Poster Presentations Session 2

IV. Environmental Microbiology

IV.P.1

Isolation and characteristics of *Salmonella enterica* and *Campylobacter spp*. from farming birds droppings

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Salmonella enterica, *Campylobacter jejuni* and *Campylobacter coli* are main pathogens infecting poultry meat. The major virulence factor of *Salmonella* and *Campylobacter* species is the functional locomotor system, which facilitates the colonization of intestines and adhesion to difficult surfaces, such as epithelium. These bacteria are responsible for food poisoning in humans, known respectively as salmonellosis and campylobacteriosis. These diseases constitute an ever increasing threat to public health, especially in EU and USA. In 2014 there were about 91 000 cases of salmonellosis, and about 240 000 cases of campylobacteriosis in EU. The main symptoms of these infections are vomiting, diarrhea and abdominal pain.

In Poland, most cases associated with food poisoning are caused by *Salmonella* strains. There were no reported cases of campylobacteriosis. However, there were no official regulations and procedures regarding monitoring of this bacteria. Since 2018, permitted number of *Campylobacter* strains are 1000 CFU/g of meat, while infective dose for human is about 500 CFU/g of meat. The aim of this study was to compare the number of *Salmonella* and *Campylobacter* strains isolated from farming birds droppings.

We have isolated *Salmonella* and *Campylobacter* strains from bird feces samples using selective media and then we used MALDI-TOF mass spectrometry for identification of *Campylobacter spp.* In case of *Salmonella* genus, we have performed sero-agglutination tests.

We have then tested bacterial sensitivity to antibiotics such as ampicillin, tetracycline, streptomycin, chloramphenicol, rifampicin or colistin and compared the results with sensitivity of laboratory strains. Bacteria may have been analyzed as resistant or sensitive to antibiotics. It depended on inhibition zone diameter. We have also analyzed bacterial sensitivity to phages from our collection in case of isolated *S. enterica* strains.

IV.P.2

Increasing bacterial contribution in the nitrification following turf disturbances of the translocated wet meadows

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To compensate for the Katowice airport build-up on highly valuable wet meadows, 1.3 hectare of turf were translocated in 2013 to the habitat garden located 15 km apart (Radzionków). This was the first case of such large turf vegetation transfer in Poland. It was unique in particular that the ecosystem salvage was combined with reclamation of the abandoned post-industrial area. Three meadows were monitored for three seasons: before the transfer and two years afterwards (2014, 2015). The domination of ammonia over nitrate, characteristic for wetlands, strongly decreased after the transfer. The content of soil ammonia dropped for a half a year after the transfer. At the same time nitrate content increased two-times and three-times two years later. This suggests increased nitrification resulting from physical disturbances at the time of translocation, oxygenation and also presumed habitat drying and humus decomposition at the receptor place. To evaluate possible changes in the nitrifying community size q-PCR of amoA gene of Bacteria (AOB) and Archaea (AOA) was performed. The Thaumarchaeota amoA gene copies number strongly dominated over bacteria before the meadows translocation. Following the turf transfer the AOB abundance was increasing each year so that it finally reached the AOA abundance two years after the transfer. The results points on increasing contribution of bacterial nitrifiers at increased soil oxygenation and nutrient enriched conditions, while waterlogged conditions are limiting bacterial nitrification for the sake of Thaumarcheota.

Keywords: ammonia; soil; amoA gene; q-PCR

IV.P.3

Metabolic pattern of microbial communities in soil treated with cefuroxime and/or inoculated with a multidrug-resistant *Pseudomonas putida* strain MC1 based on the community-level physiological profiling (CLPP)

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Among the second-generation cephalosporins (CPs), cefuroxime (XM) is the most frequently prescribed and, its consumption in many European countries constituted more than 50% of the total CP administration. Due to the fact that conventional wastewater treatment plants remove XM from wastewater partially, this antibiotic and antibiotic-resistant bacteria are introduced into soils through the agricultural usage of sewage sludge, and may affect the metabolic activity of the soil. To ascertain this impact, the community level physiological profiles (CLPPs) using the Biolog® EcoPlate™ system in the XM (XM1 – 1 mg/kg and XM10 – 10 mg/kg) and/or antibiotic-resistant *Pseudomonas putida* strain MC1 (Ps – 1.6×10^7 cells/g)-treated soils were determined during a 90-day experiment. A multifactorial analysis and the resistance (RS)/resilience (RL) concept were used to assess the potential of native microorganisms to maintain their catabolic activity under exposure of XM and/or a high level of *P. putida*. The results revealed a negative impact of XM on the metabolic activity of soil microorganisms, especially on days 1–30 as was showed by a decrease in the values of the CLPP indices, i.e. the average well-color development (AWCD), substrate richness, Shannon-Wiener index as well as the AWCDs for the six group in which the 31 carbon substrates were grouped (i.e. amines, amino acids, carbohydrates, carboxylic acids, miscellaneous and polymers). In turn, an increase in the metabolic activity of soil microorganisms was observed at the same time after the addition of the bacterial strain. These observations suggested a low initial resistance of soil microorganisms to XM and/or strain MC1. Although the negative effect of XM was transient, the application of this antibiotic into soil may temporarily pose a potential risk for soil functioning.

Keywords: cefuroxime, multidrug-resistant bacteria, Biolog EcoPlates, soil microorganisms

Acknowledgements:

This work was carried out as part of a grant financed by the National Science Centre, Poland (No. DEC-2014/15B/NZ9/04414).

IV.P.4

Effectiveness of phenol-formaldehyde (PF) resin against wood biodegradation by decay fungi

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Wood destroying basidiomycetes cause enormous damage to wooden constructions in favourable growth conditions. In this study the Silver birch (*Betula pendula*) specimens were treated with phenol-formaldehyde (PF) resin to determine its protective effectiveness against wood decay fungi. The specimens of size 20×20×5 mm^3 were vacuum impregnated with PF resin of concentrations 5, 10 and 15% in water. The leaching procedure according to standard EN 84 was performed to evaluate PF resin fixation stability. PF resin penetration in wood cell walls was determined by the dyeing with safranin stain and visual evaluation by light microscopy (LM). The durability of impregnated wood specimens was examined according to the standards EN 113 and EN 84 (leaching). The non-leached and water leached specimens were exposed to the brown rot fungus *Coniophora puteana* and the white rot fungus *Trametes versicolor* for 6 weeks. Efficient protection against wood decay fungi was reached at 5% concentration for non-leached samples, and at 10% concentration for leached samples. At these concentrations the mass loss of treated specimens was <3%. The presence of PF resin in the wood cell walls depended on the concentration of solution as a result of different intensity of safranin staining. The amount of absorbed dye on the cross-section of birch wood was reduced by an increased amount of incorporated PF resin in the cell wall.

