Poster Presentations Session 1

I. Microbial Biotechnology

I.P.1

Oxygen and thermal stress tolerance of *Zymomonas mobilis* respiratory mutants

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Zymomonas mobilis is an ethanologenic bacterium with desirable characteristics for metabolic engineering and industrial use. It contains a constitutive respiratory chain which provides NAD(P)+ regeneration, contributing to the rapid catabolism of this bacterium. Although *Z. mobilis* exerts the most important traits desired in bioproducers, improvements are still required. In recent years elevated thermotolerance has been one of directions in focus. It has been shown, that respiratory chain plays important role for the thermotolerance in *Zymomonas mobilis* [1]. In this study we examined collection of respiratory mutants of *Z. mobilis*.
Zymomonas mobilis strains Zm6 (ATCC29191) and eight of its respiratory mutant derivates were grown in four different conditions: 30°C aerobic (in shaken flasks), 30°C microaerobic (without shaking), 42°C aerobic and 42°C microaerobic, to compare the oxygen and thermal stress impact on each of these strains. Strain growth, ethanol production and oxygen consumption were monitored.
Overexpression of cytochrome c peroxidase improved growth at 30°C under microaerobic conditions. However, cytochrome c peroxidase and NADH dehydrogenase double knock-out strain showed impaired growth under 42°C aerobic conditions, when compared to the NADH dehydrogenase single knock-out. Unexpectedly, overexpression of cytochrome c peroxidase highly reduced oxygen consumption rate, pointing to yet unknown regulatory effects. NADH dehydrogenase knock-out mutation elevated thermotolerance, but knocking out the respiratory lactate dehydrogenase against the NADH dehydrogenase-deficient background reduced this effect.

Keywords: *Zymomonas mobilis*, thermal stress, respiratory chain

References:

1. Hayashi T, Furuta Y, Furukawa K (2011) *J Biosci Bioeng* **111**: 414–419. doi: 10.1016/j.jbiosc.2010.12.009 PMID: 21236727

I.P.2

Rapid intracellular lipid quantification in oleaginous yeast with FT-NIR

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In oleaginous yeasts, lipid extraction for quantification often requires strong acids and harmful organic solvents and is laborious and time consuming. Therefore, in most cases just end-point measurements of lipid accumulation are performed, and kinetics of intracellular lipid accumulation is difficult to follow. To overcome the disadvantages of lipid extraction, a less laborious and faster non-invasive method was developed for intracellular lipid quantification, using Fourier transformation near infrared (FT-NIR) spectroscopy. To build a predictive model using PLS regression, a calibration sets was constructed from spectra collected on freeze-dried cells of the oleaginous basidiomycetous yeast *Rhodotorula toruloides* CBS 14. The R^2 of the *R. toruloides* model was 98.16 and the RMSECV was 1.7. This model was then extended to also include the ascomycetous yeast *Lipomyces starkeyi* CBS 1807. The derived models were applied to predict the total fat content in freeze-dried samples of the yeasts. Species-specific differences in the FT-NIR spectra were found. The results demonstrated that the FT-NIR based lipid quantification fits very well to values obtained from usually applied lipid extraction methods. The developed FT-NIR-based lipid quantification method allows a higher throughput than traditionally used extraction methods, making it possible to in much higher resolution follow the kinetics of lipid accumulation during fermentation experiments.

Keywords: oleaginous yeast, FT-NIR, intracellular lipid, lipid extraction

I.P.3

Insight into the chitinolytic enzyme system of *Bacillus cereus* sensu lato isolates

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Surpassed only by cellulose, chitin is the second most abundant polysaccharide on earth. It occurs in insect exoskeleton, crustacean shells or the cell wall of fungi. For many microorganisms chitin is a major source of carbon and/or nitrogen. *Bacillus cereus sensu lato* comprises soil-dwelling endospore-forming bacilli which have a huge impact on human health and economy. Several studies have demonstrated that chitinases produced by *B. thuringiensis,* one of the most known members of this group, have proven to be extremely efficient tools for chitin degradation.
In this study 112 *B. cereus s.l.* strains (*B. thuringiensis, B. cereus,* and *B. mycoides*) isolated from diverse habitats in Northeastern Poland were characterized by Multilocus Sequence Typing (MLST). Chitinolytic activity of bacterial isolates was screened on the 10% colloidal chitin agar medium. Then, exo- and endochitinase activities were tested using Fluorimetric Chitinase Assay Kit. The full length coding regions of the endochitinase genes *(chiA* and *chiB*) were sequenced and compared with the phylogenetic tree constructed based on the concatenated loci from MLST.
Our results indicate that 68% of the tested strains hydrolyze the chitin polymer on minimal agar plates. Their chitinolytic enzyme system seems to be composed only from endochitinases which randomly cleave glycosidic bonds at interal siltes along the chitin chain and generating a variety of N-acetyloglucosamine (GlcNAc) oligomers. Sequence comparisons revealed four genetic types both *chiA* and *chiB* genes which corresponded well with phylogenetic relationship of soil isolates. In addition, non-chitinolytic clusters were defined, which is in line with the Cohan's definition of an ecotype.

Keywords: endochitinases, chitin degradation, MLST, *Bacillus cereus* group

Acknowledgemets
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I.P.4

Myrothecium sp. IM 6443 laccase – isolation, purification and preliminary characterization

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Laccases are blue multicopper oxidases characterized by broad substrate specificity and capability to oxidize a wide range of chemical compounds. Therefore, they have become valuable tools in many industrial processes and environmental protection.
In the presented work, we report the purification of extracellular laccase from a Cu-induced culture of ascomycetous fungus *Myrothecium* sp. IM 6443. The enzyme was precipitated by ammonium sulphate (80%) and concentrated by ultrafiltration using an *Amicon* unit (with a 10 kDa cutoff). The concentrated crude extract was purified in the FPLC System (ÄKTA start). First, it was applied to an ion exchange HiTrap Q column equilibrated with 0.05 M citrate phosphate buffer pH 7.2 and the protein was eluted in the gradient of NaCl. Fractions with the laccase activity were pooled, concentrated and loaded onto a gel filtration Superdex 200 10/300 GL column (GE Healthcare). Subsequently, the substrate specificity of the protein was determined based on the modified Eggert *et al.* (1996) method. The activity and stability of the enzyme in the presence of selected inhibitors and under different pH and temperature conditions were also examined. SDS-PAGE gel electrophoresis was performed to determine the purity of the protein and its molecular weight.
SDS-PAGE revealed the presence of one protein with a molecular mass of 77 kDa, which corresponded to the approximate weight of typical laccases**.** The enzyme showed the highest activity (measured using ABTS as a substrate) in the solution at pH 3.0 and it was the most stable at pH 7.0. The enzyme oxidized several substrates (e.g. ABTS, 2,6-dimethoxyphenol, syringaldazine, guaiacol). It was strongly inhibited by kojic acid.
The ability to oxidize a variety of substrates makes the enzyme a valuable tool in studies concerning xenobiotics elimination. The obtained results including purification and biochemical characterization of laccase will be useful in toxic textile dyes decolorization.

