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Doctoral theses in biochemistry, biology, biophysics, botany, ecology and environmental science defended in Lithuania in 2016

Compiled by Indrė LIPATOVA

THERMODYNAMICS OF ARYL-DIHYDROXYPHENYL-THIADIAZOLE BINDING TO RECOMBINANT HUMAN Hsp90 (Biochemistry)

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Dissertation defended:
14 June 2016

The heat shock protein 90 (Hsp90) is a chaperone that, while catering only for a limited clientele, manages to impact a significant fraction of proteome. Molecular mechanisms of the 6 hallmarks of cancer cells all involve Hsp90 clients. Therefore successfully drugging Hsp90 might be the strike at the heart of oncogenic processes that is needed for the development of effective anticancer therapy. A series of potential Hsp90 inhibitors, aryl-dihydroxyphenyl-thiadiazoles, has been synthesised at Vilnius University. The goal of this study was to explore the structure–thermodynamics relationship for aryl-dihydroxyphenyl-thiadiazoles binding to human Hsp90. We employed isothermal titration calorimetry in conjunction with protein denaturation profile analysis techniques in order to dissect the thermodynamic mechanism of the protein–ligand binding event. In this work we provide a detailed thermodynamic characterisation of these inhibitors binding to recombinant human Hsp90, both α and β isoforms. We show for the first time that protonation of hydroxyl residue in resorcinol-based Hsp90 inhibitors is essential for binding. Comparison of thermodynamic and structural data signified the ability of chlorine to form interactions of a non-hydrophobic nature. As generally enthalpic contribution to the ligand affinity is more difficult to improve than the entropic contribution, the mostly enthalpydriven binding of three of our compounds makes them excellent candidates for further lead development.

STRUCTURAL-FUNCTIONAL ANALYSIS OF THE PLANT SMALL RNA METHYLTRANSFERASE HEN1 (Biochemistry)

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Dissertation defended:
25 August 2016

miRNA, siRNA, piRNA are small non-coding RNAs (sRNAs) that regulate eukaryotic biological processes by mediating translational inhibition or/and decay or cleavage of the target RNA. In plants and animals sRNAs are protected from degradation by 2'-O-methylation at their 3'-terminal nucleotides, which is carried out by a family of HEN1 proteins. Arabidopsis thaliana HEN1 modifies miRNA/miRNA*, siRNA/siRNA* duplexes. Evaluation of HEN1-binding activity with various RNA, and DNA substrates showed that enzyme specifically recognises sRNA/sRNA* duplexes at the substrate binding step. Using truncated variants of HEN1 and its mutants with amino acid substitutions it is shown that both double-stranded RNA (dsRNA)-binding domains, although unequally, together with methyltransferase domain contribute to substrate binding. Based on the obtained data, a model of HEN1 interaction with sRNA/sRNA* duplex was proposed. It was determined that HEN1 peptidyl-prolyl cis-trans isomerase-like domain of a previously unknown function together with a La-motif-containing and the second dsRNA-binding domains form contacts with the dsRNA-binding protein HYL1, which together with ribonuclease DCL1, zinc finger protein SERRATE (SE) perform excision of miRNA/miRNA* duplexes from predecessors at the early stage of miRNA biogenesis. After identifiction of HEN1 interaction with HYL1and DCL1, and elucidation that HEN1 does not form contacts with SE, a model explaining processes of late stage of miRNA biogenesis was proposed.

SALT RESISTANCE MECHANISM OF HALOTOLERANT/HALOPHILIC PROKARYOTIC DNases AND HALOTOLERANCE INDUCTION FOR BOVINE DNaseI (Biochemistry)

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Dissertation defended:
7 December 2016

Analysis of the sequence data revealed that many prokaryotic DNaseI-like nucleases from halotolerant/halophilic species are multi-domain proteins. This fact led to a hypothesis that in some cases a fusion of an additional domain to the DNase domain was the key factor in evolution that enabled the activity of prokaryotic DNases at high ionic strength. In this study the hypothesis was experimentally proved by analysing halo-tolerance of one DNase from *Thioalkalivibrio* sp. K90mix (DNaseTA) and its mutants. DNaseTA is comprised of two domains: one domain is DNaseI-like and the other is a DNA-binding domain comprising two HhH (helix-hairpin-helix) motifs. It was decided to mimic *in vitro* the evolutionary step that created the natural fusion. The research revealed that this domain originated from ComEA/ComE proteins (DNA receptor of bacterial competence system) and through the course of evolution was fused with the DNaseI-like domain. In this study the domain organization of DNaseTA was mimicked by creating two fusion proteins comprising bovine DNaseI and a DNA-binding domain. Both fusions with additional DNA binding domains were demonstrated to be more salt tolerant than bovine DNaseI, albeit to a different extent. Molecular modelling data suggested that differences in tolerance for high ionic strength between the two created DNaseI fusions could be due to differences in hydrogen bonding between the fused domains and DNA, and different ability to transfer monovalent cations to solvent during DNA-protein complex formation.

TYPE II CRISPR-CAS SYSTEMS: FROM BASIC STUDIES TOWARDS GENOME EDITING (Biochemistry)

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Dissertation defended:
15 December 2016

The recent development of the Cas9 technology revolutionized the genome editing field. The simple modular organization of Cas9 complex, where specificity for the DNA target is encoded by a small crRNA and the cleavage reaction is executed by the Cas9 endonuclease, provides a versatile platform for the engineering of universal RNA-directed DNA endonucleases. In this work, we identified all molecular components required for an assembly of the functional Cas9 complex of *Streptococcus thermophilus* CRISPR3-Cas system and demonstrated targeted DNA cleavage by the reconstituted Cas9 complex *in vitro* and *in vivo*. More specifically, we showed for the first time that Cas9 complex can be used as a tool for DNA cloning and targeted genome editing by chemical transfection of the *in vitro* assembled Cas9 ribonucleoprotein complex into the cells. Aiming to understand the molecular mechanism governing DNA recognition and cleavage by the Cas9 complex, we analyzed the mechanism of R-loop formation and provided first direct evidence for directional R-loop formation, starting from PAM recognition and expanding toward the distal protospacer end. Realizing the importance of the PAM sequence for Cas9 function, we developed an assay for rapid PAM identification for newly identified Cas9 proteins and characterized Type II-C Cas9 protein of *Brevibacillus laterosporus*, expanding Cas9 toolbox for genome editing applications.

DOSE DELIVERY AND MICROENVIRONMENT DEPENDENT TRANSCRIPTOMIC PROFILES OF TUMOUR CELLS EXPOSED TO IONIZING RADIATION (Biochemistry)

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Dissertation defended:
16 December 2016

Modern radiotherapy (RT) is based on the harmful effect of ionizing radiation (IR) on the tumour tissue. The RT treatment is usually applied in fractioned doses of IR, where the oncological patients receive small (2 Gy) doses of IR every 24 hours 5 days a week. However, after a periodic exposure to a fractioned dose IR tumour cells acquire a resistance to RT. In order to evaluate the mechanisms leading to acquired radioresistance, a higher amount of current knowledge in radiobiology is based on the cellular response to single dose irradiation. It has also been noted that the cellular response to IR is dependent on the microenvironment surrounding the cell.