Keywords: Biodegradation, wood decay fungi, phenol-formaldehyde (PF) resin, protection

IV.P.5

Metagenomic analysis of the microbiota in Neris river sediments to evaluate the impact of antropogenic city pollution

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Human activities such as the burning of fossil fuels, various industrial processes and waste disposal in the city area leads to greater pollution with significant impact on human health and damage to the natural or built environment. Organic and inorganic matter from various sources ends up in nearby rivers and accumulates in the river sediments, which is an active place with high abundance of microorganisms. Bacterial communities subjected to anthropogenic chemical changes may cause increase in detoxifying species, biodiversity loss, spread of pathogens, disabling of ecological functions and other effects on the ecosystem. This study was conducted to investigate the shift in structure of sediment bacterial communities of river exposed to multiple anthropogenic contaminants and relate changes to ecological functions for potential bioremediation and bioindication. Neris river is a suitable model object to investigate the impact of pollution on the aquatic ecosystem, it crosses the capital Vilnius – one of the most urbanized cities in Lithuania. Three different anthropogenic localities were selected to sample Neris river sediments: before the city, city center and after wastewater treatment plant. The microbiome was characterized on the basis of the V3 and V4 hypervariable region of the 16S rRNA gene by using next generation sequencing platform Illumina MiSeq. The metagenome analysis of Neris river bacteria has revealed that anthropogenic pollution, and in particular wastewater treatment plant, reduces the natural biodiversity of bacteria. Furthermore, several candidate genera were identified as potential bioindicators to monitor river pollution. Also, possible bioindicative bacterial species associated with pollution effects and bioremediation processes have been identified and the prevalence of pathogenic bacteria and toxic cyanobacteria have been detected in the city area. Overall, bacterial communities could provide a useful tool for monitoring and assessing ecological state in freshwater sediments indicating anthropogenic city pollution.

Keywords: metagenome, microbiota, river sediments, pollution

IV.P.6

Various methods of isolation and characterization of *Actinobacteria* isolated from caves in the Tatra mountains

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Due to recent worldwide antibiotic resistance crisis we observe a rapid increase in searching for new substances from bacteria that might have pharmacological or industrial use. *Actinobacteria* isolated from oligotrophic ecosystems, such as caves, are very promising sources of unknown biologically active compounds. Caves are an extremely specific environment with limited access to light and nutrient resources, low temperature. Previous studies showed that cave moonmilk deposits are appropriate for the development of rare *Actinobacteria*. The complexity level of isolating these rare bacteria strains warrants a lot of studies in different research facilities all over the globe. To increase the efficiency of our efforts, our team incorporated various methods of isolating these rare bacterial strains.
In this work we have used different attempts to extract *Actinobacteria* from samples coming from sendimends developing on cave walls. Samples came from caves located in Tatra National Park, Poland. The isolation was performed on various types of enriched media, assorted temperatures, times of incubations and heat pre-treatment. We also characterised isolated bacteria (bacterial colony morphology analysis, Gram staining, streak tests regarding potential antibacterial activity). We hope that our research will arouse the interest in the *Actinobacteria* species as a potential source of novel bioactive substances, as secondary metabolites, enzymes or antibacterial compounds.

Keywords: *Actinobacteria*, isolation, caves, enriched media

IV.P.7

First insight into microbial communities of myrmecophilous insects species inhabiting nests of red wood ants, *Formica rufa* and *F. polyctena*: molecular evidence of Wolbachia endosymbiosis

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Nests of red wood ants (*Formica rufa* group) are very specific habitats, which constitute optimal living conditions for the large number of invertebrates accompanying ants, known as myrmecophiles. The aims of this study were to investigate the microbial communities of selected myrmecophilous species inhabiting anthills of *F. rufa* and *F. polyctena*, to establish the presence of known endosymbionts, to identify a diagnostic microbiome profile that would be universal for myrmecophiles associated with red wood ants and to describe the metabolic potentials of the associated microorganisms. The microbiome profiles of seven insect species were analyzed: *Atelura formicaria*, *Dendrophilus pygmaeus*, *Leptacinus formicetorum*, *Monotoma angusticollis*, *Myrmechixenus subterraneus*, *Ptenidium formicetorum* and *Thiasophila angulata*.
Taxonomy-based analysis indicated that the microbial communities of the myrmecophiles consisted of a total of 26 phyla. The most abundant phyla were *Proteobacteria* and *Actinobacteria*, and the most dominant classes were *Alphaproteobacteria*, *Actinobacteria* and *Gammaproteobacteria*, with different proportions in the microbial communities investigated. Two known endosymbionts – *Wolbachia* and *Rickettsia* – were found in all the microbiome profiles. The relationships among the microbiome profiles were complex, and no relative abundance pattern common to all the species tested was observed. Certain regularities were apparent in the profiles associated with four coleopteran myrmecophiles – *D. pygmaeus*, *L. formicetorum*, *M. angusticollis* and *M. subterraneus*. Microbial communities of all the species tested were rich in genes involved in membrane transport, replication and repair processes, translation, metabolism of amino acids and carbohydrates, and energy metabolism.

Keywords: microbial community structure, myrmecophilous insect species; 16S rRNA gene; Next Generation Sequencing

Acknowledgements:

The authors gratefully acknowledge the State Forests National Forest Holding, Poland, for funding the research and the Management of the Polesie National Park for its cooperation. The project was carried out with the cooperation of the Polesie National Park and the Maria Curie-Sklodowska University in Lublin; the numbers of the relevant agreements are: EZ.0290.1.28.2017 (between PNP and SFNFH); 6, NB 520-3/2017 (between PNP and MCSU). This work was also supported by the National Science Centre, Poland (grant no. 2014/15/N/NZ9/03882).

IV.P.8

Vibrio cholerae in the polish coastal waters of the Baltic Sea – single case or a sign of climate warming?

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Bacteria of the family *Vibrionaceae* naturally occur in marine and estuarine environments. *Vibrio cholerae*, causative agent of cholera, water-borne disease, mainly belong to serotype O1 or O139, but non-O1/non-O139 serotypes can cause gastrointestinal and extraintestinal infections. Increasing abundance of this pathogen in European coastal waters may occur as a result of climate change, e.g. rising surface water temperatures.
We obtained 8 isolates of *V. cholerae* from surface waters of Puck Bay (Baltic Sea), near marine outfall of a wastewater treatment plant. Biochemical identification of the species was confirmed by 16S rDNA sequencing. The isolates were characterized for their resistance to 13 antimicrobial agents, using the disk diffusion method. PCR was used to detect the presence of integrons and antimicrobial resistance genes. Water-borne diseases caused by *Vibrio* species are rare in Poland, but still are of major concern, thus all isolates were serotyped and analyzed for ability to produce cholera enterotoxin (CT), the main virulence factor of *V. cholerae*.
In case of four isolates serological typing was unsuccessful due to production of extensive amount of mucus by bacterial cells. Four other isolates belonged to O1 serogroup. None of the tested *V. cholerae* isolates possessed *ctx*A, *ctx*B, *ctx*AB and *tcp*A genes responsible for toxin production. Isolates were also resistant to at least one tested antibiotic, two isolates showed multidrug resistance phenotype (MDR). In all isolates we detected *int*I4 integrase gene and antibiotic resitance genes, such as bla_{OXA}, bla_{TEM}, *sul*3 and *tet*D.
V. cholerae strains are an important cause of potentially life-threatening infections. There has also been an increase in the number of reports of infections involving non-O1/non-O139 *V. cholerae*. Thus, the presence of antibiotic-resistant *V. cholerae* in Puck Bay waters (Baltic Sea, with temperate water temperatures), is of great concern and should be monitored.

Keywords: non-pathogenic *Vibrio cholerae*; antimicrobial resistance; climate change; Baltic Sea

IV.P.9

Screening for polyketide synthase and nonribosomal peptide synthetase genes in bacteria isolated from Krubera-Voronja Cave

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Antibiotic resistance of pathogenic bacteria has become a major threat to human health over the last few decades. A few different approaches are used to solve this problem, screening for new natural antimicrobials being one of them. Namely caves represent one of the most attractive environments with a strong potential for the discovery of novel antimicrobials.
The aim of our study was to identify polyketide synthase and nonribosomal peptide synthetase genes in bacterial strains isolated from Krubera-Voronja Cave. This cave is the deepest known cave in the world. Because of its depth and, consequently, strong oligotrophy, high antimicrobial activity of bacteria inhabiting this cave was expected. The main focus of our research were bacterial strains without any identifiable phenotypic bioactivity isolated from the different branches of the cave – both rarely and frequently visited. In total, 98 strains were subjected for screening using 28 PCR primer pairs targeting different polyketide synthase type I and type II as well as nonribosomal peptide synthetase genes. From 1 to 7 PCR products of the correct size were obtained using different primer pairs for almost all investigated strains. PCR products of the strains that showed multiple antimicrobial biosynthesis genes (from 4 to 7) were cloned, sequenced and analysed.