Keywords: laccase, purification, *Myrothecium* sp.

I.P.5

Biochemical characterization of novel *Staphylococcus saprophyticus* AG1 esterase belonging to procariotic hormone sensitive lipase-like enzyme family

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Background: Lipolytic enzymes of *Staphylococcus* sp. are implicated as possible virulence factors in pathogenesis of the bacteria of this genus, but, nevertheless, they can also be considered as potential industrial biocatalysts and only fragmented information is known about staphilococcal lipolytic enzymes as a biotechnological tool. In our study we found that *S. saprophyticus* AG1 exhibits high lipolytic activity and depending on environmental conditions secrets up to six different lipolytic enzymes, including hormone sensitive lipase-like esterase (Lip1) which in the present work was subjected for more detailed study.
Objectives: 1) Identification of the active lipolytic enzymes-secreting bacterium; 2) *in silico* analysis of *S. saprophyticus* AG1 lipolytic enzymes (*Lip*) coding genes and achievement of their recombinant forms, heterologous expression of Lip1 in *E. coli*; 3) determination of Lip1 biochemical properties.
Methods: Lipase-secreting bacterium was identified as *Staphylococcus saprophyticus* using 16S rRNA gene sequencing techniques and phylogenetic analysis. The species was confirmed by performing coagulase and novobiocin tests. Further, from the chromosomal DNA of *S. saprophyticus* AG1 *Lip1* gene was amplified, cloned and expressed in *E. coli* DH5α/BL21 (DE3), respectively, using pET-26b(+) vector. Target enzyme was purified by immobilized metal affinity chromatography in denaturing conditions from inclusion bodies after expression in optimal conditions determined. Biochemical properties, such as optimal temperature, pH, substrate specificity, thermostability, activity dependence on metal ions, surfactants, organic solvents, inhibitors and kinetic properties as V_{max}, K_M, k_{cat} and k_{cat}/K_M were determined using spectrophotometric assay with *p*-NP substrates. Regioselectivity (using 1,3-dipalmitoyl-2-oleoylglycerol as a substrate), hydrolysis and transesterification of natural fats were studied using thin layer chromatography.
Conclusions: Lip1 was determined to be alkaline esterase with optimal working temperature at 55°C and preference to hydrolyze short chain *p*-NP. Some of the used chemicals (metal ions, surfactants, organic solvents) hyperactivated the enzyme and its residual activity was measured to be more than 200% after the exposure to the mentioned chemicals. *Lip1* can be proposed as highly valuable catalyst with rationally designed activity and some properties using at least chemical modification.

Keywords: *Staphylococcus saprophyticus* AG1, hormone sensitvive lipase-like, esterase, biochemical characterization

I.P.6

Exploring carbohydrate active enzyme capabilities in *Euglena gracilis*

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A simple and easily accessible fluorescence-based methodology has been implemented to detect membrane-bound glycosyltransferases activities with potential replacement of traditional radiolabelled methods used in the field. Synthetic hexynyl α-D-mannopyranoside and its α-1,6-linked disaccharide counterpart were fluorescently labelled through CuAAC click chemistry with 3-azido-7-hydroxycoumarin. The resulting triazolyl-coumarin adducts, which were amenable to analysis by TLC, HPLC and mass spectrometry, proved to be acceptor substrates for α-1,6-ManT activities in mycobacterial membranes, as well as α- and β-GalT activities in trypanosomal membranes, benchmarking the potential of the fluorescent acceptor approach against earlier radiochemical assays. Following on to explore the glycobiology of the benign protozoan alga *Euglena gracilis*, α-1,3- and α-1,2-ManT activities were detected in membrane preparations, along with GlcT, Glc-P-T and GlcNAc-P-T activities. These studies serve to demonstrate the potential of readily accessible fluorescent glycans as substrates for exploring carbohydrate active enzymes.

Keywords: *Euglena gracilis*, Glycosyltransferases, Fluorescent glycans, *N*-acetylglucosamine-1-phosphate transferase, Enzyme assays

I.P.7

In Situ Product removal of 2-phenylethanol during production on lactose-containing media by yeast *Kluyveromyces lactis* DSM 70799

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2-phenylethanol (2-PE) is an aromatic compound with a rose-like odor. Due to increasing demand for natural fragrances, biotechnological production of 2-PE can be a potential alternative for chemically produced compound and a less expensive process than naturally-obtained extraction from roses. Many yeasts, including lactose utilizing *Kluyveromyces* spp., are capable of 2-PE production. *Kluyveromyces lactis* has been assigned GRAS (generally regarded as safe) and QPS (qualified presumption of safety) status. Bioconversion of lactose containing substrates such as whey or milk permeate (by-products of dairy industry) into more valuable products will contribute in reducing the negative impact on the environment.

The formation of high 2-PE concentration in the medium can cause inhibitory actions on microorganisms. To evaluate *K. lactis* DSM 70799 tolerance to 2-PE, a 96-well plater reader was used for growth observation in lactose semi-synthetic medium with added different exogenous 2-PE concentrations (0–4.08 g/L). For *K. lactis* DSM 70799 strain 4.08 g/L exogenous 2-PE concentration was found to be completely growth inhibiting.

One of the in situ product removal methods that could be effective in reducing 2-PE toxicity in the fermentative production is two-phase fermentation with vegetable oils. The 2-PE biosynthesis by *K. lactis* DSM 70799 in the presence of rapeseed oil was investigated. The increased 2-PE concentration in this fermentation system was demonstrated, but it was found that 2-PE yield strongly depends from mixing and aeration conditions, probably because oil phase complicates the oxygen availability for *K. lactis* cells.

