VI.P.1

Small rodents from recreation areas of Wrocław and its surroundings as reservoir of *Borrelia burgdorferi* s.l.

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Small rodents such as striped filed mouse (*Apodemus agrarius*), yellow-necked mouse (*Apodemus flavicollis*) and bank vole (*Myodes glareolus*) are known to be reservoirs for several genospecies of *Borrelia* spirochetes in Europe. The group of *Borrelia* sp. that can cause Lyme borreliosis now consists of at least 19 genospecies (i.e. LB group) and several of these have been detected on European continent. The knowledge of genetic diversity of agents causing borreliosis is still poor.
From September till November 2014, 196 specimens of rodents belonging to three different species (*A. agrarius*, *A. flavicollis*, *M. glareolus*) were captured in four locations representing recreation area of Wrocław (Mokry Dwór and Osobowice) and surroundings (Ruda Milicka, Sulistrowice). Samples of blood and spleen were taken and DNA isolation was conducted using commercial kits. Nested PCR reactions were performed to estimate prevalence of LB spirochetes among captured rodents. As markers, fragments of flaB, ospA and 23S genes, were used. Based on literature data sets of primers were applied. Positive samples were sequenced and identification of genospecies and molecular analysis were performed, as well as comparison of sensitivity and application of markers used for detection of *Borrelia burgodorferi* in reservoir hosts tissues.

Key words: *Borrelia burgdorferi*, rodents, vector-borne pathogens

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VI.P.2

Halophilic microbial communities inhabiting biodeteriorated historic buildings in Łódź

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Łódź – a city with 650-year history which was in the past a great industrial center of the country instead of being admired, more often forces us to focus on progressive deterioration and disturbing technical conditions of its cultural heritage. Excessive moisture and high salinity commonly affecting monuments in Łódź have resulted in the development of halophilic microorganisms that contributed to its destruction. The importance of the problem is escalated by inestimable loss of our local heritage. Hence, a need to implement effective methods for the identification and characterization of halophilic microorganisms arises to fight against their development as a step prior to proper renovation strategies. In this study we focused our attention on the search of halophilic microorganisms in deteriorated historic buildings in Łódź.
Samples (n=14) from different materials (brick and paint coatings) derived from the surrounding walls showing the salt efflorescence phenomenon, were taken from external and internal locations within the historic buildings in Łódź, Poland and pooled both for cultivation and molecular analysis. All samples were inoculated on culture media supplemented with different salt concentration (NaCl, $MgSO_4$; 0–30%) and showing different pH range (1–12). The DNA were amplified with PCR primers which flanked the 16S rRNA gene. To determine taxonomic position, gene sequencing was carried out. The obtained nucleotide sequences were compared with the National Centre of Biotechnology Information database.
The concentration of halophilic microorganisms ranged between 4.8×10^2–1.3×10^5 cfu/ml. The most abundant species were *Halobacillus* sp., *Marinococcus* sp., *Virgibacillus* sp. and *Staphylococcus* sp. (96–99% similarity) with white to orange and pink pigmentation (optimal salinity 5–15%, optimal pH 5–8). The results of the study suggested that salt deposits formed within historic buildings in Łódź offer a suitable habitat for specialized halophilic microorganisms, which are responsible for aesthetical changes as well as cracking, powdering and material loss.

Keywords: halophilic microorganisms, biodeterioration, historic buildings

VI.P.3

Effect of lysozyme on the formation of *Escherichia coli* antibiotic tolerant cells

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Bacteria have evolved many mechanisms that allow them to survive in adverse environmental conditions. One of these mechanisms is the formation of persister cells. Persister cells represent a small number of bacterial populations. In contrast to antibiotic-resistant bacteria persisters are phenotypic variants of wild type cells and become sensitive to antibiotics again after transfer to a fresh medium. They arise in response to nutrient depletion, oxidative stress or heat shock. Formation of persisters can also be induced by certain antibiotics. It is suggested that high tolerance to antibiotics is due to the inhibition of vital processes which are the targets of antibiotics. Another phenotype of antibiotic tolerant cells are L-forms which are, at least partially, deprived of their cell wall. The formation of L-forms is induced by exposure of bacteria to cell wall degrading factors (for example lysozyme) or cell wall synthesis inhibitors, including β-lactam antibiotics. L-forms can be resistant to antibiotics that inhibit cell wall synthesis.
The aim of the project is answer the question whether persister cells can arise from L-forms. This hypothesis can be supported by the fact that both persisters and L-forms are able to tolerate β-lactams and restore their antibiotic sensitivity after transfer to fresh medium. Moreover, it is known that L-forms overexpress genes, including the toxin-antitoxin module MazEF, phage shock and DNA repair/SOS response pathways, which are also induced in persisters. Both types of cells are formed in response to depletion of nutrients in the environment and other types of stress.
Our preliminary studies demonstrated that lysozyme can induce formation of persister cells in *E. coli* cultures. These results allow us to conclude that during reversion of L-forms to normal rod-shaped cells the bacteria can acquire tolerance not only to β-lactams, but to other classes of antibiotics.

Key words: L-forms, lysozyme, persister, *Escherichia coli*

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VI.P.4

New insights into the investigation of marine microbial diversity of the Black Sea surface water

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The Black Sea is unique marine ecosystem with high microbial biotechnology potential. The microbial communities are the integral part of the ecosystem and play the key role in all biological processes occurring in it. The data on the Black Sea bacterial diversity is mainly obtained by cultural methods. However few prokaryotes are capable of cultivation and it's known about candidate phyla without cultured representatives. The aim of research was to investigate the Black Sea biodiversity by metagenomics 16S rRNA analysis. Provided study is a complex investigation of the seawater microbiota based on isolation of environmental DNA from marine biome and further sequencing. Marine water was collected and filtered during July 2014 from 6 sites along the seacoast in Odessa region. Total DNA was isolated from filters. Primer design followed Kozich *et al.* (2013) with dual indexing, Read1, Read2, Index1 primers and 16S V4 variable DNA region specific primer. PCR reactions were performed to obtain a 16S clone library that was quality controlled, purified and quantified. Sequencing was performed on Illumina MiSeq platform. QIIME workflow (Caporoso *et al.*, 2010) was used for computer analysis of data. The 16S rRNA gene sequencing and bioinformatics analysis showed dense Bacterial community among samples. Obtained sequences were identified up to genus level. Taxonomic phylum Proteobacteria was the most abundant among Bacterial Domain. The composition of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Epsilonproteobacteria and Deltaproteobacteria classes varied depending on the source of sample. Sequences of Cyanobacteria, Bacteroidetes, Actinobacteria, Planctomycets and Verrucomicrobia phyla were identified in high amounts as well. The members of Fusobacteria, Tenericutes and Firmicutes phyla were less represented in samples. The results of research identified members of candidate divisions such as SR1, OD1, OP3, OP8, TM6, TM7 and others. Some of the determined bacterial species were described for other parts of World Ocean in similar metagenomics investigations.

Key words: 16S rRNA analysis, metagenomics, marine microbiota, Black Sea

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VI.P.5

Microbial quality of recreational beaches of Vlora Bay, Albania

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Vlora Bay, located in the southern part of Albanian coast is a busy tourist destination. Local beaches are not only the main attraction for visitors, but also for residents. Since this area has no sophisticated sewage system it may be contaminated by discharge of untreated sewage generated from rapid urban developments. Animals, like pets or birds can be another reason for recreational water pollution. A total of 5 sampling points along Vlora Bay beaches (Radhimë, Plazhi i Ri, Akademia e Marinës, Plazhi i Vjetër, Kabinat, Nartë) were selected and monitored during the period of January 2014 to August 2014. Samples were evaluated for faecal coliforms (FC) and faecal streptococci (FS). Akademia e Marinës beach had the highest incidence of faecal indicators (FC and FS), respectively 100% of samples, followed by Plazhi i Ri (27.3% and 45.5%), mainly during summer. Whereas, Plazhi i Vjetër, Kabinat, Radhima and Narta beach were in compliance with the Guidelines, as faecal indicators concentations remaind well below the standards. High concentration of faecal indicators, especially during summer in some of these beaches emphasizes the vitality of periodically monitoring of these areas in order to prevent bathers health risk.

Key words: recreational water pollution, faecal indicators, Vlora Bay

VI.P.6

First report of *Apophysomyces variabilis* from endangered *Chelonia mydas* nests

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The fungus *Apophysomyces variabilis* (order Mucorales) is a thermotolerant species that can cause significant infections in immunocompetent patients. This fungal pathogen causes zygomycosis, which have a worldwide distribution, in humans and animals. *Apophysomyces variabilis*, clinical and environmental strains, can be isolated from soil, decaying vegetation and contaminant environment.
The study area, Sugözü Beaches, is located at the north of Iskenderun Bay. Iskenderun Bay has become an important center for marine transportation and has the large number of industries. Also, Sugözü Beaches are one of the most important nesting beaches for endangered green turtle in Mediterranean basin. In this study, sand and unhatched eggs which contains dead embryo were taken from the nests randomly in four sub-sections (Akkum, Sugözü, Botaş, Hollanda) of Sugözü Beaches from June to September of 2014. A total of 22 sand samples were collected inside of the different nests aseptically, and then were inoculated on Saboroud Dextrose Agar medium. Fungal DNA isolation kit was used and samples were analyzed by using partial 18S rRNA gene; complete internal transcribed spacer region 1, 5.8S rDNA gene and internal transcribed spacer region 2 for Real-Time PCR. The sequences can be compared to the GenBank database by using the NCBI BLAST serve. The isolate of *Apophysomyces variabilis* was compared with all strains of *Apophysomyces* spp. Fungi was identified as *A. variabilis* (100% sequence similarity with sequence of type strain R-4746 [JN980700.1]) by morphological and molecular analysis. This is the first ecological report of *Apophysomyces variabilis* from endangered green turtle nests. The presence of this fungus in the nests might be explained in two ways: i) This species could be a new pathogen for green turtles ii) This species could be a new bioindicator of marine pollution for this region.

Key words: *Apophysomyces variabilis, Chelonia mydas,* fungal pathogens

VI.P.7

The presence of the resistant *Escherichia coli* strains in Lesser Poland surface waters

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The increasing resistance of microorganisms to antibiotics is actually one of the most important problems of medicine, the more that it is no longer only the clinical strains. Increasingly, drug-resistant bacteria are isolated from the environment outside the hospital (water, soil, air), posing a serious threat to the health of humans and animals.
The aim of the paper was to estimate the incidence of resistant strains of *Escherichia coli* in the waters of the two Lesser Poland rivers: Raba and Prądnik.
Samples were taken at 20 testing points – 10 for each of the river along its course. Microbiological water analyzes were performed by membrane filtration method. After the incubation randomly chosen *Escherichia coli* strains were isolated. After confirming the systematic assignment of isolates (API20E) the antibiotic resistance tests were performed using the disc diffusion method based on the recommendations of EUCAST and KORLD. The following antibiotics were tested: Ampicillin, Ampicillin/Sulbactam, Aztreonam, Cefamandole, Cefepime, Cefuroxime Sodium, Cephalothin, Cephazolin, Fosfomycin + Glucose-6-Phosphate, Gentamicin, Imipenem, Meropenem, Nitrofurantoin, Norfloxacin, Ofloxacin, Piperacillin, Sulfamethoxazole/Trimethoprim, Tetracycline and Ticarcillin.
Escherichia coli were present in all tested water samples. Full susceptibility testing of isolates was performed for 67 strains – 30 came from the river Prądnik and 37 from the river Raba. Over 60% of tested strains were resistant to at least one of the tested antibiotics (60% of isolates from Prądnik and 62.2% from Raba), 26.9% showed relative resistance to 5 or more antibiotics and 4.5% were susceptible to 10 and more tested compounds. The greatest strains resistance was observed in the case of ampicillin (53.7%) and minimum (<3%) for imipenem, meropenem and fosfomycin.
The presence of antibiotic-resistant bacteria in water indicates faecal pollution and constant flow of people or livestock feces. Among the studied strains dominates resistance to antibiotics commonly used in therapy. Bacteriologically contaminated river water may be a potential source of antibiotic-resistant *E. coli* strains.