The aim of this study was to evaluate the global gene and miRNA expression changes in cancer cells exposed to single and fractioned dose IR irradiation in a microenvironment-dependent manner. We have revealed that the global gene and miRNA profile of murine lung carcinoma LLC1 cells was dependent on the irradiation delivery type. Also, we have shown that gene and miRNA expression signatures in LLC1 cells, as well as gene expression signatures in human colon carcinoma DLD1 and HT29 cells, were extracellular matrix-dependent. Finally, we have demonstrated that the DLD1 and HT29 cell response to fractioned dose IR treatment was dependent on the extracellular matrix, and during the study we identified the genes involved in the cell cycle, DNA damage repair, and the immune pathway as potential molecular targets for further improvement of radiotherapy.

CORTICAL DYNAMICS OF VISUOSPATIAL PROCESSING INVESTIGATED BY EEG MICROSTATES METHOD (Biophysics)

Ingrida Antonova

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Dissertation defended:
23 March 2016

Visuospatial processing is important for normal perception of the environment and normal functioning in the surrounding environment. The involvement of brain areas in visuospatial processing is well known but the order of activation of these areas – temporal dynamics of visuospatial processing – is not known yet. The aim of this work was to investigate temporal dynamics of visuospatial information processing by electroencephalography (EEG) microstate analysis. Two experiments were carried out: 22 healthy subjects participated in the visuospatial and non-spatial judgment experiment, and 28 healthy subjects participated in the position judgment experiment with a lateralized visual stimulation. Reaction times were longer in (1) visuospatial judgment compared to non-spatial judgment, (2) for left than right visual field stimulation, and (3) when stimulated medial compared to lateral portions of the both visual fields. Microstate analysis revealed that visuospatial judgment evoked longer and stronger P1-related activity. P3-related activity differed in the onset, duration and activation between visuospatial and non-spatial judgment. Also, lateralized stimulation evokes lateralized activation related to N1 potential, and this activation is stronger for right medial stimulation. The visuospatial and non-spatial judgment task and the position judgment task are suitable to be used in EEG studies and could be used in developing screening procedures for visuospatial impairment.

THE INVESTIGATION OF MOLECULES TRANSFER INTO CELLS USING ELECTROPORATION AND ELECTROSPRAY (Biophysics)

Paulius Ruzgys

Scientific supervisor:
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Dissertation defended:
30 August 2016

Nowadays targeted and controlled drug and other exogenous molecule delivery into cells and tissues remains to be an important issue in modern medicine. Therefore there is a constant need to investigate methods that allow temporal modification of cell membrane permeability. In this study, two physical cell membrane permeabilization methods (electroporation and electrospray) have been investigated. Electroporation experiments were performed to examine small molecule delivery to cells and electrotransfection dependency on electroporation medium conductivity. Results show that efficiency of small molecule electotransfer into cells and cell electrotransfection is inversely dependent on electroporation medium conductivity. Also, the efficiency of small molecule electrotransfer into cells and electrotransfection dependency on electroporation medium viscosity was investigated showing an inverse dependency relation. It has been shown for the first time that cell electrotransfection efficiency decreases as a result of increased electroporation medium viscosity that can be partially compensated with additional low-voltage impulses. In this work a new method to increase cytotoxicity of anticancer drugs, namely electrospray, has been investigated. For the first time it was demonstrated that cytotoxicity of anticancer drugs methotrexate and cisplatin has been increased as a result of electrospray. In *ex vivo* experiments methotrexate and cisplatin significantly decreased the tumour size five days after the first electrospray experiment. During *in vivo* experiments, methotrexate decreased the tumour size 2.3 times, cisplatin 4.5 times. In contrast, the control tumour size increased by 5.4 times. Also, it has been demonstrated that electrospray can be efficiently applied for cell transfection. Using electrospray at optimised parameters cell transfection efficiency was increased up to 56%.

DEVELOPMENT OF GENETIC TRANSFORMATION SYSTEM FOR BACTERIA OF THE GENUS *GEOBACILLUS* (Biology)

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Dissertation defended:
15 January 2016

Thermophilic bacteria of the genus *Geobacillus* are valuable microorganisms that can be used in various biotechnological processes such as bioconversion and bioremediation. However, an effective employment of geobacilli is impossible without a reliable and convenient methodology for their genetic engineering. Therefore, the goal of this work was to create a new plasmids vector and to elaborate an effective method for its transfer to the cells of geobacilli. First, two new plasmids isolated from wild *Geobacillus* spp. strains were described. One of them, pGTG5, was found to be related mostly to some poorly characterized plasmids and together with these plasmids it could constitute a new family of bacterial plasmids. To date, pGTG5 is the smallest plasmid identified in bacteria belonging to the genus *Geobacillus*. Another plasmid analyzed in this work, pGTD7, is a member of the well-known pUB110 plasmid family and is suitable for construction of new shuttle vectors. The replicon of this plasmid ensures high segregational stability and provides a high copy number to it bearing vector. Further, the electrotransformation protocol for the transfer of the vector DNA to *Geobacillus stearothermophilus* NUB3621R cells was elaborated. The influence of various conditions on electrotransformation was tested and the conditions resulting in the highest number of the transformants were determined. In summary, the results of this work broaden the assortment of geobacilli vectors and facilitate genetic transformation of one of the most relevant *Geobacillus* spp. strain, *G. stearothermophilus* NUB3621R.

FORENSIC EVALUATION OF GENETIC PARAMETERS OF AUTOSOMAL AND Y CHROMOSOME STR MARKERS IN LITHUANIAN POPULATION (Biology)

Giedrė Ruzgaitė

Scientific supervisor:
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Dissertation defended:
30 March 2016

For the purpose of the development of resources and tools required for forensic DNA analysis, numerous population and forensic genetic studies are being conducted on autosomal and Y chromosome STR marker validity for forensic DNA testing in various populations. The validity assessment is based on population and forensic genetics parameters of selected autosomal and Y chromosome STR markers which are generated in the population under study. National and international population databases representing the genetic structure and diversity of the population according to various STR marker sets are being built up for statistical justification of a DNA profile match in court. In this research work we analyzed the Lithuanian population from the point of view of forensic genetics. In particular, we devoted our efforts to calculate population and forensic genetics parameters recommended by forensic genetics experts of autosomal and Y chromosome STR marker sets standardized in the jurisdictions of the United States and Europe and to prove the validity of these STR markers for forensic DNA testing in the Lithuanian population experimentally. During the research project we compiled a collection of autosomal and Y chromosome STR profiles according to the STR marker sets standardized in forensic DNA testing of the American and European jurisdictions, which meets forensic genetics requirements for autosomal and Y chromosome STR DNA database to represent the genetic structure and diversity of the Lithuanian population. In addition, we calculated population and forensic genetic parameters of selected autosomal and Y chromosome STR markers in the Lithuanian population and experimentally determined the statistical significance of the DNA evidence in Lithuanian forensic DNA testing. We used a compiled autosomal STR DNA database representing Lithuanian population and an international Y-STR DNA database representing various populations worldwide as references. The results experimentally proved the validity of these STR systems for forensic DNA testing in the Lithuanian population, introduced the experimental data necessary for the accreditation of worldwide accepted forensic DNA analysis methods in the Serology and DNA laboratory of the State Forensic Medicine Service, and set up the basis for the development of resources and tools required for forensic DNA expertise in Lithuania.

