conventional chemotherapies. However, JNK action is highly complex and context-dependent, thus requires further investigations.

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Genetics

D1. MIRNA BIOMARKERS IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disorder that results in bradykinesia and tremor due to the death of dopaminergic neurons. PD 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. Dysfunctioning miRNAs can lead to a variety of pathological events, including manifestation of neurodegenerative diseases [2]. MiRNA molecules formed in various body cells can be packaged into extracellular vesicules (EV), then infiltrate the extracellular space and travel through biological fluids providing long lasting expression of disease – related genes [3]. EV miRNAs are expecting to help differentiate diseases, their stages and their progression. Current research shows that high miR-155-5p and low miR-195-5p expression is mainly associated with PD related neuroinflammation.

The aim of this study was to determine EV expression profile of miR-155-5p and miR-195-5p in the serum of patients with PD. MiRNA expression was evaluated by age, sex, the onset of the disease, its duration, severity of symptoms, and selected method of treatment for 88 individuals with PD. Surgical treatment approach was either deep brain stimulation or gamma knife surgery, control group were treated using medications only. EV miRNAs were isolated from collected samples of blood serum, transcribed into cDNA and its expression was measured by RT-PCR. Statistical analysis was performed using GraphPad Software Inc. Prism 8.

The results revealed that high levels of miR-155 and low levels of miR-195 ($p < 0,001$) were observed in patients requiring surgical PD treatment comparing to control group. As bradykinesia manifests miR-155 ($p < 0,05$) expression increases and miR-195 ($p < 0,05$) expression diminishes. Similar changes were observed in miRNA expression profile as symptoms of tremor manifest, but these results were not statistically significant. Primary data suggest that patient groups with elevated mir-155 and decreased miR-195 expression levels experience stronger PD related symptoms and require surgical treatment. Gamma knife surgery treatment seemed to negatively affect miR-155 expression levels, however more samples are needed to confirm these findings. Neither surgical treatment affect changes in miR-195 expression prior and post surgery ($p > 0,05$). The obtained data showed that expression of miR-155 positively correlates in significant matter as the subjects age ($p < 0,01$). Statistically relevant miR-155 and miR-195 expression levels were not observed regarding gender, the onset of the disease and its duration.

In conclusion, the work demonstrates the importance of miR-155 and miR-195 molecules in the context of PD. More detailed studies of miR-155 and miR-195 expression levels are needed to support obtained primary results which could be useful in clinical practice to facilitate diagnosis, prognosis and therapeutic applications for patients with PD.

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D2. IDENTIFICATION OF LIQUID BIOPSY-BASED BIOMARKERS FOR CLEAR CELL RENAL CELL CARCINOMA DIAGNOSIS

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Introduction: Renal cell carcinoma (RCC) remains one of the most lethal urological malignancies. With the increasing use of imaging techniques, a higher number of patients who harbor RCC are identified incidentally every year [1]. The disease encompasses >10 histological and molecular subtypes, of which clear cell RCC (ccRCC) is most common and accounts for most cancer-related deaths [2]. ccRCC is classically associated with the loss of the short arm of chromosome 3 which is regarded as the critical genetic event found in the majority of patients. This genetic change has a long latency period (more than 30 years) between it's diagnosis and the development of ccRCC. The deleted region includes four tumor suppressor genes: *VHL, PBRM1, SETD2*, and *BAP1*. Early identification of ccRCC is highly important because it can impact clinical management. Considering the lack of effective non-invasive indicators, liquid biopsies could act as convenient diagnostic and prognostic biomarkers for ccRCC patients. The aim of this study was to determine mRNA levels of *VHL, PBRM1, SETD2, and BAP1* genes in the plasma of ccRCC patients in order to adapt them for ***early disease detection*** and ***risk assessment.***

Methods*: We used quantitative* reverse transcription PCR (RT-qPCR) to analyze 51 plasma samples of ccRCC patients and 30 plasma samples from people with non-malignant kidney tumors. Clinical samples were collected during 2019-2021 at National Cancer Institute.

Results: Gene expression analysis revealed that *SETD2* mRNA level was downregulated in ccRCC patients in comparison to non-cancerous cases (P = 0.049, FC = -1.66). *VHL, PBRM1*, and *BAP1* expression showed no significant differences between ccRCC patients and controls (all P > 0.05). However, we determined that *VHL* mRNA level was downregulated in T1/T2 stages of ccRCC patients (P = 0.014, FC = -1.68) but there were no significant *PBRM1, SETD2*, and *BAP1* expression differences between early and late disease stages (all P > 0.05) Also, VHL mRNA level was significantly higher in ccRCC patients who have small renal masses compared to > 4 cm diameter tumors (P = 0.026, FC = 1.77). Besides, *BAP1* mRNA level was significantly lower in ccRCC patients whose tumor spreads to the renal vein (P = 0.021, FC = -2.89).

Conclusion: Detection of *SETD2* expression changes in plasma samples could be adapted for early ccRCC diagnosis. To validate *VHL, PBRM1*, and *BAP1* genes as potential kidney cancer biomarkers, further analysis is needed.

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D3. STAT3 SIGNALING PATHWAY GENE EXPRESSION CHANGES FOR TREATMENT MONITORING IN PLASMA OF TNBC PATIENTS

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Triple negative breast cancer (TNBC) is considered the most aggressive form of breast cancer, as patients who are diagnosed with the disease have a generaly worse outcome. TNBC has a much higher recurrence and metastasis rate, due to the absence of the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) [1].The most common treatment for TNBC patients is neoadjuvant chemotherapy (NAC). While it is effective, some manage to evolve resitance, leading to a bad short-term progression-free survival rate [2]. *STAT3* is one of the most researched and investigated oncogenic transcription factors, and was shown to be highly asociated with cancer initiation, progression, metastasis and chemoresistance [3]. STAT3 is especially interesting as it is capable of eliciting the expression of cancer-related genes, but also physically and functionally cooperates with other oncogenic transcription factors. When *STAT3* is overexpresed, many of the transcriptional factors like: UPF3A, BCL-2, R-RAS-2 could also be induced to be overexpressed.. High expression of these genes is associated with worse short-term progression-free survival rate [4].

The aim of this study was to evaluate the expresion *UPF3A*, *BCL-2*, *R-RAS-2* of genes in serial plasma samples, which were reported to be apart of the STAT3 signaling pathway and to determine their associations with clinical features.

To evaluate the changes in gene expression RNA was extracted from serial blood plasma samples. Nearly 50 TNBC patient plasma was analysed by means of of quantitative reverse transcription PCR with focus on *UPF3A*, *BCL-2*, *R-RAS-2* gene expression changes. The preliminary comparative analysis showed *BCL-2* and *R-RAS-2* as upregulated after treatment with NAC, while for *UPF3A* no changes were detected

Overall, *UPF3A*, *BCL-2* and *R-RAS-2* as part of the STAT3 signaling pathway could be also involved in chemoresistance. Understading on how the expression of these genes changes during the course of treatment in TNBC patient plasma potencially could be used as non-invasive molecular biomarkers for treatment effectiveness.