Key words: water, *Escheriachia coli*, antibiotic-resistant bacteria

VI.P.8

Application of BIOLOG system for characterization of *Serratia marcescens ss marcescens* isolated from wastewaters of onsite wastewater technology (OSWT)

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Biolog™ system combine the microbial identification, functional diversity of microbial communities and phenotype microarray testing approaches. The correlation between genotypes with phenotypes, determination of metabolic and chemical sensitivity properties of cells, discover new targets for antimicrobial compounds, optimize growth and culture conditions in bioprocess development can be evaluated by the system.
The scope of the study was to apply Biolog system to identify and characterize *Serratia* strain isolated from the surface of black plastic pieces which constitute the fluidized bed filter (onsite wastewater technology, OSWT). Over the last four decades, *S. marcescens* has emerged as a human pathogen, it is involved in hospital-acquired infections (HAIs), particularly catheter-associated bacteremia, urinary tract infections and wound infections an important healthcare-associated pathogen. Most *S. marcescens* strains are resistant to several antibiotics because of the presence of R-factors, which are a type of plasmid that carry one or more genes that encode resistance; all are considered intrinsically resistant to ampicillin, macrolides, and first-generation cephalosporins (such as cephalexin). The preliminary isolation of the strain was done in the medium with tetracycline in 16 mg/l concentration. To characterize of isolated strain the following Biolog methods were applied: (1) EcoPlates microplates for evaluation of physiological profiling, (2) GEN III OmniLog® ID System for identification of isolate, and (3) phenotypic microarrays (PM) technology for evaluation of sensitivity to antibiotics (PM11 and PM12). Results were recorded using the OmniLog® and companion computer software. *Serratia* strain was identified as *Serratia marcescens ss marcescens* with similar index 0.569. PM analysis showed a gain of phenotype (resistance or positive growth) of the strain to 34 antibiotics. The loss of phenotype (sensitivity or negative growth) was only observed for 4 antibiotics: lomefloxacin (0.4 μg/ml), enoxacin (0.9 μg/ml), nalidixic acid (18 μg/ml), paromomycin (25 μg/ml). PM technology allows phenotypic testing to become a simple analysis on gene expression and allows to directly observe the consequence of a genetic change.

Key words: small wastewater facilities, Biolog system, *Serratia* spp., antibiotic resistance

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VI.P.9

Biosynthesis of silver nanoparticles (Ag-NPs) using culture supernatants of *Bacillus subtilis* and *Escherichia coli*

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During the last decade, the biosynthesis of metal nanoparticles (MeNPs) has emerged and is being developed as an alternative environmentally benign procedure. The biological methods of nanoparticle synthesis belong to new green generation processes, which are eco-friendly and are designed as credible alternative to chemical and physical methods often called "green-synthesis" or "green chemistry" procedures. Recognizing the importance of developing eco-friendly and biological methods of nanoparticles synthesis, scientists have recently started looking into research relating to the synthesis of metallic nanoparticles with the use of biosurfactants as aggregation and stabilization agents. Different commercial or bacteria produced biosurfactants have been examined as stabilizer and modifier in the synthesis of metallic nanoparticles. Various strains of *Bacillus* produce a broad spectrum of biosurfactants mainly lipopeptides from surfactin, iturin and fengicin family.

It is aimed in the present work to investigate the production of silver nanoparticles synthesized in culture supernatants of *Bacillus subtillis* T-1 producing surfactin and *Escherichia coli*, no-biosurfactant producer. The production and properties of Ag-NPs have been verified by UV-Vis and TEM techniques. The formation of silver nanoparticles from silver nitrate by the supernatants of *Bacillus subtilis* T-1 and *E. coli* cultured on LB medium was investigated. The appearance of yellowish-brown color in the reaction vessels suggested the formation of silver nanoparticles. The UV-vis spectra were recorded as a function of reaction time of the culture supernatants with silver nitrate. The nanoparticles exhibited an absorption peak around 450–500 nm after 24 h of the incubation. TEM images of studied Ag-NPs solutions produced in supernatants of *Bacillus subtilis* showed the presence of nanoparticles with broad range of sizes. Whereas, in the supernatant from the culture of *E. coli* the small amount of aggregated Ag-NPs was observed.

Key words: silver nanoparticles (Ag-NPs), biosynthesis, biosurfactants, *Bacillus subtilis*, *E. coli*

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VI.P.10

Mycological pollution of selected city beaches in zachodniopomorskie region

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In the summer increased tourist traffic can be observed on the beach. Cleanliness of water is a very important factor in deciding on a holiday destination. Maintenance of seashore facilities and assurance of safety is the responsibility of owners and local authorities. National Sanitary Inspectorate is obliged to inspect the bathing waters and monitor the sanitary-technical and epidemiological risks. The water was analyzed for the presence of coliform bacteria, faecal streptococci and *Salmonella*. Microbiological pollution can lead to closure of bathing waters on health grounds. Fungi also play an important part in the pollution, as they are highly adaptable to new environments and have low nutrient demand. Fungi can cause mycosis, a fungal infection that is easily transmitted to humans and animals indirectly or directly through contact.

The aim of the research was to assess of the microbiological pollution in the selected public beaches on e.g. bathing Klukom in Choszczno, Miedwie in Zieleniewo near Stargard Szczeciński and Głębokie in Szczecin.

The research aim was to compare the amount of microorganisms before and after the summer season. Samples of water and sand were taken from three selected guarded beaches and then tested for the presence of mould fungi (Martin glucose-peptone medium), keratinolytic fungi (Omeliański medium) and dermatophytes (Agar Sabourauda with chloramfenikol, Scharlau substrate).

The results showed that the public bathing area in examined localities was microbiologically polluted. The amount of mould fungi, keratinolytic fungi and dermatophytes in the three locations varied at different times of the season. Mostly, higher amounts of fungi were observed in the sand sampled from the beaches than in the bathing waters. Moreover, definitely there were more microorganisms detected in the second research period, that is after the summer season, which may have been caused by increased tourist traffic and more conducive environment for fungi growth. The findings indicate that the biggest amount of microorganisms could be found on the beach and in the waters of Miedwie bathing site, whereas Klukom bathing waters and beach area had the smallest presence of fungi.

Key words: fungi, city beaches, sanitary contamination

VI.P.11

The tortuous pathways of methylotrophy in the genus *Paracoccus*

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The genus *Paracoccus* (*Alphaproteobacteria*) consists of bacteria isolated from various natural and polluted environments (e.g. soil, marine sediments, seawater, biofilters, activated sludge or human tissues). Representatives of the genus show significant metabolic versatility and can switch between different growth modes (e.g. heterotrophy *vs*. chemolithoautotrophy, aerobic respiration vs. nitrate respiration). About half of the known *Paracoccus* spp. strains are described as methylotrophs, organisms utilizing C1 compounds (reduced carbon compounds containing no carbon-carbon bonds) as a sole carbon and energy source. The knowledge about methylotrophy in the genus is mostly restricted to the type strain, *P. denitrificans* Pd1222. *P. denitrificans* exemplifies so-called autotrophic methylotrophs, assimilating CO_2 derived from the oxidation of C1 compounds (methanol and methylamine) *via* the Calvin cycle. Analysis of genome sequences of more than thirty other representatives of this genus shows large variation among the strains in terms of methylotrophy metabolism. Intriguingly, the divergence concerns not only the range of oxidized C1 substrates (many strains are able to oxidize trimethylamine, dimethylamine, *N,N*-dimethylformamide or formamide), but also the central assimilatory C1 metabolism (the serine cycle instead of the Calvin cycle). Comparative genomics of strains encoding the serine cycle enzymes (*P. aminophilus* JCM 7686, *P. aminovorans* JCM 7685 and *Paracoccus* sp. N5), revealed the existence of a large methylotrophy genetic island located within extrachromosomal replicons, suggesting that C1 metabolic network in this group of bacteria was shaped by horizontal gene transfer. The homologous islands are found in the chromosomes of numerous marine bacteria of the *Roseobacter* clade, which may be the origin of the set of genes in certain *Paracoccus* spp. strains. Since the island contains genetic information which is sufficient to implement methylotrophy in non-methylotrophs, it may be an important factor in horizontal spread of the methylotrophy capability in the environment.

Keywords: methylotrophy, *Paracoccus* sp., serine cycle

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VI.P.12

Isolation and molecular characterization of extremely halophilic archaea from Turkey Tuz Lake

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Extreme halophilic bacteria are adapted to live at in various high salt locations and therefore have some interests at biotechnological area. In this study, we aimed that extreme halophilic bacteria isolation from Tuz lake. An extreme halophilic bacteria were isolated from different water and soil samples from Tuz Lake and identified biochemically and genetically. For isolation of halophilic bacteria that was collected from soil and water samples, standart saline medium were used. Mobility and morphological features of isolates were identified by biochemical tests as identity after it were approved by the molecular characterization by 16s rRNA analysis. The obtained results, Gram-negative, red pigment producing bacteria that were determined *Halobacterium salinarum* was isolated in the presence of 30% NaCl from lake. These isolates were showed catalase positive and oxidase activity. They were also able to hydrolyze casein and gelatin. This study showed the prevalence of isolated ecosystems, such as halophilic *Halobacterium salinarum* species in the Central Anatolia from Turkey Tuz lake.

Key words: halophilic bacteria, *Halobacterium salinarum*, Turkey Salt Lake

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VI.P.13

Molecular screening of vector-borne pathogens in tissues of reservoir hosts

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The rodents from diverse habitats of Lower Silesia were examined for occurrence of vector-borne pathogens of public health significance. For the detection of *Babesia microti*, *Bartonella* sp., *Borrelia burgdorferi* s.l. and *Anaplasma phagocytophilum*/*Candidatus Neoehrlichia mikurensis*, blood and spleen samples were obtained from rodent specimens represented by *Apodemus agrarius*, *Apodemus flavicollis* and *Myodes glareolus*. The trappings of animals were conducted on sites located in suburban, recreation and natural reserve areas. PCR methods, conventional and nested-PCR, were used for the detection of DNA of examined pathogens in rodents' tissues. The choice of genetic markers (18S, *fla*B, *glt*A, *msp2*, 16S genes) and primers was based on the literature data and our preliminary results. Selected PCR positive products were sequenced and used for confirmation of obtained results as well as for molecular identification. For molecular analysis own sequences and homological sequences previously deposited in GenBank were used. Additionally, ecological analysis were performed. The relationship between infection rate and kind of examined tissue, host species, trapping sites and co-occurrence of other vector-borne pathogen was analyzed. Results of our molecular screening showed the presence of all studied VBP in tissues of reservoir hosts from Lower Silesia.

Key words: vector-borne pathogens, rodents

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VI.P.14

Comparison of the conventional PCR method with real-time PCR in identification of genetic material of bacteria *Anaplasma phagocytophilum*

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Anaplasma phagocytophilum is a gram-negative, obligate intracellular bacterium from the family *Anaplasmataceae* in the order Rickettsiales. It infects neutrophils and causes granulocytic anaplasmosis in humans and animals. The disease in humans was first recorded in 1994 from the USA. Since 1995 cases of infection were reported also from Europe. The bacterium is maintained in natural foci by enzootic cycles between vector ticks of *Ixodes ricinus* complex and wild animals. For the epidemiological purposes there is a need to quick and accurate detection of pathogens in ticks, humans and animals by using molecular techniques. One of the frequently used genetic marker for the detection of *A. phagocytophilum* by PCR assay is a fragment of *msp2* gene. The *msp2* gene has high intraspecific variability and in consequence, major surface protein 2 (MSP2) exhibit high antigenic variation. The aim of this study was to compare specificity and sensitivity of conventional nested PCR method with real-time PCR for detection of *A. phagocytophilum* in material from animals.

The study material consisted of DNA isolated from tissues of wild rodents. Small mammals were captured in 2014 from areas located in south-western Poland (Lower Silesia) and belonged to three species: *Apodemus agrarius*, *Apodemus flavicollis* and *Myodes glareolus*. In order to identify DNA of *A. phagocytophilum*, fragment of *msp2* gene was used for amplification with outer pair of primers (msp2-3F, msp2-3R) and inner designed pair of primers. For evaluation of the nested PCR method, a real-time PCR was designed based on different part of *msp2* gene. The fragment of *msp2* gene used to design primers and probe for real-time PCR in these study, was not previously available in the known PCR and real-time PCR methods from literature.