Keywords: 2-phenylethanol, fermentation, *Kluyveromyces lactis*, lactose, vegetable oil

Acknowledgements:
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I.P.8

Thermophilic *Geobacillus* bacteria as a source for bacteriocin discovery

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Bacteriocins are defined as ribosomally synthesized bacterial peptides killing or inhibiting growth of other bacteria. In the era of emergence of antibiotic resistance they have been suggested as an alternative to antibiotics in medicine and veterinary against pathogenic bacteria. Growing interest on bacteriocins from extremophiles is based on their increased thermal stability and resistance to harsh conditions. Such bacteriocins could be used against spoilage microorganisms in heat-treated food production. In this study we *in silico* investigated potential of thermophilic bacteria of genus *Geobacillus* to produce bacteriocins. All to-date available *Geobacillus* genome sequences were processed using BAGEL3 and BAGEL4 web tool for identification of biosynthetic genes. Study revealed 77% of strains seem to possess biosynthetic machinery to produce 1-5 bacteriocins. In total 129 putative bacteriocin gene clusters were identified in 76 chromosome and 23 plasmid sequences. These were further analyzed and classified by their biosynthesis gene organization and precursor, modification, immunity, transport genes homologies. 32 of these clusters were sactipeptides, 37 head to tail cyclized bacteriocins and 42 lanthipeptides, making up the most common bacteriocin classes (25%, 29% and 32% respectively) in *Geobacillus*. We show that most species of the genus have good potential to produce a wide variety of antimicrobials and there is high occurrence of putative biosynthetic gene clusters suggesting *Geobacillus* bacteria could be a good source for novel bacteriocin discovery.

Keywords: bacteriocin, *Geobacillus*, antimicrobial peptides

I.P.9

Application of *Lentinula* edodes in bioconversion of pre-treated rapeseed straw

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Rapeseed straw is a common agricultural lignocellulosic residue, which can be used as a cellulose and hemicellulose source in production of bioethanol and furfural. This process generates lignin residue which must undergo further degradation in order to make furfural and bioethanol production a waste-less process.
Lignin is biologically degraded by white-rot fungi, which depolymerize it with an extracellular enzyme complex composed by phenol oxidases (laccases) and peroxidases (manganese peroxidases and lignin peroxidases). One aforementioned white-rot fungus is shiitake mushroom *Lentinula edodes* (Berk.) Pegler – an edible medicinal basidiomycete capable of selective lignin degradation. Application of these fungi in treatment of lignocellulosic waste is an approach to obtain ligninolytic enzyme complex while simultaneously decreasing quantity of residue-lignin and gaining mycelium biomass with potentially beneficial properties.
Multiple *L. edodes* mycelium strains were incubated on malt extract agar plates and as shaken-flask submerged cultures. Optimal conditions for highest mycelium biomass and enzyme yields were qualitatively and quantitatively assessed by variating incubation settings.
To evaluate the effect of pre-treated rapeseed straw on ligninolytic enzyme complex yield, various concentrations of rapeseed straw additive were introduced into culture medium.
Presence of rapeseed straw in the media noticeably increased laccase activity in submerged cultures. A positive correlation between concentration of the additive in medium and laccase activity was detected, indicating this substrate as suitable for increased enzyme complex yields.
In addition we observed a supposed negative correlation between optimal conditions for mycelium biomass increase and those regarding laccase activity.

Keywords: white-rot fungi, rapeseed straw, *Lentinula edodes*; laccase

Acknowledgements
This study was supported by University of Latvia under project "Sustainable use of natural resources in the context of climate changes" (ZD2016/AZ03) and University of Latvia, Institute of Microbiology and Biotechnology under project "A novel approach for simultaneous and waste-less production of bioethanol, furfural and other valuable compounds from local agricultural remains. (ESS2017/206/1.1.1.1/16/A/113)".

I.P.10

Improving carotenoid extraction from yeast *Rhodotorula toruloides*

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Carotenoids are red to yellow isoprenoid pigments that are naturally synthesized by plants and some microorganisms, including fungi such as the yeast species belonging to the genus *Rhodotoruloides*. These pigments are used in various industrial applications – as components of cosmetics, additives for livestock and fish. They are commonly used in food industry as food colorants and as dietary supplements for human consumption. The global market for carotenoids reached $1.5 billion in 2017 and is estimated to increase by $0.5 billion during the next four years, thus representing the high-value and demand for this substance.
We propose the use of R. toruloides as cell factory for the production of carotenoids as an alternative for chemical synthesis. Application of *R. toruloides* in production of carotenoids is time-effective, for the production of these single-celled organisms is unambiguous and compound extraction from yeast cultures is more rapid and less complicated compared to plant cells. In addition, *R. toruloides* is applicable in lipid production for various purposes, byproduct of which often are carotenoids. Establishing methods for carotenoid extraction from *R. toruloides* cultures would not only increase the overall natural pigment production but could also prove to be beneficial in lipid purification process.
Currently the methods for carotenoid extraction are not well established, especially the simultaneous carotenoid and lipid extraction. To obtain the carotenoid extracts from *R. toruloides* cells, we used polar and non-polar solvents, such as acetone, hexane and cyclohexane. This extraction method was compared to a simple acetone extraction method. Carotenoid quantification was done spectrophotometrically, and several carotenoids were identified by U-HPLC. We evaluated the lipid content of these extracts, noted carotenoid co-existence in lipid extracts and investigated the effect of extract saponification on carotenoid quantification.

Keywords: carotenoids, lipids, *Rhodotorula toruloides*

I.P.11

Influence of aditional pretreatment on glucose yield after enzymatic hydrolysis of rape straw

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Lignohemicellulose containing biomass is renewable and very promising resource for the obtaining of products with high demand. It is even more attractive if necessary for this approach biomass comes from agricultural waste. In our work new technology for joint production of furfural and bioethanol from rape straw is used. This technology consists from unique method of obtaining of furfural from hemicellulose which does not damage the remaining cellulose part. Next step of this technology is special enzymatic treatment of lignocellulose which leads to the formation of glucose. Then bioethanol is produced through fermentation process by yeast. However the use of only one type of pretreatment could not be sufficient for the obtaining of maximum glucose yield from lignocellulosic material. That is why in our work we tried to apply different additional pretreatments, such as milling, autoclaving and ultrasound for the increase of yield of glucose after enzymatic hydrolysis step. Our results showed that the use of milling and ultrasound increased glucose outcome from rape straw biomass after following enzymatic hydrolysis.