INNATE AND ADAPTIVE RESPONSES TO ACUTE COLD STRESS IN HUMANS (Biology)

Nerijus Eimantas

Scientific supervisor:
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Lithuanian Sports University

Dissertation defended:
14 April 2016

From the beginning of humankind, people have been confronted to extreme temperature fluctuations and yet they have managed to survive. It is well known that exposure to an acute cold stress (1) increases the levels of catecholamine and other stress hormones, (2) impairs vigilance, overall mood, motor, and cognitive performance, (3) increases spinal reflex excitability, and (4) suppresses or stimulates the immune system. These are physiological characteristics that are of critical importance for the survival, health, and well-being of the humans who are exposed to occupational and/or recreational extreme-cold conditions. The aim of the research was to determine the innate and adaptive response to the cold stress to physiological and psychological characteristics in humans. The first experiment reports that according to the innate reaction to acute cold stimulus, humans could be divided into two groups – fast-cooling and slow-cooling – despite their similar physical characteristics. The response to cooling of the fast-cooling group vs. the slow-cooling group was more like an insulative–hypothermic response; the slow-cooling vs. the fast-cooling group displayed a metabolic-insulative response. The most important finding is that the subjects with a lower cold strain index showed stimulation of some markers of innate immunity and suppression of markers of specific immunity. The second experiment reports that despite the greater cold strain index after acute cold exposure in subjects in the fast-cooling than in the slow-cooling group, the changes in stress markers after passive body heating to the rectal temperature of 39.5 °C did not differ between the fast-cooling and slow-cooling groups. The heat stress did not change the markers of innate and specific immunity. As expected, the data obtained in the third experiment suggest that the subjects exhibited a thermoregulatory shift from peripheral-to-central input thermoregulation, as well as from shivering to non-shivering thermogenesis.

THE FUNCTIONAL CONSIDERATION OF RENIN–ANGIOTENSIN SYSTEM IN THE HUMAN JEJUNUM (Biology)

Mantas Malinauskas

Scientific supervisor:
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Dissertation defended:
17 June 2016

The classical renin-angiotensin system (RAS), known as endocrine system, is one of the body's most powerful regulators of the body fluid balance and has a role in the cardiovascular system. There is a lack of data suggesting the presence and actions of the renin-angiotensin system (RAS) in the small intestine. The aim of the thesis was to investigate the expression and the potential functions of the components of the renin-angiotensin system (RAS) in the jejunal mucosa of healthy individuals, and in jejunal muscular layer of obese patients *in vitro*. The dissertation demonstrates that the RAS is locally expressed in the human jejunum. The present study data confirmed the presence of the following "classic" AngII receptors AT1R and AT2R, and the data of functional analyses *in vitro* suggest that the activation of AT1R inhibits SGLT1-mediated glucose transport, whereas the activation of AT2R enhances glucose transport in the jejunal mucosa of healthy individuals. Moreover, the dissertation data shows that an alternative axis AP/AngIV/IRAP of the RAS is expressed in the mucosa of the human jejunum and the musculature. The data of functional analyses suggested an AngIV-mediated stimulation of glucose absorption in the jejunal mucosa of healthy individuals. Furthermore, the pharmacological analyses *in vitro* strongly indicate the AngIV-induced contraction of both longitudinal and circular jejunal muscle layers of obese patients.

INVESTIGATION OF HUMAN MESENCHYMAL STEM CELL GENETIC CHARACTERISTICS TO ENSURE THERAPY SAFETY (Biology)

Gabrielis Kundrotas

Scientific supervisor:
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Vilnius University

Dissertation defended:
23 June 2016

Human mesenchymal stem cell (MSC) therapy holds promise for treating degenerative and immune system diseases and repair of damaged tissues. Human tissues are cryopreserved and isolated MSCs are expanded in various culture media when prepared for clinical applications. These manipulations can significantly alter the genetic characteristics of MSC. Such MSCs enter senescence state, resulting in impairment of their therapeutic properties. Moreover, such MSCs can undergo spontaneous neoplastic transformation with karyotype changes. To date, no molecular markers of MSC senescence have been available and the tumorigenic potential of MSCs has become

the most important concern for safe clinical use of MSCs. This study revealed a normal karyotype of MSCs isolated from human bone marrow and expanded *in vitro* to clinically relevant amounts under a novel protocol. MSCs became senescent during *in vitro* long-term expansion, paralleling the significant difference in expression of 13 genes and 33 miRNAs. Umbilical cord tissue controlled freezing-thawing process did not affect the genomic stability of MSCs. MSC expansion in culture medium enriched with recombinant proteins had a significant impact on the expression of genes related to the cell cycle. The novel MSC isolation and expansion technique developed during this study could be applied at institutions producing advanced-therapy medicinal products. The cellular therapy quality control could be developed on the basis of the methods used in this study.

SEX-SPECIFIC RELIABILITY AND MULTIDIMENSIONAL STABILITY OF RESPONSES TO TESTS ASSESSING NEUROMUSCULAR AND COGNITIVE FUNCTIONS (Biology)

Vaida Berneckė

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Dissertation defended:
30 June 2016

Neurophysiological and neuropsychological measures provide complementary information on both healthy and clinical populations and remain important tools for research studies, clinical diagnoses, patient outcomes, and intervention monitoring. In scientific studies, evidence must be based on valid results, with no methodological errors in the conception, design, and implementation of the study, or in the process of data analysis. In our present study, we combined three different methods (dynamometry, electrical stimulation, and surface electromyography (EMG)) to evaluate central or peripheral fatigue, or both, in a sex-specific manner, and identified test-retest cross-correlations between the characteristics measured by these methods over five identical trials. The aim of the research was to evaluate sex-specific differences in reliability and multidimensional stability response to tests to assess neuromuscular and cognitive functions. In young healthy men and women, measurements of the attention function, such as tests of complex reaction, search for image samples and attention transfer, are highly reliable over time. Despite the low reliability for CAR during prolonged MVC, all other ICCs of the neuromuscular function, such as RMS, MnF, and WPE of a surface EMG signal, maximal muscle force production, CAR from a brief and sustained MVC of the ankle plantar flexors across the five identical test-retest

trials were found to be meaningful (correlation significance of $p < 0.05$ was reached), and no significant differences were found between trials of any indices measured in young healthy women. In young healthy men, measurements of the neuromuscular function, such as RMS, MnF, and WPE of a surface EMG signal, maximal muscle force production, CAR from brief and sustained MVC of the ankle plantar flexors, are reliable between five test-retest sessions.