Key words: PCR, real-time PCR, *Anaplasma phagocytophilum*, rodents, *msp2* gene

Acknowledgements: We would like to thank Amplicon company from Wroclaw for the preparation of real-time PCR test and help in its implementation. "Project "Academy of Development as the key to strengthen human resources of the Polish economy" co-financed by the European Union under the European Social Fund".

VI.P.15

The common evolutionary history of *nodAC* genes of *Astragalus glycyphyllos* symbionts and the genus *Mesorhizobium* species symbiovar biserrulae

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Effective symbiosis between rhizobia and legumes requires several bacterial symbiotic genes, including nitrogen-fixation (*nif*) ones determining reduction of N_2 into ammonium and nodulation (*nod*) genes which encode Nod factors triggering nodule formation. In this study, we have focused on the phylogeny of *Astragalus glycyphyllos* symbionts common *nodA* and *nodC* genes which encode N-acyltransferase determining the type of N-acyl substitution on the oligosaccharide backbone of Nod factor and N-acetylglucosaminyltransferase responsible for the first step in Nod factor assembly, respectively. Phylogenetic analyses of *nodAC* gene sequences of *A. glycyphyllos* rhizobia and those of reference bacteria, available in the public databases, revealed that *nodAC* genes of *A. glycyphyllos* nodulators and those of the *Mesorhizobium* species symbiovar *biserrulae* exhibit the highest sequence identity (91–92%) and on *nodAC* gene phylogram these bacteria clustered together in monophyletic clade pointing to their common evolutionary history. These results allowed to classify *A. glycyphyllos* symbionts to the symbiovar *biserrulae* to which *Mesorhizobium ciceri*, *Mesorhizobium australicum*, and *Mesorhizobium opportunistum* bacteria belong. The considerable sequence conservation of studied symbiotic genes of *A. glycyphyllos* nodule isolates (>95%) shows that *nodAC* loci might have evolved under strong host constraints.

Key words: rhizobia, legume symbiosis, *nodAC* genes phylogeny, symbiovar

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VI.P.16

Isolation and identyfication of methanotrophs associated with peatland plants

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Wetlands and particularly peatlands are the main natural source of methane. This emission is the result of the balance between methanogenesis and methanotrophic processes and is actively affected by the wetland plants composition which can influence on CH_4 production, consumption and transport in the soil. Studies on this phenomenon indicated a significant role of methanotrophic bacteria play a in CH_4 emissions, both those free-living in the rhizosphere attached to the root surface in the form of a biofilm (rhyzoplane) and those living inside host tissues (endophytes) colonizing both the underground and the aboveground parts of plants (mainly *Sphagnum* spp.). The mechanism of associations of methanotrophs with peat forming vascular plants is poorly recognized and currently is the subject of studies of many researchers throughout the world.

We tested methanotrophic bacteria associated with *Carex* sp. and *Eriophorum* sp. originating from Moszne peatland (East Poland). Methanotrophic bacteria were isolated from plants by adding sterile fragments of each part of plant (roots, and stems) to agar mineral medium (NMS) and incubated with methane atmosphere (10% CH_4). Single colonies were streaked on new NMS agar media and after incubation transferred to liquid NMS medium. Bacterial growth dynamics were studied by optical density – OD_{600} and methane consumption. Changes of methane levels were controlled during incubation by gas chromatography technique. Characterization of methanothrophs was made by Fluorescence *In Situ* Hybridization (FISH) with Mg705, Mg84 for type I methanothrophs and Ma450 for type II methanothrophs. Identification of endophytes was performed after DNA isolation and 16 SrRNA and *mmoX* genes amplification.

Our study confirmed the presence of both types of methanotrophic bacteria (type I and II) with the predominance of type I methanothrophs. Among cultivable methanotrophs were different strains of the genus *Methylomonas* and numerous uncultured bacteria.

Key words: methanotrophs, peatland, methane

VI.P.17

Adhesive and hydrophobic properties of the selected lactic acid bacteria isolated from gastrointestinal tract of farming animals

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The aim of the study was to determine the degree of adhesion and hydrophobicity (%) of some strains of lactic acid bacteria with proven antagonistic properties against pathogenic bacteria. Research on the adhesiveness was subjected to the strains isolated from pigs and calves, and research of the hydrophobicity to the strains isolated from pigs, calves and chickens. Studies of adhesion properties and hydrophobicity were performed on the lactic acid bacteria strains obtained from the upper and lower gastrointestinal tract of calves, piglets, and chickens. Strains isolated from animals have shown antibacterial activity against pathogenic strains of *Clostridium perfringens* and *Escherichia coli*. Adhesiveness obtained strains was examined in relation to the porcine mucin and bovine. The study was also conducted to determine the existence of a correlation between adhesion and hydrophobicity. The analyzes showed that the tested strains showed a degree of adhesion in the range of 32.00–40.00% for strains obtained from calves and 34.00–40.00% for strains obtained from pigs. The hydrophobicity of tested bacteria was in the range of 31.00–44.00% for strains obtained from pigs, 26.00–42.00% for strains obtained from calves and 31.00–41.00% for strains bacteria obtained from chickens.

The positive correlation between hydrophobicity and adhesion was observed only in the case of bacteria isolated from gastrointestinal tract of pigs, however, in the case of the strains isolated from calves no correlation was detected. It was found, that the bacteria strains belonging to the same species differ in hydrophobicity properties and the adhesiveness of lactic acid bacteria is strain-dependent. The best adhesion abilities to mucin showed *Enterococcus faecalis* strains isolated from calves. Taking into account pig isolates, the best adhesion showed the strain *Leuconostoc mesenteroides*. Among tested strains the highest hydrophobicity, measured in relation to hexadecane, characterized a strain *Leuconostoc mesenteroides* isolated from piglets, a strain of *Weissella thailandensis* isolated from chickens and a strain *Lactobacillus reuteri* isolated from calves.

Key words: lactic acid bacteria, adhesion, hydrophobicity

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VI.P.18

Enzyme inhibitors and antimicrobial agents produced by the Baltic cyanobacterium *Nostoc* cf *edaphicum* CCNP 1411

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Cyanobactria inhabit almost all ecological niches. They also developed unique metabolic pathways leading to the production of compounds with a unique chemical structure and diverse biological activities. In most cases, the ecological function of the metabolites is unknown. In recent years, intensive research into the biosynthesis, chemical structure and biological activity of secondary metabolites produced by cyanobacteria has been intensified. In the cases of *Nostoc* genus, mainly the species from freshwater and terrestrial environments were analyzed.

The aim of present work was to explore the potential of the Baltic strain of *Nostoc* cf. *edaphicum* CCNP1411 to produce peptides which modify the activity of key metabolic enzymes. In addition, the antimicrobial activity of the compounds against five strains of heterotrophic bacteria was tested.

Our studies showed that *Nostoc* cf. *edaphicum* CCNP 1411 is a producer of many analogues of non-ribosomal peptides classified to cyanopeptolins and nostocyclopeptides. The chemical structures of the compounds were elucidated using LC-MS/MS. Among the compounds, some inhibitors of proteolytic enzymes (chymotrypsin, trypsin, thrombin and carboxypeptidase-A) and protein phosphatase inhibitors were identified. In antimicrobial tests, the metabolites produced by CCNP1411 strongly inhibited the growth of *Enterobacter cloacae* PP-VR 3073.

The results of our studies indicated that *Nostoc* cf. *edaphicum* CCNP 1411 isolated from the Baltic Sea is a prolific source of bioactive metabolites. However, further studies are needed to recognize their ecological significance and/or potential biotechnological application.

VI.P.19

The influence of cadmium ions on growth and survival of *Rhizobium leguminosarum* bv. *trifolii* strain Rt24.2 and its derivatives

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Heavy metals negatively influence growth, morphology, and physiology of symbiotic bacteria, commonly called rhizobia. Some metals such as cadmium could be toxic even in low concentrations and, accumulated inside cells, often leads to DNA fragmentation, disruption of S-S bonds in proteins, and inhibition of cell divisions.

In this study, the influence of cadmium ions on growth and survival of *Rhizobium leguminosarum* bv. *trifolii* wild-type strain Rt24.2, mutants defective in exopolysaccharide (EPS) synthesis (Rt2472 and Rt5819), and derivatives overproducing this polymer (Rt24.2(pBA1) and Rt24.2(pBR1)) was established. The growth of the tested strains was evaluated by the measurement of optical density after addition of different concentrations of cadmium (0.005–0.015 mM). Moreover, a wide range of cadmium concentrations (0.01 mM–50 mM) was used to determine the survival of bacterial cells. Also, the influence of the ions of this metal on EPS production and biofilm formation was examined. The analyzed strains showed different growth responses to cadmium. The growth of the wild-type strain was not inhibited even by the highest concentration of the metal used (0.015 mM). In contrast, the growth of the EPS-non-producing Rt5819 strain was inhibited by 0.01 mM concentration of cadmium ions. Addition of cadmium to the other tested strains caused a delay of cell divisions by 24 h, 48 h, or 72 h, depending on the concentration of the metal used. After the delay, the bacterial growth achieved a similar level as that in the control medium.

The study of the bacterial viability showed no significant differences in the numbers of dead cells within the range of cadmium concentrations used between the tested strains, which differed in the amounts of synthesized EPS. These data indicate that EPS plays a significant role in protection of rhizobia and suggest existence of additional defense mechanisms against cadmium stress unrelated to EPS.

Key words: *Rhizobium leguminosarum*, cadmium ions, bacterial growth, exopolysaccharide

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VI.P.20

Comparative transcriptome analysis of *Rhizobium leguminosarum* bv. *trifolii* wild-type strain Rt24.2 and its *rosR* mutant Rt2472

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Rhizobia comprise a group of gram-negative soil bacteria, which exist in the rhizosphere as motile free-living organisms or live inside specific root organs of legumes, called nodules, where they reduce atmospheric dinitrogen to ammonium. *Rhizobium leguminosarum* bv. *trifolii* produces different types of polysaccharides. Among these, extracellular polysaccharide is produced by this bacterium in the largest amounts and plays a significant protective role against stress conditions. The synthesis of this polymer is positively regulated by *rosR* encoding a global transcriptional regulator, which contains a C_2H_2-type zinc finger motif. The RosR protein also influences other cellular processes in this bacterium such as motility, envelope biogenesis, and membrane functioning. This was evidenced by a phenotype of the Rt2472 strain with a mutation in the *rosR* gene. This strain produces 3-fold less EPS, is less motile and more sensitive to several surface detergents, and shows changes in the profiles of extracellular and membrane proteins in relation to the wild-type strain Rt24.2.

In this research, a comparative transcriptome analysis of the wild-type Rt24.2 and the *rosR* mutant Rt2472 strains was performed. Using genome-wide transcriptome profiles, genes differentially expressed in these strains were examined. Our analysis revealed significant changes in the expression of 1254 genes between the mutant and the wild-type strain. Among these, 794 genes were upregulated, whereas 460 genes were downregulated in the *rosR* mutant background, indicating that RosR negatively influences expression of a majority of these genes. The RosR regulon contains genes belonging to several functional groups (COGs); however, the largest numbers of these are involved in transcription, transport and metabolism of amino acids and carbohydrates, cell motility, and signaling. These results indicate that RosR is an important global regulator influencing many cellular processes in *R. leguminosarum*.

Key words: *Rhizobium leguminosarum*, transcriptome analysis, *rosR* gene

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VI.P.21

Characterization of temperature-regulated genes in *Dickeya solani*

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Temperature is one of the most determining factors for disease outbreak and may act as a signal that activates expression of specific pathogenicity-related factors during infection. Several studies have reported thermoregulation of gene expression in plant pathogenic bacteria, but little is known about the influence of temperature on secondary metabolism and host-adaptive processes in pectinolytic bacteria.
Pectinolytic bacteria: *Pectobacterium* spp. and *Dickeya* spp. are the causative agents of blackleg and soft rot disease of potato leading to significant economic losses in agriculture worldwide. During the last decade new *Dickeya* species named *D. solani* has been isolated in many European countries. *D. solani* is more virulent and aggressive than other *Dickeya* spp. and *Pectobacterium* spp. isolated from potato in Europe so far. Aggressiveness of *D. solani* appears to increase at high temperatures.
D. solani strain IFB0099 was randomly mutagenized using a mini-Tn5 transposon. This transposon carries a promoterless *gusA* reporter gene coding for β-glucuronidase, and is therefore suitable for identification of promoter fusions preferentially expressing the reporter gene (*gusA)* at low (18°C) and high (37°C) temperatures. Out of 8000 transposon mutants: 45 mutants showed an increased reporter gene expression at 37°C, and 9 – at 18°C. Among the mutants with increased *gusA* expression at 37°C, insertions were found in genes encoding pectate lyase A, phospholipase C, regulatory protein PecM. Among the mutants induced at 18°C the insertions disrupted genes encoding protein with properties similarities to a mechano-sensitive ion channel family protein MscS and membrane proteins. No significant difference in cell growth rate, hypersensitive response in tobacco, protease, cellulase and pectinase activities between the wild-type strain and any of the tested mutants was observed. Three mutants were identified as biofilm-defective mutants and four of tested mutants exhibited a reduced virulence in a potato tuber and chicory leaves virulence test.

