Vilnius University
Students' Representation
in Life Sciences Center

COINS

International conference
of Life sciences

Abstract book

2019

CONTENT

ABOUT THE COINS 2019

The COINS 19 - 14th international conference of life sciences which gathers not only students and scholars, but also all people that are working in science fields to discuss, learn and share their scientific experience, find new partners, meet key experts and enjoy exciting programme. During the conference participants will get acquainted with scientific innovations, perspectives and most relevant topics in the fields of Molecular biology, Virology, Neurosciences, Biochemistry etc.

The COINS also gives an opportunity for BA and MA students and doctorates who are doing their scientific research to present it to a larger audience, get constructive criticism and useful advice.

The COINS 19 is an open scientific environment where everyone interested in life sciences are gathered to build partnerships as well as share and develop new ideas. Conference is based on curiosity, constructive criticism and a wish to improve.

You can find more information about the conference, lecturers, participants and the whole programme in this publication or online:

www.thecoins.eu

FOREWORD

Dear participants of The COINS 2019,

It is my great pleasure to welcome all of you to The COINS 2019 conference! Raising from the initiative of students, this conference offers incredible opportunities for all of its participants.

The COINS 2019 is the 14th international conference of life sciences organised by the Vilnius University Students' Representation. The event gathers not only students and scholars, but also various people working in the life sciences field.

In the following three days, keynote speakers, scientists and students will address various important topics in life sciences, structured in lectures, discussions and networking sessions.

I am glad that our conference gives the possibility to share your knowledge and skills along with new approaches and best practices becoming more passionate in science, expanding our horizons and making The COINS 2019 more relevant, inclusive and accessible to all.

So let's go deeper and reach higher during the conference!

Sincerely,
Coordinator of The COINS 2019
Ugnė Čėplaitė

PROGRAMME

FEBRUARY 26th, TUESDAY

08:30 - 09:30	Registration
09:30 - 10:00	**OPENING CEREMONY**
10:00 - 11:00	**THE JOURNEY TO SUCCESS** Dr. Urtė Neniškytė and habil. dr. prof. Viktoras Butkus discussion moderated by Povilas Marma
11:00 - 11:30	Coffee break

SESSION 1 - MOLECULAR BIOLOGY

11:30 - 12:15	**Single cell RNAseq as a tool for cell type discovery** Rapolas Žilionis (LT)
12:15 - 12:45	**"Vilnius-Lithuania iGEM" team**
12:45 - 13:00	**Antifungal Activities of Silver Nanoparticles Obtained by Geobacillus spp. Induced Biosynthesis** Kotryna Čekuolytė (LT) - student presentation
13:00 - 14:00	Lunch
13:00 - 13:45	**VIDEO LECTURE** **Next generation antibiotics** Prof. Ada Yonath (IL) The Nobel Prize in Chemistry 2009

14:00 - 14:45	**The impact of DNA damage, transcription stress and nutrition on aging** Prof. Jan H.J. Hoeijmakers (NL)
14:45 - 15:00	**A comparative analysis of natural and experimental Plasmodium relictum infection in Eurasian siskins (Carduelis spinus)** Elena Platonova (LT) - student presentation
11:00 - 11:30	**Coffee break**
15:15 - 16:00	**Novel computational methods for the analysis of protein structures** Dr. Kliment Olechnovic (LT)
16:00 - 16:15	**Hydration and swelling of amorphous cross-linked starch microspheres studied using Raman Spectroscopy** Jekaterina Borzova (LT) - student presentation

PROGRAMME

FEBRUARY 27th, WEDNESDAY

08:30 - 09:30 Registration

09:30 - 10:50 **PANEL DISCUSSION**
Personalized medicine - an unattainable goal or tomorrow's reality?

Participants:

Jevgenija Vienažindytė - Senior Associate at Ellex Valiunas law firm;
Eimantas Peičius - Associate Professor at the Department of Bioethics and a head of the Bioethics Centre and in Lithuanian University of Health Sciences (LUHS);
Agnė Vaitkevičienė – Member of Lithuanian Biotechnology Association (LBTA);
Rasa Sabaliauskaitė – Head of Genetic Diagnostic Laboratory at National Cancer Institute.

Moderated by **Giedrė Armalytė** – Health News Editor at Delfi Lietuva

10:50 - 11:20 Coffee break

SESSION 2 - CELL BIOLOGY

11:20 - 12:05 Microparticles in Disease
Dr. Naomi Martin (UK)

11:30 - 13:20 **COMPANY FAIR**

12:05 - 12:20	**The Effect of Stearoyl-CoA-Desaturase 1 Inhibition on Pancreatic Cancer Cells in vitro** Amon Hackney (UK) - student presentation
12:20 - 13:20	**Lunch**
13:20 - 14:05	**Electroporation as a tool for selective pasterization** Dr. Arūnas Stirkė (LT)
14:05 - 14:20	**Ethnic Differences in the Role of Microparticles on Endothelial Cell Dysfunction** Christopher Pritchard (UK) - student presentation
14:20 - 15:05	**Pluripotent stem cells: ready for clinical applications in diabetes?** Prof. Tor Henrik Semb (DK)
15:05 - 15:20	**Coffee break**

SESSION 3 - NEUROBIOLOGY

15:20 - 16:05	**Normal aging in population-based imaging cohorts** Dr. rer. medic. Christiane Jockwitz (DE)

PROGRAMME

FEBRUARY 28th, THURSDAY

08:30 - 09:30	Registration

SESSION 3 - NEUROBIOLOGY

09:30 - 10:30	The Hippocampal Cognitive Map Theory, An Update Prof. John O'Keefe (UK) The Nobel Prize in Physiology or Medicine 2014
10:30 - 11:00	Coffee break
11:00 - 11:45	Processing of periodic sounds: benefits and practical application of auditory steady-state responses Dr. Inga Griškova - Bulanova (LT)
11:45 - 12:15	„Experimentica“
12:15 - 13:15	Lunch
12:15 - 14:30	**POSTER SESSION**
14:30 - 15:00	**CLOSING CEREMONY AND AWARDS**

KEYNOTE SPEAKERS

Rapolas Žilionis

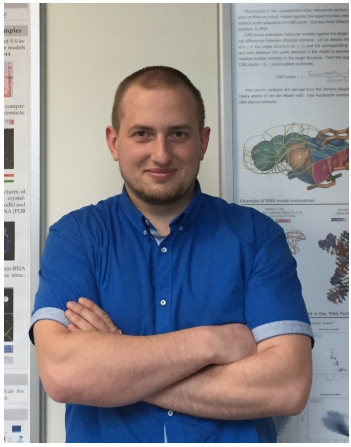
A senior PhD student in Linas Mazutis lab at Vilnius University, which is also his alma mater for BSc and MSc degrees in biochemistry.

"Single cell RNAseq as a tool for cell type discovery"

Single cell RNA sequencing (scRNA-seq) has now established itself as a powerful tool that provides information on the activity of every gene in individual cells. Due to its unbiased nature, it enables discoveries even in well studied systems. I will start my talk by providing an overview of how scRNA-seq works and how data can be interpreted in an intuitive way. I will then use two stories to illustrate the kinds of questions scRNA-seq can answer. The first story covers the unexpected discovery of a novel rare cell type with relevance to cystic fibrosis. The second story reveals how scRNA-seq can be used to better appreciate the similarities and differences between species, specifically between lung tumor infiltrating immune cells in human and mouse.

Dr. Kliment Olechnovič

A researcher in Department of Bioinformatics
in Vilnius University Life Sciences Center.



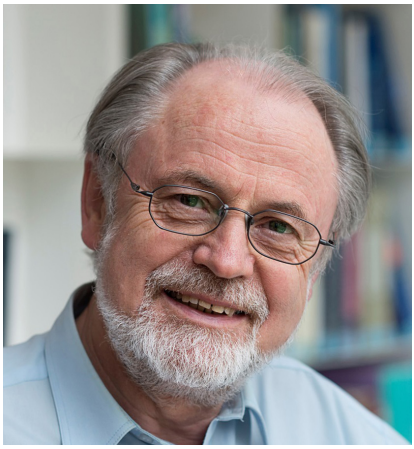
"Novel computational methods for the analysis of protein structures"

Life sciences use computer science for solving some of the main problems related to biomolecular structures: analyzing the known structures and modeling the unknown ones. The problem of predicting spatial structures of proteins from their sequences is far from being solved, but some approaches, especially homology-based modeling, are already exceedingly useful in practice. Most current protein structure prediction methods work in two stages:

1) generating a set of candidate models, i.e. predicted structures;
2) selecting the best model, i.e. the model most similar to the native (real) structure.

This talk is mainly focused on novel computational methods for improving the second stage. More specifically, it focuses on the analysis and evaluation of structural models.

Prof. Jan H.J. Hoeijmakers



A professor of Molecular Genetics Institute of Genetics Erasmus Medical Center Rotterdam The Netherlands.

"The impact of DNA damage, transcription stress and nutrition on aging"

Jan H.J. Hoeijmakers[1-3], [1]Dept. of Genetics, Erasmus MC, Rotterdam, The Netherlands, [2]Cecad Research Center, Cologne, Germany and [3]the Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands

Aging appears remarkably plastic: e.g. suppressing insulin signalling extends lifespan in numerous species. However, virtually all premature aging syndromes link with genome instability. We have generated mouse models which strikingly mimic human DNA repair deficiency syndromes and display wide-spread accelerated aging. For instance, Ercc$^{1\Delta/-}$ mice defective in four repair pathways show multi-morbidity in both proliferative and post-mitotic tissues, limiting lifespan to 4-6 month. Simultaneously, they exhibit an anti-aging 'survival response', which suppresses growth and enhances maintenance, resembling the longevity response induced by dietary restriction (DR) and providing a link with insulin signalling. Interestingly, subjecting these progeroid mutants to actual (30%) DR tripled lifespan, and drastically retarded accelerated aging, e.g. DR animals retained 50% more neurons and maintained full motoric function. The DR response in these mice resembled DR in wild type animals including reduced insulin signaling and reduced DNA damage load, explaining why DNA repair mutants overrespond to DR. Interestingly, Ercc$^{1\Delta/-}$ liver expression profiles showed gradual decline of expression preferentially of long genes, consistent with genome-wide accumulation of stochastic, transcription-blocking lesions, which affect long genes more than short ones. This phenomenon was also discovered in normal aging of post-mitotic tissues. DR largely prevented transcription stress, indicating that DR prolongs genome function. We will present phenotypes of conditional DNA repair models targeting aging to selected organs, and striking parallels with Alzheimer's disease.

Our findings support the link between DNA damage and aging, establish Ercc$^{1\Delta/-}$ mice as powerful model for identifying interventions to promote healthy aging, reveal untapped potential for reducing endogenous damage and transcription stress, provide new venues for understanding the molecular mechanism of DR, explain the aging component of all proteinopathies based on transcription stress and promote a counterintuitive DR-like therapy for human progeroid genome instability syndromes and DR-like interventions for preventing neurodegenerative diseases and ischemia reperfusion damage.

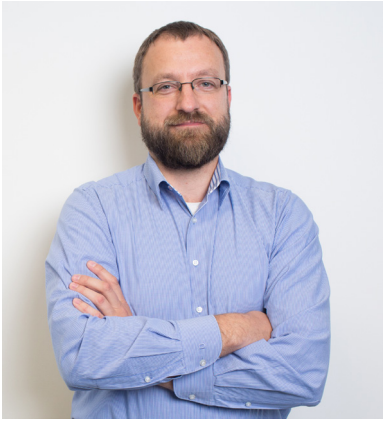
Dr. Naomi Martin

A Lecturer in Biomedical and Medical Science in the School of Allied Health Sciences, De Montfort University, Leicester, UK., and Honorary Lecturer at the University of Leicester, UK.

"Microparticles in Disease"

Microparticles (MP) are biomolecular shuttles and novel biomarkers of inflammation. MP are shed from activated cells and act as vehicles transporting effector molecules and as vectors during intercellular information exchange, influencing the pathophysiology and progression of diseases such as cardiovascular disease, cancer and diabetes. MP are known to be involved in inflammatory conditions and coagulation processes. Our work has shown that MP might be involved in the unusual susceptibility of some ethnic groups to certain diseases, as their effects on endothelial cells and cancer in vitro are differential. We are investigating integrative and alternative therapies to affect the pro-thrombotic and pro-inflammatory nature of these ethnic MP to ameliorate their effects.

Dr. Arūnas Stirkė



A head of Bioelectrochemistry Laboratory, in the Department of Material Sciences and Electrical Engineering, in the Center for Physical Sciences and Technology, Vilnius, Lithuania.

"Electroporation as a tool for selective pasterization"

Bioelectrics is a new area of science and technology where electrical stimuli are applied to biological systems. Typical targets are specific lipid vesicles, cancer cells, bacteria or fungus, or even more complex systems such as tissues or biofilms. The electric stimulus can be induced by pulsed power including high voltage and/or current pulses ranging from subnanoseconds to milliseconds. Depending on pulsed power combination different effects on cells might be obtained. Low voltage and millisecond pulses are well known in life sciences as an electroporation method for DNR, RNR or protein electrotranstection. Pulse generation systems within microsecond pulse range are typically used for electrochemotherapy, electropasterisation and electroextraction. Prospects of pulsed electric field technology application on pretreatment of food stuff for selective pasteurization purpose will be discussed.

Prof. Henrik Semb

A professor of stem cell and developmental biology of the pancreas and Executive Director of the Novo Nordisk Foundation Center For Stem Cell Biology at the Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.

"Pluripotent stem cells: ready for clinical applications in diabetes?"

Type 1 diabetes (T1D) remains one of the main health challenges in the EU and worldwide, with 6 million European citizens affected. Today, T1D accounts for 10% of all health care budgets and is responsible for a high degree of absenteeism and work loss. To bring advanced therapy in type T1D to more patients, a new cost-effective scalable source of pancreatic islets for transplantation is needed. Our objective is to build and implement a new, innovative platform for the production of human pluripotent stem cell (hPSC)-derived advanced therapy medicinal products (ATMPs) for treatment of EU citizens with T1D. We aim to establish a transferable GMP-compliant manufacturing platform based on improved protocols for generation and characterisation of new ATMPs from hPSCs. Based on an already available 1st generation ATMP, we plan the first clinical trial based on hPSC-derived beta-like cells.

KEYNOTE SPEAKERS

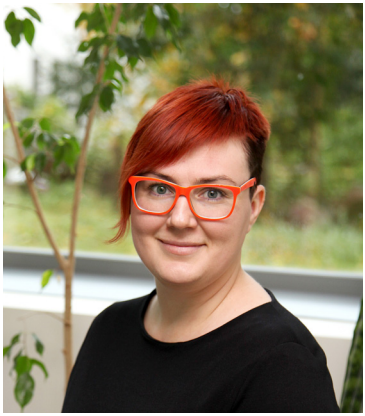
Dr. rer. medic. Christiane Jockwitz

A Post-Doc working in the Connectivity group of the INM-1 and Institute of Anatomy I, Heinrich-Heine-University Dusseldorf, Germany

"Normal aging in population-based imaging cohorts"

Our older adult population is growing, bringing along a tremendous impact on health and economic aspects of the society. Age itself is the highest risk factor for neurodegenerative diseases, such as dementia, which highlights the urgent need for a better understanding of brain aging in relation to cognitive decline. Lifespan trajectories show considerable changes in brain structure and function from early to late adulthood. In contrast, older adults are rather characterized by a high inter-individual variability, with a spectrum ranging from accelerated aging to preserved cognitive abilities until old age. However, to unravel this high variability between older adults and their relation to brain structure and function, large sample sizes are needed. Therefore, modern research investigates and identifies genetic, socioeconomic and psychosocial factors that can partially explain the high inter-individual variability of cognitive performance, brain structure and function in large population-based cohorts consisting of thousands of older adults with the ultimate goal of distinguishing normal from pathological aging. In the current state it becomes clear that age itself might not play the major role in terms of explaining differences in brain structure and function and related cognitive decline between older adults. Instead, other factors come into focus regarding the high inter-individual variability of older adults.

Dr. Inga Griskova-Bulanova

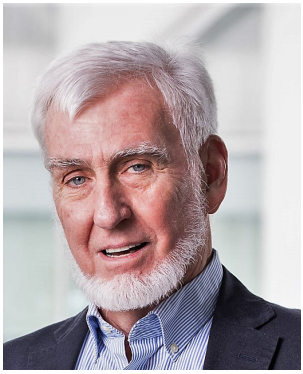


A Chief researcher leading a Brain State Research group at Vilnius University Life Science Center.

"Processing of periodic sounds: benefits and practical application of auditory steady-state responses"

The analysis of brain responses to periodic auditory stimulation with modern neuroimaging methods stands as a valuable tool to explore brain functioning in norm and pathology. One of the most widely used options is the auditory steady-state response (ASSR) approach – the method of evaluation EEG/MEG-based signal in response to periodic auditory stimulation. During the talk, an overview of the important application areas of auditory steady-state responses will be presented with particular focus on two major domains. First, the known task-modulatory effects on ASSRs, as important for neuro adaptive technologies (i.e brain-computer interface, neurofeedback) and for practical application in clinical settings, and achieved by contrasting resting state, distraction from the sound and direct attention to the sound conditions will be reviewed. This will be followed by the presentation of known ASSR changes in neuropsychiatric disorders (i.e. schizophrenia spectrum and affective spectrum disorder) with an emphasis on the relationship between the responses and cognitive functions. The importance and the advancement of the experimental settings for ASSR generation will be discussed.

Prof. John O'Keefe



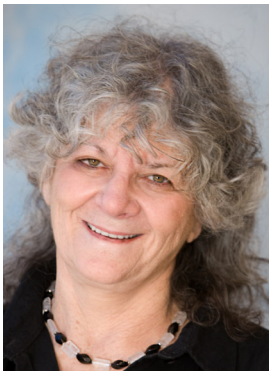
The Nobel Prize in Physiology or Medicine 2014
"for discoveries of cells that constitute
a positioning system in the brain."

"The Hippocampal Cognitive Map Theory, An Update"

The Hippocampal Formation contains different types of spatial cells (place, head-direction, boundary, and grid cells) which together make up a cognitive mapping system. The cognitive map enables an animal to locate itself and objects such as food and water in a familiar environment, and to navigate towards or away from particular locations, for example, those containing food or danger respectively. There are several behavioral testing paradigms for assessing an animal' s knowledge of spatial location and its performance on tests of spatial navigation. Foremost amongst the latter is the Morris Water Maze which, although extremely successful, has several drawbacks for the assessment of behavior and perhaps more importantly is not ideally suited for single unit recording. I will describe a new behavioral testing apparatus, the Honeycomb Maze, which overcomes many of these disadvantages. In addition to describing several spatial factors influencing successful performance on this maze, I will describe the performance of animals with hippocampal damage and discuss preliminary data on the use of the maze to study place cell activity.

In addition to providing inputs for the construction of place representations, the grid cells appear to be good candidates to provide the distance metric for the map. In the second part of my talk, I will review recent evidence from our laboratory in which we distorted the shape of the enclosure. The results suggest that the grid cells are a subset of a more extensive group of spatially periodic EC cells and that they might not be able to provide the metric for the cognitive map in all environments and under all circumstances.

Prof. Ada Yonath



The Nobel Prize in Chemistry 2009
"for studies of the structure and function of the ribosome."

VIDEO LECTURE
"Next generation antibiotics"

About speaker:

Ada Yonath focuses on the translation of the genetic code to proteins by ribosomes, on antibiotics paralyzing this biosynthetic process, on the global problems relating to antibiotic resistance, on the design novel antibiotics and on the origin of life. She graduated from Hebrew University (1964), earned her PhD from Weizmann Institute (1968) and completed postdoctoral studies at Mellon-Institute and MIT, USA. In 1971 she established the first biological-crystallography laboratory in Israel, which was the only lab of this kind in the country for almost a decade. Since then, she has been a faculty member at the Weizmann Institute, were she is also the Director of Kimmelman Center for Biomolecular Structures. In parallel, in 1978 she spent a Sabbatical year in the University of Chicago, and during 1980-2004 she headed the Max-Planck-Research-Unit for Ribosome Structure in Hamburg while collaborating with Max-Planck-Institute for Molecular Genetics in Berlin.