Keywords: polyketide synthase, nonribosomal peptide synthetase, Krubera-Voronja Cave, antibiotic resistance

Acknowledgements:

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

IV.P.10

Selection of (a waste) organic substrate for culturing sulfate reducing bacteria

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Sulfate reducing bacteria (SRB) are obligate anaerobic microorganisms that use sulfate as a terminal electron acceptor in the anaerobic oxidation of organic or inorganic substrates. Although SRB are capable of using various electron donors and carbon sources for sulfate reduction, the efficiency of the process is often limited by the degradability of the organic substrate. Industrial application of SRB in sulfate-rich wastewater treatment requires the selection of an appropriate growth substrates for SRB, necessary for maintaining high sulfate reduction efficiency, and preferably characterized by low cost and high availability.
The aim of this work was to select an efficient and cost-effective electron donor and carbon source for SRB.
Cultures of a previously constructed SRB consortium were carried out under anaerobic conditions in 100 ml bottles on a modified Postgate medium, supplemented with one of the following substrates: either simple carbon source: lactate, methanol, ethanol, acetate or one of the following organic waste materials: molasses, mixed sawdust, mushroom compost, sour whey, pine bark and brewery by-products. Samples were collected at the beginning of the experiment and every 7 days, for 21 days. The concentration of sulfates in culture supernatants was analyzed by Nanocolor® tests along with the pH.
Based on the obtained results, it was found that ethanol and lactate are the most effective electron donors and carbon sources for SRB. However, ethanol seems to be a more attractive growth substrate due to its low cost in comparison with lactate. The growth of the SRB on methanol and acetate was slow and a decrease in SRB activity was observed. Despite the wide availability of molasses and its low cost, its degradability is low without pretreatment. In the case of organic waste materials sulfate reduction rates were lower than for simple compounds, though their use in long-term experiments and industrial applications may be advantageous due to their low cost.

Keywords: sulfate reducing bacteria, electron donor, carbon source, organic waste materials

IV.P.11

Fate of antimicrobial resistance in landfill leachates

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Significant changes of solid waste management have been observed worldwide. The mechanical-biological pre-treatment (MBT) of residual (mixed) solid waste is a process often used for resources and biomass recovery prior to landfilling. Thus municipal solid waste plants (MSWPs) are usually equipped with MBT's units (sorting and composting units) and landfill prisms for remaining bulk disposal. In this study liquid by-products generated by MSWP facilities were sampled to recognize their sanitary quality as well as occurrence of emerging resistance patterns and determinants.

Bacteria were isolated from liquid by-products generated during sorting (MBT-SU), composting (MBT-CU) and landfilling processes using selective media (mFC agar) supplemented with cefotaxime (CMX). Resistance phenotypes were determined and ESBLs were confirmed according to the EUCAST recommendations. Among ESBLs the presence, localization and abundance of resistance components (genes and integrons) were analyzed using PCR technology Among 32 isolates recovered from selective agar supplemented with cefotaxime, 15 were confirmed as ESBL-producers. Among them four isolates were identified as *Klebsiella oxytoca* and eleven as *Escherichia coli*, and all originated from the liquid by-products generated by the MBT-SU. Among ESBL-producing isolates, all harboured CTX-M type 1 β-lactamase and showed resistance against tested penicillins and second-generation cephalosporins. High resistance rate was also observed to third- and fourth-generation cephalosporins, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole combination, and to aztreonam and tobramycin. Among ESBL-producing *Klebsiella* spp. additionally resistance to gentamicin (75%) and fosfomycin (25%) was detected while some ESBL-producing *E. coli* were also flourochinolones and/or amikacin resistant (20% and 8.3%, respectively). All ESBLs were multidrug-resistant (MDR) and integron-positive. Other cefotaxime-resistant, non-ESBL-producers, were affiliated to genera such as *Acinetobacter baumannii* (n=4), *Ochrobactrum anthropi* (n=1) and *Klebsiella* spp. (n=3).

The obtained results confirmed that liquid by-products generated at MSWP should not be ignored as a source of emerging resistance phenotypes.

Keywords: solid waste management liquid by-products; antimicrobials; emerging-resistance phenotypes

IV.P.12

Sea to air bacteria transfer over the Gulf of Gdańsk

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Bubbles rising in water may scavenge bacteria from the water column and transport them to the air/water interface. The bacteria scavenge is especially enhanced in saline water where rising bubbles separate ions, creating electric polarity around bubble curvatures. During that process more heavily anions are continuously collected at the upper bubble half sphere while smaller and lighter cations are gathered at the bubble bottom half sphere and within the sub-bubble vortex. Since bacteria accommodate a negative charge on the outer membranes, these are attracted to the cationic bubble bottom vortexes that form a rotating pocket collecting oppositely charged cells. When bubble bursts at the air/water interface bacteria are ejected into the air with jet droplets. Experimental measurements conducted over the Gulf of Gdańsk showed that concentrations of psychrophilic bacteria in sea water ranged from 20 to 10^6 CFU/mL, while airborne concentrations ranged from to 32 to 420 CFU/m^3. The mesophilic bacteria content in sea water ranged from 70 to 10^6 CFU/mL, while airborne concentrations ranged from 12 to 224 CFU/m^3. Conducted research confirmed high efficiency of bacteria aerosolization by bubbles rising in brackish sea water.

Keywords: bacteria scavenge, bacteria in aerosols, mechanism of bacteria aerosolization

IV.P.13

Identification of integrase genes in bacterial strains isolated from the effluent of Silesian municipal wastewater treatment plant

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Integrons are gene-capture systems that harbour antibiotic resistance genes and may provide a flexible approach for bacteria to adapt to the pressure caused by antibiotics. So far three classes of antibiotic-resistance-encoding integrons have been identified. Each class has its own integrase. Among the antibiotic–resistance integrons, class 1 integrons are the most common integron type, class 2 integrons are embedded in Tn7-family transposons and only one example of a class 3 integron is known. Effluents from urban wastewater treatment plants (WWTPs) are suspected to be among the main anthropogenic sources for antibiotics, antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) spread into the environment. The biological treatment process creates an environment potentially suitable for resistance development and spread because bacteria are continuously mixed with antibiotics at sub-inhibitory concentrations. The knowledge regarding the effects of antibiotics resistance on environmental bacteria is scare and contradictory especially with respect to develop their resistance. The WWTPs are the meeting point = hot spots of the most resistance determinants.
The aim of the study was to quality of the presence of integrase genes in effluent from the Silesian municipal wastewater treatment plant. The first stage of the research included the screening of bacterial strains using liquid and solid media supplemented with various combination of antibiotics. In the second stage, chosen antibiotic resistance bacteria isolates were identified by the Biolog system. Then, the DNA from the strains was isolated by the High Pure PCR Template Preparation Kit from Roche. Polymerase chain reaction (PCR) was done according to the literature for three integrase genes (*intI*1, *intI*2 and *intI*3).