FUNCTIONAL PROPERTIES OF *IN VITRO* MODULATED DENDRITIC CELLS (Biology)

Neringa Dobrovolskienė

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Dissertation defended:
30 June 2016

Recent studies of the immune system and cancer have shown that tumours are capable to avoid immune surveillance. Therefore, in order to effectively treat cancer, it is crucial to overcome immunosuppression and restore specific anti-tumoural response. Recently, advanced treatment strategies, such as targeted therapy and immunotherapy, have been applied. The aim of these approaches is to restore the balance of the immune system and to reprogram anti-tumoural immune response from the cancer-promoting (tolerogenic) status to the anti-cancer (immunogenic) status. Specific active cellular immunotherapy or therapeutic vaccination with autologous dendritic cells is particularly interesting. The major therapeutic purpose of these vaccines is to enhance and direct an immune response against the existing tumour. However, the data of clinical trials state that only 54% of patients respond to vaccine treatment. Since the required efficiency of vaccines has not been reached yet, it is essential to determine the optimal conditions of *in vitro* DC modulation, enabling long-term specific immune response against cancer. The aim of the study was to optimize the procedure of anti-tumoural immunogenic dendritic cells production and to identify the criteria for quality evaluation of the dendritic cell product. In this study, comprehensive evaluation of *in vitro* modulated autologous dendritic cells was performed for the first time by assessing tolerogenic and immunogenic potential and functional cytokine secretion. Also, for the first time in the production of the human dendritic cell product, novel bacterial adjuvants – Bacterial Ghosts – were used due to their dual properties as a tumour antigen transport system and as an immune adjuvant for dendritic cell maturation.

THE COLLAGENOLYTIC ACTIVITY ANALYSIS OF GEOBACILLUS THERMOLEOVORANS DSM 15325 PEPTIDASES: CHARACTERIZATION OF M3 PROTEOLYTIC ENZYMES FAMILY M3B SUBFAMILY OLIGOPEPTIDASE AND U32 PROTEOLYTIC ENZYMES FAMILY PEPTIDASE (Biology)

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Dissertation defended:
1 July 2016

A full understanding of laws of nature cannot be accomplished without comprehensive characterisation of collagenolysis as collagen is a major structural protein in organisms. The diversity of collagenolytic peptidases from non-pathogenic bacteria remains only fragmentarily characterised, whereas the characterisation of the diversity of collagenolytic peptidases from eukaryotes and pathogenic bacteria are far more comprehensive. The determined constitutive production of collagenases by moderate thermophile Geobacillus thermoleovorans DSM 15325 confirms the ability of thermophiles to be ever ready for collagen uptake. The identification of constitutive collagenolytic peptidases variety produced by geobacilli lead to a substantial development of collagen degradation model and confirms the importance of collagenolytic potential for adaptive plasticity of bacteria. Determined characteristics of M3 family M3B subfamily oligopeptidases, secreted by geobacilli, indicate the nutritional importance of oligopeptidases for bacteria during the adaption for environmental changes. Combination of oligopeptidases characteristics also outlines biotechnological applicability of this thermostable hydrolase. Determined U32 family peptidase from geobacilli characteristics ensure objective understanding of U32 family peptidases characteristics and functional importance. The ability of U32 family peptidases to specifically interact *in vitro* with dsRNA has never been determined for collagenolytic peptidases previously.

MICROBIOLOGICAL AND MOLECULAR CHARACTERIZATION OF CLINICAL CARBAPENEM-RESISTANT *PSEUDOMONAS AERUGINOSA* ISOLATES (Biology)

Greta Mikučionytė

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Dissertation defended:
23 August 2016

Multidrug resistance among *P. aeruginosa* has become a worldwide problem of major importance. The most worrisome emerging resistance feature corresponds to the increased production of carbapenem-hydrolysing β-lactamases, particularly those encoding class B carbapenemases (or metallo-β-lactamases [MBLs]). The most frequently found MBLs in *P. aeruginosa* are VIM and IMP type enzymes which confer resistance not only to carbapenems but also to almost all available β-lactams and, thus, lead to a treatment failure with increased morbidity and mortality. The aim of this work is to determine molecular mechanisms of resistance to carbapenems in *P. aeruginosa* strains isolated from different clinical specimens. Despite the increasing rate of carbapenem resistance in Lithuania, there is still a lack of a comprehensive understanding of the *P. aeruginosa* mechanisms controlling the outbreaks of antimicrobial resistance. This study was the first to describe the molecular mechanism of carbapenem resistance in *P. aeruginosa* isolates from Lithuania. Our results showed that more than 40% of imipenem- and/or meropenem-resistant isolates possessed MDR and XDR phenotypes. In this work, we report for the first time a detailed genetic analysis demonstrating that MBL VIM-2 was by far the most prevalent acquired β-lactamase in the Hospital of Lithuanian University of Health Sciences. Moreover, a high prevalence of VIM-2 was found to be driven by the dissemination of high-risk clone ST235. Thus, our work adds Lithuania to the growing list of countries documenting epidemic dissemination of this clonal lineage linked to potent β-lactamases. Additionally, our work resulted in the identification of a new GES variant, referred to as GES-27, which has not been described previously.

A COMPARATIVE STUDY OF SINOATRIAL NODE MORPHOLOGY AND INNERVATION (Biology)

Hermanas Inokaitis

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Dissertation defended:
25 August 2016

The autonomic nervous system modulates the activity of cardiac conductive system and its abnormal activity may lead to arrhythmias or even to a sudden death. The sinoatrial node is the primary pacemaking component that generates the electric impulse in the heart. Morphology and neuromorphology of sinoatrial node have been an object of interest since it was discovered. Hearts of mice, rabbits and pigs are widely used in cardio-physiological experiments. To date, there are no studies that analyze both, the distribution of pacemaker cells and their innervations in these species. Also, none of the studies examine the interspecific differences of these structures in the aforementioned cardio-physiological models. The aim of this study was to identify the limits and innervation of the sinoatrial node in the hearts of mice, rabbits, and pigs by using a multiple imunohistochemical staining for pacemaker cells and neural components. The results showed interspecific anatomical and neuroanatomical similarities and differences. The study showed that the distribution of pacemaker cells is much wider and the innervation of sinoatrial node cells is more complex than it was considered before. In addition to this, the immunohistochemical analysis of the neurons within the sinoatrial node was performed for the first time. Finally, a novel finding – nitrergic neurons that were purely positive for neuronal nitric oxide synthase – was observed in this study.

CHARACTERIZATION AND APPLICATION OF KERATINOLYTIC PEPTIDASES FROM *GEOBACILLUS* SP. AND *BACILLUS* SPP. (Biology)

Audrius Gegeckas

Scientific supervisor:
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Dissertation defended:
5 September 2016

Solid keratin-rich waste management is one of essential research area in nowadays. Conventional chemical and high thermal keratin waste decomposition methods are fully explored and not enough effective for future biotechnolgy perspectives. However, traditional keratin-rich waste decomposition methods could be replaced by environmentally-friendly and economical microbial keratin waste biodegradation methods without energy wastage and essential amino acids and nutrition elements loss. Therefore, microbial bio-decomposition are attractive approach to keratin or keratin-like waste manage without any nutrition loss in eco-friendly process environment. The aim of the dissertation work is to evaluate application possibilities of a newly

identified keratinolytic peptidases in biodegradation processes of proteinaceous materials. Increased attention has been diverted to these keratinolytic peptidases because of their important potential uses in biomedicine, pharmaceutics, cosmetics and keratin waste bioconversion industries associated to the hydrolysis of keratin. Keratinolytic proteinases are next generation molecular tools for keratin hydrolysis and production of small value-added bio-active peptides. For the first time, we identified, purified and characterized native keratinolytic peptidase (BtKER) from Bacillus thuringiensis AD-12. For the first time, we created chimeric (SynKer-TT and SynKer-TM) keratinolytic peptidases with improved physicochemical properties. Eventually, we demonstrated that metabolically active secretomes from keratinolytic microorganisms can be used for eco-friendly enzymatic keratin waste management in lab-scale system.