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D4. RESEARCH OF THE EFFECT OF SILICON DIOXIDE AND POLYSTYRENE NANOPARTICLES IN *ALLIUM CEPA* ROOT CELLS BY CYTOGENETIC AND MOLECULAR METHODS

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Nanotechnology is one of the technology fields working at a very small scale. The wide use of nanoparticles (NP) is due to their unique physical and chemical properties, such as easy-to-modify surface, strong mechanical properties, and easy synthesis, thus NP have great potential in many areas, from construction to healthcare. With the increasing use of nanoparticles, there is a growing concern about their harmful effects on the environment and living organisms. Being of extremely small size, NP can enter the cells easily and cause damage, which can later turn into mutations affecting the entire organism. We selected to study effects of silicon dioxide nanoparticles for their widespread use in manufactured products of everyday life, and in the medical field for their biodegradability and compatibility in the cellular environment [1], whilst polystyrene nanoparticles interest us because of environmental concerns.

Previous studies of silicon dioxide nanoparticles showed lethal effects on development and growth when genotoxicity occured through reactive oxygen species (ROS) [1]. Other studies revealed polystyrene nanoparticles can also cause oxidative stress in plants. NP can affect root germination when particles adhere to the peripheral root tissues and interfere with water absorption or might be absorbed and stored in various compartments of the cell [2]. For these reasons, it is important to determine the lowest concentration required to cause effects in cells.

We applied cytogenetic and molecular methods to assess the detriment of nanoparticles, either silicon dioxide or polystyrene, in onion root cells using Allium and RAPD-PCR assays. We tested various concentrations of nanoparticle solutions consisting of 10–20 nm silicon dioxide or 50–100 nm polystyrene on onion bulbs (*Allium cepa* var *Sturon*). Root growth, mitotic index and frequency of chromosome aberration in onion root cells were determined. RAPD method was applied to evaluate if nanoparticles can induce DNA polymorphism in onion. Initially, DNA was purified by three methods: CTAB and commercial Plant gDNA Purification Kits K0512 and K0791 (ThermoFisher Scientific) to determine the most suitable. End-point PCR was performed with random sequence RAPD primers and further visual analysis of PCR products was performed to conduct statistical analysis for polymorphism evaluation and differential analysis.

In summary, the observed cytotoxicity and genotoxicity results of silicon dioxide and polystyrene nanoparticles indicate what kind of damage can be caused when these nanoparticles are absorbed through plant roots and enter the cell.

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E1. SEARCH OF NEW POLYOMAVIRUSES IN EUROPEAN HAMSTER

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Our research focuses on identifying new polyomaviruses (PyVs) — nonenveloped circular double-stranded DNA viruses. These viruses infect a wide range of mammal and bird host species. Usually, they are dormant in the host organism, however, they can activate when the organism enters an immunosuppressed state, for example, after an organ transplant, causing tumours or other illnesses [1]. Historically, polyomaviruses are one of the test subjects in the study of eukaryote gene regulation, expression and tumorigenesis. One of the model viruses is the hamster polyomavirus (*Mesocricetus auratus polyomavirus 1*, HaPyV). HaPyV was the first described rodent polyomavirus in 1968, Germany, test subjects were a laboratory colony of *Mesocricetus auratus* (Syrian hamsters) affected by skin tumour. To this day, researchers are investigating the major capsid protein VP1 of HaPyV as virus-like particles (VLPs) with different applications, for example, as a novel vaccine carrier [2]. Studies and full genome sequencing are carried out on experimental infected colonies. Knowledge of naturally occurring HaPyV in wild specimens is lacking.

In this work, we decided to search for polyomaviruses in hamster kidney samples, collected from wild *Cricetus cricetus* (European hamster). These samples were provided by the Institute of Novel and Emerging Diseases in Germany. A piece was taken from all 21 kidney samples of *C. cricetus*, lysed and viral DNA was isolated from it with a viral DNA purification kit. Following that, half of each purified sample was amplified with phi29 DNA polymerase and exo-resistant random primers to increase the amount of viral DNA. Samples underwent diagnostic nested PCR with DreamTaq polymerase. The chosen PyV LTag degenerate primers' target site was the large T antigen, which is important for polyomaviruses in the early stages of replication [3]. After the PCR, 208-235 bp length fragments were extracted from gel. DNA fragments were blunted with Klenow fragment, ligated into blunt-end pJet vector with ampicillin resistance and transformed into E. Coli DHα strain competent cells. Overnight colonies of each sample were tested by colony PCR, positive clones were grown in tubes and plasmid DNA was extracted with a spin column kit. Extracted DNA was digested with BglII restriction enzyme. DNA samples were sent to the sequencing department of Life Sciences Center for Sanger sequencing.

NCBI Blast analysis confirmed that 3 European hamster samples have polyomavirus-like sequences, with ~80% sequence identity to *Mus musculus polyomavirus 2* and ~45% to *Mesocricetus auratus polyomavirus 1*. Our next step is whole genome amplification and sequencing to fully identify these PyV-like sequences as a new polyomavirus.

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E2. CITIUS, ALTIUS, FORTIUS: GENERATION OF STRONGER ZEBRAFISH CRE DRIVER LINES FOR FASTER GENE KNOCKOUT

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Spatiotemporal transgene regulation by transgenic DNA recombinases is a pivotal technology for reverse genetics in model organisms such as zebrafish with great applications for misexpression experiments. One of the most widely used methods for this purpose is Cre/lox recombination system that directionally recombines DNA at specific loxP sites, permitting swapping or removing of sequence cassettes in vivo to permanently alter transgene readout [1]. As the zebrafish embryo develops very fast and by 24 hours post-fertilization most primary organ systems are formed, key to successful experiments hinge upon efficient Cre recombinase transgene expression at different developmental times, that enables rapid disruption of candidate gene to perform loss-of-function analysis. [2]. Several promoter elements such as the heat shock protein 70-like (hsp70), beta-actin (actb), and most widespread ubiquitin (ubi) have been routinely used to drive transgene expression in zebrafish and have been successfully applied in several lox-based reporters. Although, while highly potent during early development, the recombination potency is diminished at later embryonic/adulthood stages, reducing system utilization capacity [3]. To ensure specific and effective Cre expression at distinct developmental stages new transgenic zebrafish Cre driver lines containing strong promoters are required.

The purpose of this research is to improve the efficiency of the Cre/lox recombination system in zebrafish cells by generating new transgenic lines expressing tamoxifen inducible CreERT2 recombinase.

In this study, comparative analysis of several distinct promoter elements that drive zebrafish codon-optimized 4-OHT-Tamoxifen (4-OHT)-inducible CreERT2 recombinase [4] integrated at targeted [5] or random genomic loci will be performed. Targeting vectors that contain attB or IR/DR sites required for phiC31 mediated site-specific and Sleeping Beauty (SB100x) mediated random recombination, respectively, were constructed. These constructs also contain CreERT2 sequence under the beta-actin, ubiquitin, or new generated beta-actin-enhanced ubiquitin promoters as well as a red fluorescent reporter (mRFP) driven by lens-specific gamma-crystalline promoter that leads to eye colour change upon integration. To achieve site-directed transgenesis, targeting plasmids were co-injected with phiC31 integrase-encoding mRNA into one-cell-stage embryos that harbor a previously introduced transgenic attP site in their genome (tpl102). To generate transgenic lines containing random integration, donor vectors were co-injected with SB100x transposase-encoding mRNA into one-cell-stage embryos of the wild type fish (wt). Injected embryos positive for lens RFP fluorescence at 3 days post-fertilization were raised to adulthood and outcrossed with wt.