Keywords: effluent, WWTPs, ARB, integrons

Acknowledgment:

This study was funded by National Science Centre, Poland, project no. 2017/26/M/NZ9/00071.

IV.P.14

The impact of inorganic and organic compounds of automotive brake pads onto viability of *Escherichia coli* and *Staphylococcus aureus* strains

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According to European Automobile Manufacturers' Association (ACEA) statistics, worldwide production of passenger cars is constantly rising. 77.7 million passenger cars were produced globally in 2016. Automotive brake pads are one of the main components of the braking system. The friction of surfaces causes the brake pads to break thus extracting the compounds used in its manufacturing into the environment. The subject of this research is to identify and quantify the inorganic and organic compounds of brake pads and to evaluate its effects onto viability of *E.coli* KMY-1 and *S.aureus* bacteria strains. The mineralogical composition of brake pad samples was identified by XRD (X-Ray Diffraction Spectrometry), while the elemental composition was determined by XRF (X-Ray Fluorescence Spectrometry) and EDS (Energy Dispersive Spectroscopy). ASE and HPLC-UV methods were used for the analysis of organic compounds. Particles size was measured using Dispersion technology DT1200 (USA) nanoparticles sizer. The effect of copper oxide and barium sulphate nanoparticles and main organic compounds: phenol, naphthalene and anthracene extracted from the brake pads on viability of *E.coli* KMY-1 and *S.aureus* was evaluated by minimal inhibitory concentration and inhibition zone methods.

Keywords: nanoparticles, automotive brake pads, viability, bacteria

IV.P.15

Diversity and antagonistic activity of endospore-forming bacteria of bottom sediments of the Black Sea

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Deep-sea bottom sediments of the Black Sea are an example of a bacteriologically poorly-studied habitat. The quantitative and qualitative parameters of the microbiota of the sediment data are of scientific interest because of a combination of a number of unique physico-chemical characteristics such as anoxygenity, moderate salinity, great depth, etc. The question of the presence and, if any, the taxonomical composition of an facultatively-anaerobic microbiota in these sediments is not clear. The growing need for antimicrobial compounds of new types and classes is a practical motivation to expand the search for microbial strains for screening. The aim of the work was to detect, in deep-sea bottom sediments of the Black Sea, facultative-anaerobic mesophilic endospore-forming bacteria and to identify their ability to produce antibiotic compounds. As a result of research on samples of bottom sediments from depths in the range of 888–2080 meters located in the zone of deep anaerobiosis, 150 strains of facultative-anaerobic endosporeforming bacteria have been isolated. Isolation was carried out by pasteurization followed by scattering on meat-peptone agar. The strains were identified by analyzing fatty acid spectra. All of them belonged to the families *Bacillaceae* and *Paenibacillaceae*. Among them, representatives of the genera *Bacillus*, *Brevibacillus*, *Paenibacillus*, *Lysinibacillus* were identified. Screening of antagonists was carried out by the method of radial strokes on the medium of Gauze-2. As antagonistic activity indicators, strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella enterica, Candida albicans*, *Klebsiella pneumoniae*, *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus* were used. The results indicate that 60 of 150 isolates exhibit antagonistic activity against at least one of the test strains, of which 30 exhibit a high level of antagonistic activity. Further identification of antagonistic factors by metabolomic methods is carried out.

Keywords: sea, sediments, endosporeformers, antagonism

IV.P.16

Antimicrobial Streptomyces strains from cave microbiota: a source for new drugs

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Latest forecasts suggest that by the year 2050 superbugs will be responsible for more deaths than cancers all together, which is code red for human survival instinct. In order to manage this urgent issue, new strategies and new antibiotics need to be developed. One of the possible resources in a fight might be compounds produced by cave bacteria. One of promising directions is isolation and characterization of antimicrobial compounds produced by bacterial strains isolated from cave habitats. Moonmilk speleothems host a rich microbiome, among which *Actinobacteria* represent exceedingly abundant phylum. It has been proven in recent work that the bacteria isolated from Belgium caves show antimicrobial activity.

In this study, we have evaluated 24 bacterial strains isolated from caves of the Tatra National Park. In order to evaluate their antimicrobial potential, we conducted a series of streak tests. Three of them, in particular, have shown a really promising antibacterial response to both Gram-positive and Gram-negative bacteria by inhibiting their growth. We tested metabolites production on different media to chose the most efficient one.

Keywords: *Actinobacteria*, antimicrobial compounds, moonmilk, cave bacteria

IV.P.17

The cytotoxic potential of *Bacillus cereus sensu lato* isolates is associated with their geographic origin

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Bacillus cereus sensu lato comprises soil-dwelling endospore-forming bacilli which have a huge impact on human health and economy. *B. cereus sensu stricto* (*B. cereus s.s.*), the most known member of the group, is an opportunistic pathogen commonly involved in gastrointestinal infection which can manifest in two types of symptoms, emetic and diarrhoeal. Among causative agents of diarrheal disease is cytotoxin K (CytK) belonging to the β-barrel pore-forming toxin family with necrotic and haemolytic properties.

In this study we decided to investigate presence of cytK-encoding genes (*cytK-1* and *cytK-2*) among 182 *B. cereus s.l.* strains isolated from soil samples originating from different geographic locations (Poland, Burkina Faso, and Argentina). In addition, 111 isolates were characterized by the MLST scheme according to the *B. cereus* PubMLST database. Phylogenetic tree based on the concatenated loci was constructed jointly with the MEGA7 software.

Our results showed that cytotoxic potential is higher among strains isolated from Burkina Faso (53%) and Argentina (34%) than Poland (12%). The environmental isolates were divided into six phylogenetic groups. *CytK-1* gene, originally identified in *B. cytotoxicus* strain, was not detected in any of the tested strains. While *cytK-2* gene was localized among 92.3% and 94.1% strains within clades III and IV, respectively. Our results showed a high degree of polymorphism in the *B. cereus group* and lack of clear phylogenetically divisions between species. Climates with arid hot steppe or temperate with hot summer without dry season favor the evolution of cytotoxic strains that could contaminate food products and caused seriously food poisoning.

Keywords: *Bacillus cereus sensu lato;* Cytotoxin K; climate; soil isolates

Acknowledgements:

This work was supported by National Science Centre of Poland (Grant No.: 2015/19/B/NZ8/03944)

V. Medical Microbiology

V.P.1

Bacteriological assessment of biofilms from Orbera gastric balloons in BIB treatment of obese patients

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BIB method (BioEnterics Intragastric Balloon) is one of the means of the treatment for obese patients for whom diet therapy was ineffective. It is based on the endoscopic placement of a balloon filled with saline in a patient's stomach for the period of 6–12 months. To prevent the possibility of the damage of stomach mucosa by exceedingly released H^+ ions, the patients were administered protective drugs from the group of proton-pump inhibitors.
The study was motivated by probable coating of the balloons with biofilm and potential risk of patients' infection. After obtaining desired body weight loss the balloons were removed using endoscopy and packed in sterile conditions transferred to microbiological laboratory. Both during the treatment and after balloon removal there were no signs of localized or general infection in any of the patients.
Microbiological analyses were performed on the material collected from 18 patients with initial BMI exceeding 30 kg/m^2. The surface of the balloons was covered with slimy coating. Scanning microscopy images were taken to show the structure of potential biofilm. Moreover, qualitative analyses were performed.
Microbial cultures from swabs collected from balloons surfaces did not give any colony growth on solid media. Therefore, the collected material from 8 cm^2 (according to requirements for medical devices) was initially cultured in liquid media: with soy and casein for aerobes and with sodium thioglycolate for anaerobes. Next, the samples were cultured on enrichment and selective media for G- *Enterobacteriaceae* family, staphylococci, streptococci, faecal streptococci, G+ LAB, anaerobic rod-shaped bacteria and yeasts. Species identification was determined using Api ATB (bio Merieux).
Conclusions: Body mass reduction with BIB does not pose any particular risk of infection during 6 months of observations. Microorganisms cultured from balloons surfaces were mainly yeasts *C. albicans*, next *C.glabrata* and anaerobic bacteria from *Clostridium* species and a small number of faecal streptococci and β haemolytic streptococci. Cultures microorganisms will be sequenced.