Keywords: enzymatic hydrolysis, rape straw, pretreatment

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II. Molecular Microbiology

II.P.1

Identification of the redox partner and substrates of *C. jejuni* 81116 Dsb protein – thiol oxidoreductase C8J1298.

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Bacterial Dsb enzymes are involved in formation of disulfide bonds between cysteine residues. *Campylobacter jejuni* Dsb proteins function in two pathways: oxidation (DsbA1, DsbA2, DsbB, DsbI) and isomerization. Bioinformatics analyzes identified C8J1298 and its potential redox partner C8J0565 as potential components of isomerization pathway.

The aim of this project has been to identify the substrates and the redox partner of C8J1298 protein as it is crucial to understand its role in Dsb system in *Campylobacter jejuni*.

The C8J1298 substrates was identified by "reverse purification substrates trapping" strategy. Mutated form of C8J1298 was created by site-directed mutagenesis and overexpressed in *E. coli* Rosetta strain. C8J1298-substrate complexes was purified using Medium-Pressure Liqiud Chromatography: mutated His-tagged C8J1298 was immobilized on Ni-NTA column, then WT lysate of *C. jejuni* was loaded on a column. The complexes were eluted with rising concentrations of imidazole. To verify whether C8J0565 is a redox partner of C8J1298 specific suicide recombinant plasmid was constructed. It will be used to generate *C. jejuni* 81116 Δ*c8j0565* strain by allelic exchange mutagenesis.

To identify the C8J1298 substrates its mutated version (CXXC motif changed to CXXS) was constructed. Mutation cause accumulation of mixed complexes. Purified complexes were analyzed by SDS-PAGE with or without reducing agent (DTT). Several additional protein bands appeared after DTT treatment when compared with non-reducing conditions. The proteins released from complexes will be analyzed by mass spectrometry.

The comparison of *in vivo* redox state of C8J1298 in wt and Δ*c8j0565* mutated strain (AMS trapping technique) should allow us to verify the role of C8J0565 in rereducing C8J1298.

The obtained data showed that several proteins are C8J1298 targets. Their identification will facilitate to gain insight into C8J1298 functioning.

The work was supported by the National Science Centre (grant no. 2015/17/B/NZ1/00230).

Keywords: Dsb protein, *Campylobacter jejuni*, substrates, redox partner

II.P.2

Identification of the *Campylobacter jejuni* 81116 Dsb thiol oxidoreuctases substrates by site-directed mutagenesis

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The Dsb protein family in prokaryotes catalyzes the generation of disulfide bonds between cysteine residues in nascent proteins, ensuring their proper three-dimensional structure. The mechanism of disulfide bond formation in microorganisms is extremely diverse. The Dsb oxidative pathway of *C. jejuni* consists of four extracytoplasmic proteins. Two of them (CjDsbA1andCjDsbA2) are soluble periplasmic proteins member of TRX protein family. However, they vary considerably with regard to their active sites, and in the charge distribution on the protein surface.

To get insight into the functioning of the Dsb machinery of *C. jejuni* we decided to identify CjDsbA1 and CjdsbA2 substrates using point mutated forms of both oxidoreductases and "reverse purification" substrates trapping strategy.

Mutated forms of analyzed proteins were generated by site-directed mutagenesis and overexpressed in *E. coli* Rosetta strain. Substrate-CjDsbA1/CjDsbA2 complexes were purified using Medium-Pressure Chromatography: mutated proteins with His-tag were immobilized on Ni-NTA column, then WT lysate of *C. jejuni* was loaded on column. The complexes were eluted with rising concentrations of imidazole.

The mixed complexes between disulfide oxidoreductases and their substrates are short-lived, and it is impossible to isolate them by standard biochemical methods. Mutation in the CjDsbAs active site allowed to create stable complexes of oxidoreductases with their substrates. The eluted complexes were analyzed by SDS-PAGE, with or without reducing agent. Several additional bands appeared after reducing agent treatment, as compared to the non-reducing conditions. The proteins released from complexes will be analyzed by mass spectrometry. CjDsbA2 has more restricted specificity when compared to CjDsbA1.

Keywords : *Campylobacter jejuni,* Dsb proteins, protein interactions

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The work was supported by the National Science Centre (grant no. 2015/17/B/NZ1/00230).

II.P.3

Construction and analysis of the *Helicobacter pylori* strain deprived of the *htrA* gene

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Helicobacter pylori is a widespread human pathogenic bacterium that colonizes the epithelial tissue of the stomach. Infection with *H. pylori* causes a number of diseases from chronic gastritis to stomach ulcers or even stomach cancer. To effectively colonize the host this pathogen produces a number of virulence factors, including a serine protease, $HtrA_{Hp}$. HtrA is a periplasmic protein, however its fraction is secreted extracellularly to damage gastric epithelium. Apart from its important functions in virulence, $HtrA_{Hp}$ must be engaged in functions vital for cell physiology because the $htrA_{Hp}$ gene could not be inactivated in more than 200 *H. pylori* strains so far. This indicates that $HtrA_{Hp}$ must participate in a key process for the survival of this bacterium.

We have selected a strain, WGE00, in which the $htrA_{Hp}$ gene can be inactivated. We constructed two strains, WGE01 and WGE02, in which we deleted or mutated *htrA* in its catalytic active site, respectively. The lack of the gene and its protein product in WGE01 were confirmed by the Southern and Western Blotting, respectively. The correct mutation of the catalytic active site in WGE02 was confirmed by sequencing and a casein zymography.

Taking into account difficulties in constructing the Δ*htrA* knockout mutants we expected that the constructed strains might have accumulated suppressor mutations. Therefore we performed sequencing of the whole genomes of the parental WGE00 and the *H. pylori* mutated strains. We found substitutions in the *secA* gene, coding for the SecA protein that delivers the newly synthesized polypeptides to the Sec secretion system. This result suggests that the vital intracellular function of $HtrA_{Hp}$ is associated with the process of protein export. Possibly, mutated SecA variants deliver their substrates to the Sec translocon less efficiently than the wild type protein, thus it prevents clogging of translocation system and enable *H. pylori* survival.