Among others, she is a member of the US-National-Academy-of-Sciences; Israel Academy of Sciences-and-Humanities; German Academy for Sciences (Leopoldina); European Molecular Biology Organization; Pontifical (Vatican) Academy of Sciences; Korean Academy of Sciences and Technology. She holds honorary doctorates from over 20 universities worldwide, in USA, Latin America, Europe and the Far East. Her awards include the Israel Prize; Linus Pauling Gold Medal; Albert Einstein World Award for Excellence; UNESCO-L'Oréal Award for Woman in science; Wolf Prize; the Louisa Gross Horwitz Prize; Erice Peace Prize; Indian Prime-minister medal and the Nobel Prize for Chemistry.

ORAL PRESENTATIONS

"Antifungal Activities of Silver Nanoparticles Obtained by Geobacillus spp. Induced Biosynthesis"

Kotryna Čekuolytė[1], Renata Gudiukaitė[1], Vitalij Novickij[2,3], Audrius Maneikis[4], Eglė Lastauskienė[1]

[1]Vilnius University Life Sciences Center, Institute of Biosciences, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania
[2]Institute of High Magnetic Fields, Vilnius Gediminas Technical University, Naugarduko st. 41, 03227, Vilnius, Lithuania
[3]Department of Electrical Engineering, Vilnius Gediminas Technical University, Naugarduko st. 41, 03227, Vilnius, Lithuania
[4]Faculty of Electronics, Vilnius Gediminas Technical University, Saulėtekio av. 11, LT-10223 Vilnius, Lithuania

Increasing resistance to the antifungal therapy and growing number of yeast caused skin diseases are the main problems that rise the research for the new antifungal compounds. It has long been known about antimicrobial effects of silver, thus nowadays antimicrobial characteristics of silver nanoparticles (AgNPs) are receiving more interest. Commonly used techniques for obtaining silver nanoparticles are physical and chemical synthesis, however, the biological synthesis of this nanomaterial are gaining more interest as cheaper and environmentally friendly alternative. This study reports the Geobacillus spp. strains 18, 25, 95 and 612 induced extracellular biosynthesis of AgNPs. Ag+ reduction and formation of AgNPs in all Geobacillus spp. secretomes were confirmed by Scanning Electron Microscopy (SEM) and UV-Visible (UV-vis). Obtained AgNPs were tested for their antifungal activities against pathogenic yeast (Candida lusitaniae, C. guilliermondii). The antimicrobial activities of the AgNPs were estimated by growth inhibition (100 µg/ml concentration of each AgNPs for 2 days). The synergistic effect of the AgNPs and electroporation was also evaluated (concentration of AgNPs was 5 µg/ml, parameters of electroporation were single 100 µs impulse, 2,5; 5; 7,5; 10; 12,5; 15 kV/cm electric field).

The results show that all analyzed AgNPs have antifungal effect against Candida yeast (the most effective results were received with Geobacillus spp. strain 25 AgNPs). Additionally, the synergistic effect of AgNPs and electroporation was identified. The results of this study confirms that Geobacillus spp. strains 18, 25, 95 and 612 are appropriate tool for formation of AgNPs. Furthermore, the antifungal activities of AgNPs make it possible to use them as an alternative biocontrol agents against pathogenic human yeast.

"A comparative analysis of natural and experimental Plasmodium relictum infection in Eurasian siskins (Carduelis spinus)"

E. Platonova[1], A. Mukhin[2], V. Palinauskas[1]
[1] Nature Research Centre, Institute of Ecology, Lithuania
[2] Biological Station of the Zoological Institute, Rybachy, Russia

Experimental information about development of avian Plasmodium parasites in vertebrate hosts contradicts with that found in nature. Field studies report that intensity of malarial parasites in blood almost never reach 3%, however, experimental studies show that parasitemia during primary infections in some infected birds can reach up to 80%. This could be caused by biased sampling of less active sick birds in field studies, or because of differences in parasite penetration vertebrate host body. Naturally, Plasmodium parasites are transmitted by mosquitoes after inoculation of sporozoites, while during experiments in laboratory, birds are usually inoculated with infected blood from donors.

Our objective was to obtain data about Plasmodium relictum (lineage SGS1) development in both, birds infected with sporozoites and by standard inoculation of infected donor blood. Sub-adult siskins Carduelis spinus were used into three experimental infections. Birds of the first group were infected by inoculation of infected blood with P. relictum. Siskins of the second group were infected by inoculation of sporozoites extracted from salivary glands of infected vectors. Birds in third group got infection by natural bite of infected mosquitoes.

Our results show that P. relictum (lineage SGS1) infects all inoculated birds with infected blood. Birds infected by sporozoites either via experimental inoculation of sporozoites or by natural bite exhibit partial susceptibility. The smallest amount of birds exhibited patent parasitemia from the group, which were experimentally inoculated by sporozoites. The profile of infection development was much more diverse in birds infected with sporozoites, however, we showed that in some individuals that were bitten by mosquitoes, maximum of parasitemia reached up to 80 %. This study shows that in birds bitten by infected mosquitoes parasitemia can increase as high as in birds experimentally inoculated with infected blood. These results are important for further experimental studies on avian malaria parasites and for better understanding host-parasite interactions.

"Hydration and swelling of amorphous cross-linked starch microspheres studied using Raman Spectroscopy"

Jekaterina Borzova[1], Gediminas Niaura[1]
[1]Vilnius University Life Sciences Center, Institute of Biosciences, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania

Amorphous cross-linked starch microspheres (DSM) is polymerized maltodextrin with additional crosslinks that form a porous starch network. In addition to an active substance it becomes an innovative delivery system. Inside the body it gradually breaks down releasing encapsulated substance with the end product of glucose making DSM safe drug delivery system. In 2016 Vitaly Kocherbitov and his team explored thermodynamic features of DSM during the gradual hydration using DSC, Sorption calorimetry, SEM, Optical microscopy, gravimetric swelling studies, rheology, and SAXS. They established water quantity limits when DSM undergoes phase transitions (glass transition at 18.9 wt% of water at room temperature), swelling capacity etc. However, the hydration mechanism at molecular level is still not fully understood. In this study, Raman spectroscopy was used as an ideal probe method for that purpose. C-O, C-C, C-H2, C-OH region occurring at 975-1195 cm^{-1} of mixed stretching and bending vibrations and mixed symmetry was chosen for the detailed study. Analysis of stretching C-O mode wavenumber average dependency on water content in DSM-water system showed the shift to lower wavenumber values right after the glass transition (20.8 wt% of water). This shift shows the C-O stretching vibration bond strength decrement due to the formation of hydrogen bonding. Another changes were observed in bending C-OH vibration range during the hydration; namely, the shift to higher wavenumbers and an increase of Gaussian/Lorentzian peak area value. These changes were because DSM became flexible from 18.9 wt% (glass transition) and the level of freedom increased inside the molecule thus C-OH bending vibration increased. Finally, the shift to lower wavenumbers of stretching C-C vibrational band and decrease of Gaussian/Lorentzian peak intensity showed the decrease of C-C bonding strength due to the tension inside the molecule during the hydration due to the expansion of the microsphere. In conclusion, it was demonstrated that the glass transition (18.9 wt% of water) made the structural changes possible: molecule expansion, side chain group inside the microsphere freedom of motion, water penetration inside the microsphere.

"The Effect of Stearoyl-CoA-Desaturase 1 Inhibition on Pancreatic Cancer Cells in vitro"

Hackney AB[1], Isherwood, J[1], Chung WY[1], Dennison AR[1], Martin N[1]
[1] De Montfort University, Leicester, United Kingdom

Pancreatic cancer (PC) is an aggressive cancer with poor prognosis and high mortality; it is the third biggest cancer killer in the EU. PC is characterised by lack of symptoms and Gemcitabine chemotherapy relieves symptoms but does not improve disease prognosis. There is an urgent need for advancements in PC diagnosis and treatment. Stearoyl-CoA-Desaturase 1 (SCD1) is a highly regulated enzyme responsible for desaturating fatty acids, converting Stearic acid to Oleic acid. The expression of SCD1 is upregulated in PC, indicating that it may represent a metabolic bottleneck for cancer cell metabolism and contribute to the growth and spread of PC. A PC cell line was incubated in vitro with SCD1 inhibitor (SCDi) or a vehicle control (VC) for 48 or 120hrs, cells were then counted and assayed for viability using flow cytometry. The viability of cells treated with SCDi alone (142nM) or Gemcitabine alone (Gem) (13nM) or both drugs in combination (SCDi+Gem) was assessed by MTT assay both immediately after incubation and after 48 hours recovery. Cells were also prepared and imaged SEM. No significant induction of apoptosis or necrosis was observed by either drug alone at therapeutic doses. Cellular growth after recovery (to mimic periods between chemotherapy) was significantly decreased after treatment with SCDi alone compared to SCDi+Gem (38% SCDi +/-2.19 vs 153% SCDi+Gem +/-9.45, $P<0.05$). SEM imaging revealed that SCDi treated PC cells have a flat phenotype compared to VC cells, and those treated with SCDi+Gem are less numerous, larger (20um VC vs 64um SCDi+Gem) and have a flat phenotype with many multinucleate cells.

These results indicate that SCDi might be a promising chemotherapy supplement for PC patients as it has high potency and a synergistic effect when used in combination with Gemcitabine. Further work is required to elucidate the mechanism of inhibition of PC proliferation induced by SCDi.

"Ethnic Differences in the Role of Microparticles on Endothelial Cell Dysfunction"

Pritchard C[1], Henshaw M[1], Akubueze A[1], Martin N[1]
[1] De Montfort University, Leicester, United Kingdom

Individuals of different ethnic backgrounds are susceptible to certain diseases, but the cellular and molecular reasons for this are unclear or unknown. Individuals of South Asian (SA) or Black African (BA) origin are at greater risk of developing type 2 diabetes, cardiovascular disease or certain cancers than those of British White (BW) origin. Microparticles (MP) are biologically active nanovesicles that are shed systemically from activated, tumour or apoptotic cells. Due to their transport of membrane effector proteins, MP are novel markers of inflammation and may contribute to the pathogenicity and progression of disease. The aim of this study was to assess whether MP obtained from different ethnic groups differentially affect endothelial dysfunction in vitro. MP were isolated from blood taken from healthy volunteers of different ethnic groups (BW n=6, BA n=7, SA n=3). Endothelial cell line EA.hy926 were seeded until near-confluent in 48-well plates, with either high-glucose DMEM (HG-DMEM) or low-glucose DMEM (LG-DMEM). MP were added and incubated for 48 hours. EA.hy926 were stained for apoptosis and necrosis and analysed using flow cytometry. Upon incubation with HG-DMEM, SA-MP induced significantly greater apoptosis with EA.hy926 compared to both BA and BW MP (SA 14.36±1.12% (mean±SEM) vs BA 12.54±0.82% BW 10.32±1.22%, $P<0.05$). Under the same conditions SA-MP also induced significantly more endothelial cell necrosis (SA 4.37±0.14% vs BA 3.64±0.14% BW 3.30±0.32%, $P<0.05$). In low-glucose milieu, SA-MP and BA-MP induced a higher degree of necrosis (SA 4.27±0.39% vs BA 4.05±0.121% BW 3.10±0.30% $P<0.05$), but there was no difference in the effects on apoptosis. Our data suggests that MP induce endothelial dysfunction in vitro, in an ethnically differential manner. The in vivo consequences and mechanisms of the pathophysiological consequences of this and the specific MP interactions here remain to be elucidated.

POSTER PRESENTATIONS

"Intrinsic affinity of N-substituted benzenesulfonamides to carbonic anhydrases"

Vaida Paketurytė[1], Daumantas Matulis[1]
[1] Vilnius University, Life Sciences Center

A series of benzenesulfonamides bearing N-substituents at para position and methyl groups or/and halogen atoms at ortho or meta positions were designed, synthesized, and tested as inhibitors of the twelve catalytically active human carbonic anhydrase (CA) isoforms[1-3]. The development of high affinity and selective inhibitors of CA proteins is a need in target based drug design.

We determined the observed binding affinities by fluorescent thermal shift assay, ITC, and enzymatic activity inhibition assay. However, these observed affinities cannot be used in structure-thermodynamics correlations. Therefore, the intrinsic (pH-independent and buffer-independent) binding affinities were calculated representing the interaction of sulfonamide anion (RSO2NH-) to the Zn(II)-bound water form of CA. Some compounds in this series exhibited selectivity for CA VII and CA XIII (up to 500 fold) while other compounds showed low nanomolar dissociation constants and over 10-fold selectivity for mitochondrial isoform CA VB. Determination of intrinsic affinities allowed the development of compounds as inhibitors of CA with higher affinity and selectivity to particular CA isoforms.

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"Cs^+ and Ba^{2+} as blockers of ion channels of the tonoplast of Nitellopsis obtusa"

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Electrochemical signaling pathways are crucial to many plant physiological functions. On a single cell level, excitation originates from the activity of ion channels, thus ion channel research is invaluable in obtaining knowledge about various physiological processes on a single molecule, single cell, tissue or the whole organism level. A classical research approach employs various ion channel blockers that inhibit ion current flow through ion channels. Characean macroalgae model system is particularly convenient for testing the effect of ion channel blockers. These ancestors of land plants possess giant intermodal cells that can be easily accessed via different microelectrode methods.

Single ion channel research can be carried out using patch clamp technique. A microelectrode is pushed against an area of a desirable membrane, forming a seal of high resistance (>5 GΩ) around it. Thus electric current flowing through ion channels inside the said area can be registered by a microelectrode in near-physiological conditions in real time. Plant cell wall prevents examination of the plasma membrane but vacuolar membrane (tonoplast) can be accessed easily using cytoplasmic droplet technique. Cytoplasmic droplets are made spontaneously when cell sap flows out of a "decapitated" cell into a drop of bath solution. The droplets consist of cytoplasm covered with the tonoplast, containing intact ion transport systems.

Our previously conducted experiments have confirmed that Cs^+ ions block high-conductance channels (presumably K^+ ion channels) in the tonoplast of freshwater Characaen Nitellopsis obtusa. We examined the mechanism of Cs^+ block in greater detail and its correspondence to the known model. The potential-dependent decrease of ion channel conductance was attributed to fast ion channel flickering induced by Cs^+ ions rapidly associating and dissociating from the channel pore. We also tested the action of another known K^+ ion channel blocker Ba^{2+} and confirmed that its effect is dissimilar to that of Cs^+.

The research was partly supported by Research Council of Lithuania (Project No. 09.3.3-LMT-K-712-10-0020).

"The Effect of Epigallocatechin Gallate on Sup35p Aggregation in vitro and in vivo"

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Protein aggregation into amyloid fibrils is related with neurodegenerative diseases, such as Alzheimer's, Parkinson's and prion diseases. Compounds that could inhibit such aggregation and prevent the onset or progression of these diseases can become potential drugs. A polyphenol from green tea known as epigallocatechin-3-gallate (EGCG) was shown to inhibit amyloid fibril formation of different proteins and peptides. Here, we tried to determine whether EGCG can inhibit the formation of amyloid fibrils by yeast protein Sup35p, both in vitro and in Saccharomyces cerevisiae. Thioflavin T fluorescence assay was used to follow amyloid formation of recombinant Sup35p NM domain (Sup35NM) in vitro. Amyloid formation in vivo was observed using 74-D-694 [psi-][PIN+] yeast strain. We found that EGCG clearly slows down the amyloid aggregation of Sup35NM. However, no effect of EGCG on the suppression of Sup35p prionisation in yeast cells has been detected. These findings suggest that EGCG interacts

"Identification of Amino Acids Responsible for Difference in Activity of Geobacillus spp. Lipases"

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Thermophylic Geobacillus bacteria, already described as a great source of enzymes, produce lipolytic enzymes possessing high activity in broad temperature and pH ranges, tolerance to organic solvents and finally – the ability to hydrolyse wide range of substrates, which makes them industrially attractive biocatalysts. Nowadays, enzyme engineering strategies allow us to obtain novel enzymes with better characteristics. However, the main question remains which parts of the protein should be chosen for modification, in order to get these improvements.

Aim: During this study it was aimed to apply site-directed mutagenesis for lipases GD-95 (possessing high activity) and GD-66 (possessing low activity) from two Geobacillus strains –in order to find out whether selected amino acids are responsible for huge difference in enzymatic activity.

Methods: Analysis in silico and site-directed mutagenesis methods (megaprimer and overlapping extension) were applied to incorporate several amino acids from GD-95 lipase to GD-66 and vice versa. Mutated genes were sequenced, cloned into pET-21c(+) vector, expressed in E. coli BL21 (DE3) cells and primary analysis of changed lipolytic activity was evaluated qualitatively based on hydrolysis of tributyrin in growth medium.

Results: Restriction analysis showed that target genes were successfully cloned into vector. In addition, sequencing results confirmed the incorporation of mutations (153, 154 and 247 amino acid positions) in both genes of lipases. Based on tributyrin hydrolysis by E. coli BL21 (DE3) cells carrying the recombinant lipase, expanded lipolytic activity was indicated for mutated GD-66 lipase and decreased activity was identified in the case of GD-95 mutant lipase.

Conclusions: Primary qualitative analysis confirms that amino acids localized at 153, 154 and 247 positions might actually be the main factors responsible for differing lipolytic activity in these enzymes. However, deeper physicochemical and kinetic analysis will be applied in further research for quantitative evaluation of changed activity in the mutated lipases.

"Specificity of the argonaute protein from Archeoglobus fulgidus to the 5'-end of the guide"

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Argonaute proteins (Agos) are widespread in all three domains of life (bacteria, archaea and eukaryotes), and are structurally highly conserved. In eukaryotic organisms, eAgos constitute the functional core of the RNA-silencing machinery, which is critical for regulation of gene expression, silencing of mobile genome elements, and defence against viruses. According to the latest experimental data prokaryotic Agos (pAgos) constitute an additional defence system with high versatility against invading nucleic acids. The structural organization of full-length pAgos, as well as eAgos is bilobal, composed of four domains. The N-terminal and the PIWI/Argonaute/Zwille (PAZ) domains together with the L1 and L2 linker regions constitute the N-terminal lobe, whereas the C-terminal lobe is composed of the MID and the P-element-induced wimpy testis (PIWI) domains, the latter harbouring the catalytic site of cleavage-active Agos.

pAgos are further divided into two major groups termed long and short pAgos that lack the PAZ domain, the N-terminal domain and consequently the L1 linker region. The short Argonaute protein from anarchaeon Archaeoglobus fulgidus (AfAgo) is composed of only the L2 linker region and the MID and PIWI domains and therefore corresponds to the "MID/PIWI" lobe of full-length Agos. Its PIWI domain is inactivated by mutations of active site residues. AfAgo is well crystallographically characterized with solved crystal structures of the apo protein and its complexes with RNA and DNA duplexes providing initial information on the molecular mechanism of RNA interference (RNAi) in eukaryotes. Target recognition by Agos is realized via complementarity between the Ago-bound guide and the target strands (RNA or DNA). The 5'-end of the guide strand is anchored in the evolutionarily conserved pocked of the MID domain. Eukaryotic and prokaryotic Agos usually show a preference for a specific 5'-nucleotide of the guide strand (e.g. humanAgo2 for a 5'-U, while bacterial TtAgo for a 5'-dC). Here we present biochemical and structural studies of AfAgo specificity for the 5'-end of the guide strand.