Up to 20% of mosaic embryos injected with donor plasmids and phiC31 recombinase showed positive RFP signal, while injections with SB100x showed 40% positive RFP signal. After outcrossing grown RFP+ fish with targeted integration, we have successfully found one F0 adulthood transgenic zebrafish that contains enhanced ubiquitin promoter and transmits the construct to 10% of its offspring. F1 generation embryos are currently being raised for further investigation.

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E3. OPTIMIZATION OF CULTIVATION, DNA PURIFICATION AND PCR-RAPD CONDITIONS FOR PUCCINIA SP.

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Pucciniales are parasitic rust fungi with a wide range of host plants. Genus Puccinia consists of more than 4000 different species all of which are plant pathogens [1]. Puccinia is macrocyclic with five spore stages: urediniospores, teliospores, basidiospores, pycniospores, and aeciospores (Fig. 1) that vary depending on the host plant.

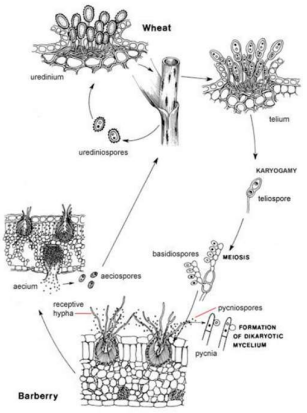

Figure 1. Fig. 1. Puccinia graminis f. sp. tritici life cycle on a primary host (wheat) and alternate host (barberry) plants [2].

Species like P. coronata Corda, P. graminis Pers, P. recondita Roberge ex Desm, and P. striiformis Westrern threaten cereal crops in Lithuania and worldwide [3]. Our research aim is to compare the genetic variation of old herbarium Puccinia specimens with the corresponding ones naturally occurring nowadays. It is like looking 30–100 years back to see if, or how much Puccinia sp. changed over the last century compared with the current species.

We received herbarium specimens of Puccinia sp. from Vilnius University herbarium (WI), and the corresponding rust species were collected in a field during the 2021 season. We selected the potato dextrose agar (PDA) medium that is commonly used for growing yeast and mould for the cultivation of rust fungus species in our research. Later, we extracted fungi DNA from spores and mycelium harvested on Petri dishes to avoid cross-contamination with the host plant DNA. This step was essential, as RAPD primers are not species or taxon-specific, so they could easily anneal to plant DNA. The DNA was purified using the CTAB method or GeneJET Genomic DNA Purification Kit K0721 (ThermoFisher Scientific, Lithuania) with some modifications. After purification, the RAPD was performed to determine the polymorphism level in the DNA sequence between modern and herbarium rust specimens.

Not all the herbarium species and those collected in summer 2021 succeeded to outgrow on PDA medium. Field species that sprouted well on the medium were P. graminis, pear rust and one undefined species, while revived herbarium specimens were mostly P. graminis. The yield of extracted DNA was greater with the conventional CTAB method compared to commercial kit. However, A260/280 ratio was insignificantly variable among all samples. Eight RAPD primers (OPA-02, OPA-03, OPB-08, OPB-15, OPB-17, OPC-08, and OPG-02) were selected for further downstream genetic diversity investigation.

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E4. MiRNA blocking potential for periodontits treatment

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Periodontitis (PD) is an inflammatory disease of tooth-supporting tissues which affects around 40% of human population. It is caused by microbial infection along with aggressive immune response against these microorganisms and can lead to tooth loss. There are several risk factors associated with this disease such as smoking, systemic diseases (e.g., rheumatoid arthritis, cardiovascular diseases, diabetes), poor dental hygiene, etc. Usually, the early stages of periodontitis do not have any symptoms, and, as a result, PD is diagnosed too late, after some damage is already done [1, 2]. Furthermore, this inflammation makes it harder for tooth implants to endure. Taken all together, it is extremely important to diagnose PD early and find a proper way to treat the disease. miRNAs are short non–coding RNAs that are involved in gene expression regulation and in the case of PD – control functions of cells involved in the immune response. This study aims to evaluate miRNA blocking potential as a therapy method for PD.

Four miRNAs (miR-140-3p, miR-145-5p, miR-146a-5p, miR-190-5p) were tested in healthy (PD–) vs. affected (PD+) people gingival crevicular fluid (GCF) (N=40). Later, miRNA expression was evaluated by conducting in vitro experiments with three types of treatment: inflammatory mediator IL-1β (IL-1β), miRNA inhibitors (INH), and both (IL+INH) and compared to a control (CT). In all cases, selected miRNAs were quantified by quantitative reverse transcription PCR (RT-qPCR).

MiRNA levels did not significantly differ between PD– and PD+ groups ($P>0.05$), but the amount of miRNA-146a-5p was significantly higher in the control/mild group compared to the moderate/severe PD group ($P=0.0277$). MiRNA changes were also associated with clinical-pathological parameter – bone loss (BL) as GCF samples from people with BL ≥ 20% compared to BL < 20% had a higher amount of miRNA-145-5p ($P=0.0398$) and lower amount of miRNA-146a-5p ($P=0.0150$). In vitro experiments revealed a statistically significant increase in miRNA-146a-5p expression after cells were treated with IL-1β ($P<0.0001$) and a decrease when miRNA-145-5p expression was compared between IL-1β and IL-1β+INH treated cells ($P=0.0028$).

The present study showed that miRNA-146a-5p changes in GCF have a connection with PD severity and in vitro results suggest the possibility of miRNA blocking to be a future approach to treat periodontitis. However, more research has to be done to confirm current findings.

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F1. DIABETES AND PREGNANCY–ASSOCIATED MIRNA ANALYSIS IN BLOOD OF WOMEN DIAGNOSED WITH GESTATIONAL DIABETES

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Gestational diabetes mellitus (GD) is characterized as a chronic hyperglycemia diagnosed during the 2nd trimester of pregnancy with a tendency to disappear after delivery [1]. This disease contributes to global diabetes epidemic by causing higher risk of type 2 diabetes mellitus (T2D) for the mother and her child [2]. Therefore, investigation of potential molecular biomarkers for the early risk assessment of both GD and T2D development is important. Epigenetic alterations, like expression differences of small regulatory RNAs, are among the most promising liquid–biopsy biomarkers in the context of diabetes [3].

The aim of this study was to analyze 10 selected microRNAs – miR-16-5p, miR-27a-3p, miR-152-3p, miR-155-5p, miR-222-3p, miR-518d-5p, miR-17-5p, miR-29a-3p, miR-195-5p and miR-499a-5p – in blood samples of women diagnosed with GD and to determine their association with diabetes-related clinical parameters.

In total, 64 women diagnosed with GD, 7 healthy pregnant and 5 non-pregnant women were included in the study. Blood samples of the GD group (129 in total) were collected at 24–28 weeks of gestation (n = 59), at 6–12 weeks after delivery (n = 28), and also from the umbilical artery (n = 21) and vein (n = 21) during the delivery. The miRNAs were quantified by means of real-time PCR, using TaqMan–based assays, after non-specific reverse transcription (RT) of all miRNAs.