Keywords: *Helicobacter pylori, serine protease, HtrA, SecA*

II.P.4

Conservation of the chromosome 2 replication synchronization mechanism in Vibrionaceae

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There is still much to learn about the maintenance of bacterial genomes, especially for the 5–10% of bacteria that have non-canonical genome structures. Usually, bacteria have one essential replicon called chromosome, but a growing number of strains have been found to host secondary chromosomes. Unlike eukaryotes, bacteria with multiple chromosomes utilize distinct replication initiation systems for each chromosome. As replication of chromosomes in such strains has to be synchronized and coupled to the cell cycle, specific synchronizing mechanisms were predicted to exist. The first such mechanism was recently discovered in the human pathogen *Vibrio cholerae*. In this bacterium replication of a unique sequence *crtS* located on chromosome 1 is required for replication initiation of chromosome 2 and, thus, its proper timing during the cell cycle. Interestingly, *crtS* relocations along chromosome 1 change the time-point of chromosome 2 replication initiation. It seems that this phenomenon may be replicated in *E. coli* to set the specific time of replication initiation of chromosome 2 mini-derivatives. In this study, the conservation of this synchronization mechanism among other closely related bacteria of the Vibrionaceae family was demonstrated. At the same time, the evolutionary diversity of chromosome 2 replication systems in this group of bacteria was studied (cross interactions between origins of replication of chromosomes 2 (*ori2*s) and *crtS* sequences originating from different species were tested, in order to track their co-evolution, as well as, to find non-interacting *ori2-crtS* pairs; incompatibility groups of *ori2*s from different Vibrionaceae were determined to find replication systems that can stably co-occur in the same cell). In the future, sets of non-cross-talking *ori2-crtS* systems could be used to establish multiple regulated replicons in *E. coli*, which may give totally new regulatory possibilities for synthetic biology.

Keywords: Vibrionaceae; chromosome 2; replication; *crtS*

II.P.5

The putative antitoxins genes of *M. tuberculosis* in RecA-independent DNA repair process

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M. tuberculosis (*Mtb*) is the most dangerous bacterial pathogen worldwide, responsible for 1,5 million deaths each year. During the course of an infection *Mtb* is exposed to oxygen and nitrogen radicals generated by the immune response cells, which cause DNA damage to the bacterial genome. Major DNA damaging events require a SOS response, with RecA as key recombinase. Previously, microarrays analysis of *Mtb* response to a strong DNA damaging agent - mitomycin C (MMC), showed induction of 112 genes, 21 of which were characterized as RecA-dependent (SOS) and further 6 partially RecA-dependent. Our RNA-Seq analysis of Δ*recA* mutant strain revealed 193 genes up or down regulated under MMC treatment, among them only 43 genes were regulated by RecA dependent manner, and 150 genes were regulated independently of RecA. We found 7 genes belonging to antitoxin family AbiE4 in Δ*recA* mutant under MMC treatment by RNA-Seq. The most upregulated genes identified by both RNA-Seq and proteomic analysis were *rv3714c* and *rv3517*.
The main goal of this project is to elucidate the mechanisms that govern RecA-independent DNA damage repair responses in Mtb.
We constructed, using homologous recombination, *M. tuberculosis* defined mutants lacking a functional *rv3714c* and *rv3517* genes and *M. tuberculosis* strains overproducing *rv3714c* and *rv3517* genes. The resulting mutants were tested in the phenotypic analysis with genotoxic factors like MMC, UV, hydrogen peroxide. The DNA damaging factors sensitivity profiles were confirmed by survival assessment (CFU) and/or minimal inhibitory concentration assay (MIC). We postulated that deficiency of *rv3714c* and *rv351,* in contrast to lack of *recA*, causes slightly changes in the susceptibility to the tested DNA damaging factors.
The future studies will head to evaluation of the function of *rv3714c* and *rv3517* genes in the DNA repair processes.

Keywords: DNA damaging, SOS response, RecA-independent DNA damage repair responses

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II.P.6

Bioactive metabolites produced by cyanobacteria strain *Nostoc edaphicum* CCNP1411

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Cyanobacteria are prolific producers of structurally unique bioactive secondary metabolites, derived from complex biochemical pathways. Vast majority of these compounds are synthesized non-ribosomally by large multimodular nonribosomal polypeptide (NRPS) or mixed polyketide (PKS)-NRPS enzymatic systems. Each of the modules is responsible for incorporation of amino acids into the forming peptide. This ability relies on domains that are assembled into modules. Low substrate specificity of the domain responsible for selection and activation of an amino acid raise the possibility of producing diverse structural variants of peptides by a given strain of cyanobacteria.
Nostoc edaphicum CCNP1411, isolated from Gulf of Gdańsk coastal waters, produces compounds characterised by cyclic structure and non-proteinogenic amino acids incorporated into its sequence which are characteristic features for non-ribosomal peptide synthesis. These bioactive metabolites belong to nostocyclopeptides and cyanopeptolines classes, as determined by mass spectrometry.
Insufficient quantity for complex analysis of these compounds, due to limitations caused by low amounts of natural products obtained from cyanobacterial biomass, inspired us to consider applications of alternative methods for peptide synthesis. Therefore, we constructed an expression system in *Escherichia coli* host, for expression of heterologous genes involved in biosynthesis of bioactive metabolites. The activity of elicited compounds were tested for cytotoxic properties on four cell lines (HDFa, HeLa, PC-3, and T-47D) to determine *in vitro* effects on cell viability, proliferation, cell cycle regulation, and cell death. Our studies allow to determine the usability and potential application of these bioactive metabolites in anti-cancer therapies.

Keywords: nostocyclopeptides; cyanopeptolines; human cell lines; molecular cloning

II.P.7

The effects of toxin-antitoxin systems to the fitness and stress tolerance of *Pseudomonas putida*

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Most bacteria contain various chromosomal toxin-antitoxin (TA) systems consisting of a harmful toxin and its corresponding antitoxin that neutralizes the toxin. TA systems have been proposed to be involved in stress management as they have been associated with growth-rate regulation, biofilm and persister cell formation, protection against phages and stabilization of genomic mobile DNA. However, as the results of different groups are highly controversial, the physiological importance of chromosomal TA systems remains unclear.