"Synthesis of 3,4,5-trisubstituted-2,6-difluorobenzenesulfonamides as selective inhibitors of human carbonic anhydrase IX"

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Carbonic anhydrases (CA) are enzymes, which catalyzes reversible reaction of water and carbon dioxide into bicarbonate. There are twelve catalytically active carbonic anhydrase isoforms in human body. Their malfunction or overexpression causes numerous diseases including cancer. CA IX shows limited expression in normal tissues but is significantly up-regulated in a variety of tumors. There are many carbonic anhydrase inhibitor classes, however the most notable one - sulfonamides due to their strong affinity to these enzymes. The Biothermodynamics and drug design department is notable in synthesis of various benzenesulfonamides. One of their best inhibitor's is 3-cyclooctylamino-2,5,6-difluoro-4-[(2-hydroxyethyl)sulfonyl]benzenesulfonamide (VD11-4-2) which showed exceptionally strong binding to CA IX. Although, this substance is a lead compound for various in vitro and in vivo investigations, it's solubility in water and selectivity towards CA IX still can be improved. In this project, we want to show synthesis and selectivity of new CA IX inhibitors, which were obtained by modifying compound VD-11-4-2. After analysis by thermal shift assay method all new substances displayed improved selectivity towards CA IX while their binding affinity decreased only slightly.

"Electric field impact on NF-kB reporter system expression"

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Nuclear factor kappaB (NF-κB) is the key regulator in immune response, infection, inflammation and apoptosis. It is known, NF-κB acts in cellular response caused either by biotic stimuli, such as cytokines, bacterial or viral antigens, either by abiotic stimuli, such as free radicals, heavy metals, ultraviolet irradiation. There is still a lack of sufficient data related to the effects of pulsed electric field on activation of intracellular signals. Exposure to pulsed electric field (PEF) damage cells by means of increased reactive oxygen species (ROS) formation. ROS bind to proteins, lipids, other biomacromolecules and change their properties causing lower survival of cells (West, 20061).

The aim of this study was to determine how activity of one of the inflammatory pathway markers NF-κB depends on exposure to PEF in mammalian cells.. Cells, containing chemically transfected plasmids with NF-κB promoter (plasmid pNF-κB-SEAP) or constitutive CMV promoter (plasmid pCMV-SEAP) as a control (the conditions were selected based on published data 2-3) were treated with rectangular electric pulses of different amplitude and fixed pulse frequency (1 Hz). Pulse duration of 100 μs was used. This study shows that activation of NF-kB signalling pathway depends on ROS formation caused by cells exposure to PEF.

"Synthesis and Anti-Cancer Activity of 1,2,3,4-Tetrahydroquinoline-Based Hydroxamic Acids"

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Cancer is a serious public health problem resulting in high morbidity, mortality, and incogitable losses every year around the world. Overall cancer survival has barely changed over the past decade, as 72 cancer therapies approved in 2002-2014 extended life of patients by only 2.1 more months in comparison to older drugs. Thus, the unquestionable need to develop new more efficient therapies remains. Hydroxamic acids possess various biological activities, such as anti-bacterial, anti-inflammatory, anti-cancer, anti-viral, anti-HCV activity, anti-parasitic, etc. and are excellent ligands for coordination chemistry. More importantly, hydroxamic acids constitute the largest class of HDAC inhibitors, among which compounds such as SAHA, CBHA, Pyroxamide, Oxamflatin and Scriptaid are undergoing clinical trials. In this work, a small library of 1,2,3,4-tetrahydroquinoline-based hydroxamic acids was synthesized employing Suzuki-Miyaura cross-coupling and alkylation reactions followed by subsequent treatment with hydroxylamine hydrochloride. The obtained final compounds were evaluated for their cytotoxicity against K562 and MCF-7 cancer cell lines. The most potent compounds displayed micromolar IC50 values against the cells.

"Synthesis of Enantiopure Heterocyclic Amino Acids Possessing 2-Amino-1,3-Thiazole Structural Unit"

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Over the past decade, peptide drugs have been receiving increasing attention from scientists because they often prove to be a preferred alternative to small molecule drugs. In order to overcome inherent weaknesses of peptide drugs and seek increased therapeutic activity, synthetic amino acids are used in peptide drug discovery. Conjugation of chiral cyclic amino acids with heteroaromatic carboxylic acids can provide an opportunity for developing novel, conformationally constrained analogues of biologically important amino acids. L-Proline is one of twenty proteinogenic amino acids and it is an important scaffold for the preparation of various functionalized heterocycles.

In the present work L-proline and related heterocyclic amino acids were used in the preparation of novel methyl 2-amino-1,3-thiazole-5-carboxylate derivatives possessing a chiral N-Boc-protected cycloaminyl substituent on their heteroaromatic ring. A series of methyl 2-amino-1,3-thiazole-5-carboxylates possessing a chiral pyrrolidin-2-yl, piperidin-2-yl or piperidin-3-yl substituent at C-4 of the heteroaromatic ring were designed and synthesized. The structures of the novel heterocyclic compounds were confirmed by 1H and 13C NMR spectroscopy investigations. The obtained chiral amino acids can potentially be used as scaffolds for the synthesis of more complex molecules with biological activity or be used as building blocks for the development of DNA-encoded chemical libraries where peptide bond formation is necessary.

This research was funded by Vipergen ApS corporation (Copenhagen, Denmark) and the European Social Fund under the No 09.3.3.-LMT-K-712-10-0272 "Development of Competences of Scientists, other Researchers and Students through Practical Research Activities"measure.

"Synthesis of Heterocyclic Amino Acids for Application as Building Blocks"

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Nowadays, generation of chemical libraries in combination with high-throughput screening enables fast identification of new lead compounds for drug discovery against various molecular and cellular targets. Ever since 1920s, which marks the beginning of insulin therapy, peptide therapeutics plays an increasingly notable role in contemporary medicine. Up to date, over 60 peptide-based drugs are approved worldwide, and many others enter clinical trials every year. In order to overcome weaknesses of natural amino acids and to seek increased therapeutic activity, nowadays synthetic amino acids are often used in peptide drug discovery.

In the present work synthesis of various heterocyclic amino acids will be presented and an overview of possible modifications on amino and carboxylic acid functional groups will be discussed. These obtained amino acids can be used as building blocks for the synthesis of more complex molecules or peptides with potential biological activity.

"Sol- gel synthesis and thermoanalytical study of Li–Al–Mo–O tartrate gel precursors"

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Sol – gel chemistry is said to have many advantages in ceramics preparation. For its excellent chemical stability and resistance for metal substrates, its applications on metals is widely used in coatings to prevent corrosion [1]. In addition, this method can produce high quality products while shaping materials in a complex geometric structures, as beginning the synthesis in aqueous stage has shown to require lower temperatures, which better control morphology of the ceramics [2]. As this method is also more ecological, cheaper and sufficient than other investigated methods, sol – gel chemistry has been widely researched and discussed in chemistry field [3; 4].

This study investigates Li–Al–Mo–O tartrate gel synthesis by aqueous sol – gel method and researches thermodynamic properties of said gel precursors. The synthesis is performed by using tartaric acid as a ligand to rapidly increase the solubility of the reaction components, which has not been studied extensively before. The chemical properties of Li–Al–Mo–O tartrate gels are analyzed using TG/DSC, XRD.

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"Antibacterial calcium alginate-based bandages"

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A large amount of fluid in the pleural cavity causes respiratory failure. In order to remove the accumulated fluid, in the pleural cavity, a drain is applied, which attaches to the skin with various bandages and is connected to a special container. Pleural liquid drainage take time, therefore, it is important to use antibacterial bandages.

This study aimed to develop calcium alginate-hyaluronic acid flexible bandages covered with gum of Chios mastic for the prevention of bacterial growth. The results showed that the presence of Chios mastic gum layer on the originally prepared bandages improved antibacterial properties. The bandages were effective against Gram-negative (Escherichia coli) and Gram-positive (Staphylococcus aureus and Staphylococcus epidermidis) bacteria.

"Synthesis and investigation of N-substituted aromatic amine for laccase activity assay"

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Laccases are multi-copper oxidases (EC 1.10.3.2), containing T1, T2 and T3 copper sites. Their natural substrates are phenolic and aromatic primary and secondary amine compounds. A lot of information is already accumulated throughout the years of their analysis (redox potentials, enzyme sources, reorganization energy, etc.). Laccases transfer one electron and proton from substrate to T1 Cu (oxidation); transfer one electron from T1 to T2/T3 Cu cluster; the T2/T3 cluster reduces one oxygen molecule to two water molecules, by using four electrons and protons. The byproduct of catalysis is water, therefore this enzyme has high potential for industrial application. Currently, the most common compounds used for this enzymes spectrophotometric activity assays are 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid under the trivial name ABTS) and 4-[[2-[(3,5-dime-

thoxy-4-oxocyclohexa-2,5-dien-1-ylidene]methyl]hydrazinyl]methylidene]-2,6-dimethoxycyclohexa-2,5-dien-1-one known as syringaldazine. Activity assays with these substrates give tolerable results, but these compounds have high price and low stability. Moreover, they are not suitable for agar-plate screening tests.

By this project, we present the synthesis and investigation of a new substrate for spectrophotometric laccase activity assay. This compound was synthesized via three reaction steps and using relevantly low price and common reagents such as p-phenylenediamine and water. The latter substrate was tested with commercially available laccase Novozym 51003 from Trametes versicolor. The results in more detail will be presented during the poster session.

"Synthesis of novel 2-substituted-6,7-dihydro-5H-pyrrolo[3,4-b]pyridine-3-carboxylates"

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Nitrogen heterocycles are prevalent in biologically active molecules and are increasingly attractive scaffolds in the development of new pharmaceuticals. Based on data pyridine is the second most commonly used nitrogen heterocycle among all U.S. FDA approved pharmaceuticals. Pyridine structure allows the molecule to interact with different groups of pharmacological receptors, thus various polyfunctionally substitutedand condensed pyridines show different pharmacological activities such as anti-inflammatory, antituberculous, anticancer, antidiabetic ones. Pyrroles are well known examples of heterocyclic compounds associated with diverse biological activities such as COX-1/COX-2 inhibitors and cytotoxic activity against a variety of marine and human tumour models. Due to the important role of pyridine derivatives in organisms, novel functionalized pyridines and their fused polycyclic ring systems, with important heterocycles such as pyrrole are continuously being sought in order to develop new biologically active agents.

In the present work the synthesis of novel bicyclic polysubstituted pyridine

derivatives was accomplished starting from commercially available N-Boc-3-pyrrolidinone which react with N,N-dimethylformamide dimethyl acetal (DMF-DMA) to give enaminone. Subsequently, the obtained enaminone was reacted with 1,3-dicarbonyls in the presence of ammonium acetate giving rise to desired 2-substituted-6,7-dihydro-5H-pyrrolo[3,4-b]pyridine-3-carboxylates. Synthesis was carried out in refluxing acetic acid or 2-propanol in the presence of catalytic amount CeCl3*7H2O-NaI at reflux temperature. The structures of the novel heterocyclic compounds were confirmed by 1H, 13C NMR, IR, MS, HRMS spectroscopy. The obtained compounds can potentially be used as scaffolds for the synthesis of more complex molecules with biological activity.

"Synthesis of novel 2H-pyrazolo[4,3-c]pyridines with anti-mitotic activity"

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Pyrazoles and their derivatives represent a class of important nitrogen-containing heterocyclic compounds that are covering a broad range of synthetic as well as natural products that display innumerable chemical, biological, agrochemical, and pharmacological properties. In a series of recent publications, we have demonstrated that pyrazole-4-carbaldehydes, carrying an alkynyl function adjacent to the formyl moiety, are valuable starting materials for the construction of condensed pyrazole systems.

The aim of this study was to synthesize variously substituted 2H-pyrazolo[4,3-c]pyridines by employing A Sonogashira-type cross-coupling reaction to

yield 3-alkynyl-1H-pyrazole-4-carbaldehydes, ethanones and propanones from the corresponding 1H-pyrazol-3-yl trifluoromethanesulfonates. Subsequent treatment of the coupling products with dry ammonia[1] under the elevated temperature and pressure afforded a versatile library of 2H-pyrazolo[4,3-c]pyridines with various substituents and 2-, 4- and 6- positions. Newly prepared 2H-pyrazolo[4,3-c]pyridines were evaluated for their cytotoxicity against K-562 and MCF-7 cancer cell lines. The most potent compounds displayed low micromolar GI50 values in both cell lines. The active compounds induced dose-dependent cell-cycle arrest in mitosis, as shown by flow cytometric analysis of DNA content and phosphorylation of histone H3 at serine-10. Moreover, biochemical assays revealed increased activities of caspases-3/7 in treated cells, specific fragmentation of PARP-1, and phosphorylation of Bcl-2, collectively confirming apoptosis as the mechanism of cell death. The mechanism of cellular action of these compounds, however, still remains unclear.

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"Urinary miRNA analysis in castration-resistant prostate cancer patients"

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Prostate cancer (PCa) is one of the leading malignancies in men and overall survival for early stages is comparably high. However, recurrent patients who are treated with androgen-deprivation therapy develop resistance and eventually progress into castration-resistant prostate cancer (CRPC). One of the approved CRPC patient management strategies is the usage of abiraterone acetate (AA), since the disease is known to have, at least in part, still active androgen signalling pathway. Recently it has been shown that microRNAs (miRNAs) play an important role in the disease occurrence and progression. As miRNAs can be detected in urine, they can be used as non-invasive predictive and prognostic biomarkers. The main goal of our study was to analyse the abundance of selected miRNAs in urine of CRPC patients and their significance in response to AA treatment prediction. Four miRNAs, namely, miR-148a, -365, -375, and -429, were analysed in 51 serial urine specimens collected from 22 CRPC patients. According to response to AA treatment without progression the patients were divided into long (>8 months; LR; N=5), medium (3-8 months; MR; N=13) and short (<3 months; SR; N=4) response groups. The abundance of extracted mature miRNAs was measured by means of quantitative reverse transcription PCR.

Comparative analysis showed higher level of urinary miR-148a in SR and MR groups vs LR suggesting possible miRNA involvement in response to AA. For further analysis, the results were compared with other urine specimens from 215 cases with localised PCa and 85 controls, analysed in our previous study (Stuopelyte et al., 2016). Detected level of urinary miR-429 in CRPC vs localised PCa and control groups was higher, while levels of miR-148a, -365 and -375 were lower in CRPC patients ($P<0.05$). Urinary miRNAs are promising as non-invasive predictive biomarkers of CRPC and have a potential to improve the disease management.

"Promoter DNA Methylation Analysis of BMP7, PCDH8 and TFAP2B Genes in Clear Cell Renal Carcinoma"

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Renal cell carcinoma (RCC) is the most lethal neoplastic disease of the urinary system with the clear cell RCC (ccRCC) as predominant subtype. Lithuania occupies the second position by kidney cancer incidence rates in Europe and has the highest mortality rate in the world. The main reason of such mortality is lack of tumor markers for early clinical diagnosis. Epigenetic alterations, such as DNA methylation are observed early during cancer development, therefore may be the valuable tool for early cancer detection. Despite high mortality rates, no such studies have ever been performed on Lithuanian RCC samples so far. This study focuses on the evaluation of DNA methylation at promoter of the BMP7, PCDH8 and TFAP2B genes in the Lithuanian RCC tissue samples.

For the investigation, DNA extraction from 99 renal cancer tissue samples and 19 non-cancerous cases was performed using standard phenol-chloroform-isoamyl alcohol method. DNA methylation level was evaluated performing DNA modification using sodium bisulfite followed by qualitative methylation-specific PCR.

The hypermethylation of BMP7, PCDH8 and TFAP2B was statistical significantly ($P < 0.05$) more frequent (16.3%, 41.8% and 44.7% respectively) in the cancerous than non-cancerous tissues, and the changes were highly specific (94%-100 %) to RCC. Aberrant BMP7 and PCDH8 methylation was more frequent in male patients ($P = 0.039$ and $P = 0.014$ respectively) as well as in large (> 4.5 cm) tumors ($P = 0.026$ and $P = 0.032$ respectively) of higher differentiation grade ($P = 0.017$ and $P = 0.012$ respectively) and stage (BMP7 only, $P = 0.023$). Meanwhile, TFAP2B was more commonly methylated in tumors with higher differentiation ($P = 0.009$) and Fuhrman ($P = 0.045$) grades.

In conclusion, promoter DNA methylation of the BMP7, PCDH8, and TFAP2B genes is related to renal carcinogenesis and may serve as the potential biomarkers for early RCC detection and prognosis of the disease progression.

"DNA methylation of metallothionein genes as a potential diagnostic biomarker of renal cell carcinoma"

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Introduction. Metallothioneins are low-weight cysteine-rich proteins responsible of metal ion homeostasis, cell proliferation and differentiation. Deregulation of metallothionein genes has been reported in various human tumors but their role in renal cell carcinoma (RCC) has been poorly investigated.

Aim. The aim of this study was to evaluate promoter DNA methylation of MT1E, MT1G and MT1M genes as potential RCC biomarkers.

Materials and methods. In total 108 tumors (RCC), 10 pericancerous (PRT) and 30 noncancerous renal tissues (NRT) were included in the methylation analysis. Extracted genomic DNA was modified by bisulfite treatment and methylation-specific PCR was used to analyze the promoter methylation status of the selected genes. Gene expression was analyzed in 52 RCC and 9 NRT samples by means of quantitative PCR after reverse transcription. Results. Methylation of MT1E was frequent in RCC as compared to NRT ($P = 0.0015$). Methylated MT1E and MT1M gene promoters were associated with larger tumor size, higher differentiation grade and Fuhrmann grade (all $P < 0.0500$). Furthermore, aberrant methylation of MT1G and MT1M was more frequent in tumors with necrotic zones ($P = 0.0116$ and $P = 0.0164$, respectively). However, no associations were observed between promoter methylation status and patients' age, gender, tumor stage and metabolic syndrome-related clinical parameters such as glucose fasting or waist circumference. Gene expression analysis showed that MT1E, MT1G and MT1M were downregulated in tumors as compared to NRT ($P = 0.0004$, $P < 0.0001$ and $P < 0.0001$, respectively). However, only lower MT1E expression levels were associated with the promoter methylation ($P = 0.0082$). The three genes were expressed at higher levels in clear cell RCC than in tumors of other histological subtypes ($P < 0.0001$, $P = 0.0005$ and $P < 0.0001$, respectively).

Conclusion. This study revealed the tumor-specific promoter methylation of

MT1E which was associated with the decreased gene expression. Further investigation is needed to validate the potential clinical value of this metallothionein gene in RCC diagnostics.

"Integrative Proteomic, Bioinformatic and Primary Cell Culture Approach Facilitates the Prediction of Anticancer Drugs"

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest forms of cancer due to the lack of diagnostic tools at the early stage and low efficiency of current chemotherapeutic approaches.

The present study combines proteomic analysis of PDAC surgical specimens and drug testing in patient derived primary cell culture in search for effective PDAC treatment. We performed high-throughput differential proteomic analysis of tissue samples taken during operations of patients with PDAC, chronic pancreatitis and those without these diseases. Differentially expressed PDAC-specific proteins enabled us to identify a set of proteins specific to pancreatic cancer but not pancreatitis patients. By comparing proteomic data to the databases of gene expression perturbation with small molecules we extrapolated a shortlist of chemotherapeutic compounds for evaluation as potential drugs for PDAC treatment: sorafenib, BGJ398, ASP2215, afatinib, 17AAG, ABT737. The efficiency of the drugs was assayed using primary patient-derived PDAC cell cultures. All of the drugs except for ABT737 significantly slowed down growth of the primary cells in most PDAC cell cultures, and the combination of BGJ398 and ASP2215 was distinguished by exceptional efficiency. Most of the drugs also inhibited cell dissemination from spheroids and cell cycle to a various extent.

The results show a promising potential of this integrative pipeline for anticancer drug discovery and evaluation.

"Glioblastoma subtyping based on target-genes expression profile"

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Background. Glioblastoma (GBM) is the most common and aggressive form of primary malignant brain cancer in adults. Glioblastoma has a high mortality rate and uniformly poor prognosis despite intensive treatment. It exhibits both histological and gene expression heterogeneity together with genetic diversity. In order to obtain the most accurate data on the tumor, not only histopathological studies but also molecular markers have been applied, which help to clarify the diagnosis, determine the origin of the tumor and apply more effective therapy. Despite recent advances, there is still a need to move toward clarifying the sets of target genes whose expression could be used as biomarkers and would give more accurate information on diagnosis, best choice of treatment, prediction of course of the disease, recurrence chance and patient survival.