Key words: plant pathogen, transposon mutagenesis, thermoregulation

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VI.P.22

Comparison of two Arctic fjords in terms of bacterial distribution in water column (Spitsbergen) during summer 2013

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The changes in climate are recently observed in Arctic marine ecosystems. In these terms the bacterial communities can be influenced to unknown extent.
Two Arctic fjords: Hornsund (77°N, 16°E) and Kongsfjorden (79°N, 12°E) are localized on the western coast of Spitsbergen. Both are influenced by warm Atlantic waters, however in Hornsund also cold Arctic waters inflow from Barents Sea is observed. Water samples were collected in July and August 2013 during polar cruise of r/v Oceania along transects in both fjords. Total bacteria number (TBN), biomass, mean cell size and cells morphology were determined with use of DAPI (4',6-diamidino-2-phenylindole) dye and direct count method. Vertical distribution of bacterial abundances and biomass was investigated in relation to physical parameters in the water columns.
Bacteria were not homogeneously distributed through the layers, higher TBN values occurred around picnocline. Bacterial concentrations were almost two times higher in Hornsund than in Kongsfjorden. Hydrological data shows increased influence of warm Atlantic waters on both fjords during summer 2013. The exact impact of this phenomenon is unknown, however we speculate that among many possible interactions, such as: glacial meltwater and particulate inorganic matter concentration, stratification due to temperature and salinity, the main factor that regulates bacterial communities is the nutrients availability. This may be caused by inflow of nourishment substances form bird colonies, which are approximately two times more numerous in Hornsund than Kongsfjorden. Also increased concentrations of organic and inorganic matter were present in Hornsund. To compare structure and composition of bacterial communities of these two fjords we plan to perform PCR-DGGE and 16S rRNA gene sequencing (MiSeq, Ilumina).

Key words: Spitsbergen, Hornsund, Kongsfjorden, bacterial abundance, bacterial biomass

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VI.P.23

Mesorhizobium loti NZP 2213 requires O-acetyl groups in LPS to survive inside *Acanthamoeba castellanii*

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We previously demonstrated that the NZP2213 strain of *Mesorhizobium loti* remained viable within free-living amoeba *Acanthamoeba castellanii* at least during the first 24 h of infection and it was able to self-release from amoeba cells. We also showed that environmental conditions influenced the association and internalization of bacteria by amoebae, the uptake of bacteria was mediated by the mannose-dependent receptor, and the protein tyrosine kinase participated in phagocytosis of the bacteria by *Acanthamoeba castellanii*.
The aim of the present study was to determine the effect of bacterial LPS (O-PS) in rhizobia-amoeba interactions using defined Tn5 mutants, Mlo-13 and 2213.1, which showed higher and impaired effectiveness in symbiosis with *Lotus corniculatus*, respectively, derived from the parental strain. Results from co-cultivation, viable counts, an antibiotic protection assay, showed that: (1) the degree of association with amoeba cells is the highest for the parental strain, (2) the uptake of the 2213 strain and Mlo-13 during the first 3 h of co-culture is at the same level, but (3) after 4h-co-cultivation the recovery of living bacterial cells was much higher for the mutant Mlo-13 than the wild strain, (4) there was no recovery of viable cells of mutant 2213.1 ineffective in *Lotus corniculatus* symbiosis, from amoebae in all the tested incubation times of co-cultivation. The inhibition phagolysosome fusion assay using NH_4Cl, followed by staining of infected amoeba cells together with plaque assay, indicated that the 2213.1 mutant was actively uptaken in phagocytosis but digested in the endocytic degradation pathway. Contrarily, the wild strain was partly digested and the Mlo-13 strain was not.
The above results indicate that LPS of the wild strain is mainly responsible for the receptor-dependent uptake of rhizobia, while whether bacteria are digested or remain viable inside amoebae depends on the LPS hydrophobicity.

Key words: endosymbiosis, *Acanthamoeba castellanii*, lipopolysaccharide, *Mesorhizobium loti*

VI.P.24

Ureolytic activity of soil bacteria in presence of petroleum derivatives and heavy metals

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Biocementation, due microbial carbonate precipitation, is convenient technique in soil mechanical improvement. Urease lead to calcium carbonate precipitation by urea hydrolysis and alkalization. However, environment pollution (e.g. petroleum, heavy metals) negatively affect on enzymatic activity of ureolytic microorganisms.
The aim of this work was determine ureolytic activity of bacteria isolated from polluted (by petroleum or metals: U, Cu, S, and Fe) soil samples (obtained from Rajskie, Kowary and "Colourful Lakelets" in Rudawski Landscape Park) in presence of heavy metals (Cu, Zn and Mn) and petroleum derivatives (kerosene, leaded petrol and heater oil). Bacteria were cultivated on solid microbiological medium supplemented with soil extract (3 days in +25°C). To investigate ureolytic activity, isolated bacterial strains were incubated in saline + urea (for activity screening with phenol red; for quantitative determination without phenol red) supplemented with petroleum derivatives (up to 20% of kerosene, leaded petrol and heater oil) or heavy metals (up to 100 mM of copper sulphate, zinc sulphate and manganese sulphate). The change of the medium color (due alkalization) was measure using spectrophotometer. Amount of ammonia ions was determined in phenol-nitroprusside assay.
From among 25 isolated bacterial strains, 7 isolates were able to urea hydrolysis in presence of petroleum derivatives or heavy metals. Ureolytic activity was diverse and dependent on added compound. From among petroleum derivatives, only leaded petrol, in 20% concentration, completely inhibit ureolytic activity. All investigated heavy metals significant inhibit urea hydrolysis. Bacteria able to decomposition of urea may be useful in biocementation in environment contaminated especially by petrol derivatives.

Key words: biocementation, urease, soil bacteria

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VI.P.25

Outer membrane proteins of bacteria as change's marker of sensitivity to silver nanoforms

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One of the alternative methods of outfighting pathogens is using the antibacterial features of silver nanoparticles (AgNPs). They possess high biological activity and they're efficient against wide spectrum of microorganisms. Department of Microbiology at the University of Wroclaw, is carrying out the research on the resistance of Gram-negative bacteria to nanoparticles, connected with outer membrane proteins (OMP) patterns. The tested strains – *Klebsiella pneumoniae* 626 and *Enterobacteraerogenes* 323 – were subject to sublethal doses of silver nanocomposites. They showed reduced susceptibility to its antimicrobial action after prolonged-exposure to AgNPs with a few differences: mainly in size, silver's content, oxidation state or its immobilization on inorganic solids. OMP of studied strains were extracted according to Murphy and Bartos methods with our own minor modifications and then the two-dimensional electrophoresis (2-DE) was performed. The protein quantification was performed with the BCA Protein Assay Kit. Electrophoregram's analysis showed differences in protein profiles between variants and wild types of each strain – disappearance, amount changes or synthesis of new OMPs. These modifications are a morphological marker used to determine the influence of each silver nanoform with physico-chemical differences on bacterial sensitivity to silver nanoforms.

Key words: outer membrane proteins (OMP), gram-negative bacteria, silver nanoforms, nanoparticles

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VI.P.26

Comparison of the intestinal microflora composition of free-range and caged chickens

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Chicken gastrointestinal track is inhabited by various bacteria, fungi and archaea. *Lactobacillus spp.* are members of the chicken intestinal micro biota. *Campylobacter* spp, pathogenic for humans, is also a normal inhabitant of the chicken gut. The composition of the chicken bacterial community can be affected by several factors such as diet, age, antibiotic administration and infection with pathogens. The aim of the study was to evaluate the influence of the different rearing system, especially, the dietary treatment of chickens on *Lactobacillus* species colonizing their gut. Chicken stool samples were collected from privately owned "backyard" flocks and from commercial broiler chicken flocks. Chicken stool samples were streaked onto selective MRS (de Man, Rogosa, and Sharpe) agar and incubated within an anaerobic atmosphere overnight at 37°C. Pure cultures were obtained. The strains were further characterized by morphological and microscopic observations. Belonging to the *Lactobacillus* genus was confirmed by molecular analysis (amplification of intergenic chromosomal DNA region between the 16S rDNA and 23S rDNA). To determine the species of the isolates the 16S rDNA genes were amplified and PCR products were sequenced. The nucleotide sequence of the PCR products were analyzed using BLAST against the nucleotide database at the NCBI website.

So far a total 50 *Lactobacillus* strains were isolated from privately own chickens. The most abundant *Lactobacillus* species are *L. salivarius*, *L. reuteri* and *L. plantarum*. In the future we intend to evaluate their adhesion and potential probiotic properties as well as their anti-*Campylobacter* activity of isolated strains.

Key words: chicken gastrointestinal track, *Lactobacillus*, 16S rDNA, *Campylobacter*

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VI.P.27

Influence of surfactants on environmental and collection bacterial strains

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Surfactants are compounds commonly used, both in the household and in different branches of industry, primarily in the process of cleaning and disinfection. They have a several positive features, where the most important is to reduce the surface tension of water, what facilitates the cleaning of various types of surfaces. In the present work two compounds were used as biocides: hexamethylene-1,6-bis-(*N*,*N*-dimethyl-*N*-dodecylammonium bromide) belongs to the gemini surfactant, and its single analogue – dodecyl(trimethyl)ammonium bromide (DTAB). Two fold dilution method was used to determine the minimum concentration of compounds (MIC) which inhibit the develop of bacteria: *St. epidermidis* (ATCC 12228 and environmental strain), *Ps. aeruginosa* (ATCC 85327 and environmental strain), and *Brochothrix thermosphacta* (ATCC 11509 and environmental strain). The viability of cells in liquid cultures in TSB medium (Merck, Germany) with addition of these substances in concentrations ¼ MIC and MIC were also determined.

The obtained results show that DTAB inhibits the growth of bacteria in the concentration 0.126–1.01 microM/mL, and gemini surfactant is active in 0.0018–0.0145 microM/mL. Both compounds in MIC value reduced the number of cells of all strains of more than 5 log. Decrease the concentration of both single and double surfactant to 1/4 MIC caused inhibition of cell growth of all the strains for 4–8 hours. After this time, further cell growth continued up to the level of 10^7–10^8 cfu/ml after 48 hours.

It was found that gemini surfactant is active in more than 17–140-fold lower concentrations than its monomeric analog. Bacteria strains isolated from natural environment are less sensitive on testing biocides than collecting strains. The use of too low concentration of biocides can limit the growth of bacteria, but often only for a short period of time. Later microorganisms can adapt to adverse environmental conditions and begin to produce defense mechanisms.

Key words: gemini surfactants, antibacterial activity, *Brochothrix, Pseudomonas*

VI.P.28

Biocontrol of phytopathogenic bacteria and insect pests using antagonistic bacteria from the genus *Bacillus*

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The most powerful antiphytopathogenic activity poses bacteria of the same ecological niche as pathogen. The aim of the research was to elaborate biopreparation for plant protection active against phytopathogenic bacteria and insect pests based on the bacilli strains.

Active antagonistic strain *Bacillus megaterium* ONU500 was isolated from the fermented mustard leaves – traditional Vietnamese meal. Species identification was carried out using morpho-tinctorial properties, API and fatty acid gas chromatography (MIDI-Sherlock automatically identification system).