In the GD group, higher levels of miR-16-5p, miR-152-3p, miR-155-5p, miR-222-3p, miR-17-5p and miR-195-5p were detected in blood collected after delivery as compared to the pregnancy (Fig. 1, all $p < 0.05$). Also, miR-29a-3p level was higher in GD group during pregnancy as compared to healthy non-pregnant women, whereas miR-195-5p showed the opposite association when compared to healthy pregnant group (all $p < 0.05$). Furthermore, the levels of miR-16-5p, miR-27a-3p, miR-152-3p, miR-17-5p, miR-29a-3p and miR-499a-5p after delivery were higher than in healthy non-pregnant women (Fig. 1, all $p < 0.05$). Interestingly, the miR-195-5p levels in blood of the umbilical artery in the GD group were lower than in healthy women ($p = 0.04$), while miR-518d-5p was undetectable in the majority of samples, except in blood of the umbilical artery of GD women.

Various associations with clinical parameters were also observed in the GD group. MiR-16-5p, miR-17-5p and miR-29a-3p levels during pregnancy correlated with the body mass index, while miR-155-5p after delivery was associated with women's age (all $p < 0.05$). Furthermore, miR-27a-3p and miR-155-5p amounts during pregnancy correlated with plasma glucose levels, as well as miR-17-5p and miR-499a-5p amounts in blood from the umbilical vein (all $p < 0.05$). Besides, miR-29a-3p levels in blood from umbilical artery and vein were associated with high– and low–density lipoproteins, respectively (both $p < 0.05$).

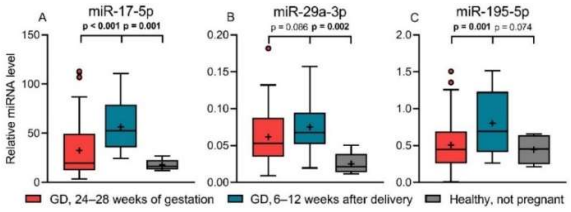

Fig. 1. Comparison of miRNA blood levels among the study groups.

The present study revealed the differences in levels of the tested miRNAs among the GD women and control groups as well as their associations with clinical indicators, suggesting these miRNAs as potential biomarkers for GD. However, larger independent studies would be necessary to validate these miRNAs for potential clinical use.

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F2. INVESTIGATION OF THE ASSOCIATION OF BARLEY TWEAKY SPIKE MUTATION WITH AUXIN BIOSYNTHESIS PATHWAYS USING COMPARATIVE GENOME ANALYSIS

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Barley is the fourth highest yielding cereal in the world, with the ability to grow in a wide range of climates and soil conditions thanks to targeted breeding. Constantly changing environmental conditions call for more adaptable/fertile varieties, and mutant research is one way of finding new targets. The tweaky spike (tw) pleiotropic mutant, developed by chemical mutagenesis, is characterised by alterations in various parts of the spike, increased free radicals and altered amino acid composition in the grain, as well as immune deficiency and genetic instability [1]. Because of these properties, tw mutants have no direct economic value, but some features of their pleiotropic complex are directly related to fertility and product quality, so exploration of tw mutation genetic determination and regulation may expand the range of genetic manipulation targets.

Previous work has already identified abnormalities related to the phytohormone auxin in tw mutants[2][3]. The aim of this study is to determine and reconfirm the correlation between auxin and tweaky spike mutants and to identify mutations in the auxin biosynthetic pathway that could potentially induce tw phenotype. The effect of auxin on spikelets was investigated by treating 3-4 leaf stage shoots with the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) and observing the resulting changes in flower parts. In addition, genes involved in auxin biosynthesis were selected and their homologues in barley were found. Potentially deleterious gene variants were selected by comparative genome analysis.

Treatment of tw mutants with 2,4-D reduced the expressivity of traits characteristic to tw pleiotropic complex: organ number variations and frequency of lodicule transformations compared to Wild Type ($p < 0.05$). Previous studies also show that exposure to auxin in tw mutants reduced the frequency of 'crowned tips' and 'crown with gaps' [3]. Furthermore, the concentration of indole compounds, including auxin, in the leaves of tw mutants was found to be significantly different from the Wild Type [2]. A genome comparison between tw2 and Wild Type identified 4 genes in auxin biosynthesis pathway potentially harmful mutations.

Figure 1. Wild-type (A) and tweaky spike mutants (B-D) of common barley (Hordeum vulgare L.).

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F3. URINARY MIRNA ASOSCIATION WITH TREATMENT RESISTANCE IN TRIPLE-NEGATIVE BREAST CANCER

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Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype and accounts ~12-17% of all breast cancer types. It lacks expression of 3 receptors: progesterone, estrogen and human epidermal growth factor 2. Due to the lack of therapeutic targets and its heterogeneity TNBC has very limited treatment options [1]. In recent years it has been shown that many different microRNAs (miRNAs) are frequently dysregulated in cancer cells and human body fluids (e.g., urine) [2]. Moreover, it has been discovered that ~30% of nonurinary molecules are passed through the kidneys which suggests that urine could be used as a promising resourse for non-invasisve biomarker research for nonurological cancers such as TNBC [3]. In addition to this, individualized comprehensive treatment strategy based on non-invasive biomarkers such as urinary miRNAs could be useful in two ways: it could help in treatment prediction and choosing the best possible option for prolonged progression free survival of TNBC patients.

The aim of this research was to identify miRNAs which are associated with chemoresistance in TNBC and could help predict the response to treatment.

In order to evaluate miRNAs expression differences in serial urine samples, we used reverse transcription quantitative PCR (RT-qPCR). In our going research, in more than 50 serial TNBC samples 4 miRNAs urinary levels were examined (miR-200a, miR-210, miR-125a, miR-454). The samples were collected before and 6 months after the treatment of paclitaxel combined with carboplatin, or 4 cycles of doxorubicin combined with cyclophosphamide. Interim analysis showed that all miRNAs (miR-200a, miR-210, miR-125a, miR-454) were upregulated before treatment and after 6 months of treatment all of them were downregulated. However, not all of them showed significant differences comparing before and after treatment groups. This suggests our analysed urinary miRNAs could be attractive candidate biomarkers for response to chemotherapy monitoring in TNBC.

Overall, miRNA-200a, miRNA-210, miRNA-125a and miRNA-454 are involved in chemotherapy resistance mechanisms. Identification of these miRNAs level differences in TNBC patients' urine specimens could be used as a completely non-invasive method which would help predict the efficacy of treatment.

Keywords: TNBC; urine; miRNA; chemoresistance

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F4. IDENTIFICATION OF CANDIDATE GENES ASSOCIATED WITH DIFFERENTIATION GRADE OF OVARIAN CARCINOMA

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Ovarian cancer is a heterogeneous gynecologic malignancy that describes different types of neoplasms, the most common being epithelial ovarian carcinoma [1]. After diagnosis patients' clinical information such as age, lymph node status, metastatic status, pathological T stage, is gathered for tumor molecular pattern identification and precise analysis. Combining clinical tumor description with gene expression datasets can lead us to the identification of new genetic biomarkers associated with specific clinical characteristics such as histologic differentiation grade. We aimed to investigate gene expression differences in ovarian carcinomas compared to normal tissue considering differentiation grade as a clinical factor.