The aim of this study was to identify potential target genes associated with the pathogenesis of glioblastoma, evaluate their suitability for glioblastoma subtyping, based on publication and TCGA database analysis, determine the relation between subtypes and clinical data of the patients.

Materials and methods. In total 61 glioblastoma samples were used for this study. Brain tumour tissue specimens were snap-frozen in liquid nitrogen after dissection and stored until analysis. Tumour RNA was purified from frozen tissue using the mirVana™ Isolation Kit. The expression analysis of 16 target genes and 5 housekeeping genes was performed using reverse transcriptase real-time PCR method with SYBR-Green I dye. Data were analysed and visualized using IBM SPSS Statistics (version 20), R software and GraphPad Prism 7.0.

Results. In total 16 GMB subtype associated genes were selected applying TCGA GBM datasets analysis. Using generated algorithm glioblastoma samples were divided into one of three subtypes (classical, mesenchymal or proneural) with average accuracy of 78% based on selected gene expression profile. Subtype characteristics and correlation with patient clinical data such as age at diagnosis or outcome were in line with previous studies.

"Urinary Prostate-Specific Membrane Antigen (PSMA): a novel biomarker for early prostate cancer detection"

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In recent decades, the high increase of prostate cancer (PCa) incidence has been highly linked with the wide use of serum Prostate-specific antigen (PSA) test for early detection. However, due to low specificity, PSA test shows little benefit in mortality rates and causes overtreatment. Because of limitations of PSA screening, new diagnostic and prognostic biomarkers for prostate cancer and new, more specific tests, based on more than one biomarker, are emerging. One of the most promising emerging PCa biomarkers is Prostate-specific membrane antigen (PSMA), overexpressed in prostate cancer as well as in neovasculature of other solid tumors. PSMA is already used as a biomarker in positron emission tomography (PET) imaging for primary PCa localization and staging. Also, PSMA transcript can be detectable in urinary sediments, which may be useful in creating new non-invasive molecular test to diagnose PCa and predict disease progression.

In this study, we used quantitative PCR (qPCR) to evaluate mRNA expression levels of PSMA in 73 early stage PCa tissue samples and 48 early stage PCa urinary sediment samples, from different cohorts. The urinary samples were obtained before prostatectomy without prostate massage and stored without RNA inhibitors, thus lowering the test cost. Although we could not detect significant difference in gene expression between PCa of pT2 and pT3 stages or significant correlation with clinical features (age, tumor volume, PSA test score, Gleason score, biochemical recurrence, presence of TMPRSS2:ERG gene fusion and other) in PCa tissue samples, we detected statistically significant differences and correlation of PSMA ($p=0.0094$) and disease stage in urinary sediment samples. In conclusion, urinary PSMA could be used as a non-invasive biomarker for early PCa detection and staging.

"Molecular mechanisms of cancer metastasis"

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Metastasis is the leading cause of death in cancer patients. Early diagnosis of the progression of tumour before the formation of metastasis would make cancer treatment more efficient. However, that requires identification of potential molecular biomarkers that are specific to different stages of tumour metastasis. The previous study at VU Institute of Biosciences and National Cancer Institute revealed a large number of miRNA that are potentially related to non-small cells lung cancer metastasis (Stankevicius, 2016, BMC Cancer).

The aim of this study is to further investigate the involvement of miRNAs in metastasis by knocking down genes encoding these miRNAs. We carried out a bioinformatical target analysis and pathway enrichment analysis of miRNAs that have been identified in the study. The list of miRNAs was narrowed down to 19 miRNAs. Three of them - mmu-miR-195a-5p, mmu-miR-207, mmu-miR-500-3p - were selected for further functional analysis. In order to delete genomic sequences encoding these miRNAs, we constructed CRISPR/Cas9 vectors containing the individual sgRNAs specifically targeting selected microRNAs. The CRISPR/Cas9 constructs were transfected into LLC1 cells and expanded accordingly for further analysis - detection and confirmation of deletions generated by CRISPR/Cas9 editing. Functional analysis of generated knock-down cell lines will be carried out to investigate the lung cancer metastasis candidate miRNAs that have been identified by genome-wide and bioinformatical analyses.

"DNA Methylation Biomarkers of Clear Cell Renal Carcinoma"

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Renal cell carcinoma (RCC) affects nearly 300,000 worldwide each year and is responsible for nearly 100,000 deaths annually. Clear cell RCC (ccRCC), the most common form of sporadic RCC, often presents with synchronous metastatic disease that correlates with poor prognosis. Lithuania is in the 2nd place in Europe according to RCC incidence and has the highest RCC mortality rate in the world. Despite of high mortality, neither genetic nor epigenetic studies have ever been performed on Lithuanian RCC samples so far in order to find new valuable biomarkers (BMs) for early cancer diagnosis and/or prognosis.

This study aims to explore the epigenomic profile of ccRCC cases with indolent and aggressive phenotypes and to define the epigenetic changes that may serve as potential BMs for the early diagnosis of RCC and prognosis of disease aggressiveness. Genome-wide DNA methylation profiling was performed using two-color Human DNA Methylation 1×244K Microarrays. Samples (N=22) from indolent, progressive and metastatic ccRCC cases as well as paired non-cancerous tissue samples were analyzed. Selected genes were validated in a larger cohort (N=118) by methylation-sensitive PCR. Statistical analysis was performed using GeneSpring GX v14.9 and STATISTICA8 softwares. In the comparison of cancerous and non-cancerous renal tissue samples, significant methylation differences (fold-change ≥ 1.5; $P \leq 0.05$) were identified in numerous genes even in the initial non-aggressive stage of ccRCC. For the initial validation, 6 protein-coding novel genes, associated with various cellular processes related to the cancer development, were selected. The specificity (94-100%) of these markers for RCC and association with patient clinicopathological characteristics, including tumor size, stage, Furhman and differentiation grade, tumor invasion and necrosis has been confirmed.

In conclusions, DNA methylation-based BMs may be utilized for early diagnosis of RCC and prognosis of disease aggressiveness.

"Prognostic potential of microRNAs expression levels in different grade glioma"

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Background Gliomas are among the most common Central Nervous System tumours distinguished by their progressive development and short survival. One of the most problematic glioma tumours is glioblastoma which is characterised as a highly heterogeneous type of cancer in both histological and molecular manner. In order to maximize the treatment strategy and facilitate accurate glioma diagnosis, it is crucial to understand gliomas epigenetic changes and predict patients' course of the disease after the surgery by its molecular signature. Intensive world-wide studies in epigenetics have shown various non-coding regulatory RNA molecules involved in oncogenesis. Some of the most promising epigenetic regulators, associated with cancer development and prognosis are long non-coding RNAs and small non-coding RNAs like small nucleolar RNAs (snoRNA) or micro RNAs (miRNA). Various miRNAs have both a tumour inhibiting and stimulatory properties in the process of post-transcriptional gene expression regulation. In addition, miRNA expression alterations in various types of cancer play an important role in various signalling pathways associated with tumour invasiveness, malignancy and regrowth. However, an accurate set of miRNAs which could determine diagnostically and prognostically informative molecular signature in gliomas' is still unknown.

Materials and methods Expression levels of 11 miRNAs: miR-34a, miR-93, miR-181b, miR-181d, miR-221, miR-17, miR-143, miR-335, miR-21, miR-193a, miR-148a were investigated in 53 post-surgical, different malignancies glioma tissue samples. Samples were stored in liquid nitrogen (-196°C) until miRNA extraction using mechanical grinding, ultrasonic homogenisation and mirVana isolation kit. Micro RNAs expression levels were detected using RT-qPCR with TaqMan assays.

Results In this study, we established that changes in miR-181d, miR-21 and

miR-148a expression correlates with glioma tumour volume. Also, different miR-21 expression levels significantly correlated with glioma patients' survival time performing Kaplan-Meier survival analysis. In addition, Student's t-test distinguished other potential miRNAs which expression levels could indicate glioma malignancy.

"Functional analysis of miR-376 family miRNAs in murine Lewis lung carcinoma LLC1 cells"

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MicroRNAs are small non-coding RNAs that play key roles in the regulation of cellular pathways associated with cancer development. Deregulation of miR-376 family consisting of miR-376a, miR-376b and miR-376c has been associated with many cancer types but the exact functions of these miRNAs still remains unclear. Previously we demonstrated that the expression of miR-376 family miRNAs is deregulated in murine Lewis lung carcinoma LLC1 cells grown under 3D culture conditions suggesting that miR-376 family could be involved in communication between cancer cells and surrounding microenvironment.

In this study we established modified LLC1 cell sublines carrying monoallelic deletion of miR-376 gene cluster using CRISPR/Cas9 system. RT–qPCR analysis confirmed that genome editing significantly reduced miR-376 family miRNA expression. Furthermore, reduced expression of selected miRNAs correlated with increased LLC1 proliferation and altered cell cycle. Next, in silico analysis revealed that the most significantly altered functional categories were enriched in miR376 family target genes related to cellular adhesion and cancer cell stemness pathways. Finally, we exposed that the expression changes of miR-376 target genes including integrins and BMP signaling molecules inversely correlated with miR-376 family expression in a microenvironment dependent manner.

To conclude, our findings suggest new insights of miR-376 family miRNAs in molecular regulation of lung cancer development.

"Proton pump inhibitors as doxorubicin transport modulators in 2D and 3D cancer cell cultures"

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Increased extracellular acidity of tumor causes basic drugs ionization. Positive charge reduces drug ability to permeate cellular membrane and reach the target site, thus limiting drug efficacy [1]. Proton pump inhibitors (PPIs) are a group of drugs that are used to reduce gastric acid production [2]. Recent studies show that they can reduce extracellular acidity and increase basic drug delivery to cancer cells [3].

The aim of our study was to evaluate the influence of two PPIs (omeprazole and lansoprazole) on doxorubicin (DOX) delivery to monolayer cultured 4T1 murine breast cancer cells and spheroids. Effect of PPIs on cell viability was evaluated by MTT assay. 3D cell cultures were formed using 3D Bioprinting method [4]. DOX penetration into monolayer cultured cancer cells and spheroids was assessed using fluorescence microscopy at pH 6.0 and 7.4.

Results. Among tested PPIs omeprazole showed no effect on the 4T1 cell viability after 24 h. Lansoprazole reduced cell viability (EC50 value after 24 h was 158.3 ± 11 μM) but the cytotoxicity of this compound was 800-fold lower than DOX. Omeprazole and lansoprazole had no effect on the delivery of DOX at pH 7.4 but in acidic conditions both compounds increased the amount of drug in cancer cells and their nucleus. Lansoprazole also increased the penetration of doxorubicin in cancer cell spheroids. However, after longer period (8 h) the effect was no more visible. Conclusion. Omeprazole and especially lansoprazole increased DOX penetration in 2D and 3D cancer cell cultures and are promising transport modulators of basic drugs.

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"Chondrocyte Cultures and Chondrogenic Differentiation of Human Mesenchymal Stem Cells in Chondroitin-Sulphate-Based Three-Dimensional Scaffolds"

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Human articular cartilage has a weak ability to restore lesions leading to the development of progressive degenerative diseases such as osteoarthritis (OA). Unfortunately, currently there is no effective treatment for OA, and methods used to diagnose and predict the residual cartilage regeneration potential of chondrocytes for the further purposeful regulation of targeted chondrogenesis, are far from sufficient. Bone marrow mesenchymal stem cells (BMMSCs) are the most widely used tool to repair damaged cartilage, as these cells have a strong ability to differentiate into chondrogenic lineage. However, to date, no effective chondrogenic differentiation technologies of the BMMSCs have been developed that could effectively restore damaged human cartilage, whereas extracellular matrix is crucial for efficient function of articular cartilage. Therefore, three-dimensional systems are important in cartilage tissue engineering due to close cell-cell and cell-matrix interactions, which mimics natural environment found in vivo.

The aim of this study is to evaluate efficiency of an implantable chondroitin-sulphate hydrogel stimulating chondrogenic differentiation of BMMSCs and chondrocytes using different stimulatory factors. We isolated mesenchymal stem cells and chondrocytes from human bone marrows and cartilage, respectively, and cultured in chondroitin-sulphate hydrogels for chondrogenesis.

Results showed that these scaffolds have stimulated cell growth and chondrogenic differentiation. Chondrogenic differentiation was evaluated by the intensity of production of hyaline cartilage extracellular matrix proteins (proteoglycans, collagen type II) by staining histological sections with Safranin-O and immunohistological labeling with antibodies against collagen type II. Gene expres-

sion analysis showed that during chondrogenic differentiation in hydrogels, cells actively synthesized collagens type I and, most importantly, type II, and early transcription factor of chondrogenesis SOX9. Also, we have evaluated the influence of chondrogenesis stimulating factors – prolopirone and taurine on cell proliferation. Prolopirone and taurine promoted chondrocyte proliferation, but had no effect on proliferation of BMMSCs.

In summary, chondroitin-sulphate-based hydrogels seem suitable for engineering of human cartilage tissue. The nearest future plans include evaluation of prolopirone ant taurine effect on chondrogenesis in chondroitin-sulphate hydrogels.

"Regulation of Mesenchymal Stem Cells and Their Chondrogenic Differentiation Potential via Voltage-Operated Calcium Channels"

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The degradation of cartilage tissue caused by trauma or chronic and progressive degenerative joint diseases (e.g., osteoarthritis) has become a global problem for which no efficient therapy is available nowadays. Chondrocytes have limited ability to regenerate damaged tissue therefore cell-based therapies using mesenchymal stem cells (MSCs) seem promising candidates for cartilage regeneration. During MSCs differentiation, many processes are modulated by a variety of hormones, cytokines or genes. Calcium ions (Ca^{2+}) play a crucial role in many of these processes' regulation. Furthermore, the increase of intracellular Ca^{2+} was shown to improve cell differentiation potential. Ca^{2+} can enter cells through many calcium specific channels, however voltage-operated calcium channels VOCCs have attracted an exclusive interest due to their high susceptibility to mechanical load.

The aim of this study was to evaluate intracellular Ca^{2+} influence in human MSCs, isolated from two different sources – bone marrow (BMMSCs) and menstrual blood (MenSCs) before and during chondrogenic differentiation and com-

pare them to chondrocytes, extracted from human cartilage tissue. MenSCs were selected because of their easy, non-invasive isolation and potential to be used in MSCs therapies. Cells were treated with Ca^{2+} channel regulators - classical VOCC antagonist nifedipine and agonists BayK8644. Cell proliferation capacity was analyzed using cell proliferation kit 8 (CCK-8). Intracellular Ca^{2+} concentration was established using fluorescent dye Cal-520 (flow cytometry). Chondrogenic differentiation was evaluated by Safranin and Collagen II antibody staining as well as SOX9 gene expression analysis (RT_PCR).

Our results demonstrate differences of intracellular Ca^{2+} concentration and its regulation in MenSCs, BMMSCs and chondrocytes. Nifedipine downregulated proliferation capacity in all cell types, whereas agonist BayK8644 stimulated it. Chondrogenic differentiation capacity was similar in MenSCs, BMMSCs and chondrocytes. Nifedipine and BayK8644 stimulated all three cell types chondrogenesis, according to Safranin and Collagen type II staining and SOX9 gene expression.

In conclusion, we demonstrate that Ca^{2+} levels were different in all three cell types - chondrocytes, MenSCs and BMMSCs, however, VOCCs modulators had similar responses on their proliferation and chondrogenic differentiation. Stimulation of chondrogenesis by nifedipine suggests that VOCC inhibition could be beneficial for cartilage regeneration in OA.

"Modified methods of chondrogenic differentiation for human dermal fibroblast-derived induced pluripotent stem cells (hDF-hiPSCs)"

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Human articular cartilage has limited capacity for its self-repair, which leads to serious joint cartilage degradation disease – osteoarthritis (OA). Cell-based cartilage regeneration therapies are proposed as a promising regenerative medicine tool. Due to large self-renewal capacity and no ethical controversies,

induced pluripotent stem cells seems attractive tool for potential of stem cell therapies. So far, several differentiations of hDF-hiPSCs into chondrocytes has been reported, however currently methods via embryoid body (EB) formation, pellet culture (PC) and mesenchymal stem -like cells are not optimal and require further improvement.

For this study, we derived hiPSCs from several human dermal fibroblasts and differentiated into chondrocyte-like cells in the presence of chondrogenic medium supplemented with diverse combination of growth factors. The protocols established here allow efficient and simple production of chondrocyte-like cells. The cells obtained through these protocols were evaluated via histochemical staining with Safranin-O and immunohistochemical Collagen type II staining. The most effective method is direct differentiation of hiPSCs in turn via pellet culture system. Growth factors Wnt3a and Activin A play crucial role in the beginning of differentiation (mesodermal stage) while combination of TGF-ß1, GDF5, bFGF2 and BMP-2 resulted in the most efficient chondrogenic differentiation.

"Effect of 3D PLA scaffolds on new bone formation"

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Introduction. The concept of bone regeneration is described as a process by which a bone defect is filled with a donor bone tissue or a bone substitute. More than 2 million bone transplant procedures are carried out each year, making bone the second most commonly transplanted tissue in the world. A promising method of bone augmentation is use of 3D composite scaffold to promote biological cellular responses and regulating cell adhesion, proliferation, migration and differentiation.

Aim. To evaluate the impact of polylactic acid (PLA) scaffolds' on bone regeneration.

Materials and Methods. 3D scaffolds were composed from transparent PLA filaments. PLA scaffolds were printed using 3D FFF printer. Pore size of each scaffold was 450 µm with porosity of 58 %. The scaffolds were sterilised using ethylene oxide gas. 3D PLA scaffold (study group) and Geistlich Bio – Oss® bone

substitute (positive control) impact on bone regeneration was evaluated by using standardised critical size bone defect on Wistar rats cranial bone in vivo (8 animals in each group). After 8 weeks bone defects were investigated by micro-computed tomography and histological analysis. The license was issued by the Animal Research Ethics Committee, Licence No. G2-40, 2016-03-18. Statistical analysis was performed by SPSS, v. 17 program. The level of significance was set to 0.05. Results. A qualitative histological analysis showed PLA scaffolds' good biodegradability without significant inflammatory reaction. Connective bone tissue and lymphocytes are present in histological samples of PLA filled bone defects. Examination of micro-computed tomography images revealed that newly formed bone in bone defect samples filled with PLA scaffolds was 2,5 (1,04) mm^3, in Geistlich Bio-Oss® filled samples 3,2 (0,45) mm^3. There was no significant difference between groups.

Conclusion. 3D PLA scaffolds could be a promising material for bone regeneration, augmentation and may be used as graft substitutes in reconstructive surgery.

"Regulation of inflammatory responses in synovial mesenchymal stem cell populations of patients with rheumatoid arthritis"

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Rheumatoid arthritis (RA) is characterized as a chronic inflammatory progressive joint disease that affects about 0.5 - 1% of population. In RA, synovium turns from stable and relatively a cellular tissue into hyperplastic invasive tissue rich in immunocompetent cells. There is evidence that inflammatory environment can cause dynamic changes in the properties of synovial mesenchymal stem cells (MSC) and lead to a destructive process, as well as actively contribute to inflammation during RA. Nucleotide-binding oligomerisation domain-like receptor (NLR) containing a PYRIN domain 1 (NLRP1) and NLRP3 inflammasomes

as well as Toll-like receptors (TLR) seem important in the pathogenesis of chronic autoimmune joint diseases such as RA.

Aim of the current study was to characterize inflammatory responses in synovial MSC populations from patients with different articular pathologies.

Methods: Synovial tissue samples were collected from patient knee joints with different damage: early arthritis (EA) (duration <12 months), healthy controls (HC) (after meniscus tear due to trauma), osteoarthritis (OA) and RA, (Lithuanian Ethics Committee permission was obtained). The isolated cells were cultured in monolayer until 90% of confluence and expression of TLR1, TLR2, TLR4, NLRP1 and NLRP3 inflammasomes genes were analysed by qRT-PCR after 24h stimulation with tumour necrosis factor α (TNFα), lipoteichoic acid (LTA) and lipopolysaccharide (LPS). SMSC characterized by flow cytometry and differentiation experiments.