In *Pseudomonas putida*, out of 15 predicted TA systems, only GraTA has been thoroughly characterized [1–3] and some data has been presented about MazEF [4] and MqsRA [5] systems. In this work, we examined if the deletion of multiple TA systems from the genome of *P. putida* had any effect on the bacteria's fitness and stress tolerance. Our data shows that deletion of 13 TA systems does not affect *P. putida*'s fitness and stress tolerance considerably. We did not observe any effect on *P. putida*'s growth rate in rich medium or on LB solid medium containing different chemicals. Neither did TA system deficiency affect the amount of antibiotic tolerant persister cells or biofilm production. Still, little effect was detected on the mutant's growth rate when growing in LB liquid medium containing either nitroquinoline or paraquat compared to wild type strain. Due to the absence of notable fitness effects, we tested whether the predicted toxins have maintained their toxicity. Deletion analysis of antitoxin genes revealed that most of the toxins are toxic or moderately active, hence indicating that TA systems are functional. Further studies are in progress to determine the importance of TA systems in certain growth conditions or in a mixed bacterial consortium.

Keywords: Toxin-antitoxin system, *Pseudomonas putida*, stress tolerance, fitness

References:
1. Tamman H, Ainelo A, Tagel M, Hõrak R (2016) Stability of the GraA Antitoxin Depends on Growth Phase, ATP Level, and Global Regulator MexT. *J Bacteriol* **198**: 787–796.
2. Tamman H, Ainelo A, Ainsaar K, Hõrak R (2014) A moderate toxin, GraT, modulates growth rate and stress tolerance of *Pseudomonas putida*. *J Bacteriol* **196**: 157–169.
3. Ainelo A, Tamman H, Leppik M, Remme J, Hõrak R (2016) The toxin GraT inhibits ribosome biogenesis. *Mol Microbiol* **100**: 719–734.
4. Miyamoto T, Kato Y, Sekiguchi Y, Tsuneda S, Noda N (2016) Characterization of MazF-mediated sequence-specific RNA cleavage in *Pseudomonas putida* using massive parallel sequencing. *PloS One* **11**: e0149494.
5. Sun C, Guo Y, Tang K, Wen Z, Li B, et al. (2017) MqsR/MqsA toxin/antitoxin system regulates persistence and biofilm formation in *Pseudomonas putida* KT2440. *Frontiers Microbiol* **8**: 840.

II.P.8

An analysis of transcription of Anti0945 – a small antisense RNA of *Listeria monocytogenes*

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Listeria monocytogenes (*Lm*) is an intracellular pathogen, which is responsible for a foodborne infections in humans and different animal species. Regulation of transcription by small RNA (sRNA), was the subject of numerous studies in *Lm*. Thanks to advancements in sequencing methods it was possible to identify more than 250 sRNAs, including about 100 antisense RNAs (asRNA). AsRNA are crucial transcripts in bacterial stress response and virulence. Recent NGS analysis allowed identification of Anti0945 as the asRNA complimentary to 3' end of *lmo0945* transcript. Both Anti0945 and its target gene are located in ferritin operon in *Lm*, but their physiological function and mechanism of action is still unknown.

The aim of the study was to characterize profile of transcription of Anti0945 in *Lm* in different stress conditions. The study was started from construction of the transcriptional fusion of *anti0945* promoter and *lacZ* gene in pTCV vector and subsequent analysis of β-galactosidase expression from *anti0945* promoter in *Lm*. In the β-galactosidase assay no transcriptional activity of *anti0945* promoter was observed, suggesting overall low level of transcription of Anti0945 in *Lm*. For further research more sensitive northern blot method was applied. Results from northern blot analysis has indicated that Anti0945 is produced in *Lm* in both optimal and stress conditions. However, the significantly increased amount of this transcript is observed in the stationary phase of growth, suggesting particular role of Anti0945 in starvation. Interestingly, northern blot analysis has shown that size of the Anti0945 transcript is more than 200 nt that is much larger than previously described on the basis of NGS analysis (115 nt). Furthermore, primer extension analysis revealed that transcription start site of Anti0945 differ from that designated on the basis of NGS analysis. These results emphasize the need to verify the results of NGS research with more detailed molecular analyses.

Keywords: *Listeria monocytogenes*; antisense RNA; expression; Anti0945

II.P.9

Evolution of bacterial populations in *m*-cresol-containing environment

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Due to human activities there are many toxic aromatic compounds called xenobiotics in the environment. Xenobiotics are persistent in the environment for a very long time because of their chemical structure and stability. Since most of these aromatic compounds contain foreign structures there are missing necessary catabolic pathways in microbes for the degradation of these compounds. Despite of that there has been isolated many bacterial strains that are capable to degrade such compounds. Microbial ability to evolve to new environmental conditions and their metabolic versatility contributes to the evolution of new catabolic pathways which enables to use new aromatic compounds as an energy and carbon source.

In this work we carried out laboratory evolution experiment with constructed *Pseudomonas putida* strains that degrade toxic phenolic compound called *m*-cresol (Cre^+ phenotype). The aim of this experiment was to examine what kinds of changes could occur in bacterial populations throughout the laboratory evolution experiment which enabled bacteria to degrade or tolerate *m*-cresol more efficiently. Bacteria were cultivated in glucose-containing medium in the presence of 5 mM *m*-cresol (335 to 427 generations). *P. putida* wild-type strain which did not carry genes necessary for the degradation of *m*-cresol was used as a control population. In order to evaluate impact of increased mutation frequency on the evolution of bacterial populations in the presence of *m*-cresol, *P. putida* Cre^+ lineages deficient in DNA mismatch repair (lack of functional *mutS* gene) were also included in the evolution experiment. We wanted to investigate whether *mutS*-deficient bacterial population could evolve and adapt more efficiently to these environmental conditions compared with bacterial population which had lower mutation frequency. Initial screenings of the evolved lineages implied that despite the frequency of mutations, in each lineage there are subpopulations evolved via different evolutionary trajectories either leading to increased efficiency of *m*-cresol catabolism or higher tolerance to this substrate with concomitant loss of corresponding catabolic genes. These results indicate that a "bet-hedging" strategy could be prevailing in populations of *P. putida* when exposed to toxic phenolic pollutants.

Keywords: aromatic compounds, adaptive laboratory evolution, evolution of bacteria

II.P.10

The role of ferritin operon genes of human pathogen *Listeria monocytogenes* in infection of mice macrophages P388D1

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Listeria monocytogenes is food-borne human pathogen, an etiological agent of disease called listeriosis (with high mortality among infected individuals), leading an intracellular lifestyle during the pathogenesis process. After entry to the non-phagocytic cells such as epithelial cells or uptake by phagocytic cells like monocytes *L. monocytogenes* is able to growth inside them and spread to another cells.