Keywords: intragastric balloon. infection, safety

V.P.2

The contribution of MSMEG4305 protein in the synthesis of vitamin B12 in *Mycobacterium smegmatis*

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Introduction: *Mycobacterium smegmatis* is used as a model organism to study mycobacterial physiology and gene regulation. The genome of this organism contains the MSMEG_4305 gene coding for a two-domain protein. This gene is present across *Mycobacterium* but absent in other species of bacteria. Sequence similarity analysis indicates that the C 'terminal domain is the CobC domain, predicted to be involved in the synthesis of vitamin B12, while the N'terminal domain encodes an RNase H. The function of RNase H domain has been confirmed *in vivo*, however the function of CobC domain remains elusive. **Aim:** The aim of this project is to determine the possible role of MSMEG_4305 protein in the process of vitamin B12 biosynthesis in *M. smegmatis* cells.
Materials and Methods: The ability to synthesize the B12 molecule by *M. smegmatis* was investigated thru analysing the formation of methylfolate trap resulting in hypersensitivity to sulfonamide and thru phenotypic analysis. We compared the growth of three gene deficient mutants MSMEG4305, MSMEG3873 (*cobIJ*, gene with confirmed role in vitamin B12 synthesis) and MSMEG4305/3873 with wild type *M. smegmatis* mc^2 155. We used flow cytometry to count bacterial cells and compared this data with the number of colony forming units (CFU). Flow cytometry was used also to determine cell size.
Results: We observed a hypersensitivity to sulfonamide of all of the analysed mutants on the medium enriched with sulfonamide. We noticed also significant differences between the cell length of all analysed strains of *M. smegmatis* compared with wild type. Supplementation of the medium with vitamin B12 restored the phenotype of the mutants to the wild type *M. smegmatis*. **Conclusions:** Our results suggest that MSMEG4305 is involved in vitamin B12 synthesis in *M. smegmatis*, though further research is needed to confirm our hypothesis.

Keywords: *Mycobacterium smegmatis*, vitamin B12, sulfamethazine sodium salt, flow cytometry

V.P.3

Detection of the epitopes of the enolase protein from *Streptococcus agalactiae*

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S. agalactiae (GBS) is an opportunistic pathogen which can cause severe infections in newborns. Rapid immunogenic assay based on highly specific epitopes could constitute a good alternative to widely used cultivation methods. The aim of the study was mapping of the epitopes of the enolase protein of GBS.
A homogenate of proteins of various GBS strains (n=180) has been separated in Western Blot. Immunoreactivity was tested in the presence of umbilical cord blood sera from GBS-positive patients (N=20) and GBS-negative patients (N=14) divided according to CDC guidelines. A few highly immunoreactive GBS proteins have been detected, however one of them – enolase (47.4 kDa), has been choosen for further analysis, which included the bioinformatic prediction of the most likely epitopes. The most probable epitopes were synthetized on polyethylene pins by PEPSCAN method. Immunoreactivity was examined in presence of GBS-positive and GBS-negative sera. The most immunoreactive peptides have been subjected to a modified synthesis based on cutting off consecutive amino acids from the N-end and the C-end to obtain the shortest highly immunoreactive peptide specifically recognized by human antibodies. According to Western Blot followed by a bioinformatics analysis, 32 most likely epitopes of enolase had been subjected to PEPSCAN synthesis, however two of them: RAAADYLEVPLYSYLG and MIALDGTPNKG, demonstrated the highest immunoreactivity and specificity to antibodies. Therefore, they were classified as the epitopes. The presented results constitute the basis of patent proceedings nos. P.404498 and P.424214.
The identified epitopes demonstrated features qualifying them as good candidates for an innovative subunit vaccine against GBS as well as a marker in the immunodiagnostic assay for GBS infection.

Keywords: *Streptococcus agalactiae*, epitopes, immunoreactive proteins, enolase

Acknowledgements:

This study was supported by the National Science Centre in Poland within the framework of project grant no. 2013/09/B/NZ6/00801 and by the National Centre for Research and Development within the framework of project grant no. DZP/TANGO2/395/2016.

V.P.4

Antimicrobial resistance of Gram-negative bacteria in Lithuanian healthcare institutions: analysis of clinical cases

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Use of antibiotics, which started in 20th century, now is a cause for resistance to antimicrobial agents and spreading of resistance genetic elements in different bacterial species. Bacteria's ability to develop different resistance mechanisms by using their huge genome flexibility and adaptivity to changing environment conditions was insufficiently evaluated and now is a cause of antimicrobial resistance.
Research was done with gram-negative bacteria, that were isolated from human blood – *Escherichia coli, Klebsiella pneumoniae, Acinetobacter spp.* group and *Pseudomonas aeruginosa*. During the research Gram-negative bacteria's resistance to antimicrobial agents was evaluated by disc diffusion and Rosco disk/tablet diffusion methods, resistance genes were identified by PCR (according to literature study) and the genotyping of isolates was performed using BOX-PCR and the results were analyzed by creating dendrograms with TreeCon software.
The results show that all of *K. pneumoniae* and *E. coli* samples, which were analyzed during the research were resistant to one, two or three III – generation cephalosporins, 6 *K. pneumoniae* isolates were resistant to carbapenems. Meanwhile all cultures of *P. aeruginosa* and *Acinetobacter spp.* were resistant to one or few carbapenems. According to these results, resistance genes, which were responsible for resistance mechanisms, were found. The isolates with the identical BOX-PCR electrophoretic profiles were identified for all tested Gram-negative pathogens suggesting that all four pathogens are involved in the intra- and/or interhospital dissemination between the Lithuanian healthcare institutions.
Clinical analysis of resistance genes distributed between different hospitals of Lithuania was made according to genotyping results and dendrograms. During the research it was discovered that only a few of the hospital samples were from the same strain, but all the samples were genetically very close to one another. The level of the transmissions differed between pathogens, and the worst case was detected for *Acinetobacter spp.* followed by *Escherichia coli.*

Keywords: β-lactam resistance, Gram-negative bacteria, nosocomial infections

V.P.5

Impact of biological or nutritional treatment on the number of *Candida* in the stool of children with Crohn's disease

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Introduction: It is supossed that Crohn's disease may be the result of excessive immune response directed against microorganisms naturally occur in the gastrointestinal tract or as a consequence of intestinal dysbiosis. Most of the previous studies have focused mainly on the participation of bacterial flora in the pathogenesis of CD, but did not take into account the share of fungi of the genus *Candida*, which are also part of the natural microbiota of the digestive tract. Moreover, it is interesting whether biological or nutritional treatment resulting in quickly remission, contribute to the normalization of the gut mycobiome.
Aim: The aim of this study was a quantitative assessment of the composition of *Candida* genus in children with CD relative to healthy and impact of biological therapy or nutritional treatment on quantitative changes within gut mycobiome.
Material and Methods: The material subjected to the analysis were stool samples taken from children: – with CD, before and after anti-TNF alpha antibodies therapy (n=14) – with CD, before and after the nutritional treatment (n=48) – healthy, being a control group (n=17) The fungal's DNA was isolated from the samples, as a template for amplification by the quantitative polymerase chain reaction (qPCR) in order to determine the number of *Candida* cells in the individual groups.
Results: In the group of biological treated patiens, before the starting therapy, number of *Candida* sp. (CFU/g) was significantly higher (9.74×10^{17} CFU/g) in relation to the control group (9.35×10^{10} CFU/g, p=0.011). After treatment the number of CFU/g was clearly reduced to the value 5.91×10^{11} and was comparable with the number of fungi colonizing healthy children (p=1.0). There were not observed these correlations in the group of nutritional treated patients.
Conclusion: In group of biological treated patients number of *Candida* was a significantly higher compared to the control group. Biological treatment led to a significant reduction in the number of *Candida* fungi in the gastrointestinal tract of children with Crohn's disease. Nutritional treatment did not impact for reduce the colonization of the fungi.