BIOLOGICAL SIGNIFICANCE OF IGF2BP1 GENE EXPRESSION IN t(12;21)(p13;q22)-POSITIVE LEUKEMIA MODEL (Biology)

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Dissertation defended:
29 September 2016

IGF2BP1 belongs to a family of evolutionary highly-conserved regulatory RNA-binding proteins with an oncofetal expression pattern. While the growing body of data supports the involvement of IGF2BP1 and other family members in different types of epithelial and soft tissue tumours, still little is known about their role in malignant hematopoiesis. The data from this research demonstrate that overexpression of IGF2BP1 is a unique and characteristic marker of t(12;21)(p13;q22) ETV6/RUNX1-positive acute lymphoblastic leukemia (ALL), but not of other acute and chronic malignancies of myeloid and lymphoid origin. This study also presents a novel finding of the involvement of IGF2BP1 protein in ETV6/RUNX1-mediated leukemogenesis as ETV6/RUNX1 and STAT3 transcripts are newly identified targets of IGF2BP1 in REH cells – a model cell line of this ALL entity. Downregulation of IGF2BP1 attenuates proliferation and survival of REH cells and augments sensitivity to treatment with STAT3 selective inhibitor S3I-201. This study frames a potential role of IGF2BP1 protein in ETV6/RUNX1-mediated leukemogenesis and justifies further research in this field.

SKELETAL MUSCLE MASS AND SPECIFIC FORCE: EFFECTS OF GENETIC BACKGROUND AND MYOSTATIN DYSFUNCTION IN RESPONSE TO HYPERTROPHIC AND ATROPHIC STIMULI IN MICE (Biology)

Petras Minderis

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Dissertation defended:
29 September 2016

The skeletal muscle is an abundant tissue comprising 40–50% of the body mass in humans. Its main role is to generate force for posture maintenance and performance of daily movements, but it also acts as a metabolically active organ. The aim of this research was to examine the effects of genetic background and myostatin dysfunction on specific force and mass of skeletal muscles after muscle hypertrophy and atrophy. Conclusions: Study 1: 1. Skeletal muscles of mouse strains vary significantly in muscle mass and force generation capacity. Specific muscle force, however, does not change with increase in body size and as a result of muscle growth during maturation. 2. DUH background shows lower specific muscle force compared to both BEH+/+ and C57BL/6J backgrounds. Study 2: 1. Myostatin dysfunction is associated with increased muscle mass, but reduced specific muscle force. 2. Myostatin dysfunction is also associated with reduction in hypertrophy and impairment in force generation capacity of soleus muscle after functional overload. Study 3: 1. Skeletal muscle atrophy after food deprivation is dependent on myostatin dysfunction, genetic background and muscle properties. Myostatin dysfunction is associated with a greater loss of muscle mass. C57BL/6J background is more susceptible to muscle atrophy than BEH+/+ background under these conditions. 2. After food deprivation, the slower contracting soleus shows less severe muscle atrophy, but greater loss of specific muscle force compared to a faster contracting extensor digitorum longus.

ROLE OF CUGBP2 AND HuR MEDIATED POST-TRANSCRIPTIONAL REGULATION IN ANTI-CANCER DRUGS RESISTANCE OF PANCREATIC ADENOCARCINOMA (Biology)

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30 September 2016

Pancreatic cancer is the eighth-ninth most frequent cause of cancer death worldwide, and the fourth-fifth in developed countries. As patients with pancreatic cancer have a median survival of six months from diagnosis, recent efforts have focused on detecting disease at an earlier stage as a way to improve survival. Despite the sustained effort to develop new agents, none of the currently available chemotherapeutic agents have an objective response rate higher than 10%. The unchanged outcomes of pancreatic cancer treatment over the decades mandate further research to develop novel therapeutic agents. Post-transcriptional regulation is a very important mechanism to control gene expression in changing environments and is increasingly recognised to be a powerful, general determinant of the quantitative changes in proteomes, and, therefore, a driving force for cell phenotypes. Gene expression is intricately regulated at the post-transcriptional level by RNA-binding proteins (RBPs) via their interactions with mRNA during development. The impact of RBPs on the expression of cancer-associated genes is well recognised. The aim of this study is to assess the effects of post-transcriptional regulation in the human pancreatic cancer cell lines through the expression of cytoprotective molecules and *in vitro* response to gemcitabine (GEM) treatment.

AEROBIC CAPACITY AND STRENGTH RELIANCE ON TRAINING PROGRAMS IN PERSONS WITH SPINAL CORD INJURY (Biology)

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Dissertation defended:
30 September 2016

People with spinal cord injury (SCI) often suffer from some motor, sensory, and autonomic impairments. It predisposes individuals to multisystem dysfunction, leading to an increased likelihood of a range of related secondary complications defined as medical consequences that can cause functional limitations. The degenerative process may be reversible through exercise training. The aim of this study is to analyze the aerobic capacity of and strength reliance on training programs in persons with spinal cord injury. Conclusions: (1) During moderate intensity workload, persons with spinal cord injury involved in sports indicated faster oxygen consumption kinetics than participants with spinal cord injury not involved in sports. Persons with the spinal

cord injury involved in sports showed higher system ability to adapt to energy demands associated with the exercise load. (2) Before participating in the endurance training program, the wheelchair basketball squad had already attained relatively high levels of aerobic fitness. Nevertheless, the high-volume, moderate-intensity, short-term training program, which evolved over the two-week period, resulted in an improvement in the athletes' aerobic endurance. (3) Swimmers with paraplegia involved in 12-week combined strength on dry land and resistance training in the water program improved strength, swimming performance and stroke parameters in comparison with swimmers training only in the water.

SACCHAROMYCES CEREVISIAE K2 KILLER SYSTEM: INTERPLAY BETWEEN KILLING AND RESISTANCE (Biology)

Juliana Lukša

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30 September 2016

The rapid propagation of viruses, their variability, and adaptation to different hosts are critical factors for the spread of pathogens and a threat to health, economic and agricultural progress. Currently, extensive studies of yeast viral killer systems are underway in order to determine the molecular-physiological characteristics of the secreted toxins, to reveal the mechanisms of killing-immunity formation, to establish the interaction of virus or its agent to the host cell, and to define this process affecting the abiotic and biotic environment. One of the most developing areas of functional genomics is the establishment of host factors supporting or inhibiting the replication of the virus and conditioning the action of viral products. For this type of research, *S. cerevisiae* yeasts and their viral-originated killer system are routinely selected as a model organism. The aim of the research was to investigate the impact of the external environment on the functioning of *Saccharomyces cerevisiae* K2 toxin and to establish the importance of target-cell genetic factors for efficient cytotoxic activity and resistance formation. In this study, the mechanism of the action of *S. cerevisiae* K2 toxin at the cell wall level was revealed, the influence of external environment changes to the functioning of K2 protein was evaluated, and the cellular genetic factors important for killing process and those involved in resistance to K2 toxin formation were identified.