One of the proteins involved in *L. monocytogenes* virulence is ferritin (Fri), encoded by gene *lmo0943*. Besides virulence this protein is also involved in adaptation to different environmental stress conditions e.g. oxidative stress or iron limitation and mediating in β-lactam tolerance. Analysis of genomic surrounding showed operon organisation of gene *lmo0943* and four downstream genes. Such arrangement and first experiments indicate the potential role of genes *lmo0944*, *lmo0945*, *lmo0946* and *lhrC-5* in *L. monocytogenes* fitness in variety of conditions especially when it comes to virulence and pathogenesis process.

The aim of the study was verification of ferritin genes involvement in first stages of *L. monocytogenes* infection. Accordingly, mutants in each ferritin operon gene were obtained and their ability to survive and multiplicate inside mice macrophages P388D1 were examined. After infection, bacteria where isolated from the macrophages at specified time points and enumerated after their incubation on agar plates.

The results of the study indicate that after short time of infection there was no significant differences between the examined strains in comparison to the wild type strain of *L. monocytogenes* suggesting that ferritin operon genes are not involved in surviving and escape from primary phagocytic vacuole of macrophage cells. However the difference in bacteria number isolated after longer time of infection were observed, especially in case of mutants in genes *fri*, *lmo0946* and *lhrC-5*. These results indicate that ferritin operon genes are important for efficient multiplication of *L. monocytogenes* inside infected mice macrophages.

Keywords: *Listeria monocytogenes*, ferritin operon, pathogenesis

II.P.11

Contribution of the Hfq chaperone of *Listeria monocytogenes* to regulation of transcription and translation of ferritin operon genes

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Listeria monocytogenes (*Lm*) is the Gram-positive human pathogen, which shows ability to survive in wide range of stress conditions. Hfq-dependent regulation of gene expression of many species of pathogenic bacteria is crucial for adaptation to a variety of stress conditions, including those associated with virulence and antibiotic resistance. While the role of chaperone Hfq in Gram-negative bacteria is well known, the function of this protein in Gram-positive bacteria remains unclear. Ferritin-like protein (Fri) plays crucial role in adaptation of *Lm* to multiple stresses. The *fri* is the first gene of a five-gene operon which consists of poorly characterized genes *lmo0944*, *lmo0945*, *lmo0946* and *lhrC5* – small non-coding RNA interacting with Hfq. Except of *lhrC5*, there is no data concerning possible involvement of Hfq in regulation of expression of ferritin operon genes.
The aim of the study was investigate the contribution of the Hfq to regulation of expression of ferritin operon genes in different stress conditions. For this purpose transcriptional and translational fusions of the promoter of each gene from ferritin operon and *lacZ* reporter gene were constructed using pTCV-*lac* and pCK-*lac* vector, respectively. The received derivatives of pTCV-*lac* and pCK-*lac* vectors were introduced into wild-type *Lm* and Δ*hfq* mutant strain. The strains were cultivated in BHI medium supplemented with the analysed stress factors and subsequently β-galactosidase activity was determined. The β-galactosidase assay revealed decreased level of transcription of *lmo0944* and *lmo0945* genes in Δ*hfq* mutant background in osmotic, acidic and alkaline stress conditions, while *hfq* mutation had none effect on transcription of *fri* and *lmo0946* genes. The study showed also decreased level of translation of *lmo0944* gene in Δ*hfq* mutant background at low temperature and oxidative stress conditions.
These results indicate that in different stress conditions Hfq affects transcription or translation of ferritin operon genes of *Lm*.

Keywords: chaperon Hfq, gene expression, β-galactosidase assay, *Listeria monocytogenes*

II.P.12

The chromosome of an opportunistic pathogen *Paracoccus yeei* CCUG 32053 (*Alphaproteobacteria*) contains a novel type of integrative and conjugative elements

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The main carriers of foreign DNA in horizontal gene transfer (HGT) are mobile genetic elements (MGEs) that have extremely diverse genetic structures and properties. An interesting group of MGEs are integrative and conjugative elements (ICEs), which combine some features of transposons, plasmids and bacteriophages. These elements are characterized by the presence of a recombination system encoding a phage related integrase responsible for ICE integration-excision, and a conjugal transfer system. ICEs usually occur in the chromosome-integrated form. They may, however, be excised from the genome and form autonomous circular forms (dsDNA) that mimic plasmids, although they lack their own replication systems. Such circular forms of ICE may be reintegrated into the host genome or transferred via conjugal transfer to another bacterial cell, where they integrate within the recipient genome. Comparative genomic analysis of several strains of the species *Paracoccus yeei* allowed us to identify two putative ICEs in the chromosome of strain CCUG 32053. Highly homologous regions were found in the genomes of distantly phylogenetically related *Alphaproteobacteria* but not in other *Paracoccus* spp. This strongly suggests that these are true mobile elements capable of horizontal transmission. Although the ICEs differ in size and structure, they share some common features. Both encode (i) serine or tyrosine family recombinases, (ii) components of plasmid conjugation machinery, (iii) putative REP_3 domain-containing proteins related to plasmid replication initiators (REP module), and (iv) putative plasmid partitioning proteins (PAR). The presence of REP and PAR *loci* may suggest that the ICEs originate from integrated plasmids. We confirmed that the predicted REP regions contain replication systems and showed that they are functional in several *Paracoccus* spp., but not in *P. yeei*. This strongly suggests that the identified elements have a dual nature and, depending on the host strains, may be maintained as autonomous replicons or integrated ICE-like elements.