Keywords: Crohn's disease, Candida, enteral nutrition, biological treatment

V.P.6

Role of carbapenemase-producing *E. coli* (OXA-48) and *Acanthamoeba* in Urinary Tract Infections

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Urinary tract infections (UTIs) are one of the most common hospitals and community-acquired diseases in the UK. The re occurrence of UTIs is not uncommon especially with the presence of *E. coli*. Carbapenemase-producing *E. coli* (OXA-48) has become an increasing problem worldwide due to its multi-drug resistance. A recent study has confirmed the presence of *Acanthamoeba*, the free-living amoebae in urine samples collected from critically ill patients. It has been confirmed that the interaction between *E. coli* K1 and *Acanthamoeba* resulted in growth and survival of *E. coli* in *Acanthamoeba* leading to enhanced bacterial virulence. Therefore, it is reasonable to hypothesise that *Acanthamoeba* can possibly plays a role in increasing carbapenemase-producing *E. coli* virulence and also its increasing role in UTIs.
The aim of the current project was to study the virulence factors of carbapenemase-producing *E. coli* (OXA-48), and to investigate the presence of *Acanthamoeba* in urine samples and its interaction with the pathogenic bacteria. Moreover, the cytotoxic effect of both microorganisms on the TERT-NHUC urothelial cell line was investigated.
The results showed that OXA-48 is carrying several genes encoded for its virulence. The interaction between OXA-48 resulted in growth, survival and multiplication of this pathogen inside *Acanthamoeba*. It has also been confirmed that OXA-48 induced high cytotoxic effect on TERT-NHUC cell line compared with the sensitive *E. coli* control. This effect was significantly higher using 10 bacteria per cell when compared with 100. This could have a significant impact on UTI and its re occurrence.

Keywords: OXA-48, *E. coli*, *Acanthamoeba,* UTIs

V.P.7

FTIR-microspectroscopy of small quantities of biosamples using the miniature diamond-anvil cell

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It is well known that Fourier transform infrared (FTIR) spectroscopy techniques and data analyses are widely available and easy to use for different studies of various biosamples. Due to relatively low sample requirements those are frequently used in biomedical science as label-free methods providing objective and reliable diagnostic information in a relatively non-invasive manner. However, sometimes cell cultivation or even more – purification of cell components or excretes may be costly. Thus, the possibility to even more reduce the required sample amount would be of high value. In this study we demonstrate a novel method for analysis of small quantities of biosamples by FTIR-microscopy of dry sample films using diamond-anvil cell (DAC) in comparison to more convenient high throughput screening (HTS).

Human colorectal cancer cell lines SW480 and SW620 derived from primary and metastatic tumour from a single patient were cultured under hypoxic conditions (1% oxygen, 94% N_2, 5% CO_2), and used as test sample for comparison and evaluation of DAC versus HTS. HTS spectra were acquired using HTS-XT microplate reader while DAC spectra using Hyperion microscope (both Bruker Optics, Germany). Data were analyzed using Bruker software Opus 6.5.

Approximately 200'000 cells are required to gain a good quality spectrum using HTS. By use of DAC we were able to significantly reduce the number of cells needed. From less than 2'000 cells we were able to gain multiple spectra, while maintaining the same intensity and quality as with HTS. FTIR absorption spectra from both methods showed no significant spectral differences. Thus, we conclude that the DAC could be especially useful when dealing with limited biosample quantities like working with biofluids, their components and extracellular matrix.

Keywords: FTIR-spectroscopy, small biosample, diamond anvil cell, high throughput screening

V.P.8

Antimicrobial peptides involvement in insect immune response to fungal α-1,3-glucan

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Antimicrobial peptides are important components of innate immunity in different groups of animals. They are involved in combating bacterial and fungal infections. *Aspergillus niger* is an opportunistic fungal pathogen which is particularly dangerous for immunodeficient patients. Studies on the pathogenicity of many fungi, including *A. niger*, are carried out using a model organism, the greater wax moth *Galleria mellonella*.

The cell wall of *A. niger* consists of many polysaccharides, such as chitin, β-glucans and α-1,3-glucan. Our studies performed using *G. mellonella* larvae demonstrated that α-1,3-glucan isolated from *A. niger* cell wall is recognized by insect immune system and triggers different types of immune reactions. In response to recognition of α-1,3-glucan the level of antimicrobial peptides in larval hemolymph increased, even up to 14-times in the case of defensin (galiomycin), which has antifungal activity. Real Time qPCR showed that immune-relevant genes expression was considerably higher in fat body of α-1,3-glucan-challenged *G. mellonella* in comparison to the control ones. Up to four times increased level of galiomycin, gallerimycin and IMPI transcripts was noticed, genes playing a role as antifungal factors in insect immunity.

All of these results indicate that different antimicrobial peptides are engaged by *G. mellonella* immune system in response to α-1,3-glucan. In addition, high level of peptides with antifungal activity detected after α-1,3-glucan immunization, indicates the specificity of insect immune system.

Keywords: antimicrobial peptides, glucan, innate immunity, *Galleria mellonella*

Acknowledgements:

The work was supported by grant 2013/09/N/NZ6/00838 from the National Science Centre (Kraków, Poland).

VI.P.1

The reserach of tetraphenylphosphonium and ethidium interaction with *Listeria monocytogenes* bacteria

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Antimicrobial resistance is a constantly growing worldwide problem. A key part of antimicrobial resistance is multidrug resistance efflux pumps. Because of these pumps *Listeria monocytogenes* is a multidrug resistant pathogen, not sensitive to many antimicrobial compounds. It is very important to understand the mechanisms how could we regulate the activity of efflux pumps, because *L. monocytogenes* is an opportunistic foodborne Gram-positive pathogen causing serious human infections. It is not easy to develop new antimicrobial compounds which would not be a substrate of efflux pumps in addition the sides effects of new compounds are unknown so the knowledge about the inhibition of antibiotics efflux out of cells could increase the effectiveness of treatment.

We used potentiometric and fluorescence methods to assay the inhibition of *L. monocytogenes* efflux pumps. We used tetraphenylphosphonium, which is a substrate of these pumps, selective electrode to register the inhibition of efflux. In parallel, the intensity of ethidium fluorescence was determined. We used inhibitors of different families of efflux pumps, such as chlorpromazine, verapamil, reserpine. Also we explored the effect of Phe-Arg-β-naphthylamide (PAβN) and 1-(1-Naphthylmethyl) piperazine which have not yet been used against gram-positive bacteria.

To assess the influence of potential inhibitors on the interaction of *L. monocytogenes* cells with efflux indicator Tetraphenylphosphonium (TPP^+) ions.