Antagonistic activity investigation was carried out *in vitro* (agar-holes method) and *in vivo*. During the research *in vitro* and *in vivo* there were used 18 strains of phytopathogenic bacteria from the species *Erwinia carotovora* and *Rhizobium tumefaciens* as indicators and as infecting agents respectively. *In vitro* minimal inhibition zone diameter was 20 mm. Experiments *in vivo* on potato tubers and carrot discs showed inhibition effect of the culture *B. megaterium* ONU500 in 100% of cases. It was shown also statistically significant increase of germinative capacity on 10% and of root-top mass (87–91%) after the tomato seeds soaking in 1% *B. megaterium* ONU500 culture suspension.

Using methods of multifactor mathematical analysis culture medium was optimized. Cultivation of the strain *B. megaterium* ONU500 in this medium during *in vitro* experiments resulted in the increasing of inhibition zones size from 50–70% depending from the indicator strain sensitivity.

For the strain *B. megaterium* ONU500 entomocidic activity against *Bradysia pilistriata* was also shown. Maximal entomocidic activity (100% lethality) was shown during the three-time suspension treatment. Biopreparation based on the strain *B. megaterium* ONU500 posses antagonistic activity against causative agents of soft rot and bacterial cancer, against insect pests and also stimulate tomato seeds germination and seedlings formation.

Key words: *Bacillus*, biocontrol, phytopathogens

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VI.P.29

Endophytic methanotrophs identification from different species *Sphagnum* by direct PCR kit

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In the past decade molecular techniques have provided a means for detecting the presence of virtually any type of bacteria in environmental samples, independent by to them the ability of culture in the laboratory. Most of these methods requires the isolation of bacterial DNA. However, isolation of bacterial DNA is very often problematic of even impossible. The solution of this problem is use a set of direct PCR kit, which is designed to direct DNA amplification from the different plant samples without any DNA prior isolation.
In our study methanotrophic endophytes inhabiting three types of *Sphagnum* species: *S. magellanicum, S. fallax, S. flexuosum* were tested. In the first step of preparation the surface of plant material was sterilized to eliminate the assistance of epiphytic microorganisms, after that was lysed, blended and centrifuged to precipitate the debris of big particles. As negative control the fragment of plant not blende was used. Detection of methanotrophic bacteria was performed with genetic marker – *pmo*A gene. Next, cloning of PCR product obtained was carried.
A positive result of *pmo*A gene detection was obtained for all tested *Sphagnum* endophytes. Molecular analysis of the *pmo*A gene sequences were done directly in the plant material. The presence of *Methylobacter* sp. belonging to groups A1, A2, *Methylomonas* sp. – group B and constituting an independent line A3 were confirmed.
This advantage of this method is the lack of need for isolating DNA, the possibility of *in situ* biodiversity studies, as well as reduced costs of reagents.

Key words: endophytic bacteria, methane, methanotrophs

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VI.P.30

Degradation of alkyl sulfates by psychrotolerant bacteria

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Synthetic anionic surfactants such as linear alkyl sulfates, e.g. sodium dodecyl sulfate (SDS), are extensively used as components of a variety of household and industrial detergent formulations. Significant quantities of surfactants and their derivatives released in the environment after use may exert harmful effects such as the remobilisation of organic pollutants and the inhibition of biological activity. The problem of surfactant pollution is especially severe in the arctic environments, where the rate of biodegradation is low due to temperature effect. Here we describe the ability of the psychrotolerant arctic strain *Psychrobacter* sp. DAB_AL62B to degrade and metabolize biocidal SDS.
The strain, isolated from ornithogenic deposits in Spitsbergen (Svalbard Archipelago, Norway), was found to harbour a 34.5-kb plasmid, pP62BP1, which carries i.a. a phenotypic module, named SLF. It consists of four open reading frames (*slfCHSL*) encoding catabolic enzymes participating in the alkyl sulfates metabolism, and a gene (*slfR*) for transcriptional regulator of AraC/XylS family.
Even though SDS at the concentration as low as 0.17 mM exerts a bacteriostatic effect on several tested *Psychrobacter* spp. strains, the pP62BP1-containing strain is capable of the complete SDS degradation in these conditions. The crucial enzyme of the SLF module, the alkyl sulfatase SlfS, was also found to hydrolyze its homologues of 8, 10, and 16 carbon atoms in the aliphatic chain. The molecular analysis of SLF module revealed that the *slfCHSL* genes form an operon, whose activity is determined by the SlfR protein acting as a negative transcriptional regulator.
To the best of our knowledge, the studied genetic module is the first compact plasmid-borne alkyl sulfate degradation module known and analyzed to date.

Key words: alkyl sulfate, *Psychrobacter* spp., plasmid

VI.P.31

Genetic, biochemical and spectroscopic characterization of bacterial species isolated from entomopathogenic nematodes

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The pathogenicity of entomopathogenic nematodes (EPNs) is largely due to the presence of bacteria. Many species of bacteria creates a non-specific mutualistic relationships with EPN. These bacteria have not yet been fully described. The studied nematode strains were isolated from soil samples obtained from different regions of Poland. The bacteria isolated from EPNs were passaged on Trypticase Soy Agar and next examined by biochemical methods (ureolytic activity), genetic tests (16S rRNA gene sequencing) and infrared spectroscopy (IR). The ureolytic activity was tested on Christensen medium using the plate method. The IR spectra was measured using Attenuated Total Reflection Fourier Transform Infrared Spectroscopy technique (ATR/FT-IR). The first derivatives of the bacterial IR spectra were used for Principal Component Analysis (PCA). A total number of 20 bacterial strains were isolated. These bacteria are members of six different species. *Pseudomonas* sp. accounted for the greatest number of the isolated microorganisms (8 out of 20). The IR spectrum range 1200–750 cm^{-1} was selected for bacterial differentiation. Analysis of the IR window suggests that the tested bacteria are divided into two distinct clusters. Cluster I includes *Acinetobacter* sp., *Delftia* sp., *Pseudochrobactrum* sp. and some of *Pseudomonas* sp. strains. Cluster II includes *Achromobacter* sp., *Enterobacter* sp. and other *Pseudomonas* sp. strains. Cluster I is very consistent. In contrast, cluster II is highly incoherent. Six isolates exhibited ureolytic activity. The strongly ureolytic bacteria occupy very similar positions in PCA. The wave numbers strongly correlated with this biochemical characteristic were: 1108 cm^{-1}, 1109 cm^{-1}, 1221 cm^{-1}, 1286 cm^{-1} and 1440 cm^{-1}. Spectroscopic studies and mathematical analysis enabled the classification of bacterial strains into ureolytic and non-ureolytic group.

Key words: entomopathogenic nematodes, infrared spectroscopy

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VI.P.32

Wild living animals as zoonotic reservoir for microsporidia *Enterocytozoon bieneusi* and *Encephalitozoon* spp.

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Microsporidia are obligate intracellular parasites significant for public health, especially for immunocompromised patients as cause of opportunistic infections. *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *E. cuniculi* and *E. hellem* are the most common species among 14 microsporidian identified, by now, as human pathogens. Considerable genetic diversity within *E. bieneusi* has been found with over 100 genotypes identified based on the ITS nucleotide sequences. Microsporidia infect a wide range of vertebrate host, including domestic, livestock and wild animals and humans. The epidemiology, ecology and clinical impact of microsporidian infection in animals in human is poorly understood. The main objective of the present study was to determine the occurrence of *Enterocytozoon bieneusi* and *Encephalitozoon* spp. in wild rodents and birds. Fecal and spleen samples of wild living rodents (*Apodemus* spp., *Myodes glareolus*) and fecal samples of rooks (*Corvus frugilegus*) were collected for surveys form areas located in Lower Silesia, Poland. PCR amplification was performed on set of nested primers amplifying the ITS region of the rRNA gene of *E. bieneusi* and *Encephalitozoon* spp. Genetic markers and primers were chosen based on the literature data. Selected PCR positive products were purified and sequenced. BLAST searches were conducted in order to elucidate any homologies with previously deposited sequences in GenBank. Several samples showed high similarity to described sequences of pathogenic microsporidia.

Key words: microsporidia, zoonotic reservoir, wildlife

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VI.P.33

Antimicrobial resistance of *Pseudomonas* spp. isolated from wastewater and wastewater impacted marine coastal zone

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In this study species distribution and antimicrobial susceptibility of cultivated *Pseudomonas* spp. was studied in influent (INF), effluent (EFF) and marine outfall (MOut) of wastewater treatment plant (WWTP). The susceptibility was tested against 8 antimicrobial classes, active against *Pseudomonas* spp.: aminoglycosides, carbapenems, broad-spectrum cephalosporins from the 3rd and 4th generation, extended-spectrum penicillins, as well as their combination with the β-lactamase inhibitors, monobactams, fluoroquinolones, and polymyxins. Among identified species, resistance to all antimicrobials but colistin was shown by *P. putida*, the predominant species in all sampling points. In other species resistance was observed mainly against ceftazidime, ticarcillin, ticarcillin-clavulanate and aztreonam, although some isolates of *P. aeruginosa*, *P. fluorescens, P. pseudoalcaligenes* and *P. protogens* showed multidrug-resistance (MDR) phenotype. Among *P. putida* resistance to β-lactams and to fluoroquinolones as well as multidrug resistance become more prevalent after wastewater treatment, but resistance rate decreased in marine water samples and some phenotypes were lost. Obtained data, however, suggests that *Pseudomonas* spp. are equipped or are able to acquire a wide range of antibiotic resistance mechanisms, thus should be monitored as possible vectors of resistance dissemination.

Key words: *Pseudomonas* spp., species distribution, antimicrobial susceptibility, wastewater, marine outfall

VI.P.34

Prevalence and antimicrobial resistance of *Listeria monocytogenes* from meat products in Poland

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Listeria monocytogenes is the main causal agent of listeriosis, a well-documented food-borne illness. Although the incidence of listeriosis is low, it is a major public health concern worldwide because *L. monocytogenes* is capable of causing severe infections.

The aim of the study was to characterize strains of *L. monocytogenes* isolated from meat products collected under official control and monitoring in Poland and to determine the susceptibility of *L. monocytogenes* isolates to various antimicrobial agents. The samples were tested for the presence of *L. monocytogenes* by the laboratories of Sanitary and Epidemiological Stations, according to the procedure PN EN ISO 11290-1:1999+A1:2005. The isolates were sent to the National Reference Laboratory for *L. monoctytogenes* for confirmation and further analysis. *L. monocytogenes* molecular serotyping was perform using multiplex PCR. *L. monocytogenes* isolates were screened for susceptibility to a panel of antimicrobials on Mueller-Hinton agar by a disc diffusion method described in the CLSI.

A total of 72 *L. monocytogenes* isolates from food samples: 41 – sausages, 27 – delicatessen with meat and 4- meat samples were examined. Fifty-five (76%) of *L. monocytogenes* isolates were resistant to ampicillin and 3 (4%) isolates to amoxycillin/clavulanic acid. All *L. monoctytogenes* isolates were sensitive to gentamicin, chloramphenicol, ciprofloxacin, meropenem, sulphamethoxazole-trimethoprim, erythromycin and tetracycline. No multiple resistance was observed in *L. monocytogenes*.

Serotyping of *L. monocytogenes* strains by multiplex PCR showed the highest incidence of serotype 1/2a-3a serotype (IIa) – 53% of all isolates, 19% was defined as serotype 1/2c-3c (IIc), 14% as 1/2b-3b-7 (IIb) and 8% as 4ab-4b-4d-4e (molecular group: IVb). Any isolate of serotype 4a-4c (molecular group IVa) was not found.

The results of the present study indicate that *L. monocytogenes* isolated from meat products most often represented molecular group IIa and were sensitive to common antibiotics (except ampicillin and amoxycillin/clavulanic acid).

Key words: *Listeria monocytogenes*, meat products, antimicrobial resistance

VI.P.35

The occurrence and characteristic of *Escherichia coli* O157 in retail food in Poland in 2010–2012

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Shiga toxin-producing *Escherichia coli* (STEC) is a zoonotic food- and waterborne pathogen that is a serious public health concern because of its propensity to cause outbreaks, hemorrhagic colitis, and the potentially fatal hemolytic-uremic syndrome (HUS). The O157:H7 serotype of enterohemorrhagic STEC is the most common and is well recognized for its high virulence in human populations. Meat and vegetables have been implicated in outbreaks of *E. coli* O157:H7 in most parts of the world.