To explore this, we obtained gene expression profiles of ovarian carcinoma samples from the NCBI Gene Expression Omnibus database (GEO) (Fig. 1). Datasets extracted from GEO contained clinical information on tumor differentiation grade. Only well-differentiated (WD) and poorly-differentiated (PD) grades were used (G1-2 as WD and G3-4 as PD) in the analysis. Firstly, we compared mRNA expression levels of microdissected tumor tissue samples to normal surface ovarian epithelial cells for differential gene expression analysis. We determined gene expression changes to be significant with $|\log2FC| \geq 2$ and Padj.<0.05. Secondly, we employed the analysis of clinical and gene mRNA expression data to assess the influence of tumor differentiation grade on gene expression. Out of almost 1500 genes which expression was significantly up or down-regulated in both WD and PD tumors compared to normal surface ovarian epithelial cells, 205 genes were identified as significantly up-regulated in WD tumors and down-regulated in PD tumors. Other 35 genes were identified as significantly up-regulated in PD tumors and down-regulated in WD tumors. This result shows a potential for tumor histologic differentiation grade biomarker identification. Our analysis of gene profiles from multiple ovarian carcinoma datasets combined with tumor differentiation grades reveals that changes in some gene expression might specifically depend on such clinical factor as differentiation grade. However, further analysis of the identified genes is required to assess their significance as potential biomarkers of differentiation grade in ovarian cancer. These results are also expected to contribute to the improvement of an ovarian cancer grading system.

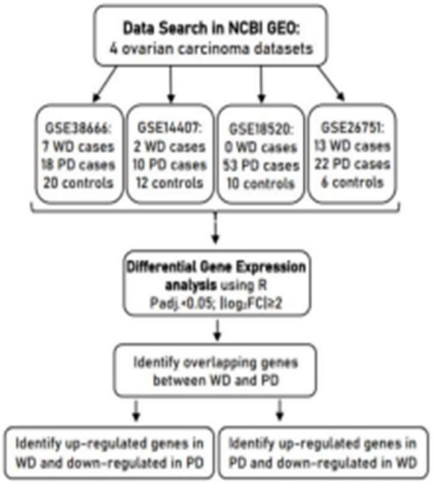

Figure 1. Schematic representation of differential expression analysis steps.

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G1. ANALYSIS OF *IN VITRO* GENOTOXICITY OF SILICA NANOPARTICLES IN HUMAN LYMPHOCYTES

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Silica nanoparticles (NPs) are used in vast array of industries: as an additive for the manufacture of rubber and plastics, as a strengthening filler for concrete or as a platform for biomedical applications [1], so it is very important to evaluate safety and possible genotoxicity of these NPs.

In this study we evaluate cytotoxicity and genotoxicity of 10-20 nm silica nanoparticles to human lymphocyte cultures *in vitro*. Short-term (1 h incubation) and longer-term (24 h incubation) effect on cell viability and DNA damage was analyzed. The cytotoxicity test used in this study was trypan blue exclusion test. Genotoxicity was determined using Comet assay or single-cell gell electrophoresis [2].

In this experiment, lymphocytes from five healthy donors were treated with SiO_2 nanoparticles at concentrations of 10-500 μg/ml. After 1 hour incubation, no significant decrease in cell viability was observed. However, after 24-hour incubation, we observed that the viability of cells exposed to silica nanoparticles above 300 μg/ml was less than 50%, so the effect of 24 h incubation was further studied only up to a concentration of 300 μg/ml, because the recommended concentration of nanoparticles ranges from non-toxic to about 60% viability [3]. In comet assay, 3 out of 5 donors were insensitive to tested SiO_2 nanoparticle concentrations after 1-hour incubation. However, when cells were exposed to silica NPs for 24-hours, a sudden increase in the amount of DNA in comet`s tail was observed at 150 μg/ml concentration. Statistical analysis of the data showed that silica nanoparticles concentrations from 150 μg/ml to 300 μg/ml caused a significant increase in the extent of DNA damage in all 5 donors ($p < 0{,}00001$).

Taken all together, silica nanoparticles can be genotoxic in human lymphocytes *in vitro* after a longer-term incubation. This raises concern about safety of use of SiO_2 nanoparticles and requires for other major endpoints, such as gene mutations or chromosomal aberrations, to be evaluated.

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G2. IDENTIFICATION OF A CANDIDATE CARDIAC ENHANCER OF THE *TBX5A* GENE IN ZEBRAFISH (*DANIO RERIO*)

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Tbx5a (T – box transcription factor 5a) is a gene that regulates the development of heart and forelimb tissue. Mutations in this gene cause Holt-Oram syndrome in humans, a condition characterized by heart and forelimb development abnormalities. Zebrafish embryos containing *tbx5* mutations similarly display a complete absence of pectoral fins and defects in heart development. *Tbx5* gene function is conservative in vertebrae therefore zebrafish become an attractive model organism to study enhancer function due to their transparent embryos which allow direct *in vivo* imaging [1]. ATAC-seq, chromatin immunoprecipitation, whole-genome bisulfite sequencing and chromosome conformation capture methods are often used to generate maps of regulatory elements in zebrafish. Characterization of such regulatory elements is usually achieved by performing a reporter assay in which a putative regulatory element is tested to determine its ability to activate the gene promoter. Using CRISPR/Cas9 genome editing system it is possible to remove the putative enhancer sequence from zebrafish genome and monitor if the enhancer-associated gene expression has changed [2]. Three cardiac and two forelimb enhancers associated with *in vivo* expression of *tbx5* have already been identified. Given the importance of this gene little is known about its enhancers. A potential tbx5 fin enhancer, CNS12, was identified in the downstream region of *tbx5* locus [3]. In contrast, a double knockout study proved that the discovered putative fin enhancers (including CNS12) were unessential in limb development [4]. Therefore, it remains unclear which sequences can be identified as tbx5a forelimb enhancers. The aim of this work was to carry out an assay on a conservative sequence downstream of the *tbx5a* gene locus and characterize it.

Two reporter plasmids containing the putative enhancer with a minimal promoter and a reporter encoding either a red (RFP) or green (GFP) fluorescent protein were created. The RFP gene was controlled by the Hsp70 and the GFP gene was controlled by the cFos minimal promoter. The mentioned constructs were flanked by two Tol2 inverted terminal repeats and co-injected with Tol2 mRNA into 1-cell stage zebrafish embryos. Two additional plasmids without the putative enhancer were created and used for control injections. Fluorescent reporter expression was monitored 72 hours post fertilization (hpf).

Results have shown that our constructs induce a fluorescent signal in zebrafish hearts. Constructs with the putative enhancer sequence resulted in 75.4% of injected embryos being RFP and 68% being GFP positive in the heart. Embryos injected with the RFP control plasmid showed a 4% positive fluorescent signal in the heart. Whereas the injected GFP control plasmid had 25% of the affected embryos display a positive fluorescent signal in the heart. It is important to notice that no fluorescent signal was detected in any of the injected embryo's pectoral fins, indicating that the tested enhancer is not active during pectoral fin development.

In summary, we have identified a new conserved zebrafish enhancer which likely contributes to regulation of *tbx5a* expression during heart development.