Conclusions. TPP+ strongly inhibits the intensity of respiration of *L. monocytogenes* cells while ethidium – not. We determined that all of used inhibitors increase the accumulation of efflux pumps substrate. In addition, we observed that PAβN and NMP inhibit the efflux of tetraphenylphosphonium but not ethidium. The results obtained from electrochemical analysis whith PAβN selective electrode showed that *L. monocytogenes* bacteria accumulate a large amount of this compound.

Keywords: efflux pumps, *Listeria monocytogenes*, Tetraphenylphosphonium, ethidium

Acknowledgements:

We thank prof. S. Fanning (University College Dublin, Ireland) for *L. monocytogenes* cells.

VI.P.2

The $HtrA_{Hp}$ protein protects the *Helicobacter pylori* cells from consequences of stressful environmental conditions

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Helicobacter pylori is a Gram negative bacterium that colonizes the mucus layer covering the gastric epithelium of humans. Chronic infection with *H. pylori* is associated with increased risk for development of gastric and duodenal ulcers or even of gastric adenocarcinoma and mucose-associated lymphoid tissue (MALT) lymphoma. Effective colonization of the host is facilitated by a number of virulence factors, including a serine protease, $HtrA_{Hp}$. This protein is involved in damaging of the intercellular epithelial junctions by cleavage of E-cadherin, claudin-8 and occludin. In the *H. pylori* cell $HtrA_{Hp}$ must play a vital role, as the attempts to inactivate the $htrA_{Hp}$ gene in approximately 200 *H. pylori* strains were unsuccessful thus far.

After many unsuccessful attempts we finally inactivated the $htrA_{Hp}$ gene and constructed the WGE01 strain (*H. pylori* Δ*htrA*) which allowed for a direct analysis of the Δ*htrA* phenotypes in *H. pylori* grown under physiological and stressful conditions. We found that the non-stressed *H. pylori* Δ*htrA* cells did not show growth defects and the all tested strains (mutated and control) grew on solid media equally well. However, when bacteria were challenged with certain stressful conditions, a lack of HtrA turned out to affect cell viability. *H. pylori* Δ*htrA* showed a high sensitivity to elevated temperatures, changes of pH values, presence of ionic osmotica and treatment with antibiotic puromycin. Interestingly, the lack of $HtrA_{Hp}$ not always increased sensitivity to stressful agents, e.g. oxidative stress caused by hydrogen peroxide.

The majority of stressful conditions used in this work disturb folding of proteins. Therefore, the observed phenotypes allow us to propose that $HtrA_{Hp}$ is engaged in the protein quality control in the *H. pylori* cells, a function which is particularly important under stressful conditions, including these provided by the host's defense mechanisms.

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

VII.P.1

Involvement of the HtrA family members, DegP and DegS, in virulence of the potato pathogen *Dickeya solani*

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Dickeya solani is a plant pathogenic bacterium that causes soft rot and black leg in *Solanum tuberosum*. Due to its high aggressiveness and ability to infect over a wide range of temperatures it causes significant loss of potato crops annually (e.g. 25 mln € in the Netherlands in 2005).

Infection of plant tissues and subsequent dissemination of bacteria are associated with a variety of environmental stresses. Bacterial envelope is particularly prone to damage by adverse environmental conditions; hence an efficient extracytoplasmic stress response is crucial for bacteria to survive in the host and develop disease symptoms.

The HtrA family of proteins is known to play important roles in the stressed bacterial cells. In a model bacterium, *Escherichia coli,* DegP is a serine protease/chaperone that is regarded as one of the most important elements of the extracytoplasmic protein quality control system that is responsible for folding and/or removal of misfolded proteins. DegS is a protease that is involved in regulation of the sigma E dependent expression of the extracytoplasmic stress response genes. Together these proteins help to maintain proteostasis in the cellular envelope. The role of DegP and DegS in *D. solani* has not been studied yet.

To examine the importance of the DegP and DegS proteases we cultured derivatives of *D. solani* IPO 2222 strain deprived of the functional *degP* or *degS* genes and checked their ability to product major virulence factors: cellulases, pectinases, proteases and siderophores as well as and their motility. Next, we examined effectiveness of plant tissue infection on potato tuber, potato slices, and chicory leaves models. The virulence determinants were produced with similar efficiency in all cases, however *D. solani degS* was less infective in potato slice tests.

Keywords: *Dickeya solani*, DegP, DegS, potato

VIII. Food Microbiology

VIII.P.1

Physico-chemical and sensory properties of yogurt processed from cow's milk and soymilk alone and in combination

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Fermented soymilk was reported to contain no lactose or cholesterol, and its proteins have a greater antioxidative ability in preventing lipid oxidation, compared to casein. In this study, yogurt was processed by inoculation of cow's milk and soymilk used alone and in combination (ratio 1/1 v/v) with freeze-dried culture of Lactobacillus bulgaricus and Streptococcus thermophilus. Three samples from each processed yogurt were evaluated for physico-chemical and organoleptic properties using a control. Sensory test was carried out by sensory panel testing three yogurts prepared and a commercial yogurt SOUMMAM. Yogurts were scooped into small cups with random 3 digit codes. The pH values of yogurt samples ranged from 4.65±0.03 in cow's milk yogurt, 4.60±0.05 in soymilk yogurt, and 4.64± 0.01 in the mix. Soymilk yogurt was low in Dornic acidity (81.66±1°D). Fat and total dry extract were highest in cow's milk yogurt: 31±1 g/l and 106.6±0.2 g/l, respectively. There was significant difference (P<0.05) in the protein content between cow's milk yogurt (3.1±0.2 g/l) and soymilk yogurt (3.47±0.01 g/l). The results indicate that the addition of soymilk to cow's milk improved the physicochemical properties as well as sensory characteristics of yogurt, resulting in enhanced health-benefit ingredients and consumers' preferences. Soymilk yogurt alone or in combination with cow's milk yogurt can be adopted as substitute to cow's milk yogurt especially by the low income earners due to its cheaper raw materials, and as protein supplement at household level.

Keywords: Cow's milk, soymilk, yogurt, physico-chemical and sensory properties

VIII.P.2

Bioactive compounds in fried cottage cheese with *Galactomyces geotrichum* mould

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Now, the use of bioactive compounds in food is an important branch of food technology. Some microorganisms characterising the microflora of food products is able to produce bioactive components. The cheese's microflora consists of starter cultures and non-starter cultures - often not yet characterised, but which affect the taste and aroma of the finished product.
Wielkopolska fried cottage cheese is a popular product in Wielkopolska. Among the isolated microorganisms from the samples taken from various stages of the production of fried cheese were tested and strains of *Galactomyces geotrichum* mould was found, which are common in various dairy products. Proteomic analysis of cellular biomass and culture was carried out using the HPLC/LC-MS technique and the MASCOT database. Among the identified proteins, three were associated with the biosynthesis of bioactive compounds: polyunsaturated fatty acids, ergosterol and B vitamins. Medium and culture conditions were optimised to increase the production of the specified compounds. Analysing the quantities of synthesised products, GC and HPLC techniques were used. The increase in the production of polyunsaturated fatty acids was determined by the presence of rapeseed oil as a source of carbon and vitamin B12 in the medium. On the basis of the obtained results, a model fried cottage cheese was prepared with the addition of *G. geotrichum* cells, rapeseed oil and vitamin B12. The obtained product was characterised by larger amounts of bioactive compounds tested compared to traditional cheese, without additional ingredients.

Keywords: *Galactomyces geotrichum*, bioactive compounds, fried cottage cheese

Acknowledgements:

This study was funded by National Science Centre, Poland (grant number 2015/17/N/NZ9/00960).