In 2010–2012 official control studies were carried in Poland to detect the presence *E. coli* O157 in food samples. A total of 4096 food samples were collected, including 3596 samples of meat and 500 samples of vegetables. *E. coli* O157 were isolated from 178 samples. To the National Reference Laboratory for *E. coli* 14 strains were sent for confirmation. These strains were subjected to further studies. Analysis by PCR was used to detect the presence of the verotoxin genes (*stx1, stx2*), the attaching and effacing gene (*eaeA).* Antibiotic susceptibility was assessed using the disk diffusion method on Mueller-Hinton agar according to the criteria defined by the CLSI.

None of the isolates *E. coli* O157 possessed the genes encoding *stx1* and *stx2*. Of the 14 isolates 9 contained the *eaeA* genes. Four (27%) of *E. coli* O157 isolates were resistant to ampicillin, one (7%) to sulfonamides, five (36%) to strepomicin and tetracycline, two (14%) thrimetoprime. All *E. coli* O157 isolates were sensitive to aztreonam, amoxycillin/clavulanic acid, cefepim, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, ertrapenem, gentamicin, imipenem, nalidixic acid. Two isolates were resistant to one antimicrobial agent, two isolates were resistant to two antibiotics and four isolates resistant to three antibiotics.

Key words: *Escherichia coli* O157, meat, vegetables, antibiotic resistance

VI.P.36

Novel methods for extraction and purification of plasmid DNA from aquatic environments

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Metagenomics offers a potential tool for molecular research of genomes of non – cultured microbial communities with the goal of better understanding of the microbial ecology as well as answering the increasing biotechnological demands for novel enzymes and biomolecules (Schmeisser *et al.*, 2007).

Nowadays, plasmids are a very attractive target for metagenomics research. As an extrachromosomal mobile genetic elements, they have the ability of carrying different genes such as these encoding antibiotic (Cummings *et al.*, 2011) or heavy metal resistance and they represent a wide resource that enable poorly adapted bacteria to survive and gain new functions (Wright 2007; Schlüter *et al.*, 2007).

The aim of this research is to devise an effective method for extraction and purification of plasmid DNA from samples originating from different aquatics environments that will be followed by NGS (New Generation Sequencing). Samples from aquatic environments are a very specific and difficult for plasmid DNA isolation because of the dispersion of microorganisms as well as the capacity of the sample needed. Samples used in this project were collected from the municipal wastewater, municipal river and unpolluted lake. During study we plan to check whether the type of environment may influence the diversity of genes encoding antibiotic and heavy metal resistance genes. Using this knowledge we plan to determine the biodiversity and the potential for spreading and transfer horizontally genetic information among unrelated species of bacteria.

Obtained sequences of plasmid DNA will be analysed to find genes encoding antibiotic and heavy metal resistance. They will be subsequently compared to sequences deposited in databases in attempt to find replication origins of sequenced plasmids. In the future, this information may be used for construction of new plasmids with potential use in molecular biology and biotechnology.

Key words: metagenomics, plasmids DNA, aquatic environments

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VI.P.37

Microbiological quality of atmospheric air in the center of Bydgoszcz

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Air pollution has become a major cause of concern in the world. Since the self-cleaning ability of the air is limited, it is necessary to monitor air quality and to take measures against excessive air pollution.
This study evaluates microbial air quality in the center of Bydgoszcz. Air sampling by impaction was conducted at four sampling sites in a seasonal cycle.
The results indicate the dominance of mold fungi (77%). Heterotrophic mesophilic bacteria were the second most numerous (19%). Mannitol-positive staphylococci, actinomycetes and *Pseudomonas fluorescsens* accounted for a small percentage of the total number of microorganisms (2%, 1%, and 1%, respectively). The following genera contributed to the population of mold fungi: *Cladosporium spp* (73%), *Penicilium* (11%), *Fusarium* (9%), *Alternaria* (6%), *Aspergillus* (1%).
According to Polish Standard air contamination with microorganisms belonging to all investigated groups did not exceed limit values. The number of the investigated microorganisms varied seasonally and depended on the sampling site.

Key words: microbial air contamination, mold fungi, heterotrophic bacteria, actinomycetes

VI.P.38

Genetic diversity of *Trifolium rubens* nodule rhizobial isolates

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The *Rhizobium*-legume symbioses vary in both specificity of the host range and the diversity of bacterial species nodulating a given host plant. *Trifolium rubens* is rare ornamental legume plant, which is nodulated by *Rhizobium* spp. Up to now, the symbiotic interactions of *T. rubens* and microsymbionts that nodulate this plant were not studied. The main objective of this study was to determine the taxonomic status of *T. rubens* microsymbionts. The genomic diversity of 64 nodule isolates was determined by plasmid content analysis, ERIC-PCR and BOX-PCR. The selected, diverse strains were further genetically characterized by study 16S rDNA sequence polymorphism and multilocus sequence analysis (MLSA) some of the housekeeping and symbiotic genes. The subsequent phylogenic analyses showed that most of the *T. rubens* nodule isolates belong are closely related to *R. leguminosarum* species and are able to nodulate efficiently, besides *T. rubens,* different varieties of *Trifolium pratense. Vicia* sp. (vetch) was not nodulated by the sampled rhizobia. Unexpectedly, from among studied microsymbionts, the individual strains closely related to *R. galegae* and *R. skiernievicense* species were found. The metabolic profiles of the selected microsymbionts showed differences in the use of carbon and energy sources. Currently, further experiments are conducted to study the host range specificity of *T. rubens* microsymbionts.

Key words: *Trifolium rubens*, microsymbionts, phylogeny, metabolic pattern

VI.P.39

Quantitative variability in microbial community in constructed wetlands purifying wastewater containing pharmaceutical substances

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Pharmaceutical substances and their residues are present in an increased amount in the environment. Therefore, attempts are being performed to remove them by using different processes. It turns out, that due to their refractive character, pharmaceuticals are not removed by conventional wastewater treatment system with activated sludge, because they are not biodegradable, indicated that the apply wastewater treatment system are insufficient. It occurs, the greatest problem relates to anti-inflammatory drugs (often available without a prescription) and antibiotics. Among these processes the techniques based on natural phenomena that occur in wetland ecosystems, called a technical scale constructed wetlands become more and more important. The information about a relatively high efficiency of these methods of degrading pharmaceutical compounds appears in the recent literature. Bacteria adhere to the filler material in the wetland (plant roots – if they are present, and solid particles) and create biofilm. This biofilm structure is responsible for the majority of the fundamental transformations and degradation of contaminants that are found in the wastewater. Any kind of pollution has a potential impact on the structure of the bacterial communities, involved in wastewater treatment processes. Changes in the structure of the community can reduce the effectiveness of the purification process or even inhibited it completely. Tool recognized as extremely useful in studies of the structure of bacterial community is PCR-DGGE (Polymerase Chain Reaction – Denaturing Gradient Gel Electrophoresis). In the study we attempted to determine the effect of pharmaceuticals dispensed in the wastewater supply constructed wetland – diclofenac and sulfamethoxazole for the entire structure of the bacterial community, and ammonia oxidizing bacteria (AOB), which are considered to be crucial in leading the process of nitrification. The study showed that presence of the plants and pharmaceuticals in medium influences the genotypic structure of the community, changing dominant genotype and the level of diversity in microbial community compared with the period, where no pharmaceutical was dosed.

Key words: microbial community, PCR-DGGE, pharmaceuticals substances, constructed wetlands

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VI.P.40

Description of a species-specific microbiome of co-cultured non-marine ostracods from southern Africa

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Natural environments are organized in well balanced ecosystems where organisms from all domains of life coexist. This delicate balance of any ecosystem is maintained thanks to the multiple and diverse interactions between inhabitants. Microorganisms are the most abundant group of organisms that inhabits the largest number of environments. Despite their number, microorganisms have been largely overlooked in descriptions of ecosystems. Recently, rapid progress in metagenomics revealed richness of microbial species associated with specific environments and organisms. Microbiome analysis revealed its specificity towards the host in vertebrates, where studies have been conducted in laboratory conditions or on clinical samples. Research on animal-associated microbiome in natural environments is more complicated due to lack of control and numerous variables that can affect the outcome of studies, which in turn often argue between species-specific and general microbiome of studied animals.

Here we present data supporting the hypothesis of species-specific microbiome in freshwater ostracods (Crustacea: Ostracoda), which have been raised from dry mud collected from a salt pan in the Kalahari Basin (southern Africa), and subsequently cultured together in a semi self-sustaining culture under laboratory conditions. We have utilized high resolution melt (HRM) analysis to estimate differences between *Sclerocypris sarsii* and *Potamocypris mastigophora* microbiome. Results indicate that both ostracods inhabiting the same, artificial water tank with the original sediment harbour distinct composition of bacterial species. HRM results have been backed by cloning and sequencing the library of 16S PCR amplicons created on samples from corresponding species. Our results suggest possible interactions between specific bacteria and ostracod species, which can have further biological implications.

Key words: microbiome, HRM, ostracods

VI.P.41

Abiotic factors in microbial induced mineralization

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Biomineralization is a widespread phenomenon, occurring in different natural environments such as soil, sedimentary and metamorphic rocks, freshwater, oceans and saline lakes. Microbial induced calcium carbonate precipitation is one of the biomineralization types closely dependent on the parameters of the microenvironment. Minerals are precipitated as a product of the interaction between environmental and biological activity and the system has very little control via microbial cells. Moreover, any changes in the microenvironment will have an effect on the biominerals precipitated.
The aim of this study is to determine the influence of abiotic factors such as the pH value of the culture medium, temperature, type of substrate, and the calcium concentration on the calcite precipitation process.
The studies were conducted on six environmental strains isolated from building materials and historical objects: *Bacillus atrophaeus, B. muralis, B. mycoides, B. subtilis, B. weihenstephanensis* and *Arthrobacter sulfureus*. The cultivation of bacteria were carried out in liquid medium at 28°C for 5 days with continuous aeration at 150 rpm. At an interval, from 24 hours up to 5 days, the biomass of bacteria, the concentration of calcium ions and the growth of crystals were measured. The crystals' morphology and structure were determined using a scanning electron microscope with EDS for microanalysis.
Our results showed that the pH value, source, and concentration of calcium ions influenced the structure, morphology and amount of precipitated calcium carbonate. The calcium acetate was the most preferred calcium source and the pH value of culture media above 8.0 was the most appropriate for the biomineralization process. Moreover, the structure, morphology and size of biocrystals depended not only on the environmental conditions, but also on the features of the bacterial strain.

Key words: microbial induced mineralization, calcite, abiotic factors

VI.P.42

Metabolic biodiversity of *Coprinus comatus* using Biolog System

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Coprinus comatus, the shaggy mane mushroom, is cultivated as delicious and highly nutritious edible species by Chinese people in recent years. Beside its culinary value *C. comatus* is considered as medicinal mushroom because of its antioxidant, antitumor, antidiabetic, immunomodulating, hypolipidemic, antibacterial, and antinematode properties.
Although the genus *Coprinus* include many species of edible and medicinal mushrooms, these mushrooms are often poorly characterized or intractable to genetic analysis, and there are many gaps to be filled in the current knowledge on their taxonomy and biology.
Bearing in mind that metabolic features are becoming increasingly important in fungal taxonomic studies, the aim of the present work was to determine the intraspecific diversity of *Coprinus comatus* using biochemical profiling tools. In addition, we investigated the usefulness of these methods for establishing the metabolic relationships between *C. comatus* strains.
Biolog FF MicroPlates were applied to obtain data on utilization of 95 carbon sources and mycelial growth. The analysis allowed comparison of functional diversity of the fungal strains and revealed a broad variability within the analyzed *Coprinus* species based on substrate utilization profiles. Significant differences have been shown in substrate richness values. There is no clear correlation in metabolic preferences of the analysed strains to a particular group of substrates. However, most fungal strains were easily capable of carbohydrates and amino acids utilization. All the isolates were grouped into two major clusters at a 100% similarity level, which then were arranged in subclusters. In general, the strains from group A comprise all slowly metabolizing strains that used fewer substrates (2–7), than the isolates from group B.
The Biolog experiments have demonstrated to be a good profiling technology for studying the diversity in shaggy manes due to metabolic differences and proved all the *C. comatus* strains might be considered individually.