Results: Trilineage differentiation potential towards chondrogenetic, osteogenic and adipogenic lineages confirmed their mesenchymal stem cell characteristics of isolated cells. In addition, flow cytometry analysis showed typical expression of MSC surface markers, including CD44, CD90, CD73, CD105, and no expression of hematopoietic stem cell markers. There were no changes in TLR1 and TLR4 expression in response to stimulation with all inflammatory agents in all patient groups. They decreased expression of NLPR1 while upregulated expression of NLRP3 and TLR2 genes, TNFα being most efficient. The highest upregulation of TLR2 was observed in RA and EA patients. Expression levels of other genes showed high variation between all patients, disrespectfully to diagnosis.

Conclusion: In the present study, we demonstrate that inflammatory stimuli have different effects on the expression of TLRs and inflammasome genes in SMSC. Furthermore, those genes responded differently in patients with arthritis of different etiologies, supporting involvement of SMSC in the development of inflammatory process during RA.

"Modern-Day Air Pollution Effects on Lung Tissue Structure"

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Introduction. Air pollution is now fully acknowledged to be a significant public health problem, responsible for a growing range of health effects. The respiratory system becomes the main target of the harmful effects of air pollutants. In children and adults, both short- and long-term exposure to air pollution can lead to reduced lung function, respiratory infections, asthma, chronic obstructive pulmonary disease (COPD) and lung cancer. Modern societies understand dangers of polluted air and are in search of potential biomarkers that can detect the effects of air pollution on lungs and seek to find an effective way to treat the diseases. We hypothesize that post-translational modifications of structural proteins, e.g. citrullination, may be a potential candidate biomarker. Consequently, we have designed the study in which mice were exposed to diesel exhaust and citrullinated protein levels in mice blood serum were evaluated. Also the ongoing protein modifications and inflammation in post-mortem human lungs were assessed.

Materials and methods. To assess the effects of diesel exhaust an in vivo study was designed. Mice (n=10) were subjected to everyday 2-hour exposure to diesel exhaust for 14 days. Control mice were treated the same way without diesel exhaust. The citrulline levels within blood serum were evaluated by ELISA. Levels of inflammation and citrullination related markers were also investigated in post-mortem human lungs by immunohistochemistry of formalin-fixed and paraffin-embedded tissues.

Results. In vivo study confirms our own data from in vitro and reveals diesel exhaust initiated inflammatory shift. In addition, high levels of homocitrulline and citrulline were observed in exposed mice blood serum. Up-regulated citrulline and lung peptidyl arginine deiminase 4 (PAD4), citrullination associated enzyme, levels were observed in post-mortem human lungs related to parenchymal destruction.

Conclusions. Subacute exposure to diesel exhaust tend to increase the post-translational modifications in mice and air pollution modifies certain structural proteins in human lungs. Such structural changes of proteins may lead a pathways to lost/gain function of affected molecules and also propagate autoimmune processes within the lung. Keywords—Air pollution, citrullination, lungs

"Study on zona pellucida's and whole whole envelope's thickness to oocytes fertilization"

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Objectives. Nowadays, one of the most significant young people health problems is infertility. One of the main reasons of failed oocytes fertilization is listed as too thick zona pellucida. This study focuses on evaluating correlation between zona pellucida and whole oocytes envelope thickness and oocytes fertilization frequency, comparing fertilization frequency among in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) methods. Methods and Materials. This study enrolled 1797 oocytes from 189 women undergoing to the IVF/ICSI treatment. NIS-Elements F software was used in order to measure zona pellucida thickness and whole envelope thickness. 1210 oocytes were fertilized using IVF (117 women), for remaining 587 oocytes (72 women) ICSI was performed. Results. This analysis showed statistically significant difference in zona pellucida thickness among two groups: thickness in IVF group was 10,73 ± 0,58μm and in ICSI group was 10,84 ± 0,63μm (P <0,05). However, there were no significant differences in whole oocytes envelope's thickness between these groups. Study showed that in IVF group, thickness of whole envelope is statistically significant among younger patients (35 years old or younger, 101,45 ± 9,53μm) and older patients (more than 35 years old, 96,35 ± 8,83μm) (P<0,05). Moreover, after IVF zona pellucida in non-fertilized oocytes was thicker than in normal fertilized (2PN): in non-fertilized oocytes zona pellucida thickness was 11,08 ± 0,69μm and in normal fertilized oocytes it was 10,62 ± 0,50μm (P<0,05). Fisher's exact probability test revealed significant differences in 2PN fertilization ratio between patients in IVF (68,4%) and ICSI (76,1%) groups (P<0,05). Conclusion. Our findings showed that oocytes with thicker zona pellucida fails to fertilize more often compared with oocytes with thinner zona pellucida.
Keywords: zona pellucida thickness, infertility, in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), 2PN fertilization.

"3D Cell Culture Engineering by Using Natural, Biologically Compatible Hydrogels and Recombinant Morphogens"

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Introduction: Eph receptors are transmembrane receptor tyrosine kinases (RTK) and interact with ephrin ligands, which are also transmembrane proteins. Unlike other RTK whose ligands are usually secreted, soluble and capable of travelling at different distances, Eph receptors and their ephrin ligands require physical interaction or as it called cell-to-cell interaction. These molecules are associated with various physiological and pathological processes including embryogenesis, tissue formation, neuronal and vascular networks development, cell migration, cancer and other diseases [1; 2; 3]. Although research over the past few years has made it possible to understand structures, interaction and signal pathways, it is still very difficult to replicate most of the processes in living organisms using only in vitro systems.

Aim: To create in vitro 3D cell model by using hydrogels, genetically modified ephrin ligands and cell spheroids, which allows study cell-to-cell interaction and migration mechanisms.

Materials and methods: Engineered recombinant ephrinB2-Fc, ephrinA2-Fc, ephrinB1-Fc, containing extracellular receptor binding domain of ephrins fused with Fc portion of human immunoglobulin G (IgG) were expressed in human 293 fibroblasts using calcium phosphate and Lipofectamin 2000 transfection methods. The calcium phosphate transfection system was optimized using HEPES buffers with different pH values (6,9; 6,95; 7,0) and expression plasmid with green fluorescent protein. The expression system for engineered recombinant eprinB2-Fc has been established in adhesive and suspension cultures of human 293 fibroblasts and Fc fusion proteins have been purified using Mab Select Sure media and immunoaffinity chromatography. The panel of different cell types for expression

of EphA2, B2 and B4 receptors were tested by qPCR. Efficient 3D cell migration and cell-cell interaction model has been created by using a purified recombinant engineered ephrin-Fc proteins, cell spheroids and fibrin hydrogels [4].

Results: The recombinant ephrin-Fc protein induced EphB2 and EphB4 tyrosine kinase activity and stimulated human mesenchymal cell migration in a 3D microtissue system. Conclusions: The expressed recombinant ephrinB2-Fc, ephrinA2-Fc and ephrinB1-Fc proteins effectively activate Eph receptors and induce migration of mesenchymal cells in fibrin 3D hydrogels. Results of this research could be applied in the future studies in regenerative medicine.

"Effects of testosterone on apoptosis and mitophagy in Leber's hereditary optic neuropathy"

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Leber's hereditary optic neuropathy (LHON) is the most common mitochondrial disease, caused by point mutations in mitochondrial DNA (mtDNA). The majority of diagnosed LHON cases are caused by a point mutation at position 11778 in mitochondrial genome. LHON mainly affects young men in their 20s and 30s with usually poor visual prognosis. It remains unexplained why men are more likely to develop the disease and why only retinal ganglion cells are affected.

In this study for the first time a cell model was used to investigate the influence of testosterone on different cell death mechanisms: apoptosis and mitophagy. It was found that cells with m.11778G>A were significantly more susceptible to nucleosome formation and effector caspase activation which serve as hallmarks of apoptotic cell death. Cells with the mutation were found to express higher levels of mitophagic receptors Bnip3 and Bnip3L/Nix in medium with testosterone. Moreover, the cells with the mutation exhibited greater mitochondrial mass, which can be explained by decreased cell survival machinery caused by mutations in the mtDNA. Observed decreased cell survival was supported by the observed increase in apoptotic cell death. Autophagy was analysed after inhibition with Bafilomycin A1 (Baf A1).

The results indicate impairment in autophagy in LHON cells due to observed lower levels of autophagosome marker LC3-II. The observed impaired lower autophagic flux in mutant cells correlated with the increased levels of Bnip3 and Bnip3L/Nix in mutant cells as a result of an insufficient autophagic flux.

"The impact of Wnt signaling for autophagy in chemoresistant colorectal cancer cells HCT116"

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Colorectal cancer is one of the most common malignancies worldwide and the third leading cause of cancer related deaths. Acquired chemoresistance is one of the main causes that limit efficiency of the chemotherapeutic treatment. Wnt signaling is one of the key pathways regulating stemness and cell fate decisions during development. Activation of Wnt signaling is a hallmark of colorectal cancer. It has been shown that Wnt signaling is upregulated in chemoresistant sublines of colorectal cancer cells HCT116. Autophagy is an important process for cell survival, it can promote cell survival after treatment with chemotherapeutic drugs.

The aim of this study was to evaluate the impact of Wnt signaling on autophagy process after 5-fluorouracil and oxaliplatin treatment in chemoresistant and sensitive sublines of HCT116 cells. The amount of autophagic membranes and autophagy flux was estimated by the extent of LC3B protein lipidation which occurs during autophagy. We have demonstrated that Wnt pathway inhibition diminishes the autophagic flux.

"The effects of 5-Fluorouracil and Oxaliplatin on Autophagy in Chemoresistant Colorectal Carcinoma Cells HCT116"

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Intrinsic and acquired chemoresistance limit efficiency of anticancer treatment. Autophagy is one of cellular processes that can have the impact for acquisition and maintenance of chemoresistance. Autophagy has multiple roles in cancer: at early cancer development stage it can suppress tumor initiation and during later stages of cancerogenesis it can help cells to survive stressful conditions.

In this study we sought to reveal effect of 5-fluorouracil (5-FU) and oxaliplatin (OxaPt) on autophagy of chemoresistant sublines of HCT116 cells. We have found that treatment with 5-FU and OxaPt decreases ATG7 and ATG12 protein levels. 5-FU and OxaPt have the opposite effects on autophagosomes formation in HCT116 cells: 5-FU decreases and OxaPt increases the amount of autophagosomes. Autophagic flux depended on the drug being used: 5-FU decreased autophagic flux in all tested cell lines, whereas OxaPt induced changes in autophagic flux depended on concentration and cell line. After silencing of the core autophagy genes, viability of control cells increased but this phenomenon was not observed after the exposure with either 5-FU or OxaPt.

"Characterization of phenotypic sub-populations of triple negative breast cancer cell line MDA-MB-231"

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Triple negative breast cancer (TNBC) is considered as the most aggressive form of breast cancer. In most cases, this type of tumor is highly resistant to chemotherapy [1]. The resistance of TNBC tumors to chemotherapeutic agents is related to the phenotypic heterogeneity of tumor cell populations due to different cell localization in the tumor, their interaction with tumor microenvironment cells, various secreted factors, etc. [2]. Scientists have shown that breast cancer cell lines are composed of phenotypically different populations of cancer cells that differ in their response to anticancer drugs, rate of proliferation, migration, and morphological differences. Identifying the differences between populations and their response to anticancer drugs could help to predict the tumor resistance to chemotherapy and help rationalize the choice of appropriate antineoplastic drugs, paying attention to the nature and relationship of the phenotypic populations of the tumor [3].

The aim of the study was to isolate phenotypically different cell sub-populations (sublines) from the commercial MDA-MB-231 cell line. Isolation was performed by multiple dilutions of the cell suspension and passage to a 96 well microplate. Based on the morphological differences of separate colonies, those cell sublines that were most distinct in appearance and in the density of the colony were selected. Also, sublines have been characterized by the expression of the CD133 receptor (immunofluorescence staining), their susceptibility to anticancer drugs doxorubicin (DOX) and paclitaxel (PTX) (MTT assay), and by ability to migrate (wound healing assay).

Results. The expression of the CD133 receptor was more than 30% higher in the subline E7 compared to the commercial cell line MDA-MB-231. The susceptibility of cell sublines to the tested anticancer drugs was different. DOX most significantly reduced the viability of cell subline F5 (EC50 value after 72 hours was 58.9 ± 7.2 μM, whereas EC50 value for MDA-MB-231 cell line was 140.0 ± 23.2 μM). Subline H2 was the most resistant to DOX (EC50 value after 72 hours was 158.7 ± 33.3 μM). All sublines were from 2 to 4 times more resistant to PTX compared to the commercial line.

The results of the cell migration study showed that majority of isolated sublines possessed higher ability to migrate compared to the parent line MDA-MB-231. The migration rate of sublines B7, F7, G5 and H2 was from 4 to 8 times higher in comparison to MDA-MB-231 line. Conclusion. Sublines isolated from MDA-MB-231 show significant resistance to anticancer drugs DOX and PTX and higher migration ability compared to the commercial cell line.

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"Research of receptor and its ligand interaction on cell surface"

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One of the most important areas in pharmacy, medicine and biotechnology is interacting molecules research, which allow to establish more effective medicines for disease treatment. This involve for cell-cell communication important receptors, cell adhesion molecules, channels ant transporters. These molecules ensure the survival or death of cells, regulate their proliferation and differentiation, and help to spread to other parts of the body. Cell surface molecules expression disorders due to the resulting mutations can cause the signal transmission failure to the cell, the insufficient interaction of molecules and are the common cause of cancer, syndromes or other diseases. Developing drugs for the treatment of diseases, it is important to find out the characteristics of the interaction between receptor and its ligand, how many surface receptors have to be activated for signal transmission in cells, stability or duration of the interaction.

Currently existing technologies enable us to investigate molecules interaction characteristics on artificial surfaces and to obtain sufficiently accurate data. However there are few methods available to test molecules on the cell surface, though total internal reflection fluorescence (TIRF) microscopy is more and more frequently chosen for interaction between receptors and ligands observations with high resolution. One of the advantages of this method is the ability to perform in vivo observations. It is important to investigate the interaction between receptor and ligand in living cells, as the environment and receptor mobility in the membrane have a significant influence on biomolecules interaction. Using the TIRF method it would be possible to observe the changes in the cell after the signal transmission, to measure the duration of the interaction between the receptor and the ligand, to evaluate interaction stability and how many receptor molecules are

interacting with ligand. The data obtained in such experiments can be compared to the studies performed in artificial systems and may be used to develop new drugs.

The aim of this study is to create mammalian cells model and technique for receptor and its ligand interactions research in living cells membrane. The TIRF and Forster resonance energy transfer (FRET) method will be used to perform observations of receptor-ligand interaction.

"Genome Mining for Nonribosomal Peptide Synthetase and Polyketide Synthase Genes in Two Paenibacillus sp. Strains from Krubera-Voronja Cave"

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Paenibacillus sp. 23TSA30-6 and Paenibacillus sp. 28ISP30-2 strains were obtained from one of the deepest caves on Earth – Krubera-Voronja Cave. Oligotrophic environment suggests that microorganisms in this cave might possess novel bioactive compounds. Paenibacillus bacteria are known to produce various bioactive compounds such as nonribosomal peptides (NRPs) or polyketides (PKs) enabling them to compete with other microorganisms in harsh environment. Nonribosomal peptide synthetases (NRPS) and polyketide synthases (PKS) are involved in biosynthesis of NRPs, PKs and their hybrid compounds with antimicrobial, antifungal, antiparasitic or antitumor features.

The aim of this study was to determine genetic potential of Paenibacillus sp. strains 23TSA30-6 and 28ISP30-2 from Krubera-Voronja cave to produce NRPs and PKs using in silico-based methods. Predicted biosynthetic gene clusters (BGC) were further analysed to identify their domain organization.

An investigation of draft genome sequences from Paenibacillus sp. 23TSA30-6 and Paenibacillus sp. 28ISP30-2 revealed that these strains have undiscovered potential in secondary metabolites synthesis. Using AntiSMASH 4.0 web-based software in Paenibacillus sp. 23TSA30-6 genome sequence we predicted 59 BGC clusters - 22 possibly involved in NRPs biosynthesis, 1 in hybrid PKs/NRPs biosynthesis. Paenibacillus sp. 28ISP30-2 is predicted to code 55 BGC clusters, 15 of them possibly belong to NRP. Some of the analysed clusters show

similarity to known BGC and might be involved in biosynthesis of polymyxin, fusaricidin, paenilarvins, tridecaptin, pelgipeptin, calicheamicin or related compounds. However, majority of clusters show no significant similarity to any known BGC suggesting that our strains could be a good source for novel bioactive compounds identification.

This work was supported by the Research Council of Lithuania (grant No. S-MIP-17-21).

"Antibacterial effect of antimicrobial peptides derived from lactic acid bacteria"

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Background and objectives: Nowadays more and more resources are spent to finding effective and environmentally friendly ways to protect food industry and agricultures products from loses caused by molds, yeast and bacteria. Lactic acid bacteria (LAB) and their supernatants are quite promising tools for a control of growth of bacteria and fungi. LAB produce various antimicrobial agents, such as organic and fatty acids, hydrogen peroxide, diacetyl, carbon dioxide, antimicrobial peptides – antifungal compounds and bacteriocins. In our study we used lactic acid bacteria cultures newly isolated from different sources: sour cow and gout milk, pickled vegetables, bread sourdough.

Methods: We applied microbiological analysis to determine the influence of antibacterial activity of LAB supernatants on two sensitive strains Pseudomonas aeruginosa (ATCC 27853) and Staphylococcus epidermidis (ATCC 12228). Antimicrobial activity evaluation has been accomplished with the agar well diffusion method. LAB cultures were grown in MRS broth at 37 °C for 48 hours in aerobic or anaerobic conditions and sensitive strains were grown on LB agar at 37 °C temperature.

Conclusions: The analysis of an influence of aerobic and anaerobic conditions on the growth and antimicrobial activity of LAB isolates in 37 °C temperature showed that a biomass growth depends on bacteria isolate, not on growth's conditions, and the highest antimicrobial activity was determined in the station-

ary growth phase and it correlated with biomass quantity. The evaluation of thermostability of antimicrobial compounds showed that their activity remained from -20 to 100 °C. The genetic analysis of LAB isolates allowed to confirmed that they have bacteriocin-encoding genes in their genomes.

"Degradation of Polyether Polyurethane by Soil Bacteria"

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Polyurethane has a wide range of various applications. Owing to its physical properties and resilience to ageing and degeneration, it is used to manufacture mattresses, thermal insulation, surface coatings and many more products. However, managing waste, that contain polyurethane, is a great challenge. Currently, there is no bio-based polyurethane utilisation methods or technologies, although, some polyurethanes, like polyester type, are susceptible to microbial degradation. Polyether polyurethane is far more resilient to microbial degradation and there is limited amount of information on its biodegradation [1].

The aim of this research is to develop a method for identification of polyether polyurethane degrading microorganisms and to investigate enzymes, which might be involved in polyether polyurethane biodegradation. Microorganisms from Lithuanian soil were isolated by screening their ability to grow on minimum cultivation medium enriched with polyurethane as carbon and nitrogen sources. Eight strains of bacteria were isolated as potential polyurethane degraders and they were identified as Bacillus cereus, Achromobacter denitrifinacs, Pseudomonas putida and Lysinibacillus sp., using phylogenetic analysis of sequenced 16S ribosome gene DNA. In parallel, Lysinibacillus sp. urethanase protein was expressed in E.coli as possible polyurethane degrading enzyme. The obtained results in more detail will be presented during the poster session.

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"The search of collagen-like endospore surface proteins in bacteria of the genus Geobacillus"

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Background: Bacillus anthracis BclA and BclB endospore glycoproteins are one of the well-studied bacterial collagen-like proteins (CLP)s, which attract the attention of researchers because of the potential to apply them in the biomedical field. Thermophilic bacteria of the genus Geobacillus are phylogenetically affiliated with bacilli and they are source of many thermostable biotechnologically relevant proteins.

Aim: In this study, we have set a goal to determine if BclA and BclB homologous proteins are encoded in genomes of geobacilli and to choose a candidate for further research.