VIII.P.3

Occurrence of virulence–associated genes in *Arcobacter butzleri* and *Arcobacter cryaerophilus* isolates within the Czech Republic

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Bacteria of the *Arcobacter* genus, originating mainly from food and water, are dreaded germs for humans as well as animals. However, the virulence of these bacteria has not been fully elucidated yet. This study looked at the occurrence of eight virulence-associated factors (*ciaB*, *cj1349*, *pldA*, *irgA*, *hecA*, *tlyA*, *mvi*N, *hecB*) in a total of 80 isolates of *A. butzleri* and 22 isolates of *A. cryaerophilus*. A polymerase chain reaction using specific primers was used to detect these virulence–associated genes. The presence of all genes in the isolates of *A. butzleri* (98.8% *ciaB*, 95.0% *cj1349*, 98.8% *pldA*, 22.5% *irgA*, 31.3% *hecA*, 95.0% *tlyA*, 97.5% *mvi*N, 38.8% *hecB*), and *A. cryaerophilus* (95.5% *ciaB*, 0.0% *cj1349*, 9.1% *pldA*, 0.0% *irgA*, 0.0% *hecA*, 31.8% *tlyA*, 90.9% *mvi*N, 0.0% *hecB*) was monitored. Among the tested isolates, there were 13 isolates (12.7%) of *A. butzleri*, in which the presence of all eight virulence–associated genes was recorded in the genome. In contrast, in one *A. cryaerophilus* strain, none of the observed genes were detected. The presence of *ciaB* and *mvi*N genes was significantly more frequent in *A. cryaerophilus* isolates than other genes ($P < 0.05$). In general, more virulence-associated genes have been detected in *A. butzleri* isolates compared to *A. cryaerophilus*. The most common gene combination (*ciaB*, *cj1349*, *pldA*, *tlyA*, *mvi*N) was detected in case of 39 isolates. In 50.0% of *A. butzleri* isolates derived from clinical samples, all eight virulence-associated genes were significantly more frequently detected ($P<0.05$). The *tlyA* gene was significantly more frequent occurred in *A. butzleri* isolates from meat and water samples, and *irgA* and *hecB* genes in clinical samples. Therefore, our study provides information about occurrence of virulence-associated genes in genome of *Arcobacter*-isolates. Our results indicate high incidence of virulence-associated genes in *Arcobacter* genomes and hence potentially pathogenic properties of the studied strains.

Keywords: *Arcobacter*, virulence-associated genes, polymerase chain reaction

Acknowledgements:

This work was supported by the Department of Biological and Biochemical Science, Faculty of Chemical Technology, University of Pardubice, Czech Republic [grant SGS_2017_004].

IX. Microbial Physiology

IX.P.1

Impact of carbon source on acetic acid stress in different *Kluyveromyces marxianus* strains

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Kluyveromyces marxianus is non-conventional food grade yeast capable of consuming a wide spectrum of substrates. Besides the above mentioned this yeast offers other great benefits including a high growth rate, thermotolerance and possess high activity of β-galactosidase. However, several limitations have been reported that hamper a wider industrial usage of *K. marxianus*, for example, the relatively low ethanol tolerance and weak-acid intolerance.

Acetic acid is weak acid with pK_a 4.76. It is used as food preservative and is also one of the fermentation by-products. In undissociated state acetate diffuse into the cell cytosole and leave up negative effect on yeast metabolism and cell growth.

The aim of this work was to test and compare ability of different *K. marxianus* strains (DSM 5422, DSM 4906, DSM 5418, CBS 712 and CBS 6556) to grow at pH 4.4 at different acetic acid concentrations on various sugars.

The inhibition of *K. marxianus* biomass growth by acetate was studied. The growth inhibition degree was correlated with undissociated acetic acid concentration in medium. The increasing of the concentration of acetate results in lag-phase prolongation and decreasing in growth yield. It is important to note that in the case when maltose was used as carbon source the growth of all strains was inhibited in the presence of acetate at pH 4.4. Very similar pattern we see in the case of CBS 712 when this strain is cultivated in lactose medium. When glucose and galactose mixture was used, the growth curves were similar to them obtained on glucose medium. In another four strains in the case of lactose lag-phase duration is at least twice shorter compared to CBS 712.

Keywords: *Kluyveromyces marxianus*; acetic acid; stress tolerance

Acknowledgements:

This study was supported by University of Latvia under project "Sustainable use of nature resources in the context of climate changes" (No. AAP2016/B041).

IX.P.2

The role of *Candida albicans'* lanosterol 14α-demethylase in response to treatment with antibacterial drugs

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Antibacterial drugs inhibit the growth of bacteria by targeting prokaryotic metabolism, including key enzymes, 70S ribosomes and components of the bacterial cell wall. During antibacterial treatment, eukaryotic cells (the infected host cells and human yeast microflora) are exposed to the drugs. *Candida albicans* is a yeast-like fungus, natural component of human microflora, which under immunosuppression causes opportunistic infections. *C. albicans* cells become resistant to antifungals due to overproduction of membrane drug efflux transporters (mainly Cdr1p belonging to ATP-binding cassette (ABC) family), or changes in expression of genes involved in ergosterol biosynthesis (mainly *ERG11* gene, encoding lanosterol 14α-demethylase).

The aim of the present study was to check the influence of selected antibacterial drugs on viability of *C. albicans*' mutants deficient in either MDR transporters or *ERG11* gene. We conclude that MDR transporters do not play a significant role in the stress response to the drugs, however deletion of *ERG11* gene causes sensitivity of *C. albicans* to three of the tested drugs (gentamycin, tobramycin and chloramfenicol). Erg11p, the product of *ERG11* gene, is the primary target for azole antifungals, so we checked whether antibacterial drugs display synergistic effect with azoles. Additionally, we identified that the mechanisms of toxicity of selected antibacterials towards *erg11*⊿ mutant causes hyperpolarization of the plasma membrane and decrease of the cell hydrophobocity. We conclude that tested by us antibacterial drugs display activity against eukaryotic *C. albicans'* cells under inhibition of Erg11p production hence opens up new research possibilities to combat the drugs resistance of *C. albicans*.

Keywords: *Candida albicans*; antibacterial drugs; lanosterol 14α-demethylase; plasma membrane potential

X.P.1

Reasearch of the effect of β-glucan on peritoneal adherent cell population

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Cell wall components of pathogenic microorganisms in mammals are recognized through pattern recognition receptors – PRR and exhibit immunomodulating properties. Structures such as β-glucans, manans and their complexes bind to PRR receptors: Dectin-1, CR3, TLR, and activate innate and acquired immune responses to pathogen. Acting as immune system activators through cellular cytotoxicity these components can prevent oncogenesis.
β-glucans have positive effect on immune system, however these polysacharides also exhibit adverse effects causing immune cell apoptosis. To identify β-glucan iduced apoptosis two fractions of these polisacharides were extracted from *S. cerevisiae* cell wall using hot water and alkaline extraction methods. Interacting with mice peritoneum adherent cell population, consisted of macrophages and dendritic cells, both β-glucan fractions were dynamically internalized, which led to immune cell activation. Investigation of the β-glucan effect on caspase-3/7 and 8 activity, showed a concentration dependent activation of caspase-3/7 by both hot water and alkaline extracted β-glucan. Study showed that alkaline extracted β-glucan did not result in immune cell apoptosis, whereas hot water isolated polisacharide caused single cell apoptosis.

Keywords: β-glucan; immune cell activation; apoptosis