GENETIC STRUCTURE, ADAPTATION AND INVASIVENESS OF DAISY FLEABANE (*ERIGERON ANNUUS* (L.) PERS.) POPULATIONS (Biology)

Virginija Tunaitienė

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Dissertation defended:
21 October 2016

Biological invasion by alien species is recognized as a significant component of global environmental change in our planet. The rate of spread of invasive species is growing due to increasing migration and international trade. The number of invasive species in Europe is growing almost exponentially. Invasive plant species cause not only serious environmental, economic, but also human health impacts. One of the invasive species, daisy fleabane (*Erigeron annuus* (L.) Pers.), is the subject of this dissertation. It originated from the eastern part of North America. It seems likely it was introduced to Lithuania from Western Europe at the end of the 19th century as an ornamental plant. The genetic and genotypic structure of Lithuanian invasive *E. annuus* populations was evaluated using two types of DNA markers (RAPD and ISSR). ISSR analysis showed the existence of *E. annuus* genetic diversity gradient in the part of the invasive European range and revealed statistically significant genetic differentiation between *E. annuus* populations occurring in disturbed and stable habitats. Although *E. annuus* shows high phenotypic plasticity in its morphology and phenology, which helps to survive and spread in new habitats, a genetically determined local adaptation occurs in some populations.

ENGINEERING AND BIOCATALYTIC PROPERTIES OF LIPASES AND ESTERASES PRODUCED BY *GEOBACILLUS* BACTERIA (Biology)

Renata Gudiukaitė

Scientific supervisor:
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Prof. Dr. Donaldas Jonas Čitavičius,
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Dissertation defended:
4 November 2016

Lipases and esterases produced by *Geobacillus* bacteria is a promising and important area in basic research and industrial applications. In this work the significance of Asp371, Phe375, and Tyr376 from C- terminal region for the efficient functionality of *Geobacillus* sp. 95 lipase (GD-95) was shown for the first time and a new carboxylesterase (GDEst-95) produced by *Geobacillus* sp. 95 strain with a molecular size of 55 kDa was identified. In further experiments GDEst-95 esterase together with GD-95 lipase were used for the construction of the first fused lipolytic chimeric biocatalyst GDEst-lip. Because of their physicochemical and kinetic properties GDEst-95 esterase and fused GDEst-lip enzyme have a high potential for application in various industrial areas. This work also demonstrated that identical domain fusion strategy (GDLip-lip and GDEst-est) is useful for creating new

biocatalysts. It was shown that usage of several fused domains can modulate the activity and physicochemical characteristics of target enzymes for industrial applications. In this study, three new *Geobacillus* lipases were also identified. These proteins expanded the existing information about physicochemical properties of *Geobacillus* lipases. Furthermore, genes of *Geobacillus* lipases were subjected to DNA shuffling and epPCR experiments to create more thermostable and more thermoactive lipolytic enzymes.

THE DISTRIBUTION OF ARTIFICIAL RADIONUCLIDES IN DIFFERENT COMPONENTS OF AQUATIC AND TERRESTRIAL ECOSYSTEM UNDER VARIOUS ENVIRONMENTAL CONDITIONS (Biology)

Olga Jefanova

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Dissertation defended:
18 November 2016

The aim of this study is to determine the peculiarities of the accumulation and distribution of ^{137}Cs, ^{60}Co, ^{54}Mn and ^{40}K in biotic and abiotic environmental components of aquatic and terrestrial ecosystems and to assess the biological effects on test organisms caused by ^{137}Cs and the sum of various stressors arising from the nuclear facilities. This study aims to determine (1) the accumulation and distribution of artificial radionuclides in biotic and abiotic components of Lake Drūkšiai as the cooling pond of the Ignalina NPP (Nuclear Power Plant), (2) the components of the terrestrial ecosystem in the Ignalina NPP region after its official decommissioning and of terrestrial ecosystems of three Lithuanian districts that were contaminated to varying degrees after the Chernobyl accident. One of the aims of the study was to describe the existing radioecological condition of the system in the basin of the Neris-the Nemunas-the flooded Nemunas meadows-the Curinian Lagoon before the commissioning of the NPP in Belarus. For assessing the long-term radioecological situation of terrestrial ecosystems, it is proposed to use moss from forest habitats. It sensitively represents the environmental contamination with radionuclides. These research data are important to assess the ecotoxicological situation in Lake Drūkšiai after the shutdown of Ignalina NPP. For the first time the investigation of the effects of ^{137}Cs on the membrane bioelectrical properties of *Nitellopsis obtusa* cells was performed.

GENETIC VARIABILITY OF *IXODES* TICKS IN BALTIC COUNTRIES (Biology)

Eglė Galdikaitė-Brazienė

Scientific supervisor:
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Dissertation defended:
16 December 2016

Two closely related tick species, *Ixodes ricinus* (Linnaeus, 1758) and *Ixodes persulcatus* (Schulze, 1930), play a major role in the transmission of tick-borne diseases, in particular Lyme borreliosis, tick-borne encephalitis, anaplasmosis, babesiosis, tularaemia, and rickettsiosis in Europe including the Baltic countries. The Baltic countries are situated in a unique area, right on the edge of *I. persulcatus* distribution range, sympatric for both (*I. ricinus* and *I. persulcatus*) tick species. It has been proposed that sympatry of vectors has an effect on the groups of pathogens biologically associated with them: *I. persulcatus* ticks in the zone of sympatry significantly enhance *Borrelia* circulation among the *I. ricinus* ticks and pathogens exchange tick vectors. There were no data on the genetic variability of *I. ricinus* ticks in allopatric and sympatric zones in the Baltic countries. The comparison between *Ixodes ricinus* genetic variability, using mtDNA control region, 16S rRNA, and cytb gene sequence analysis, in sympatric and allopatric zones for the first time show differences between *I. ricinus* ticks in these zones, revealing a high number of specific haplotypes in each zone. The genetic diversity of *I. ricinus* ticks was for the first time compared in Norway and the rest of Europe, showing Norwegian ticks genetically separated. *Ixodes inopinatus* (Estrada-Peña, Nava and Petney, 2014) tick species was identified for the first time in Lithuania, Latvia, Estonia, and Norway.