Methods: To identify genes of interest, we have searched genomes of geobacilli available at NCBI database. Genomes encoding at least one of the searched genes was further analyzed to determine if they encode homologs of a glycosyltransferase. Further, using primers designed to amplify the gene of BclB homologous protein we have performed a PCR from genomes of 24 strains of Geobacillus and Parageobacillus (laboratory collection).

Results: In silico analysis revealed that geobacilli do not encode BclA-similar proteins. However, proteins containing conserved BclB C-terminal domain were found in genomes of many different Geobacillus strains. Collagen-like sequences composed of 3 – 79 Gly-Xaa-Yaa repeats were identified adjacent to this domain. About a half of the strains besides BclB homolog encodes also a putative glycosyltransferase, homologous to an enzyme which in B. anthracis glycosylates CLPs and supposedly contributes to its thermostability. Finally, we performed PCR analysis to examine which of 24 thermophilic strains encodes BclB homologs. For further analysis, we have chosen the protein encoded by G. lithuanicus N-3, because the highest Gly-Xaa-Yaa repeats number in its sequence.

Conclusion: Thermophilic bacteria of the genus Geobacillus encodes some CLPs, similar to those of B. anthracis. Identified CLPs encoded by these bacteria are supposed to be more thermostable, however their features should be determined experimentally in further studies.

"Development of expression systems for ß-carbonic anhydrase from Bacillus mojavensis"

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Greenhouse effect is a warming process of the earth's surface. This process is indicated as one of the main causes of atmosphere heats up, weather changes and coastal areas flood [1]. The gases which increase greenhouse effect are water vapour, methane, ozone and carbon dioxide [2]. The essential thing is that during the last millennium the emission of carbon dioxide is constantly growing and the level of CO2 are currently around 409 ppm, which expected to reach 550 ppm in the next 30-80 years. Therefore, it is necessary to develop methods to reduce the concentration of CO2 in the atmosphere [3]. One of way is to use the enzyme ß-carbonic anhydrase (ß-CA). This enzyme has a unique feature – is able to catalyze reversibly CO2 hydratation; and the fixed CO2 can be converted into various useful products (acrylates, polycarbonates, stable polymers, etc.) [4, 5].

By this study we were aiming to develop expression systems for bacterial ß-CA from Bacillus mojavensis in Escherichia coli and Saccharomyces cerevisiae. These expression systems are based on different signaling peptides, inducible promoters, resistance systems, etc. For expression in E.coli the plasmid containing target gene was created and fused with a leader sequence of signaling peptide pelB, expecting the transfer of such protein to the periplasmic space and possible further secretion to the extracellular space. For extracellular protein secretion in S.cerevisiae, signaling peptide α factor was fused with the target gene. The obtained results of ß-CA expression systems will be more presented in detail during the poster session.

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"Conversion of lignocellulosic waste into the 2nd generation bioethanol"

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Due to large CO_2 emissions from fossil fuels and increasing oil prices, biofuels have contributed significantly to the renewable energy goals and ambitious greenhouse gas emission reduction targets in Europe [1]. One of the most common biofuels today is bioethanol derived from crops rich in sugar or starch through natural fermentation and is often called 'first generation' (1st generation) bioethanol. However, as the 1st generation feedstock supply is limited, expensive and competes with food resources, 'second generation' (2nd generation) bioethanol from lignocellulosic biomass is seen as an attractive alternative for future large scale bioethanol production [2].

Biological cellulosic ethanol production consists of two stages. The upstream stage involves technologies that convert lignocellulose to fermentable carbohydrates, mainly glucose. Downstream technologies convert carbohydrates to ethanol and extract it from the medium [3]. In this study, we were aiming to optimize the enzymatic lignocellulose hydrolysis stage using the commercial enzyme cocktail, which includes six enzymes, for example cellulases, xylanases, pectinases, etc. Prior to hydrolysis, birch wood chips were pretreated using steam explosion. The hydrolysis of the pretreated biomass was optimized for temperature, pH, enzyme concentration and time. The resulting hydrolysate, containing glucose, was subsequently used as a fermentation medium for Saccharomyces cerevisiae yeast to produce ethanol. The results obtained in this study will be discussed in more detail during the poster session.

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"In silico screening and analysis of bacteriocins in two Paenibacillus sp. strains from Krubera-Voronja Cave"

Rimvydė Čepaitė [1], Nomeda Kuisienė [1], Jolanta Lebedeva [1]
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Bacteriocins are ribosomally synthesized antimicrobial peptides produced by bacteria to inhibit or kill closely related strains. These compounds may be used as food preservatives or become a potential drug candidate by replacing antibiotics in the age of multi-drug resistance. Species of the genus Paenibacillus are known to produce a variety of such antimicrobial compounds. This feature makes these organisms decent candidates for bacteriocin research.

In order to detect potential bacteriocin clusters in draft genome sequences of Paenibacillus sp. strains 28ISP30-2 and 23TSA30-6 from Krubera-Voronja Cave we used a web-based software tool BAGEL3. Gene clusters of interest were classified according to the class and function of encoded bacteriocins and results were further analysed.

Genome mining of strain 28ISP30-2 showed the presence of three bacteriocin gene clusters belonging to sactipeptide, lasso peptide, and putative class I lanthipeptide lacking known core bacteriocin gene. The strain 23TSA30-6 was found to encode four bacteriocins and modifying proteins - two lanthipeptides of class I, lanthipeptide of class II, and lasso peptide. All bacteriocins encoding genes were surrounded by different number of additional genes encoding modification, immunity, and regulatory proteins. It was noticed a clear similarity between the strains: genes encoding lasso peptides were found in both genomes and both were surrounded by the same number of additional genes encoding modification and immunity peptides. Furthermore, genes encoding lanthipeptide modification share strong similarity, while sequences of predicted structural peptides are different.

This analysis revealed that investigated strains have fine potential to produce a variety of antimicrobials signifying that paenibacilli from Krubera-Voronja Cave might be a good source for novel bacteriocin discovery.

This work was supported by the Research Council of Lithuania (grant No. S-MIP-17-21).

"Biocatalytic Synthesis of Indigo Dimethanols and Indigo Dicarboxaldehydes"

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Introduction: Indigo is one of the oldest pigments used in the dyeing industry. With current methods available, we are able to modify the indigo molecule and create a variety of pigments with novel applications. In order to overcome the hazardous effects of chemical indigo modifications, enzymatic synthesis was used to obtain new variants of indigo. Nevertheless, the selection of indigoids with desirable chemical modifications as well as enzymes capable of producing such pigments is still insufficient.

Aim: The aim of this study was to synthesize a set of novel indigoid pigments by using bacterial monooxygenases.

Methods: Metagenomic DNA was extracted from different soil samples and used for the construction of metagenomic libraries. These libraries were screened in Escherichia coli for the ability to convert different indole molecules to corresponding indigoid pigments. Target gene was cloned to expression vectors and the substrate specificity of recombinant enzyme was analyzed by TLC and HPLC/MS.

Results: Using a direct screening of metagenomic libraries, we selected a single clone which was able to convert indole methanols (indole-4-methanol, indole-5-methanol, indole-6-methanol and indole-7-methanol) as well as indole carboxaldehydes (indole-4-carboxaldehyde, indole-5-carboxaldehyde, indole-6-carboxaldehyde and indole-7-carboxaldehyde) to corresponding indigo dimethanols and indigo dicarboxaldehydes. A gene encoding a 495 aa long NAD(P)/FAD-dependent monooxygenase active towards aforementioned indole derivatives was identified and sequenced. It was shown, that whole cells harboring the recombinant monooxygenase were applicable for synthesis of indigoid pigments.

Conclusions: In this work, production of novel indigoid pigments by enzymatic oxidation of indole derivatives is described. A NAD(P)/FAD-dependent monooxygenase, selected from metagenome converts indole methanols and indole carboxaldehydes to corresponding indigoid pigments. To date, production of such pigments was not reported neither by chemical nor enzymatic methods.

"Analysis into the possible biomineralization using Staphylococcus sp. H6 and Arthrobacter sp. G7 strains"

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With daily advances being made in many industrial and scientific fields, huge emphasis is placed on environmentally friendly solutions for different applications. One such solution is biomineralization – a process in which the metabolism of microorganisms is used to create a microenvironment in which precipitation of various inorganic minerals can occur[1]. This process is commonly referred as MICP or MICCP (microbially induced calcium carbonate precipitation). The production of these biominerals invited scientists worldwide for harnessing this capability of microbes for various bioengineering applications[2].The precipitation of carbonates via urea hydrolysis by ureolytic bacteria is the most straightforward and most easily controlled mechanism of MICCP with potential to produce high amounts of carbonates in short period of time[2]. During bacterial metabolism urea is hydrolyzed to produce ammonium and carbonate, which reacts with water to produce ammonium and bicarbonate, after which bicarbonate reacts with calcium ions to produce calcium carbonate. This process can be used improve the structural strength of concrete constructions, reduce soil erosion, remove heavy metals and radionuclides, sequester carbon dioxide and calcium from the environment.

In this work we used two Gram-positive bacteria isolates: Arthrobacter sp. G7 and Staphylococcus sp. H6 and analyzed their potential use for MICCP by both using calcium carbonate precipitation broth and complexometric titration. After growth in calcium carbonate precipitation broth, centrifuged precipitate was analyzed using scanning electron microscopy. In all test E. coli cells were used as control.

The results we received show that both strains, and especially Staphylococcus sp. H6, are perspective tools for use in MICCP.

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"Metagenomic analysis of the microbiota in urban river sediments to evaluate the impact of anthropogenic city pollution"

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Human activities in the city area leads to greater pollution with significant impact on human health and damage to the natural or built environment. Organic and inorganic matter from various sources ends up in nearby rivers and accumulates in the river sediments, which is an active place with high abundance of microorganisms. Anthropogenic pollution is an important factor to shape the microbial communities that eventually could lead into spread of pathogens and ecological functions disable.

This study was conducted to investigate the shift in structure of sediment bacterial communities of river exposed to multiple anthropogenic contaminants for potential of bioindication in urban ecology. Neris river is a suitable model object to investigate the impact of pollution on the aquatic ecosystem, it crosses the capital Vilnius - one of the most urbanized cities in Lithuania. Three different anthropogenic sites were selected and six samples were collected from Neris river sediments: upstream the city, downstream city center and wastewater treatment plant. The microbiome was characterized on the basis of the V3 and V4 hypervariable regions of the 16S rRNA gene by using next generation sequencing platform Illumina MiSeq. The metagenome analysis of Neris river bacteria has revealed several uniquely found and pathogenic genera from Nostocales and Spirochaetales order as candidate bioindicators to monitor river pollution. Furthermore, potentially pathogenic bacterial genera Flavobacterium and Clostridium was domi-

nant in anthropogenic impact sites.

Overall, bacterial communities could provide a useful tool for monitoring and assessing ecological state in freshwater sediments while indicating anthropogenic city pollution.

"Pantoea sp. infecting phage vB_PagS_MED16 - a putative representative of a new genus within the family Siphoviridae"

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We present here the report on the molecular characterization of a broad-temperature range bacteriophage vB_PagS_MED16 (below referred to by its shorter common laboratory name, MED16), active on Pantoea agglomerans. Based on the results of TEM analysis, phage MED16 belongs to the family Siphoviridae and has an isometric head (B1 morphotype) about 64 nm in diameter and a non-contractile flexible tail about 145 nm in length. The host range determination test revealed that out of 20 bacterial strains tested, only Pantoea agglomerans isolate BSL was sensitive to MED16. Plating tests revealed that phage can form clear plaques surrounded by opaque halo zone in the temperature range of 4 to 36°C. The 46.103 bp genome of MED16 has a G+C content of 55.1% and contains 73 probable protein encoding genes and no genes for tRNA. Comparative sequence analysis revealed that 23 out of 73 MED16 ORFs encode unique proteins that have no reliable identity to database entries. Based on homology to biologically defined proteins, 34 ORFs of MED16 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 10 virion proteins, including 8 that were predicted by bioinformatics approaches. Phylogenetic analysis, based on the alignment of the essential structural and functional genes, revealed that phage MED16 is the most closely related to phages infecting bacteria from the different genera of Enterobacteria, but no close phylogenetic relationship between MED16

and phages infecting Pantoea sp. has been observed. In addition, based on the results of comparative genome sequence analysis conducted during this study, bacteriophage MED16 cannot be assigned to any genus currently recognized by ICTV and potentially represents a new one within the family of Siphoviridae.

"Identification of Certain Major Groups of Bacterial Community Composition in Microalgae Unialgal Culture"

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In recent years, algae have received growing attention for their industrial exploitation. Initially, microalgae were used to produce a wide range of metabolites such as proteins, lipids, carbohydrates, carotenoids or vitamins for health purposes, foods and cosmetics. Now, microalgae have an extensive application potential in the renewable energy, waste-water cleaning industries and even in bio pharmaceuticals. It is known, that microalgae and bacteria have subsequent interactions with each other since the early stages of evolution. Various studies identified the presence of the specific algae and bacteria interactions. These interactions are categorised into nutrient exchange, signal transduction and gene transfer. From this perspective, algea/bacteria specific interactions could be mutualistic or even parasitic. Therefore, to understand algae/ bacteria specific interactions, we need to identify a direct profile of each micro-organism. Standardly 16S ribosomal RNA gene sequencing are common amplicon sequencing methods used to identify and compare bacteria. Problems remain in that the sequences in some databases are not accurate. However, a major limitation of 16S rRNA gene sequencing is the high sequence similarity between some bacteria species. In these circumstances, alternative tests may be required for identification of these species, therefore, a new generation analysis tool such as mass spectrometry contributing to rapid, trustful bacteria identification is required.

"A Novel High Molecular Weight Bacteriocin produced by a Thermophilic Bacterium"

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We have revealed that G. stearothermophilus 15 secretes a novel high molecular weight antibacterial protein geobacillin 26, which belongs to the III class of bacteriocins. We successfully purified native bacteriocin and determined its amino acid sequence and structural gene based on MS/MS analysis and genome mining. Geobacillin 26 has no sequence similarities to any known function proteins. This is the first report of high molecular weight bacteriocin produced by thermophilic bacteria. Usually, producers of III class bacteriocins encode proteins responsible for immunity to the bacteriocin. The genomic context of geo26 does not include any genes that might encode proteins related to the latter function. The cell wall lysis assay confirmed that geobacillin 26 is not a cell wall degrading enzyme, as some III class bacteriocins, most probably it is killing cells by non-lytic mode of action. Geobacillin 26 is the first bacteriocin of this class, which has activity against thermophilic bacteria. Moreover, it has narrow antibacterial spectrum against some thermophilic (Para)Geobacillus sp. strains.

We determined the MIC value of geobacillin 26 inhibiting growth of thermophilic bacterium P. genomospecies 1 NUB36187 – 620 nM (16.3 μg/mL). It is thermo-labile bacteriocin, its antibacterial activity is reduced by 50% after incubation at 60˚C and completely lost after incubation at 90°C temperature. This study revealed the function of hypothetical protein encoded in G. stearothermophilus 15. Geobacillin 26 does not contain any conservative amino acid sequences and domain structures characteristic to other bacteriocins. Hypothetical proteins sharing sequence similarity with this novel bacteriocin are encoded in other thermophilic Geobacillus sp., Anoxybacillus sp. or mesophilic Bacillus sp. bacteria. Geobacillin 26 is very interesting subject for research on novel antibacterial proteins, which potentially may have a new mode of action.

Further studies could focus on the characterization of antibacterial activity mechanisms of the bacteriocin, its targets in the cell or receptors. Moreover, geobacillin 26 could be applied as antibacterial agent against other thermophilic bacteria, which are undesirable in some food or biotechnological industry.

"Pulsed Electric Field Effects on the Expression of Cell Wall Components in Yeast Saccharomyces cerevisiae"

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Yeast Saccharomyces cerevisiae is a eukaryotic single-cell microorganism that has long been used in the food industry as well as in the production of heterologous proteins. One of abiotic methods used to facilitate technological processes is pulsed electric field (PEF). It is known that PEF can increase membrane permeability, but the cellular response to PEF effects is poorly investigated. Recent studies have reported that PEF also changes in cell wall structure.

The purpose of this study was to investigate the effect of pulsed electric field on the isogenic yeast Saccharomyces cerevisiae strains and the expression of cell wall components. Early (OD600nm = 2) and late (OD600nm = 7) exponential growth phase yeast cells (WT, MNN4, MNN9) were used in this study. Yeast cells were exposed to single pulse (τ = 300 µs) when electric field strength was 4 kV/cm. The effect of PEF on the expression of yeast wall components was assessed by alcian blue binding. It is known that the amount of bound dye by the cell is proportional to the amount of mannosyl phosphate in the cell wall. Wild-type yeast cells in the early and late exponential growth phases have shown to exhibit similar dye binding levels (3.75 ± 1.92 µg in early; 3.45 ± 2.35 µg in late). When the WT cells were exposed to an electric field of 4 kV/cm, the amount of bound dye in the exponential phase increased significantly. After reaching the early exponential phase after PEF exposure, cells have bound 8.03 ± 2.73 µg of dye and 13.83 ± 1.01 µg in late. Exposure to PEF increased mannosyl phosphate content of cell wall in both early (2.2 times more) and late (3.8 times more) exponential growth phases, whereas in MNN4 and MNN9 cells it remained unchanged.

We conclude that phosphorylation of the cell wall mannan is associated with a cellular response to stress induced by the pulsed electric field. 6 hours after exposure to PEF, the amount of mannosyl phosphate in cell walls increased 3.8 times compared to control cells.

"Bacteriophage vB_PagS_AAS23: a new Pantoea spp. infecting representative within the family Siphoviridae"

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One of the most predominant bacteria in a variety of phyllospheres are the members of the genera Erwinia and their close relatives - Pantoea. These genera comprise both pathogenic and beneficial species of bacteria and more than a half of almost eighty Erwinia phages described to date have been also found to infect certain strains of Pantoea. Moreover, despite the fact that siphoviruses are the most common group of phages in nature, only several Erwinia and Pantoea-infecting siphophages have been characterized to date.

In this study, we present a characterization of a novel low-temperature Pantoea phage vB_PagS_AAS23 (AAS23) that has been isolated in Lithuania from Thicket shadbush berries using Pantoea agglomerans strain as the host for phage propagation. Based on TEM results, phage AAS23 belongs to the family Siphoviridae and has an isometric head about 66.7±3.0 nm in diameter and a non-contractile flexible tail about 146.3±8.9 nm in length. The host range determination test revealed that out of 20 bacterial strains tested, only Pantoea agglomerans isolate AUR was sensitive to AAS23. Plating tests revealed that phage can form clear plaques surrounded by opaque halo zone in the temperature range of 4 to 32°C. The 51.170 bp genome of AAS23 has a G+C content of 47.6% and contains 92 probable protein encoding genes and no genes for tRNA. Comparative sequence analysis revealed that 27 out of 92 AAS23 ORFs encode unique proteins that have no reliable identity to database entries. Of the remaining 65 proteins, based on search of protein homologs in databases, 37 ORFs of AAS23 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 14 virion proteins, including 12 that were predicted by bioinformatics approaches.

Results of this study not only extend our knowledge about Pantoea infecting

viruses but also imply that aforementioned low-temperature range phage potentially could be used as phage-based biocontrol agent to regulate plant, animal or human pathogens.

"Endotoxin removal from biological solutions"

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Endotoxins are one of the major gram-negative bacteria toxins. Endotoxins can cause septic shock, which affects low blood pressure and multiple-organ dysfunction. Since endotoxins cause toxic effects for humans, it is necessary to remove them to use the product for therapeutic purposes. Despite acquired knowledge about LPS, their removal from biological substances during the production of pharmaceuticals remained one of the major problems. Endotoxins are not removed during normal bacterial removal processes. Affinity chromatography is most often used to remove endotoxins from pharmaceuticals. Immobilized poly-L-lysine Sepharose, DEAE Sepharose or immobilized antimicrobial peptides (AMP), usually polymyxin B, are common resins for the affinity chromatography. Due to the tightened requirements for pharmaceutical products, there is a need for new, more effective endotoxin elimination methods.