Key words: Biolog, fungal diversity, *Coprinus comatus*, shaggy mane mushroom

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VI.P.43

Production of linear cyanobactins by *Lyngbya aestuarii* from the Baltic Sea

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Cyanobacteria of the *Lyngbya* genus (Oscillatoriales) live in marine, fresh and brackish environments worldwide. They are well-known producers of bioactive metabolites, including toxins and potential pharmaceuticals. In the cases of the Baltic species from *Lyngbya* genus, no reports on the production of bioactive metabolites or their diversity have been published.

The aim of the present work was to characterized the structure of peptides produced by four Baltic isolates (CCNP 1314, 1315, 1316, 1324) classified to *Lyngbya aestuarii*. For the purpose of the study, liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) was used. The structures of *Lyngbya* metabolites were characterized on the basis of their fragmentation spectra. The LC-MS/MS analyses revealed high metabolic similarity of the strains CCNP 1315 and CCNP 1316 and different metabolite profile of CCNP 1314. In three *Lyngbya* strains (CCNP 1315, 1316 and 1324) different variants of linear cyanobactins were detected. These compounds belong to ribosomally produced peptides and are characterized by a prenylated N-terminus and methylated C-terminus.

Although the gene clusters responsible for the production of the cyclic cyanobactins have been found in the genomes of 10% of the analyzed cyanobacterial taxa, so far, the presence of the linear structures of the peptides has been reported only from *Microcystis aeruginosa*. In our work, for the first time the production of the peptides by *Lyngbya* was demonstrated. Most of the identified cyanobactins represent novel structures of the compounds.

Key words: *Lyngbya,* metabolites, linear cyanobactins, ribosomally, peptides

VI.P.44

Influence of silver nanoparticles on growth and metabolism of moulds

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Nowadays, the usage of silver in the form of nanoparticles (AgNPs) is widespread and affects almost all industrial branches. The high susceptibility of moulds to silver nanoparticles is surprising, considering their known resistance to various disinfectants. The lack of published studies on the mechanisms of this sensitivity leans to the attempts of their knowledge and explanation. Hence, the aim of the study was to determine the influence of AgNPs on the growth and metabolism of moulds. The AgNPs influence on fungi biomass was determined by gravimetric analysis, organic acids production by HPLC and extracellular enzymes by API Zym test. The silver nanoparticles preparation (10–80 nm, 90 ppm) was obtained by chemical method of $AgNO_3$ reduction by sodium citrate, with PVP as an anti-aggregation factor. It was added to culture medium in the minimal inhibitory concentration (MIC) of each strain. Studies were performed using pure culture collection strains: *A. niger, P. chrysogenum*. The presence of AgNPs in the medium decreases the mould biomass by 45–50% after 14 days of incubation, reduces the growth rate and accelerates the entry into the stationary phase by 7 days. The AgNPs changes extracellular enzymes production (alkaline and acid phosphatase, esterase, phosphohydrolase, α- and β-galactosidase, α- and β-glucosidase). Silver nanoparticles reduces the organic acid production: oxalic by 65–76%, citric by 21–83%, malic by 17–32% and succinic by 24–87%. It was proven that the application of silver nanoparticles may inhibit the growth and metabolic activity of moulds.

Key words: silver nanoparticles, moulds, metabolism

VI.P.45

The metabolic characteristics of fungi isolated from environments with low and variable temperatures

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The aim of this study was the characteristics of biochemical properties of fungi isolated from environments with low or variable temperature. The SF-P2 (BIOLOG) microplates were used to determine the metabolic profile of strains in two temperatures 6 and 28°C. Wells of microplates contain 95 sources of carbon and water as a control. The carbon sources are grouped into carbohydrates, polymers, carboxylic and keto acids, amino acids, amines/amides, and other. The fungi grow in wells containing carbon sources that it can utilize, forming turbidity in those wells as compared to the reference well.
The biological material consisted of strains of *Alternaria alternata*, *Alternaria brassicae*, and *Pleospora papaveracea* isolated from food low temperature storage rooms, frozen dumplings malt, and historical brick and wooden surfaces, moreover *Thelebolus microsporus*, *Geomyces* sp., *Phoma sclerotioides*, *Leptosphaeria sydowii* isolated from Antarctic soil. A total of 14 strains were examined.
It was demonstrated the differences in the biochemical activity, depending on the origin of strains and incubation temperature. At a temperature of 6°C the higher activity in substrates utilization noted for Antarctic strains, particularly for *Thelebolus microsporus* G2 and *Geomyces* sp. G6. For other strains utilization of carbohydrate and polymers was observed. Among the Antarctic strains only *Thelebolus microsporus* G2 and *Geomyces* sp. G2 are able to growth at 28°C, but their biochemical activity in this temperature was very low. The activity of strains isolated from low and variable temperatures was higher in 28°C. The most number of substrates was utilized by *Alternaria brassicae*. The other strains mainly metabolized carbohydrates, amino acids and polymers. Cluster analysis of the results obtained at a temperature of 6°C allowed to distinguish two main groups of fungi according to their origin of isolation. The strains from Antarctic soil consisted of one group. Obtained results provided that the Antarctic strains are psychrophiles.

Key words: fungi, BIOLOG microplates, low temperature

VI.P.46

Occurrence of aflatoxins and ochratoxin A in some spicies commercialized in Poland

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Aflatoxins are secondary metabolites produced by fungi, particularly *Aspergillus flavus* and *Aspergillus parasiticus*, which were found as contaminant in a wide variety of food matrices. The consumption of food with high level of aflatoxins can cause acute and chronic adverse effects on health which include immunosuppresion hepatic disorder and cancer. Ochratoxin A (OTA) is a secondary metabolite that is produced by some species of *Aspergillus* and *Penicillium*. OTA is commonly found in cereals, coffee, spices and dried fruits and is classified as a potential Group 2B carcinogen by the International Agency for Research on Cancer.
Several factors affect mycotoxin occurrence, the most important being plant genotype, fungus strain and environmental conditions.
A total of 49 spice samples were collected from different stores of Eastern Poland. The analysis was conducted using an enzyme-linked immunosorbent assay (ELISA) method, Ridascreen Total Aflatoxin and Ridascreen Ochratoxin A (R-Biopharm).
The analysis showed that 100% of samples were contaminated with OTA and a low level of total aflatoxins contamination was found in the samples. The average concentration for total aflatoxins was 21.78 μg/kg, far above the maximum threshold admitted by European legislation (10 μg/kg for total aflatoxins). The highest incidence of OTA was found in allspice and it amounted 194.77 μg/kg. Moreover, 60% spice samples contaminated by OTA exceeded the threshold admitted by the European Regulation.

Key words: mycotoxins, spices

VI.P.47

Sensitivity of environmental bacterial and fungal strains to quaternary ammonium salts

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Quaternary ammonium salts (QAS) have been widely used in disinfection of water, surfaces and instruments, textile, leather and food industry because of their relatively low toxicity, broad antimicrobial spectrum, non-volality and chemical stability. Due to these advantages QAS are also used in the restoration and could be applied on historical material.

The aim of the research was to determine the sensitivity of six environmental bacterial and fungal strains to biocides containing quaternary ammonium salts. In the study sensitivity to two biocides of bacteria *Pseudomonas fluorescens*, *Staphylococcus equorum*, *Bacillus cereus* and moulds *Chaetomium globosum*, *Penicillium citreonigrum*, *Cladosporium cladosporoides*, all isolated from historical wood were tested. The biocides were obtained commercially and marked as A (didecyldimethylammonium chloride <5.0%, alkyldimethylammonium oxide <5.0%) and B (didecyldimethylammonium chloride >9.5%, citric acid 2.0%, propiconazole 0.5%, 2-(methoksymethylethoxy)propanol 0.5%). In order to assess the tested strains sensitivity, microorganisms were inoculated (bacteria – 10^8 CFU/ml; moulds spores – 10^7 spores/ml) on the wood samples (50 mm×20 mm×10 mm) and incubated for up to 28 days in a climatic chamber at 28°C and 80% relative humidity. Afterwards, biocides were applied in triplicate at an interval of 24 hours, at concentrations 1.5%, 3%, 6%, v/v (biocide A) and 10%, 20%, 30%, v/v (biocide B). Antimicrobial activity was determined by imprinting wood samples on TSA medium (Merck) for bacteria, and on MEA (Merck) for moulds.

Both tested biocides showed high antimicrobial activity against *Staphylococcus eqourum* and *Bacillus cereus*, at the lowest concentrations tested after a single application. Whereas, *Pseudomonas fluorescens* was less sensitive and requires the use of higher concentrations of both biocides. For all strains of moulds the biocides showed low antifungal activity in the tested concentrations.

Key words: antimicrobial activity, biocides, quaternary ammonium salts

VI.P.48

Effect of essential oils on hydrophobic properties of environmental *Candida* strains

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Both clinical and environmental *Candida* strains, especially in structure of biofilm, may cause significant health problems. Processes of colonization of biotic and abiotic surfaces and biofilm formation depends inter alia on hydrophobic properties of *Candida* spp.

The aim of the research was to determine the effectiveness of chosen essential oils in reduction of hydrophobic properties of environmental *C. rugosa*, *C. famata* and *C. krusei* isolates. Collection strain *C. albicans* ATCC 10231 was used for comparison. Cell surface hydrophobicity (CSH) was determined by the microbial adhesion to hydrocarbon test (MATH) to p-xylene in PBS buffer. The results were presented as hydrophobicity index IH, i.e. the percentage of cells in the xylene layer (adhered cells). Within the study, thyme (*Thymus vulgaris* L.), clove (*Syzygium aromaticum* Merill & Perry) and tea tree (*Melaleuca alternifolia* Cheel) oils were used. Essential oils (EOs) were applied in their MIC concentrations (0.031–0.25%, v/v) specific for the particular strain and oil.

CSH values of strains tested were high and ranged from 76.4% to 91.2%, with the highest value for *C. rugosa*. Statistically significant decrease of hydrophobicity indexes was observed after application of tea tree oil for *C. krusei*, clove oil – *C. albicans*, and all EOs tested – *C. rugosa*. Only in the case of *C. famata* isolate EOs used solely did not affect its hydrophobic properties. To determine the interactions of EOs, their mixtures (1:1, 1:2 and 2:1, v/v) were applied. The essential oils' mixtures combination reduced hydrophobicity of *C. rugosa* and *C. albicans*. For the other two strains hydrophobic properties were significantly decreased only in the presence of thyme and clove oils (1:1) – *C. krusei*, and tea tree and thyme oils (1:1, 2:1) – *C. famata*. The interactions indexes of EOs in combinations indicate their additive or indifferent effect.

Key words: *Candida* spp., hydrophobic properties, essential oils

VI.P.49

Activity of sulphate reducing bacteria isolated from oilfield waters (Flysch Carpathians SE Poland)

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Sulphate-reducing bacteria were isolated from selected oilfield waters in the Flysch Carpathians of south-eastern Poland. Organisms were incubated using the *microcosms* method with application of two media: minimal medium and modified Postgate C medium with yeast extract or trisodium citrate or monocyclic hydrocarbons from the BTEX group (benzene, toluene, ethylbenzene, and xylene) as the sole carbon source. Activity of sulphidogenic microorganism communities was noted only on the Postgate C medium. Beside active sulphate reduction – max. 70%, c. 74% biodegradation of organic compounds was also observed in the cultures. The highest content of sulphate reducing bacteria (SRB) in the COD (c. 83%) was noted in cultures, in which trisodium citrate and yeast extract were applied as the sole carbon source.

Molecular analysis indicated not only the presence of SRB such as *Desulfobacterium autothrophicum*, *Desulfovibrio desulfuricans*, but also other microorganisms e.g. *Geobacter metallireducens*. All these taxa are obligatory or facultative anaerobes, with metabolism linked mostly with elemental sulphur and/or its oxidized forms, as well as iron.

Analysis of the mineral composition of the residues confirmed the presence of mineral phases which are typical for reducing environments. Among these minerals were recorded elemental sulphur and carbonates (calcite and rare vaterite) that can result from biochemical reactions performed by microorganisms. Based on the obtained results, it is concluded that the physical and chemical properties of the oilfield waters are favourable for the growth and development of sulphidogenic microorganism assemblages and mineral-forming processes conducted by them.