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G3. GENE EXPRESSION ANALYSIS OF HISTONE DEMETHYLASES AND METHYLTRANSFERASES IN PROSTATE CANCER

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Prostate cancer (PCa) is a malignancy of the male urinary system with the 2nd highest incidence rate worldwide [1]. A large proportion of PCa is latent, never destined to progress, whereas other cases are aggressive and significantly affect patients' life. Therefore, it is of high importance to identify which PCa cases need immediate treatment while others could undergo active surveillance. Prostate-specific antigen (PSA) screening remains as a primary method for PCa diagnostics; however, it lacks prognostic value and specificity for other prostatic diseases, such as benign prostate hyperplasia (BPH), and can lead to unnecessary treatment [2]. Abnormal changes in expression of epigenetic regulatory genes have emerged as one of the major research areas in search of potential cancer biomarkers, including PCa [3]. Aberrant histone methylation is one of the characteristic features during carcinogenesis, which may be due to the altered expression of regulatory genes, like histone lysine methyltransferases (KMTs) and demethylases (KDMs). Therefore, histone methylation regulators might be utilized as perspective biomarkers for more accurate diagnostics and, especially, prognosis of further PCa development.

The present study aimed to analyze the expression of 8 histone methylation-associated genes – KDM3A, KDM5D, KMT5A, KDM7B, KMT1E, WDR77, KDM5A and KDM4B – in PCa tissues, as potential biomarkers, and determine their associations with patients' clinical-pathological characteristics.

Gene expression analysis was performed in 64 early-stage (pT ≤ 3) PCa, 25 noncancerous prostate tissues (NPT) and 16 BPH samples. Only samples with RNA integrity number (RIN) ≥7.0 were used for the molecular analysis. Total RNA fraction was used for the synthesis of complementary DNA. The transcripts of interest were quantified by means of TaqMan-based real-time PCR (qPCR).

The results of our study revealed differences in the expression of selected genes associated with histone methylation in PCa as compared to NPT and BPH. The expression of KDM3A in tumors and NPT was higher than in BPH ($p = 0.0184$ and $p = 0.0275$, respectively), but it did not differ between PCa and NPT ($p > 0.0500$). KMT5A was highly expressed in BPH as compared to both PCa and NPT (both $p < 0.0001$), whereas KDM7B had generally low expression in all samples, nevertheless, it differed significantly between NPT and BPH ($p = 0.0206$). WDR77 had low expression in BPH tissues compared to both PCa and NPT ($p = 0.0439$ and $p = 0.0198$, respectively), whereas KDM4B expression was significantly higher in PCa and NPT compared to BPH (both $p < 0.0001$). No associations were found between analyzed genes expression and serum PSA levels, patient's age, prostate mass, tumor stage or biochemical disease recurrence (all $p > 0.0500$).

The results of the current study revealed significant differences in the expression of KMT5A, KDM3A, KDM7B, WDR77 and KDM4B genes in prostatic tissues of different pathology, which suggest their potential utility for improved PCa diagnostics. However, neither of the genes showed prognostic value, what might had been associated with the lack of advanced PCa cases in the cohort. Therefore, additional investigation in larger independent cohorts is needed for further elucidation of the role of histone methylation regulatory genes in PCa.

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G4. MULTIOMICS ANALYSIS OF NEUROBLASTOMA CELLS REVEALS A DIVERSITY OF MALIGNANT TRANSFORMATIONS

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Neuroblastoma (NB) is a pediatric cancer of the developing sympathetic nervous system that exhibits a significant variation in the stage of differentiation and cell composition of tumors. Global loss of DNA methylation and genomic 5-hydroxymethylcytosine (5hmC) is a hallmark of human cancers, including NB. We used our recently developed single-base resolution approaches, hmTOP-seq [1] and uTOP-seq [2], for the construction of

5hmC maps and identification of large partially methylated domains (PMDs) in different NB cell subpopulations. To investigate the cellular heterogeneity of NB we analyzed two parental cell lines SK-N-BE(2) and LA-N-1, and their clonal sublines, BE(2)-C (I-type) and BE(2)-M17 (N-type), and LA1-55n (N-type) and LA1-5s (S-type),

respectively, in a conventional and oxygen depleted environment, which often occurs in tumors. The combined analysis of 5hmC profiles and transcriptome defined a link between hypoxic gene expression and 5hmC changes, suggesting epigenetic 5hmC functions in response to oxygen deprivation. It also revealed distinct characteristics of different NB cell lineages and stages of their malignant transformation. We determined the distinctive molecular pathway signatures of I-type BE(2)-C cells when compared with N-type BE(2)-M17 cells of the same tumor origin and suggested their more aggressive and less differentiated state. Notably, we found that the 5hmCH-containing cell-specific gene sets of S-type LA1-5s and hypoxic I-type BE(2)-C cells showed associations with poor survival of NB patients. The analysis of the cell-type-specific PMD distribution highlighted differences in the global genome organization among I-type and N-type cells that were ascribed to the same lineage identity by transcription factor networks and, on the contrary, pointed out to the similarities between I-type and S-type cells.

Collectively, we demonstrated a high informativeness of the integrative epigenomic and transcriptomic research in investigating the mechanisms that regulate cell identities and developmental stages of NB cells [3]. Such multiomics analysis, as compared with mutational studies, opens new ways for the identification of novel neuroblastoma-associated features which bring prognostic and therapeutic value in treating this aggressive pediatric disease.

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H1. NOVEL TRUNCATING VARIANT IN DYNC1H1 LEADING TO INTELLECTUAL DISABILITY AND AUTISM

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Introduction. Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social interaction and communication, and repetitive and restrictive patterns of behavior or interests. Abnormal brain development, such as synaptic and myelin dysfunction, is involved in the pathogenesis of ASD. Many brain abnormalities associated with autism are associated with pathogenic variants in microtubule-associated protein genes [1]. Pathogenic variants in one of those genes, *DYNC1H1*, compromise expression or motility of the dynein complex of the human cytoplasmic dynein heavy chain and can be the cause of neurological diseases. Both mouse and human data show that the dynein heavy chain is essential for the normal function of the nervous system, and even single conservative amino acid substitutions in this > 500-kDa protein can result in neurological abnormalities [2].

Methods. Exome sequencing was applied to analyze the DNA of the affected individual and both his parents. The DNA of a healthy brother was analyzed by Sanger sequencing. To determine the effect of the identified splice site variant on mRNA structure, the sample of the proband's mRNA was isolated from blood and Sanger sequencing was performed. Bioinformatical analysis was applied to determine the changes on protein level. Clinical evaluation of a Lithuanian patient with truncating variant in *DYNC1H1* gene was performed. To better characterize the variable syndromes associated with *DYNC1H1*, detailed analysis of the clinical and molecular data of reported patients in the medical literature was performed. In total 24 publications with 100 patients harboring 80 different *DYNC1H1* variants have been identified between 2010 and 2021.