One of the possible new methods is the use of antimicrobial peptides for endotoxin removal. During the last decades, a number of studies have been carried out to develop a new technique that effectively eliminates endotoxins. One of the innovations is the magnetic separation method using paramagnetic particles with immobilized natural or synthetic ligands. Some products on the market use synthetic and natural cationic lipopeptides and paramagnetic particle conjugates for the removal of bacteria and endotoxins from solutions.

In this study, different classes of amphiphilic cationic peptides and their conjugates with paramagnetic beads will be investigated.

"The screening for antibiotic resistance genes in Stenotrophomonas maltophilia and Chryseobacterium spp. of soil origin"

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Today one of the biggest threats to global health is increasing resistance to antibiotics. The use of antibiotics in medicine and farming has grown drastically during the last 70 years, causing the bacteria to become more and more resistant to antibiotics. The accumulation of resistance genes leads to multi drug resistant (MDR) bacteria, which often cause lethal infections, as there are no known drugs to treat these diseases. Identifying the resistance genes and exploring resistance mechanisms is crucial in development of treatment strategies against resistant bacteria.

While being in use for less than a century by humans, antibiotic resistance is ancient phenomena dating back to hundreds of millions of years of resistance gene evolution and distribution. The genes naturally possessed by environmental microorganisms can be transferred between bacteria but it is not yet clear how resistance genes travel: from natural environment to clinical settings or vice versa. Such transactions are thought to happen through opportunistic pathogens – bacteria usually found in natural environment, like soil, water, anthropologic structures, but in cases of immunodeficiency such bacteria can cause severe infections. In order to determine the paths of antibiotic resistance genes it is necessary to compare genes and their mechanisms of action in natural and clinical environments.

In this work we were screening for antibiotic resistance genes using functional gene libraries from opportunistic pathogens Stenotrophomonas maltophilia and Chryseobacterium spp. of soil origin. Genes conferring resistance to aminoglycosides, beta-lactams and tetracycline were found. Aminoglycoside resistance was caused by two different aminoglycoside phosphotransferases from S. maltophilia. Chryseobacterium spp. bacteria were found to have a tetracycline MFS transporter and two different metallo beta-lactamases. Only one of the aminoglycoside phosphotransferases APH(3') had an annotated homologue, while other genes could be considered novel versions of antibiotic resistance gene families.

"Application of E. coli auxotrophic host and synthetic nucleosides for a selection of hydrolases from metagenomic libraries"

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Novel powerful techniques for the functional metagenome screening remains an interesting and promising option, since it offers a possibility of discovering enzymes with unique properties and distinctive scaffolds applicable for further the evolution in vitro.The goal of this work were to develop a high-throughput methods for the selection of the esterases and amidohydrolases from metagenomes. The selection of hydrolases based on the uridine auxotrophy of Escherichia coli strain DH10B ΔpyrFEC and the acylated derivatives of uridine or amidated deoxycytidine. For the selection of estrases 2',3',5'-O-acetyluridine (TAU) or 2',3',5'-O-hexanoyluridine (THU) was used as the sole source of uridine supporting growth only of those recombinants, which can complement the uracil auxotrophy of the E. coli DH10B ΔpyrFEC::Km strain by hydrolysing TAU or THU to uridine[1]. For the selection of amidohydrolase, N4-benzoyl-2'-deoxycytidine was used as the sole source of uridine.

The advantages of the proposed selection system:i) it is a HTS method that allows for a rapid (1–4 days) processing of large (meta)genome libraries (more than million clones on a single agarplate) with low number of false positives,ii) it permits the selection of hydrolases belonging to different protein families, iii) it is flexible since, by using different substrates, it allows the identification of enzymes with the desired properties.

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"Are insects nutritious? Comparison of insect/ meat nutritional values"

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In the face of global exponential human population growth and climate change it is necessary to search for alternative protein rich and environmentally friendly food sources. Entomophagy is insect consumption for food. It can be the way to partly increase human population carrying capacity and do lower ecological impact to the environment. Production of insects proteins is more cost-effective and energy-efficient than common livestock farming or aquaculture. Insects are poikilothermic (cold blood) organisms and comparing to common livestock, homeothermic (warm blood) organisms, have more effective feed mass assimilation and convertion to live biomass. It means that to growth a mass unit of insect requires far more less feed to use comparing to, for example, the pork or chicken. During the last few years interest of edible insect farming is growing very rapidly and very first attempts to cultivate insects for food were made in the Nature Research Centre, Vilnius, Lithuania. However, still the knowledge in this field is based on insect farmers' experience mainly, but not on scientific studies. Can insect products compete or even surpass common livestock by their energetic and nutritional values? We address this question and present comparative data in the present report.

"Interaction between top predator and mesopredator estimated by diet analysis and field experiment"

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Large predator may kill and consume smaller predator and this form of

predation termed as superpredation. The White-tailed Eagle Haliaeetus albicilla is the largest avian predator in temperate and boreal latitudes of Europe recently returned to breed all over its range after DDT caused decline. The main prey are fishes and waterfowl, but recently observed predation on smaller raptors, mainly Common Buzzard Buteo buteo and its nestlings.

Study aim was to estimate interactions between these predators. First, analysing prey material collected during White-tailed Eagle nestling ringing we report about the White-tailed Eagle predation on raptors including Common Buzzard. Second, we performed field experiment near nests of Common Buzzard to test how it respond to appearance of powerful predator dummy and playback calls during breeding period in area inhabited by White-tailed Eagle and in area where eagle not breed yet. The raptors consisted 1.8% out of all prey items identified (n = 1705), 29 items out of 31 raptor individuals were nestlings of Common Buzzard. During field experiment, Common Buzzards responded with alarm calls and attacks/mobbing to White-tailed Eagle, but brood defence behaviour was observed only during half of trials. The individuality of Common Buzzard pair best explained brood defence behaviour. Anti-predator behaviour increased through breeding season and reached peak in late breeding stage, which agrees parental investment hypothesis. Common Buzzard response to predator, however, was similar in area inhabited and not inhabited by eagle.

In conclusion, results indicate that top predator White-tailed Eagle irregularly prey on broods of locally most abundant raptor Common Buzzard, but not on adult individuals, and this sources of prey could be easy to access because of low, breeding season-dependent brood defence behaviour of mesopredator parents.

"Analysis of Silurian Graptolite Extinction Pattern in Four Lithuanian Core Sections Using Optimal Linear Estimation Method"

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Introduction: Graptolites where colonial animals, which lived in Paleozoic era, and were abundant in oceans thus forming a major part of the zooplankton of Ordovician and Silurian periods. Their patterns of fossil appearance are widely

used for chronostratigraphic correlation and for dividing higher chronozones into subdivisions: era -> period -> epoch -> stage ~> graptolithe biochronozone. Therefore, it is very important to know when did species appeared and became extinct, however exact time is always unknown, but it should be close before first appearance datums (FAD) or close after last appearance datums (LAD). There are various methods of inferring the time of extinction. In this study we used Optimal Linear Estimation (OLE) method to estimate local extinctions. In addition to that, by comparing sequences of LADs and OLE predictions of different graptolite species, we can guess if species became extinct stepwise, or their pattern of extinction was more sudden, i. e. mass extinction like (sudden extinction of a group of species). Inferring times of extinctions can help to correlate them with main biogeologic and isotope excursion events of the Silurian period.

Aim: The aim of our research is to find out if mid-Silurian graptolites from Lithuanian stratigraphical section exhibit stepwise or sudden and synchronous pattern of extinction.

Materials and methods: Data we used contains depths of various graptolite species records from four cores of boreholes from Lithuania (Kurtuvėnai-161, Šiūpyliai-69, Vilkaviškis-131, Viduklė-61), reaching middle Silurian (Homerian stage) intervals (ca. 430-427 m.y. ago) at various depths in different boreholes, from ca. 1.35-1.25 to ca. 1.1-0.9 kilometers, containing from 10 to 25 different taxa. For estimation of final extinctions of species we used Optimal Linear Estimation (OLE) method which uses k most recent sighting times (Roberts & Solow, 2003). Here we used it for depths of microfossil records. We used all records which had >2 occurrences for every species and for every core interval separately.

Results: In Kurtuvėnai-161 core section we applied OLE for nine graptolite spiecies: Mg. flemingii (LAD depth - 1336.1, OLE depth - 1332.9), T. testis (1333.6, 1327.8), P. dubius pseudodubius (1312.0, 1308.3), P. lodenicensis (1314.0, 1308.0), P. dubius parvus (1319.0, 1314.1), P. dubius frequens (1305.5, 1303.5), P. virbalensis (1303.8, 1303.3), C. praedeubeli (1304.8, 1304.2), C. deubeli (1297.8, 1295.8). Obtained estimations are sparse and suggest a pattern of stepwise extinction. Estimation of final extinction of graptolites in Šiūpyliai-69, Vilkaviškis-131 and Viduklė-61 core sections has shown similar pattern as in Kurtuvėnai-161. In addition to that, pairwise comparisons of order of species extinctions were performed using Spearman's rho test. Mean of LADs rho's is 0.71 and mean of OLEs rho's is 0.66. Conclusion: Optimal linear estimation analysis suggests that graptolite species in four Lithuanian core sections became extinct in stepwise pattern. In order to better understand the principle of mid-Silurian graptolite extinctions it is necessary

to produce estimations of graptolite extinctions from other regions and compare them.

Acknowledgments: This research was funded by a grant 09.3.3.-LMT-K-712-10-0207

"Assessment of genetic diversity of Lithuanian summer barley cultivars using conserved DNA-derived polymorphism markers"

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The aim of this study was to determine the genetic diversity of eleven summer barley varieties using conserved DNA-based CDDP (conserved DNA-derived polymorphism) markers that are targeted to well-known gene families, and to link the CDDP profiles with particular qualitative and quantitative characteristics.

At the initial step of the present study, five functional CDDP primers (WRKY-R3, MYB1, ERF3, KNOX3, ABP1-3) were selected. Collectively, they generated 68 DNA fragments on average in each of tested barley breed. The average level of polymorphism in analyzed breeds was 22.8%. The greatest level of CDDP polymorphism was observed in 'Luokė' that reflects its adaptability to various types of soil and wide range of environmental conditions. Genetic distance between analyzed breeds varied from 0,265 to 0,581. UPGMA dendrogram, based on genetic distances showed that breeds are not genetically pure but individuals of each breed form separate groups. In UPGMA dendrogram, based on five CDDP primers, breeds formed two clusters, reflecting their resistance to lodging. Positive correlation between number of KNOX family gene copies in genomes and height of plants was determined, and it may be related with role of KNOX genes to metabolism of gibberellins in plants.

"The Study of Long-Term Technogenic Soil Genotoxicity by RAPD Molecular Markers Using Trad 4430 Clone"

Stela Pervenytė[1], Tatjana Čėsnienė[1], Raimondas Šiukšta[1]
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Soil contamination with heavy metals is among the most dangerous consequences of industrial activity which may cause a negative effect on biological systems. Organisms, such as plants, contacting the soil directly, have the highest chance of such an effect. Due to the long-term industrial pollution, ten soil samples from the "Skaiteks" industrial site in Vilnius and three soil samples from "Elnias" and "Vairas" industrial sites in Šiauliai were selected as a research objects. These soils are classified as hazardous and very dangerous soils under the total soil contamination index Zd.

The aim of this study was to assess the genotoxic effect of technogenic soil on plants by RAPD (random amplified polymorphic DNA) molecular markers.16 RAPD primers were selected for this study. The RAPD method was optimized by changing the RAMP parameter - temperature increase rate of primer annealing.

The study was carried out using Tradescantia 4430 clone plants, all of which are of the same genotype, so it does not affect the occurrence of DNA polymorphisms in different plants. RAPD profiles of one to six months-exposed Tradescantia clone 4430 plants were compared to unexposed plants. Genotoxic effects were assessed by changes in DNA structure – emergence or extinction of DNA fragments that indicate the DNA damage (DNA adducts, nicks and other types of mutations). Increased number of amplification products in agarose gel was revealed by changing the RAMP parameter. Fragments of low molecular weight separated especially well in the case of 5% RAMP. Only monomorphic RAPD profiles were obtained in all cases of our study, therefore, the genotoxic effect of contaminated soils was not detected in the Tradescantia plants by molecular RAPD markers with selected primers.

"Investigation of the impact of technogenically contaminated soil using the test-system of Vicia faba chlorophyll morphosis"

Vėjūnė Pukenytė[1], Raimondas Šiukšta[1], Tatjana Čėsnienė[1]
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Soil pollution is toxic to ecosystems and especially to organisms that directly contact with it. The soil is usually polluted with heavy metals, some of which in low concentrations are essential for living organisms, but excessive amounts of them may become genotoxic. Thus, the effect of heavy metals on living organisms is among the main topics of the environmental sciences.

In the present study, genotoxicity of contaminated soil collected from the territories of the decommissioned "Grąžtai" and "Rimeda" factories was studied using test-system of cobalt-induced chlorophyll morphosis in Vicia faba cv. 'Aušra'. Experiment consisted of two treatment modes – control group, when seeds were soaked in distilled water for 12 hours, and Co-treated group, when seeds were soaked in 7.5 mM $Co(NO_3)_2$ solution for 12 hours. After 4 weeks of growth in control and contaminated soil samples, plants were divided into 4 phenotypic groups according to the severity of chlorophyll loss, and biochemical oxidative stress markers and gene family-specific CDDP (conserved DNA-derived polymorphism) markers were used to evaluate heavy metals-induced damage in V. faba plants. The UPGMA clustering using CDDP markers revealed that polymorphic profiles from plants grown in contaminated soil are more similar to each other than those obtained from the plants grown in control soil, and group into one cluster. Heavy metals in contaminated soil also reduced the amount of ascorbic acid in V. faba leaves, compared with plants grown in the control soil.

The concentration of total carotenoids and chlorophylls only insignificantly differed between the corresponding phenotypic groups of plants grown in different types of soil, but their content was significantly lower in plants with intermediate and yellow phenotype in comparison to that found in plants of unaffected phenotype. In addition, cobalt-treated plants (NV, T, G groups) possessed an increased lipid peroxidation level, what indicate that Co-exposed plants suffer from oxidative stress.

"Vitamin D Receptor Gene Polymorphism Distribution and Methylation Analysis of Vitamin D Metabolic Pathway Genes in Lithuanian Rheumatoid Arthritis Patients"

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Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune multi-factorial disease, which adversely affects quality of life. Multiple environmental and genetic factors have been associated with increased risk for RA. Recent data shows that the association between disease activity or functional scores and low levels of vitamin D (vitD) are statistically significant. Active form of vitD (calcitriol) acts via specific vitD receptors (VDR) and regulates more than 2000 gene expression; it also has an immunomodulatory action that suppresses inflammation.

One of the aims of this study is to explore genetic polymorphisms which are at high importance in vitamin D metabolism (VDR gene), evaluate differences in disease affected subjects and healthy controls, also to assess whether VDR polymorphisms are associated with susceptibility to rheumatoid arthritis (RA) and/or correlates with other clinical features of disease (disease activity scores, low vitD concentration, ect.). More recently, a role for vitD in regulating DNA methylation has been identified as an additional mechanism of modulation of gene expression. However, is not yet clear how methylation status influences vitD metabolism and response pathways.

This study additionally explores relationship between methylation status of vitD-related genes, vitD levels and its association with RA. Since now blood sam-

ples from 106 RA patients and 39 healthy controls were collected. Four VDR gene polymorphisms BsmI, FokI, ApaI, and TaqI are assessed using real-time PCR instrument and TaqMan genotyping assays. DNA methylation pattern was determined by pyrosequencing.

Therefore, the work will provide first data on VDR gene polymorphism distribution in Lithuanian RA patients and evaluate the effect of several vitD-related genes methylation on RA susceptibility.

"Isolation and Characterization of Outer-Membrane Vesicles from Opportunistic Pathogen Acinetobacter baumannii"

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Gram-negative bacterium Acinetobacter baumannii is recognized among most dangerous microorganisms in health care settings worldwide. This opportunistic pathogen causes variety of nosocomial infections to immunocompromised patients. Due to its ability to acquire multidrug resistance and persist in clinical environment, infections caused by A. baumannii are difficult to cure. An inefficiency of antibiotics against this pathogen encourages the development of alternative treatments.

A. baumannii secretes nano-spherical structures called outer-membrane vesicles (OMVs) which contain variety of bacterial molecules. Most abundant proteins found in OMVs are outer-membrane porin OmpA and ß-lactamase AmpC. OmpA acts as multivirulent factor and possibly influence OMVs biogenesis, while AmpC contributes to antimicrobial resistance of A. baumannii. Therefore outer-membrane vesicles are considered to play an important role in A. baumannii pathogenesis. Due to high prevalence of bacterial antigens on the surface, OMVs are one of the most promising vaccine candidates against A. baumannii.

The aim of this work was to isolate and characterize outer-membrane vesicles from clinical A. baumannii strain. For this purpose we isolated OMVs from A.baumannii clinical strain and ompA gene knockout mutant. OMVs were visualized using transmission electron microscopy (TEM). Quantity and protein content of OMVs were measured using Bradford assay and SDS-PAGE. Detection of OmpA

was performed using Western blot. AmpC ß-lactamase activity using nitrocefin assay was performed as well.

According to our results, A. baumannii ΔompA mutant produced ~3 times more OMVs comparing with wild-type strain and showed differences in OMVs protein profiles. Also both OMVs from wild-type and ompA deletion mutant contained active ß-lactamase AmpC. However absence of OmpA resulted in slower hydrolysis of nitrocefin.

In conclusion, we confirmed that OmpA plays an important role in biogenesis of outer-membrane vesicles secreted by clinical A. baumannii strain and possibly contributes to the bacterial resistance to ß-lactam antibiotics.

"The antibacterial activity of silver nanoparticles"

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In recent years, multidrug resistant (MDR) microorganisms have become a major challenge towards successful treatment of various infectious diseases. Unresponsiveness of MDR microbes to various antimicrobial drugs often leads to increased time and cost of treatment and higher rates of morbidity and mortality. These problems have led to the growth of interest in research of alternative antimicrobial treatments free of resistance and high cost. One of the most promising methods for managing resistant microorganisms is treatment with silver nanoparticles (AgNPs). It has been proved that interaction with AgNPs has lethal effect on both Gram-positive and Gram-negative microorganisms including multidrug resistant microorganisms [1]. There are four main modes of silver nanoparticles action against various microorganisms which include AgNPs adhesion to microbial cells and penetration inside the cells, modulation of transmembrane transport and generation of reactive oxygen species (ROS). Moreover, it was demonstrated that bactericidal efficiency of AgNPs highly depends on their size making 5-10 nm nanoparticles the most lethal to different microorganisms [2].

In this work, we have tested the antimicrobial effects of 7 nm silver nanoparticles, manufactured by "Rho nano", UAB. AgNPs were produced by using innova-

tive liquid jet in vacuum method. Prepared particles were characterised by transmission electron microscopy and atomic absorption methods. To assess the ability of AgNPs to inhibit the growth of the bacteria, minimum inhibitory concentrations (MIC) of the particles were determined by microdilution method [3].

The experiments were performed with E. coli DH5α strain bacteria. AgNPs used in these experiments were suspended in ethanol, H2O or polyvinylpyrrolidone (PVP) solution. We have observed that nanoparticles suspended in aqueous solutions tended to aggregate and this process decreased their ability to inhibit bacterial growth. In order to solve this problem AgNPs were suspended in PVP solution which reduces aggregation process.

Results have shown that E. coli treatment with AgNPs was lethal to the bacteria. Furthermore, as expected AgNPs suspended in PVP solution demonstrated lower MIC values than AgNPs suspended in ethanol or aqueous solutions.

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"Picomolar inhibitors of carbonic anhydrase: importance of inhibition and binding assays"

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Human carbonic anhydrases (CAs) are targets for drug design due to their role in numerous diseases such as glaucoma, epilepsy, and cancer. Clinically used CA inhibitors–drugs are relatively weak and non-selective for human CA isoforms thus exhibiting toxic side effects. Further drug development should lead to compounds with picomolar affinities and significant selectivities. Currently, the Ki of CA inhibitors is usuallydetermined by the stopped-flow CO_2 hydration assay, the method that directly follows inhibition of CA enzymatic activity. However, the assay has limitations, such as largely unknown concentration of CO_2 and the inability to determine the Ki below several nM. The widely used direct binding assay, iso-

thermal titration calorimetry, also does not determine the Kd below several nM. In contrast, the thermal shift assay can accurately determine picomolar affinities.