THE APPLICATION OF *HCN2* EXPRESSING HUMAN MESENCHYMAL STEM CELLS FOR THE DEVELOPMENT OF BIOLOGICAL PACEMAKER (Biology)

Ieva Bružauskaitė

Scientific supervisor:
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Dissertation defended:
22 December 2016

More than 50% of various types of heart dysfunctions become the main cause of death in Lithuania annually. Most of them are associated with an abnormal electric function of the heart, which subsequently causes rhythm disorders. The development of a functional biological pacemaker could become an alternative treatment to electronic heart implants. The aim of this study was to characterize the vital and functional features of human mesenchymal stem cells transfected with *HCN2* gene and their ability to form intercellular connections through the pores of chosen scaffolds for the development a functional biological pacemaker. This scientific study evaluated the suitability of HCN2 expressing BM-MSC for the generation of a biological pacemaker and

focused on the analysis of cell proliferation, migration, viability and growth on artificial scaffolds. Significant changes in proliferation, migration and cell cycle were identified in *HCN2*-transfected BM-MSC. The impact of *HCN2* channel on the expression of transcription factors regulating cell cycle has for the first time been evaluated by microarrays. The functional (electrical) activity between *HCN2*-transfected stem cells and other cells through formed gap junctions and tunneling nanotubes has been also observed. The main problem in the development of a functional biological pacemaker is cell migration from the injected area. In our opinion, one of the most effective solution could be the closure of the *HCN2*-transfected cells into microscaffold hereby fixing them at rhythm generation side, which prevents migration of cells but maintains their long-term viability and functioning. The best suited size and shape of pores was estimated preventing migration of transfected MSC through Kapton® scaffold, but allowing their contact with the cells residing on the other scaffold side of the scaffold.

PHOTOPHYSIOLOGICAL REACTIONS OF GARDEN CRESS LEAVES IN SIMULATED MICROGRAVITY CONDITIONS (Biology)

Regina Losinska-Sičiūnienė

Scientific supervisor:
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Dissertation defended:
29 December 2016

Gravity and light are two of the most important natural environmental factors: the first is the least-changing factor on the Earth, and the other is constantly changing. The influence of these factors on the spatial orientation, growth, development and productivity of plants is undeniable. Microgravity (MG) is a factor leading to the abiotic stress and physiological, biochemical and gene expression in plants, whose long-term influence on plant growth, development and productivity are still difficult to predict. The aim of the research was to evaluate photophysiological reactions of the leaves of garden cress seedlings to differential and complex illumination by light spectrum components in simulated microgravity conditions. Summing up the obtained results, it can be suggested that complex red and blue lighting in MG conditions had a negligible effect on the spread of the leaves. MG conditions reinforced the response to the R+B lighting and directed the leaves to the light source. Complex red and far-red lighting in MG and 1g environment had no effect on the spatial orientation and the phototropical response of the leaves. The data confirmed the assumption that differential blue, far-red, and complex red with a blue effect has a stronger tropical expression in MG

conditions. These data reveal that the blue light stimulates a positive phototropic reaction to cotyledons regardless of the gravitational environment, but in MG conditions the leaves were moving much faster. The UV–A radiation has a phototropic effect on the leaves, which reveals under MG conditions. In normal gravity conditions, the movement of the leaf toward the light stimulus is not significant. MG conditions increase the effect of the UV–A radiation on the leaves. Short-term lighting of UV–A+B in normal gravity conditions had a slight positive effect on the leaves. Such lighting revealed a positive phototropic effect in MG conditions, although at the beginning of the test the leaves were moving in the opposite direction to the light source.

INVESTIGATION OF THE INFLUENCE OF YEAST *SACCHAROMYCES CEREVISIAE* KILLER TOXIN K2 ON THE ENVIRONMENTAL MICROORGANISMS (Ecology and environmental)

Irma Orentaitė

Scientific supervisor:
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Dissertation defended:
10 June 2016

K2 toxin-producing yeast *Saccharomyces cerevisiae* is widely used in wine industry, but the toxin has been little studied. It is known that toxin K2 kills sensitive yeasts, but our results indicate that it also has a negative effect on the growth of gram-positive bacteria. To investigate the K2 toxin influence on environmental microorganisms further, we adapted a robotic system and performed a genome-wide screening procedure to detect candidates of mutants involved in K2 resistance/hypersensitivity. We indicated that *S. cerevisiae* genes associated with the cell envelope and its biogenesis, stress signaling, pH or ion homeostasis are important for the action of K2 toxin. The mitochondrial dysfunction leads to increased resistance to this toxin. We focused on the physiological changes induced by K2 toxin on a non-toxin-producing yeast strain as well as K1, K2 and K28 killer strains. Potentiometric measurements were adjusted to observe that K2 toxin immediately acts on the sensitive cells leading to membrane permeabilization and the efflux of potassium ions. This correlated with reduced respiration activity, lowered intracellular ATP content, and decrease in cell viability. However, we did not detect any significant ATP leakage from the cells treated by killer toxin K2. The killing effect of K2 toxin is connected to the observed differences in respiratory activities between the killer strains and the non-toxin-producing strain at a low pH. This might have practical consequences in wine industry: K2 toxin producing yeast could be beneficial in controlling contaminating yeasts and non-beneficial causing sluggish fermentation.

FORMATION OF GENETIC STRUCTURE OF RACCOON DOGS (*NYCTEREUTES PROCYONOIDES*) POPULATION IN THE INVADED TERRITORIES AND THEIR IMPACT ON ECOSYSTEM (Ecology and environmental)

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Scientific supervisor:
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Dissertation defended:
28 June 2016

The raccoon dog *Nyctereutes procyonoides* (Gray, 1834) is a newly established alien species that has had a long and complicated history of introduction and immigration within Europe. Detailed studies of genetic diversity, population structure, phylogeography, and their impact on the environment of raccoon dogs are lacking. Nothing is known about genetic diversity and population structure of invasive raccoon dogs inhabiting colonized areas in the Baltic region. The aim of the research was to investigate the genetic structure of raccoon dog populations in invaded territories and assess the impact on the ecosystems. The analysis of the mtDNA control region in raccoon dogs demonstrated that the raccoon dog population from Lithuania has a higher genetic diversity compared with those from the places of secondary introduction, but a lower genetic diversity compared with raccoon dogs from autochthonous populations. For the first time we have investigated the impact of the anthropogenic pressure on the formation of the population structure and the genetic diversity of raccoon dogs using microsatellite markers. The results indicated a high level of genetic variation observed within subpopulations, whereas a low level of variation portioned among subpopulations and showed significant differences between west-east subpopulations. The findings of the study showed that raccoon dogs can be a vector for a variety of pathogens: *Babesia microti*, *Rickettsia helvetica*, *Rickettsia monacensis*, *Bartonella* spp., *Borrelia afzelii*, *B. myiamotoi, B. valaisiana* and *Anaplasma phagocytophilyum*.

DRINKING AND RECREATIONAL WATER CONTAMINATION BY MICROORGANISMS IN KAUNAS AND MARIJAMPOLĖ REGIONS (Ecology and environmental)

Daiva Staradumskytė

Scientific supervisor:
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Dissertation defended:
28 June 2016

Water is the most important source of life for humans and wildlife. Drinking water pollution is a major problem in Lithuania and the rest of the world. Private water wells and wells used for central supply in rural areas are particularly sensitive to pollution. Lithuania has sufficient water resources, but it is very important to ensure good quality of water. As a result of pollution, water quality is deteriorating. This restricts the use of water and poses a threat to our health and the functioning of aquatic ecosystems. Furthermore, this reduces the number of water sources available for consumption. Earlier studies conducted in Lithuania evaluated the microbiological quality of drinking water only on the basis of the legislation on quality requirements for drinking water (HN 24: 2003), i.e. it was evaluated according to the three microbiological indicators: coliform bacteria, *Escherichia coli*, and faecal enterococci (*Enterococcus faecium*) count (microbiologically clean water should not contain any microorganisms). In addition to these three indicators of faecal water contamination, the present research identifies, for the first time in Lithuania, other microorganisms, such as *Pseudomonas* spp., *Aeromonas* spp., *Acinetobacte*r spp., and bacteria of CDC group No-1 using molecular methods.