Results. Analysis of DNA samples revealed the heterozygous donor splice site variant NC_000014.9(NM_001376.5):c.6405+1G>C in *DYNC1H1* as *de novo* in the proband's DNA. PCR of the proband's cDNA resulted in two different fragments. Sanger sequencing revealed retaining intron 31 in one of them, presumably leading to a frameshift and premature stop codon (NP_001367.2:p.(Ile2136LeufsTer20)) affecting the ATPase domain of the protein. The patient has an intellectual disability and autism diagnosed since the age of 4. There are mild neurological signs, such as symmetrically reduced patellar and ankle reflexes without any pathological reflexes, low muscle tone, mild ataxia. The patients reported in literature (n=100) have a broad spectrum of clinical features, with overlapping manifestations and phenotypes ranging from neurological impairments to severe intellectual disability. Three main phenotypes are associated with *DYNC1H1* pathogenic variants: spinal muscular atrophy with lower extremity dominance (n=60), malformations of cortical development presenting as retardation syndrome 13 (MRAD13; n= 14), Charcot-Marie-Tooth disease (n=7). Up to 70% of reported patients have intellectual disability, while autism spectrum disorder was found in only three patients. Most variants reported are *de novo* missense mutations. There is only one loss-of-function variant reported, possibly a nonsense mutation resulting in severely truncated protein or causing mRNA decay [3]. This patient was diagnosed with atypical autism at the age of 5, obsessive-compulsive behaviors at 12 and was globally developmentally delayed.

Conclusions. With the novel variant causing MRAD13 syndrome, which is often associated with severe intellectual disability and epilepsy, we show that DYNC1H1 truncating variant in the ATPase domain may lead to intellectual disability in combination with autism, in the absence of evident neurological signs.

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H2. Comparison of informativity of chloroplast microsatellite and nuclear genetic markers of *Lemna minor* and application in genetic diversity studies

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Duckweed (L. minor) is one of the most common aquatic plant species in Lithuania and around the world. This plant is small, floats freely on the water surface, has simple genetic structure, and express high potential to adapt to variable environmental conditions. As the genome size of L. minor is not stable due to the relatively large number of repeat sequences it leads to enhanced genetic diversity within and among populations. To compare informativity of newly constructed molecular markers including fragments of non-coding chloroplast sequences encompassing microsatellite repeats and nuclear genetic markers encoding proteins we applied these markers in the study of genetic diversity of wild populations of L. minor in Lithuania.

Genetic diversity of wild type L. minor samples from Nemunas River near Kaunas (4 samples) and Neris River near Vilnius (8 samples) was studied based on sequencing and analysis of DNA fragments of chloroplast genome including microsatellite sequences surrounded by non-coding regions and fragments of antioxidant genes (GPx, Cat, APx) including promoters, introns and exons regions.

L. minor samples representing populations of Neris and Nemunas rivers grouped in two clusters in NJ phylogenetic trees constructed using microsatellite markers indicating correlation between geographic and genetic distances revealed by non-coding chloroplast sequences.

AMOVA test results revealed that the inter-population diversity identified using microsatellite markers is higher (30% (L4), 29% (L14), 33% (L16)) compared to genetic markers encompassing fragments of antioxidant genes (5% (GPx6), 10% (GPx7), 15 % (Cat4-Cat4b), 10% (Cat7), 19% (APx1-APx2)).

Seven genetic markers representing nuclear genes (GPx6, GPx7, Cat4b, Cat4b, Cat7, APx1, APx2) were identified as variable enough to be used in the studies of intraspecific genetic diversity of wild type L. minor clones in relation to formation of population genetic structure impacted by different environmental factors.

Keywords: Lemna minor, glutathione peroxidase, catalase, ascorbate peroxidase, microsatellite markers, phylogeny

H3. THE ROLE OF FUNCTIONAL POLYMORPHISMS IN OXIDATIVE STRESS-RELATED GENES ON LARYNGEAL SQUAMOUS CELL CARCINOMA

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Oxidative stress has a key role in laryngeal carcinogenesis as well as laryngeal squamous cell carcinoma (LSCC) progression [1]. One of the oxidative stress-related protein NFE2L2 mediate protection against oxidants itself and controls the expression of other antioxidant response elements–dependent genes [2]. Another gene involved in antioxidant protection, p21 also plays multiple functions in cell cycle arrest, apoptosis, and transcriptional regulation [3]. Higher p21 and NFE2L2 facilitate squamous cell carcinoma progression and metastasis [4, 5]. Genetic variation in oxidative stress-related genes may alter the coded protein level and impact the pathogenesis of LSCC. The present study investigated the association of functional single-nucleotide polymorphisms (SNP) in the *p21* and *NFE2L2* genes with LSCC clinicopathological features.

The current study investigated the association of the fourth functional SNP in the *NFE2L2* (rs10183914, rs35652124) and *p21* (rs1801270, rs1059234) genes with the LSCC clinicopathological features. Genomic DNA and clinical data were collected from 261 Eastern European (Lithuanian) patients with LSCC. Genotyping of SNPs was performed using TaqMan Genotyping assays. The associations of polymorphisms with clinicopathologic variables primary tumor stage (T1 and T2/T3 and T4), lymph node status (positive/negative), distant metastasis status (positive/negative), and tumor grade (G1 and G2/G3) were evaluated by Pearson's chi-square or Fishers's exact test.

All studied genotypes were in Hardy-Weinberg equilibrium and had the same distribution as the 1,000 Genomes project Phase 3 dataset for the European population. Evaluating the associations between studied polymorphisms and clinicopathological variables it was found, that carrying of T allele (TT+CT vs CC) in *NFE2L2* rs35652124 was associated with positive lymph node status (Odds ratio (OR) 2.21; 95% confidence interval (CI) 1.03-6.74; p=0.041), positive distant metastasis status (OR 4.12; 95%CI 1.01-6.89; p=0.045) and high tumor grade (OR 3.65; 95%CI 2.10-7.32; p=0.034). Significant associations were found between *p21* gene polymorphisms rs1059234 minor allele T carrying (TT+CT vs CC) and advanced tumor stage (OR 0.37; 95%CI 0.02-0.68; p=0.002). The study also shows that carrying of minor A allele (AA+CA vs CC) in another *p21* gene polymorphism rs1801270 was associated with advanced tumor stage (OR 0.43; 95%CI 0.24-0.79; p=0.006). *p21* polymorphism C allele (CC+CA vs AA) was associated with high cancer grade (OR 0.18; 95%CI 0.02-0.43; p=0.043).

According to the present study, there is a correlation between polymorphisms in *p21* and *NFE2L2* genes and LSCC clinicopathological features. Functional SNPs in these genes may have the potential to operate as markers contributing to the assessment of LSCC clinical characteristics.

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Immunology

H4. DIFFERENCE BETWEEN UPTAKE OF SOLUBLE AND LIPOSOMAL DOXORUBICIN IN LYMPHOMA CELLS

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New types of cancer treatment are discovered almost every year, but some particular drugs, that were discovered 50 years ago, are still in routine use in terms of effectiveness against specific types of tumors [1]. One of such drugs is doxorubicin (DOX), whose mechanisms includes DNA intercalation and topoisomerase II inhibition, resulting in cell death, which mostly occurs in tumor cells. It can also cause dose-limiting cardiotoxicity [2]. Because of its structure and size, the liposomal version of the drug (L-DOX) is considered to keep tumor selectivity. This is suppoed to function via more fragil tumor blood vessels (EPR mechanism). Thus, drug is slowly released into the extracellular space, avoiding the often lethal cardiotoxic side effect [3]. However, the mechanism of doxorubicin release from liposomes is still not completely understood. We hypothesise that after a short term exposure to tumor cells, liposomal doxorubicin cannot accumulate in target cells quickly enough in order to be present in media in sufficient amount compared to conventional soluble DOX.