The inhibitor dose-response curves were analyzed using Hill and Morrison equations demonstrating that only the Morrison model is applicable for the determination of tight-binding inhibitor Ki. The measurements of interactions between ten inhibitors and seven CA isoforms showed the limitations and advantages of all three techniques. Inhibitor 61 exhibited the Kd of 50 pM and was highly selective towards human CA IX, an isoform which is nearly absent in healthy human, but highly overexpressed in numerous cancers. Combination of inhibition and binding techniques is necessary for precise determination of CA–high-affinity inhibitor (such as 6) interactions and future drug design[2].

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"Functional Screen for the Antimicrobial Resistance Genes in the Soil from Intensive and Ecological Agriculture Farms"

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Background: Soil is a large, poorly explored reservoir of antimicrobial resistance genes. Due to naturally occurring antimicrobial compounds and their widespread use in clinical environment as well as industry and agriculture, soil is an excellent medium for resistance genes to emerge and spread. Bacterial resistance acquired via these genes, results in decreased effectiveness of antimicrobial compounds, making bacterial infections more difficult to treat. In this work we performed functional gene screening in the soil from intensive and ecological farms to identify genes conferring resistance to clinically relevant antimicrobial compounds.

Materials and methods: The genomic DNA libraries in pBluescript plasmid were constructed using DNA that was extracted from soil samples from intensive and ecological farms. Functional gene screening was performed using E. coli strain BL-21 as a host with the following antimicrobial compounds: ß-lactams (imipenem, cefuroxime), aminoglycosides (streptomycin, kanamycin, gentamicin), chloramphenicol, ciprofloxacin, tetracycline and benzalkonium chloride. The obtained resistant clones were further analyzed by sequencing and bioinformatic methods.

Results: Similarly sized libraries of 0,482 Gb (average insert size of 3,64 kb) and 0,609 Gb (average insert size of 2,24 kb) were constructed from intensive and ecological agriculture farms, respectively. Our analysis determined that DNA libraries from both farms soil resulted in clones displaying resistance to the kanamycin only. Further analysis of DNA library from intensive farm soil revealed that the resistant clone contained a plasmid with the insert coding for a GNAT family N-acetyltransferase. All other obtained resistant clones to benzalkonium chloride, imipenem and cefuroxime were determined to be false positives.

Conclusion: Genomic DNA libraries from soil from intensive and ecological farms contained the resistance determinants to kanamycin only.

"Identification of the Toxin-Antitoxin Systems in the Opportunistic Pathogen Stenotrophomonas maltophilia"

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Bacterial toxin-antitoxin (TA) systems are small genetic elements, coding a stable protein toxin and an unstable molecule, neutralizing its toxic effect – antitoxin. In stress conditions (e.g. starvation, antibiotic pressure, host immune system attack) the unstable antitoxin molecule is degraded and the toxin inhibits the main cellular processes – DNA replication or protein, cell wall, ATP synthesis. TA systems are potentially associated with the virulence traits of pathogenic bacteria, such as persistence, biofilm formation, host colonization. Furthermore, TA systems are proposed as new targets for antimicrobial therapy especially important for the multidrug-resistant pathogen treatment.

Stenotrophomonas maltophilia is an environmental bacterium found in aqueous habitats, the rhizosphere of plants, on animals, in foods. In clinical settings S. maltophilia is known as an opportunistic multidrug-resistant nosocomial pathogen causing respiratory tract, bloodstream, urinary tract infections. At present there is no information about the TA systems of this pathogen, thus our goal is to identify and characterize the TA systems of S. maltophilia.

Bioinformatic analysis was performed on 21 S. maltophilia genome sequences available to this date and 50 putative TA systems were predicted. 7 genes pairs best matching TA systems criteria were selected for further analysis. All selected TA systems were detected in clinical or environmental S. maltophilia isolates from laboratory collection. Interestingly, the frequency and spread of detected TA systems differed from bioinformatic analysis predictions. Detection results showed that several selected TA systems are present only in clinical S. maltophilia bacteria and are not found in environmental S. maltophilia isolates.

"Temperature Dependent Changes in Structure and Seeding Potential of Amyloid Fibrils"

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Introduction: Amyloids are self-assembled and highly ordered peptide or protein aggregates, which are usually rich in beta-sheet structures. Their formation is linked to several neurodegenerative diseases, such as Alzheimer's, Parkinson's or prion diseases. Recently is has been shown that prolonged incubation may induce structural changes in amyloid fibrils.

Methods: Prion proteins (PrP) were incubated at 37 °C in a 2 M GuHCl, pH 6 buffer with a final concentration of 0.5 mg/ml for 3 days with constant sample rotation at 10 rpm. The generated fibril samples were additionally incubated at 60 °C for different amounts of time. Each sample was sonicated and their seeding potential, as well as Thioflavin T binding ability and fibril stability were tested at different denaturant concentrations.

Results: PrP fibril incubation lead to an increased stability under higher denaturant concentrations, suggesting a change in their structure upon incubation at a higher temperature. There was also a noticeable difference in their ThT

binding capacity, as incubation resulted in a sizable shift of ThT fluorescence emissions. Finally, the seeding potential was affected negatively at lower and positively at higher denaturant concentrations.

Conclusions: The results of PrP fibril incubation at an elevated temperature all point towards a restructurization into higher stability amyloid assemblies, suggesting that temperature and time are an important factor not only during the initial aggregation of amyloid proteins, but also after the fibrils are already generated.

"Immunological Biomarkers for Diabetes Management"

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Study background: Monitoring of diabetes and improving life style for such patients is a big challenge for current healthcare. To address this global health issue, we have investigated immunological serum and salivary biomarkers for diabetes using molecular arrays, namely cytokine and growth factor kit I from Randox Laboratories Ltd, London, UK. This pilot study was aimed to explore immunological biomarkers for diabetes using serum and saliva from diabetic volunteers and healthy controls with the aim to detect cytokine patterns characteristic for diabetic subjects before, during and after specially designed combined exercise routine consisting of resistance and aerobic exercise.

Methods: Serum and saliva samples from diabetic individuals and controls have been processed to remove cells and then stored at -20C for the duration of the project. Randox biochip assay was performed to test EDF, IFNgamma, Il-1alpha, Il-1beta, IL-2, IL-4, IL-6, IL-8, IL-10 TNFalpha, MCP-1 and VEGF levels before, during and after the exercise routine of six weeks.

Results: The study showed that individual responses to exercise varied greatly, with serum VEGF and MCP-1 showing highest values and best correlation for all individuals tested. Serum cytokine secretion was detected to be in the following ranges (pg/ml): IL-2 – 0-2.47; IL-4 – 1.32-2.97; IL-6 – 0.88-3.71; IL-8 – 1.23-3.71; IL-10 – 0.76-2.36; VEGF – 14.2-93.41; IFNgamma – 0-1.83; TNFalpha – 1.44-2.94; IL1alpha – 0-0.51; IL1beta – 0-0.83; MCP1 – 121.29-265.01 and EGF – 0-3.01.

Further work: The study suggested that saliva can potentially be used for monitoring of diabetes as a less invasive method as compared to serum. As more data needed, we are going to expand this pilot project. Saliva sample collection could also be used to establish a data bank; this would improve research statistics and diabetes phenotyping. Our further work will also include research on improving diabetes monitoring questionnaires which could better reflect life styles and psycho-, neuro-, endocrine and immune health.

"IP-10 and sCD14 in urine as potential biomarkers of childhood tuberculosis"

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Introduction. Tuberculosis (TB) caused by Mycobacterium tuberculosis (M.tb) is still a serious health problem. The major challenge is pediatric TB, to which, according to the WHO, about one million children fall each year. Due to the non-specificity of the clinical symptoms of TB in children and the low diagnostic value of available tests, there is an urgent need to find new solutions for better and faster diagnosis of the disease in this age group.

Aim. The aim of the study was to assess the potential value of IP-10 chemokine and sCD14 protein in urine of M.tb-infected and uninfected children in diagnosing pediatric TB.

Methods. The study involved 220 urine samples collected from children (1) with active lung TB, (2) latent M.tb infection, and (3) healthy children. The levels of IP-10 and sCD14 in urine samples were evaluated immunoenzymatically. Results. A significant increase in urine sCD14 level in M.tb-infected individuals, both with active and latent TB ($p<0.05$), was found. An urine concentration of IP-10 the group of children with M.tb infection was also higher than in the controls, however the observed difference was not statistically significant.

Conclusion. The results suggest a potential value of urine sCD14 in the diagnosis of active or latent M.tb infection in the pediatric population.

Supported by: the UL Student' grant 2018 for Lidia Żukowska and the NCN grant No 2016/21/B/NZ7/01771

"Molecular identification of vector-borne pathogens in domestic dogs"

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Climate change has already impacted the transmission of a wide range of vector-borne diseases in Europe. During the past decade, vector-borne diseases have been continuously spreading in Baltic countries including Lithuania. Dirofilaria infections are mosquito-borne parasitic infections mainly of dogs and, in Europe, they are caused by D. immitis and D. repens. Accurate identification of Dirofilaria species in dogs is clinically important, because of the zoonotic concerns and therapeutic implications. Many of dogs are infected annually with dangerous tick-transmitted diseases: canine anaplasmosis and Lyme borreliosis. Infection with A. phagocytophilum in dogs is mostly asymptomatic or characterized by non-specific clinical signs, therefore especially important to use appropriate methods for early diagnosis of pathogens. Lyme borreliosis, or Lyme disease, is caused by spirochaetes that belong to the group Borrelia burgdorferi sensu lato. The number of reported borreliosis cases in Europe has continued to increase.

We aimed to investigate the presence of mosquito- and tick-borne pathogens in domestic dogs using molecular DNA analysis methods. In total 138 blood samples from domestic dogs were collected in Lithuania during 2016-2018. DNA was isolated from EDTA-anticoagulated whole blood. Discrimination between six different species of canine microfilariae was done based on amplification of partial internal transcribed spacer region 2 (ITS2) of the ribosomal DNA. To verify D. repens, we used species specific PCR based on cytochrome c oxidase subunit I (COI) gene. PCR results were evaluated by agarose gel electrophoresis. For identification of Anaplasma phagocytophilum and Borrelia spp. was used real-time-PCR with TaqMan probe and specific primers.

Results of molecular analysis demonstrated the presence of DNA of D. repens in 5 %, Anaplasma phagocytophilum in 96 %, and Borellia spp. in 28 % of

examined dogs. Our findings show necessary further investigation to evaluate prevalence of vector-borne infections in dogs in Lithuania using modern effective diagnostic methods which will allow effective treatment of diseases.

This research is funded by the European Social Fund under the No. 09.3.3.-LMT-K-712-10-0287 "Development of Competences of Scientists, other Researchers and Students through Practical Research Activities" measure

"Functional characterization of septicolysin from the opportunistic pathogen Acinetobacter baumannii"

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One of the biggest threats in health care settings is the Gram negative opportunistic pathogen Acinetobacter baumannii which causes a variety of infections in immunosuppressed patients. Its ability to persist in clinical environment and multidrug resistance profile makes A. baumannii one of the most challenging nocosomial pathogens. Therefore, identification of the virulent features is for understanding of the pathogenesis mechanisms displayed by A. baumannii. Frequently virulence genes are acquired during horizontal gene transfer, e.g. plasmids or transposable elements. spl gene is located in A. baumannii plasmid pAB120 and possibly could be described as one of the putative virulence genes, since the product of this gene -septicolysin- has homology to the pore-forming toxins in other pathogenic bacteria.

The aim of this work was to assess the importance of septicolysin in A. baumannii pathogenesis. In order to determine, whether septicolysin could be a pore-forming virulence factor, spl gene from plasmid pAB120 was cloned into pUC_gm_AcORI and pUC_gm_AcORI_Ptac vectors. These constructs were further transformed into septicolysin-lacking A. baumannii strain. Resulting strains were tested for the hemolytic activity. Cytotoxicity on the mice lung epithelial cells LLC1 was determined by measuring cell viability by trypan blue uptake. C. elegans fertility assay was performed to identify if septicolysin increases A. baumannii virulence in vivo.

According to our results, we did not observe any differences in hemolysis and cytotoxicity comparing A. baumannii wild type strain and the strain with introduced spl gene. Also, spl gene did not have an impact on nematodes fertility. In conclusion, septicolysin didn't demonstrate the expected properties of pore-forming toxins in the tested models. However, further investigations using purified septicolysin are needed in order to determine its virulence.

"Prevalence of Mycoplasma haemofelis and Candidatus Mycoplasma haemominutum Pathogens in Shelter and Pet Cats in Lithuania"

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Hemoplasmas, hemotropic mycoplasmas, are causative agents of infectious anemia in animals. Also infections with novel haemoplasma species have been described in humans, as well as infections with species that have possibly originated in animals, including cats, raising the possibility of zoonotic infections. Although previously uncommon, haemoplasma has become quite frequent in Europe during the past decade, becoming one of the emerging infectious pathogens. In the last few years an increasing number of cases with a wide variety of clinical signs have been recorded throughout the continent. In Lithuania the identification of these pathogens in veterinarian clinics is based on a microscopic analysis of blood smears revealing haemoplasma on the surface of the erythrocytes, but this is known to be very insensitive for diagnosis, and cytology cannot easily differentiate haemoplasma species. The use of molecular techniques is necessary to detect and identify Mycoplasma species effectively.

In the present study, we examined prevalence of two haemoplasma species, Mycoplasma haemofelis and Candidatus Mycoplasma haemominutum, both causative of infectious anemia in cats.

The aim of the present study was to identify prevalence of Mycoplasma hae-

mofelis and Candidatus Mycoplasma haemominutum in shelter and pet cats using molecular detection methods. Total of 162 cats bloods samples collected from shelter and pet cats were analyzed. DNA was isolated from EDTA-anticoagulant whole blood. Detection of Mycoplasma was performed using Real-Time and conventional PCR targeting a 600-bp region of the 16S rRNA gene. PCR products were sequenced and then analyzed using BLAST and Mega software. Molecular analysis allowed detection of Mycoplasma DNA in 11,111% (18/162) of cats – 22,222% (4/18) in shelter cats and 9,722% (14/144) in cats brought to pet clinic. Sequence analysis of Mycoplasma isolates revealed the presence of Candidatus Mycoplasma haemominutum and Mycoplasma haemofelis bacteria, all M. haemofelis sequences were identical and M. haemominutum sequences showed heterogeneity with variability in six differente sites.

The results of the present study provide knowledge of the distribution of Mycoplasma genotypes in cats in Lithuania, and show the necessity to use a molecular analysis for an accurate diagnosis of these pathogens.

This research is funded by the European Social Fund under the No. 09.3.3-LMT-K-712-10-0291 "Development of Competences of Scientists, other Researchers and Students through Practical Research Activities" measure.

"Development, Characterization and Application of Monoclonal Antibodies against Phleum pratense Allergens"

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Allergy is type I hypersensitivity reaction to the environmental antigens that usually cause little or no problem in most people. Grass pollen is one of the most important allergen sources causing allergic rhinitis.

This study aimed to develop, characterize and apply monoclonal antibodies (MAbs) against timothy grass (Phleum pratense) pollen allergens. Allergen extract and its components were used for the immunization of mice. It was shown that timothy grass extract induces the immune response in immunized mice. After hybridization of mouse spleen cells with myeloma cells, ten hybridoma cell lines

producing high affinity MAbs of IgG isotype were produced. Six of them produced MAbs against timothy grass allergen component Phl p 1 and four – against the component Phl p 5. The MAbs were conjugated with horseradish peroxidase (HRP) and tested in a competitive ELISA. The MAbs were grouped according to their recognized epitopes. By using MAb pairs from different groups, sandwich ELISA systems for the quantitation of Phl p 1 and Phl p 5 components were developed. They were optimized for the highest sensitivity and used for the analysis of allergen extracts. It is important to analyse the content of allergen extracts, because they comprise a broad range of proteins and other substances. It was determined that timothy grass allergen extracts of different producers contain 0,86 - 1,43 % of allergen components Phl p 1 and Phl p 5.

": Characterization of novel toxin-antitoxin systems in the cyanobacterium Aphanizomenon flos-aquae"

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Toxin-antitoxin (TA) systems are genetic elements found in prokaryotes and are hypothesized to play an important regulatory role in many cellular processes. The common trait of TA systems is that they suppress cell growth in response to stressful conditions, such as for example, nutrient limitation or antibiotic treatment. TA systems are composed of two closely located genes – a toxin which is toxic for the bacteria itself and an antitoxin, which disables toxin's activity or expression. Based on their their genetic structure and regulation, TA systems are subdivided into five classes. In class II TA system both the toxin and the antitoxin are small proteins, which form a protein-protein complex resulting in toxin neutralization. The neutralization of antitoxin by strain specific drugs could lead to toxin dependent cell growth inhibition. Therefore, identification of new toxin-antitoxin systems in the genomes of human health and environmentally relevant bacteria could create new targets for mitigating harmful effects caused by these microorganisms.

In this study we aim to characterize new type II toxin-antitoxin systems

in the genome of the filamentous cyanobacterium Aphanizomenon flos-aquae, which form harmful algal blooms in many fresh- and brackish water ecosystems, including the Baltic Sea and the Curonian Lagoon. An unusually arranged type II TA system, found with TA finder 2.0 software, consisted of a large HipA-like toxin and an antitoxin, which contained a DNA binding domain. Genes of the predicted system were cloned into two inducible vectors to be expressed in an E. coli expression strain. Induction of the following toxin and antitoxin genes produced bacteria growth inhibition or restoration respectively, which was proportional to the inductors concentration. This serves as a great indication of a new possible type II TA-system, although a deeper investigation is still needed.

"Impact of exogenous GA3 and TIBA treatment on Heracleum sosnowskyi seed vigour"

Tautvydas Žalnierius[1], Dalia Koryznienė[1], Sigita Jurkonienė[1]
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Sosnowsky's hogweed (Heracleum sosnowskyi Manden.) is invasive plant entered into list of Invasive Alien Species of Union concern. It is highly toxic plant to humans and well established in seven EU countries where transforms the landscape. Sosnowsky's hogweed propagates only by dry-fruits i.e. seeds and produce them once in lifetime. Well known the important role of gibberellins and auxins on seed development of fleshy fruits.

To figure out the role of gibberellic acid (GA3) in dry-fruit i.e. seed development, we applied Sosnowsky's hogweed's unpollinated ovaries in satellite and stem branch umbels by different concentrations (0.07 mM, 0.14 mM, 0.28 mM, 0.43 mM) of exogenous GA3. GA3 treatment didn't have a significant effect on size of H. sosnowskyi seeds, however it caused changes in shape. Longitudinal sections of mericarps and SEM micrographs of embryos revealed that embryos after GA3 (0.43 mM) treatment were at torpedo stage, whilst mature embryos in control seeds had been observed. Furthermore, we disclosed that GA3 treatment significantly reduced the germination rate in situ conditions of H. sosnowskyi mericarps from 98.0% to 16.5% in control and GA3 (0.43 mM) treatments respectively. Auxin transport inhibitor (1,3,5-triiodobenzoic acid, TIBA) was used to affect auxin and gibberellin crosstalk in fruit development. Inhibition of auxin transport from the

apical shoot by TIBA decreased seed germination rate in field conditions (98.1% and 29.8%; control and TIBA), the auxin effect on seeds' sufficient development was negated by indoleacetic acid (IAA) application on unpollinated ovaries right after the TIBA treatment (germination rate 96.1%).

All these results suggest that exogenous GA3 application has influence on dry Sosnowsky's hogweed seeds development and affects germination rate. Thus, it could be assumed that GA3 could be used to inhibit the spread of this invasive plant.

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The book of Abstract layout by Milda Girdenytė.