Keywords: *Paracoccus yeei*, *Alphaproteobacteria*, integrative element, ICE

III. Virology and Phages

III.P.1

Express-analysis of bacteria-phage interaction

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For successful reproduction bacteriophage have to deliver genome into bacterial cytoplasm without destroying the host cell envelope, and to lyse the host in a controlled manner at the end of infection cycle. Viral genome delivery as well as effective lysis are dependent on energy state of the cell. We obtain information on virus entry and phage-induced lysis from permeability changes of the host membranes and the energy state of cells using electrochemical methods of analysis. The entire viral one-step growth cycle could be analyzed directly in the infection medium and electrochemical monitoring of the infection process directly reflects the state of cells without introduction of biases related to the process of sampling, e.g., changes of oxygen level in the medium during filtration or centrifugation, delay of the data acquisition. Electrochemical on-line monitoring of the infection allows additional analyses of samples taken directly from the vessel, i. e., O.D. measurements, determinations of ATP level, counting of bacteria and phages. Aeration of the infection mixture can be controlled by the rotation rate of a magnetic stirrer and/or using different stirring bars. Accumulated amount and the rate of leakage of intracellular K^+ reflects the permeability of the plasma membrane (PM), tetraphenylphosphonium (TPP^+) ions are used to follow the PM polarization and to evaluate the selectivity of phage-induced channels, as TPP^+ accumulation in the cytoplasm is membrane voltage ($\Delta\Psi$)-dependent. Monitoring of pH allows to evaluate the intensity of energy metabolism and to detect changes in PM permeability to H^+ ions during the infection. Phenyldicarbaundecaborane (PCB^-) ions are used to determine the amount of inactivated cells, as the lipophilic anions accumulate in membranes of metabolically inactivated bacteria. Such electrochemical system of express-analysis allows to evaluate the sensitivity of bacteria to virus, to characterize the infection cycle, to detect factors affecting the efficiency of infection.

Keywords: phage entry, infected cell lysis, electrochemical analysis, membrane voltage

III.P.2

Replication level of ectromelia virus in fibroblasts depends on mitochondrial network morphology

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Ectromelia virus (ECTV) belongs to the *Poxviridae* family and is the causative agent of mousepox. Our previous study indicated that ECTV infection of L929 and RAW 264.7 cells affects morphological and functional status of mitochondria, leading to the mitochondrial network disorganization and fragmentation. Therefore, we speculate that ECTV may change the mitochondrial network morphology for its own benefits. For that reason, the aim of the present study was to investigate whether intentional changes in mitochondrial network morphology affect the level of ECTV replication in L929 cells. Two different methods were used. The first method is based on the transfection with siRNA that interferes with mRNA of mitochondrial fission protein Drp1 (effect: mitochondrial network elongation) or with mRNA of mitochondrial fusion protein Mfn1 (effect: mitochondrial network fragmentation). The second method uses the chemical inhibitors: CCCP – inhibitor of oxidative phosphorylation (effect: mitochondrial fragmentation) and mdivi-1 – selective inhibitor of Drp1 (effect: mitochondrial elongation). L929 cells were treated with siRNA or inhibitor and then infected with ECTV. ECTV titer was determined by plaque assay on Vero cells. Our findings show that the highest titer of ECTV was observed in the untreated infected cells compared to Drp1/Mfn1 siRNA- and CCCP/mdivi-1-treated cells at 10 hour post infection. The virus titer in Mfn1 siRNA- and CCCP-treated cells drastically decreases, suggesting that mitochondrial network fragmentation leads to loss of mitochondrial activity needed for sufficient ECTV replication. Additionally, hyper-elongation of mitochondria (after Drp1 siRNA or mdivi-1 treatment) also contributed to decline of viral titer compared to untreated cells, but not as intensely as in cells with fragmented organelles. We hypothesize that elongated mitochondria present increased interaction between MAVS protein and the other proteins involved in the antiviral signaling pathway leading to extensive production of type I IFNs and proinflammatory cytokines resulting in the limitation of ECTV replication.

Keywords: ECTV, mitochondrial network, mitochondrial dynamics

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III.P.3

Methods of isolation of bacteriophages infecting *Salmonella enterica* from excrements of farming birds

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Infections of digestive system are common among the human infections in Poland. In this group, infections caused by different serovars of *Salmonella enterica* are in domination. *S. enterica* is able to exist in many different environments such as soil, water, fodder and animal intestines. The source of infection is consummation of the food containing these bacteria, e.g. poultry meat and eggs. The only widely available method to prevent *Salmonella enterica* spread in Poland are antibiotics added to animal food. However, this causes the progressive increase in number of antibiotic resistant bacteria which leads to reduction of effectiveness of treatment. Due to this, we have to find alternative ways to work against infections caused by *S. enterica*. One of them are the naturally existing viruses which are able to infect and to lyse the bacteria – bacteriophages.
The first aim of our work was to compare different methods of isolation of bacteriophages from excrements of farming birds and to find out which one is the most effective. The second aim was to characterize isolated bacteriophages (plaque and capsid morphology, host range, temperature sensitivity, chemical compounds sensitivity, pH sensitivity). The results showed that there are differences in bacteriophages isolated through different methods. After analyzing the results, some of isolated bacteriophages were found as potentially valuable components of phage cocktails.
Due to the high success of phage therapy for humans, it could also be used for farming birds in order to prevent the spread of pathogenic bacteria. This may give us a possibility to overcome antibiotic resistant bacteria.

Keywords: Bacteriophages isolations, Farming birds, *Salmonella enterica*, Phage therapy

III.P.4

Expression of the components of the non-canonical NF-κB signaling in RAW 264.7 murine macrophage cell line infected with ectromelia virus

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Nuclear factor (NF)-κB is a family of transcription factors that orchestrate innate and adaptive immune responses, including antiviral immunity. Since viral pathogens have evolved numerous mechanisms to overcome antiviral effector mechanisms, both canonical and non-canonical NF-κB signaling pathway components are among cellular targets for viral pathogens. The non-canonical NF-κB signaling has been attributed to adaptive immunity regulation and is exploited by oncogenic viruses, which persistently activate NF-κB to counteract apoptosis. However, the emerging role of the non-canonical NF-κB signaling in innate immunity is under investigation. Therefore, studying the influence of non-oncogenic viruses on the non-canonical NF-κB signaling may uncover new mechanisms of antiviral immunity modulation. The aim of our research was to evaluate the impact of murine poxvirus, ectromelia virus (ECTV), on the cellular content of the components of the non-canonical NF-κB signaling in RAW 264.7 murine macrophage cell line. Immunoblot analysis of mock- and ECTV-infected cells treated with poly(I:C), Pam3CSK4, LPS O55:B5/IFN-γ, and LPS O111:B4/IFN-γ was performed on total cell extracts obtained at 18 hours post infection (hpi). NIK, RelB and p100/p52 proteins were detected by chemiluminescence. Analysis of protein content in ECTV-infected cells shows the decrease in RelB and p100/p52 protein level in RAW 264.7 cells upon ECTV infection. These findings suggest that ECTV may actively influence non-canonical NF-κB signaling.

Keywords: antiviral immunity, ectromelia virus, non-canonical NF-κB signaling

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