In this study, we subjected live and permeabilized mouse lymphoma (SL2) cells to 10 µg/ml concentration of both soluble and liposomal doxorubicin for short exposure times – from 10 seconds to 2 hours. After each exposure, cells were analysed by flow cytometry and their cellular uptake of the drug was compared. Results show that, in live cells, the uptake of soluble DOX starts increasing from 2 minute incubation time up until 2 hours, with approximately 30 times increase in cellular concentration. But with L DOX, we did not notice any significant changes with increasing exposure duration – only the initial concentration change in drug uptake was noticable. With permeabilized cells, there were also no significant changes in cellular drug uptake while increasing exposure time.

These results suggest that drug release from liposomal doxorubicin occurs slowly and needs longer exposure times in order to increase internalization of the drug into cancer cells. This property of L-DOX may be adapted in effective prolonged cancer treatment.

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I1. EXPOSITION OF SARS-COV-2 OMICRON VARIANT S PROTEIN PEPTIDE ON HAMSTER POLYOMAVIRUS VIRUS-LIKE PARTICLES

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On 11 March 2020, WHO declared COVID-19 outbreak as a pandemic. Since December 2019, 5.4 million deaths were caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Recently emerged Omicron variant is already causing majority of COVID-19 cases worldwide. Due to 37 amino acid mutations in S protein this variant was shown to evade antibody-mediated immunity to an unprecedented level, thus causing high infection rates even in immunised individuals. Furthermore, Omicron-related high case incidence creates a qPCR-based testing shortage. Rapid and available testing is yet one of the most important measures for the epidemic control.

To tackle this problem, we have an aim to create monoclonal antibodies against SARS-CoV-2 Omicron variant S protein by using chimeric hamster polyomavirus (*Mesocricetus auratus polyomavirus 1*, HaPyV) VLP (virus-like particles). Major capsid protein VP1 of HaPyV is able to form VLPs with inserted viral and non-viral epitopes and protein segments. Also, HaPyV–VP1-derived chimeric VLPs are capable to induce high insert-specific and long-lasting antibody responses, even without adjuvant co-application [2,3,4].

First of all, an oligonucleotide duplex encoding the 10 amino acid (aa) long Omicron S protein peptide was inserted into the HaPyV-VP1-encoding sequence into previously defined optimal positions #1 (aa 80-89) and #4 (aa 288-295) (Fig. 1) restriction-cloned into the expression cassette of the yeast vector pFX7. Valid chimeric VP1 construcs were confirmed by colony PCR, restriction analysis and sequencing. Yeast *Saccharomyces cerevisiae* cells were transformed with pFX7-HaPyV-VP1-Omicron position #1 and #4 constructs and VLP formation was confirmed by ultracentrifugation through saccharose cushion followed by SDS-PAGE. Further, chimeric VLPs will be purified, analysed using electron microscopy and used for mice immunisation in order to produce this Omicron S protein epitope specific antibodies, which could be used for diagnostic purposes.

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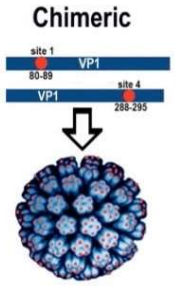

Figure 1. Insertion sites of HaPyV-VP1 used for the generation of chimeric VLPs [2] .

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STUDY OF POTENTIAL NEW DRUG COMBINATIONS FOR TREATMENT OF RESISTANT LEUKEMIA CASES

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Acute Myeloid Leukemia (AML) is the second most frequent type of hematologic malignancy diagnosed in adults. The cells affected belong to the myeloid bloodline. Typically, due to rapid progression, expensive and ineffective standard conventional treatment, elderly patients' survival rates are below 30 %. The tragical survival rates are due to excessive rates of chemoresistance to the standard treatment. For example, over 60 % of elderly patients didn't induce medical aid because of recurrence, and more than 85 % of patients failed the treatment [1].

As follows, there is a need for improvements to existing strategies to handle elderly patients and most important chemotherapy-resistant patients. We believe that the target drugs such as Metformin and Venetoclax can be combined with the conventional treatment and prolong the lifespan of the patients.

We aimed to investigate the anti-malignant effects of the BCL-2 inhibitor Venetoclax [2] and oxidative phosphorylation inhibitor Metformin [3] alone and in combination with current treatment (Cytarabine and Idarubicin) in in vitro cultivated cells. For this study we used the KG-1 cell line which is a stem-like AML cell line. We incubated KG-1 cells with Metformin alone 10 μM and in the combination with Cytarabine 30 nM with Idarubicin 8 nM for 96 hours. We demonstrated that Metformin has not shown any statistically significant difference compared to control and conventional treatment respectively in the viability and proliferation analysis. Although we found that the combination of drugs slightly induces the accumulation of early apoptotic cells, thus the gradual entering to the late apoptosis. Plus, we demonstrated, that Metformin alone increases the number of cells in the G0G1 cell cycle stage and combination slightly induces the accumulation in G2M phase. For the other half of the experiments, we use 5 nM of Venetoclax and the same concentration of conventional drugs. We proved, that only in combination with chemotherapeutics, Venetoclax showed statistically significant difference, which was almost double the effect on both proliferation and viability. Interestingly, the distribution of the cells in the apoptosis analysis indicated, that there is an unfavorable ratio of late and early apoptotic cells. Moreover, we demonstrated, that the combination of agents, distributed the cells in cell cycle analysis was very similar to the Metformin, but with the detectible difference in increase of Interphase.

We can conclude, that both Metformin and Venetoclax in fusion with existing treatment leads to the reduction of viability and rates of proliferation. In addition, both combinations have shown changes in apoptosis and cell cycle analysis, which can be favorable toward the patients.

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Statins as modulators of membrane lipid organization

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Lipid rafts are the dynamic structures composed of proteins and lipids which are free-floating within liquid disordered bilayer of cell membranes but can also cluster to form larger, ordered platforms. They are composed of sphingolipids and cholesterol in outer leaflet and connected to phospholipids and cholesterol in inner leaflet. They have been recognized as an important concentrating platform for protein receptors and are involved in regulating apoptosis, redox balance, intracellular signalling and immune response. Modulation of membrane lipid composition and organization is currently developing as an effective therapeutic strategy against a wide range of diseases, including cancer.

Pneumolysin is a cholesterol-dependent cytolysin (CDC) whose pore forming mechanism largely depend on membrane cholesterol. It disrupts lipid bilayer inducing ions leakage and osmotic shock. This mechanism is shared with Melittin, an active component in the venom of European honeybee *Apis mellifera.* In current studies, we investigated the inverse cholesterol dependency between these two toxins. Depletion of cholesterol using methyl-β-cyclodextrin reduced Pneumolysin activity but increased the damage caused by Melittin. Statins are HMG-CoA reductase inhibitors and are widely used for treating hypercholesterolemia. They are gathering increasing attention for their pleiotropic effects which are beyond cholesterol lowering. Short preincubation of lipophilic simvastatin showed similar results to the cholesterol-depletion ones, as lipophilic statins potentially alters membrane lipid organization and structure, clustering lipid raft microdomains. The results were observed using hepatocarcinoma cell line and techniques such as cell survival assay and immunofluorescence using specific antibodies. Our data suggest that both depletion of cholesterol and altering lipid bilayer organization can change cells sensitivity to toxins.

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