COUPLING OF CAPILLARY ELECTROPHORESIS WITH REACTION DETECTION FOR EVALUATION OF COMPOUNDS REDUCING OXIDATIVE STRESS (Ecology and environmental)

Mantas Stankevičius

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Dissertation defended:
30 June 2016

Coupling capillary electrophoresis with reaction detection is very useful since it would offer a possibility to assess complex natural mixtures and would allow evaluation of antioxidant activity of separate components in an automated manner and micro-format. It is expected to be a fast, inexpensive, and non-complex process that would allow effective investigation and classification of antioxidants in herbs, food products, food supplements, pharmaceuticals, and biological objects. Three unique interface set-ups of capillary electrophoresis and radical scavenging reaction detection have been created and optimized. The first interface was created using stainless steel cross connection. The second

interface was created by immersing two coaxially inserted capillaries of different diameter into the vial with the radical reagent. The third interface was created by connecting two capillaries and creating a gap between them. Radical scavenging activity on-line method has been performed by capillary electrophoresis-based analysis coupled with reaction detection for the first time. This method is created with the aim of using less environment-polluting chemical reagents and is suitable for quantitative and qualitative evaluation of potential antioxidants in food products, food supplements, biotechnological products, plants, cell cultures, and pharmaceutical products, which are closely related to the oxidative stress of the biological systems.

IMPACT OF THE KEYSTONE SPECIES, THE EURASIAN BEAVER (*CASTOR FIBER*), ON HABITAT STRUCTURE AND ITS SIGNIFICANCE TO MAMMALS (Ecology and environmental)

Arūnas Samas

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Dissertation defended:
26 September 2016

The Eurasian beaver (*Castor fiber*) is a representative of the family Castoridae that includes two living species of the genus *Castor*. Beavers are the keystone species, also deservedly known as the ecosystem engineers, for the ability to create new habitats and to change the existing landscape. The biology of the Eurasian beaver has been well studied, but there is still a lack of knowledge about the ecology of the species: the relationship with the surrounding environment and biota. These studies were carried out in hilly morainic landscape of eastern Lithuania. The effect of the Eurasian beaver on the habitat structure was analysed through the prism of its impact on water bodies and terrestrial ecosystems. The density of beaver sites, which reaches 26.1 bst./1000 hectares, is one of the greatest in Lithuania and the extent of the impact can reach up to 12% of the study area. Also, the impact of the Eurasian beaver on different groups of mammals (ungulates, medium-size predators, semi-aquatic and small mammals) has been studied. It was found that the roe deer (*Capreolus capreolus*), the red fox (*Vulpes vulpes*), all semi-aquatic and small mammals typical of forest habitats, such as the bank vole (*Clethrionomys glareolus*) and the yellow-necked mouse (*Apodemus flavicollis*) have greatest benefits from the Eurasian beaver. The roe deer benefits from the increased heterogeneity of the landscape, while the semi-aquatic and small mammals benefit from such beaver infrastructure elements as lodges and burrows.

BIRCH SP. HEALTH AND GROWTH UNDER CURRENT AND SIMULATED CLIMATE CONDITIONS (Ecology and environmental)

Valda Araminienė

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Dissertation defended:
14 October 2016

This dissertation investigated how *Betula pendula* Roth, the most common birch species in Lithuania, would grow under changed climatic conditions and how it would respond to a different intensity of foliar damage. The responses of silver birch seedlings to the damages of different intensity and type and their ability to recover were determined. The compensatory growth opportunities, morphological changes, and physiological processes of defoliated birch seedlings were investigated. The studies have shown that defoliated silver birch seedlings were able to compensate for the lost foliage during one growing season, and a severe foliage loss caused stronger compensatory growth.

NON-INDIGENOUS SPECIES IN THE MEDITERRANEAN MARINE PROTECTED AREAS: DIVERSITY, DISTRIBUTION AND IMPACTS (Ecology and environmental)

Martina Marič

Scientific supervisor:
Prof. Habil. Dr. Sergei Olenin, Klaipėda University

Dissertation defended:
2 December 2016

The aim of the study is to assess the diversity and distribution of non-indigenous species (NIS) and their impacts in Mediterranean Marine Protected Areas (MPAs). The Mediterranean Sea is heavily affected by biological invasions and marine protected areas are not immune to NIS introductions and spread. However, the effects of MPAs on the establishment and spreading of NIS are generally unrecognized. In this study, the distribution of NIS and the effect of MPAs on the establishment and spread of NIS was assessed using rapid assessment, inside and outside some selected Mediterranean MPAs. Furthermore, trophic interactions between the indigenous benthic taxa and the non-indigenous species such as the green alga *Caulerpa cylindracea*, the red alga *Asparagopsis taxiformis*, the crab *Percnon gibbesi* and the sea hare *Aplysia dactylomela* were investigated using stable isotope rations and subsequent isotopic populations metrics in order to determine the role of NIS in invaded ecosystems and the possible impacts it has on indigenous taxa.

LIFE-HISTORY TRAITS, FUNCTIONAL ROLE AND GENETIC VARIABILITY OF *POTAMOPYRGUS ANTIPODARUM* (J. E. GRAY, 1853) IN LITHUANIAN MESOTROPHIC LAKE ECOSYSTEMS (Ecology and environmental)

Rokas Butkus

Scientific supervisor:
Prof. Dr. Kęstutis Arbačiauskas,
Nature Research Center

Dissertation defended:
16 December 2016

Invasive species are one of the most important factors that not only affect biodiversity of local species, but also have a significant effect on the structure and functioning of the native community. There are four non-indigenous freshwater mollusc species recorded in Lithuanian inland waters, and two of them are considered invasive species. One of the invasive species is *Potamopyrgus antipodarum*, which is rapidly spreading and having a hight demographic potential species in an invasive range. Despite the fact that the species was firstly recorded in the Curonian lagoon, more than 60 years ago, the data on species distribution in Lithuanian inland waters, genetic variability, life-history and population traits and functional role in lake ecosystems is very limited. During this study, it was estimated that species is rapidly spreading across the Lithuanian inland waters – during the last 6 years the number of invaded ecosystems increased from 2 to 18. Also, this study indicated that according to mitochondrial and microsatellite DNA populations of *P. antipodarum* are monomorphic. As it was expected, studies in mesotrophic lake ecosystems revealed, that New Zealand mudsnail is primary consumer which relies mainly on littoral primary production. Also the study indicated hight density and secondary production of the snail for Lithuanian freshwater ecosystems. Both density and secondary production varied not only throughout the year, but also between the lakes. It was also estimated, that despite benthivorous fish species consumed the snail in laboratory conditions, these fishes avoid feeding on *P. antipodarum* and can hardly significantly contribute to the regulation of its density in field during the initial stages of the invasion.