Key words: sulphate-reducing bacteria, oilfield waters, carbonates, biogenic minerals

VI.P.50

Spatial distribution and abundance of potentially human pathogenic microorganisms in sand of the recreational marine beach

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In the recent years the increasing bacterial contamination of the coastal seawaters and the beach sand has become a potentially global problem limiting their recreational functions for millions of domestic and foreign tourists. Along with fecal bacteria, also potentially pathogenic bacteria which normal habitat is not the marine environment are introduced to the seawaters and the beach sand The accumulation of those pathogenic bacteria in the seawater and the beach sand poses a hazard to bathers for whom an infective dose of pathogen colonises a suitable growth site in the body and leads to a disease.

The occurrence and the distribution of potentially human pathogenic bacteria such as *Aeromonas*, *Pseudomonas aeruginosa*, *Staphylococcus* and *Vibrio* – like organisms and fungi *Candida albicans* in the sand of the recreational marine beach were studied. The research was carried on the sandy sea beach in Ustka, one of the most popular resorts in the central coast of the Baltic Sea. The dry and the wet sand and the seawater samples were collected once per month at the Eastern Beach and the Western Beach The samples of sand were obtained from two sites along a profile perpendicular to the shoreline. The wet sand was collected from a site situated at the waterline and the dry sand was collected from a site located a sheltered place among the dunes. Sand core samples were divided into two sections: 0–5 cm and 10–15 cm.

The highest mean number among these four studied groups of bacteria constituted *Aeromonas* – like organisms and the lowest *Staphylococcus* – like organisms. The dry sand was inhabited by the highest number of all studied potentially pathogenic microorganisms.

Within a year, the number of the studied microorganisms inhabiting the sand showed considerable monthly changes. There were differences in the abundance of potentially pathogenic bacteria between the surface and the subsurface sand layers with a clear trend for the decrease in the number of the studied bacteria within the deeper layers of the sand.

VI.P.51

Effect of creosote oil fumes on soil enzymatic activity – a preliminary study

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The aim of study was to determine effect of creosote oil fumes on activity of some soil enzymes involved in carbon cycle: dehydrogenases (EC 1.1.1.x), catalase (EC 1.11.1.6), o-diphenol oxidase (EC 1.10.3.1), lipase (3.1.1.3), β-glucosidase (EC 3.2.1.21); in phosphorus cycle: alkaline phosphomonoesterase (EC 3.1.3.1), acid phosphomonoesterase (EC 3.1.3.2), phosphodiesterase (EC 3.1.4.1), phosphotriesterase (EC 3.1.8.1), inorganic pyrophosphatase (EC 3.6.1.1); and in nitrogen cycle: urease (EC 3.5.1.5), proteases (EC 3.4.4.x) and nitrate reductase (EC 1.6.6.1).
Creosote oil is a product of coal tar distillation. It is an efficient and toxic wood preservation chemical, which consists of hundreds of organic compounds, most of which are detrimental to the environment. Soil enzymatic activities are recognized as a more sensitive bioindicator used for measuring the effect of soil pollution on microbial community.
The experiment was carried out on loamy sand and sandy loam with organic carbon content of 8.7 and 10.9 $g \cdot kg^{-1}$. The soil samples were placed with ground wooden railway sleepers impregnated with creosote oil in exsiccators for 21 days. The references were soil samples without impact of creosote oil fumes. After 21 days soil enzyme activities in control soils and in soils treated with creosote oil fumes were measured spectrophotometrically.
Obtain results shown a decrease effect of creosote oil fumes on activity of all measured enzymes involved in nitrogen cycle in both soil types. The inhibitions of enzymes involved in phosphorus cycle caused by creosote oil fumes were observed only in sandy loam. Among enzymes involved in carbon cycle, the dehydrogenase, lipase and catalase activities were increased, and *o*-diphenol activity was decreased in both soil types treated with creosote oil fumes.

Key words: creosote oil, enzymatic activity, fumes, soil

VI.P.52

Alicyclobacillus sp. biofilm formation

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Acidothermophilic bacteria *Alicyclobacillus* sp. are recognized as a threat for fruit processing industry as their presence in fruit based products (juices and juice concentrates) is more frequently observed. Alicyclobacilli are aerobic sporulating bacteria which spores withstand unfavorable conditions (elevated temperature and high acidity) what causes difficulties in their removal from industrial environments in food industry. Although their general characteristics and biochemical properties have been widely studied, the ability of *Alicyclobacillus* sp. to establish biofilm in industrial installations still remain unexplored.
This study considers *Alicyclobacillus* sp. biofilm formation capability on glass surface.
Three environmental isolates previously identified as *Alicyclobacillus* sp. and *Alicyclobacillus acidoterrestris* DSM 3922 reference strain have been inoculated into liquid BAT medium containing glass slides and incubated in 45°C for 4, 24, 48, 72 hours with and without agitation. After incubation period the number of biofilm-associated cells was determined by swab technique both for rinsed and untreated glass surfaces and quantified by count plate method.
All tested bacterial strains expressed ability to colonize glass surface even after 4 hours of incubation. It was found that agitation accelerated biofilm formation. The average range of biofilm production reached 3.0–3.7 log CFU/cm^2. The mature biofilms were formed after 48 hours of incubation and their levels did not considerably change within the time.
The study proved *Alicyclobacillus* sp. attachment capability onto glass surface showing that 2 days of incubation in favorable conditions is sufficient enough to form biofilm structure. There were any statistically important differences between the tested strains observed.

Key words: *Alicyclobacillus* sp., biofilm, glass surface

VI.P.53

Distribution of genes for virulence factors and a profile of endotoxin in *Aeromonas* spp. strains isolated from cultured fish

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Aeromonas spp. are ubiquitous water-borne bacteria responsible for economic losses in fish farming plants. The common carp and the rainbow trout are popular food fish in Poland and should be considered as a source of *Aeromonas* contaminations. We asked whether virulence factors associated with human diseases such as toxins and extracellular enzymes are widespread among *Aeromonas* isolated from cultured fish, as well as what the profile and the structural diversity of lipopolysaccharide (LPS), the main immunodominant glycolipid of the outer membrane of these gram-negative bacteria, is.

SDS-PAGE analysis of LPS showed that a majority of the isolates synthesized O-antigen containing S-form LPS. Neutral and amino-6-deoxyhexoses, hexoses, aminohexoses, two isomers of heptose and Kdo were revealed as the main components of the carbohydrate portion of the endotoxin. PCR-based detection of virulence genes for cytotoxic enterotoxin (*act*), aerolysin/haemolysin (*aerA*), cytotonic enterotoxins (*ast, alt*), serine protease (*ser*), and glycerophospholipid:cholesterol acetyltransferase (*gcaT*) was performed. The studies demonstrated that all strains harboured at least two of four toxin genes. The *aerA* and *act* were the most frequently found toxin genes among all the isolates. PCR amplification was also attempted for a 330-bp region of *alt* and a 440-bp region of *ast*; it revealed that about 30% of the isolates harboured these genes. Primers for the amplification of the 350-bp and 240-bp regions of the *ser* and the *gcaT* genes, respectively, yielded PCR products of extracellular enzymes from approx. 60% to 100% of isolates.

Both surface molecules, such as LPS, and extracellularly secreted toxins and enzymes are the main factors associated with *Aeromonas* pathogenicity. The distribution profile of virulence-related genes among the isolates from the cultured fish confirms that *Aeromonas* are genetically heterogeneous. Moreover, when they harbor aerolysin/hemolysin genes together with structural attributes such as O-antigen containing LPS molecules, they can be considered potential foodborne pathogens.

Key words: *Aeromonas*, virulence factors, PCR

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VI.P.54

Biodiversity of microorganisms in the soil contaminated with petroleum substances

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The aim of the study was to determine the effect of soil contamination with petrol and diesel oil on soil microorganisms abundance (MA) and its biological activity, expressed by respiration activity (RA), dehydrogenase (DHA) and catalase (CAT) activities. Microbial identification was realized with use of molecular methods. Soil material for investigation was extracted from the surface (0–20 cm), subsurface (20–40 cm) and subsoil (40–60 cm) layers of agricultural exploited *Mollic Gleysol*. Under laboratory conditions to soil samples enrichment with petroleum substances (petrol and diesel) in the following doses: 0, 1, 3, 5, 10, 15 g/10 g fresh soil were added and incubation was performed (7 d, 20°C). RA was determined as a concentration of CO_2 in the head space (GC Varian). Microorganisms abundance by Koch platelet method on agar (BTL) was realized. DNA was extracted by Sambrook and Russell method (with own modification) and primers 27f/1492r were used for microbial identification Enzymes activities were tested with the use of 2.3.5-TTC as a substrate and by back-titrating residual H_2O_2 with $KMnO_4$ for DHA and CAT, respectively.

Our results demonstrated that between tested petroleum substances – petrol is more harmful for soil biology than diesel, what was confirmed by notification of lower values (by c.a. 70%) of each biological factors (RA, DHA, CAT, MA) treated with petrol in relation to diesel contamination. The most sensitive on petroleum contamination seemed to be RA and DHA. As a major autochthonic bacteria being present in soil contaminated with petrol oil species belonging to the genera *Rhodococcus* sp., *R. erythropolis*, *R. qingshengi* and *R. globerulus* were noted, whereas in combination treated with diesel oil besides representatives of *Rhodococcus* genera also *Staphylococcus* sp., and *S. haemolyticus* have been identified.

Key words: petroleum substances, soil microbial activities, microorganisms identification

VI.P.55

Phenotypic and genotypic characterization of *Enterococcus* sp. strains isolated from humans and turkeys

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Enterococci are a natural component of the intestinal flora of many organisms, including humans and birds. As opportunistic pathogens, can cause fatal infections of the urinary tract and endocarditis in humans, while in poultry joint disease, sepsis and falls in the first week of life. Abuse of antibiotics in human and veterinary medicine has led to the development of multidrug resistant strains.

The aim of this study was to determine the antimicrobials susceptibility and genetic mechanism of resistance in collected strains, as well the ability of enterococci to biofilm formation.

The research covered 112 *Enterococcus* strains – 56 isolated from humans and 56 from turkeys. Samples were collected as rectal or cloacal swabs. Among the investigated isolates *E. faecalis* was detected in 53.6% (turkey strains) and in 44.6% (human strains). *E. faecium* was identified in 41.1% and in 7.1% of turkey and human isolates, respectively. Resistance to more than 4 antimicrobials (tetracycline, erythromycin, ciprofloxacin and vancomycin) showed 33.9% of all analyzed strains. Simultaneous resistance to tetracycline and erythromycin occurred in 83% of the human and turkey strains, otherwise 70.5% of all strains showed resistance to ciprofloxacin. Our study revealed, that 51.8% of turkey and 58.9% of human strains were resistant to vancomycin.

Tetracycline resistance gene – *tetM* was detected in 83% of all analyzed strains, while *tetO* gene was found in 53.6% of human and only in 8.9% of turkey strains. Vancomycin resistance gene (*vanA*) was detected in 7 strains (6 isolated from turkeys and 1 from human). *ErmB* gene (resistance to macrolide) was detected in 55.4% of all isolates (67.9% of turkey and 42.9% of human strains), and *ermA* gene was detected in 16.1% of turkey and in 3.6% of human isolates. All the strains had the ability to biofilm formation. A stronger biofilm was formed by strains isolated from humans than birds. It is also noted that multidrug resistance strains had a tendency to form more intensive biofilms.

Key words: *Enterococcus*, resistance genes, multidrug resistance, biofilm

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The identification of antimicrobials mechanisms of resistance in *Campylobacter* isolated from poultry

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In the developing countries *Campylobacter jejuni* and *Campylobacter coli* are the most common causative agents of bacterial gastroenteritis worldwide. Natural host of *Campylobacter* is mainly poultry. Contaminated, undercooked meat can lead to human infections. As a results, there may occur fatal complications such as Guillain-Barré syndrome. The over-use of antimicrobials in animal production causes an increases in bacterial resistance. This poses a problem in antibiotic treatment in humans with gastroenteritis caused also by another bacteria.

The aim of this study was to compare the antimicrobial resistance phenotypes and resistance genes in *Campylobacter* spp. isolated from slaughter poultry [n=45] in Poland. *Campylobacter jejuni* [n=41] and *Campylobacter coli* [n=4] isolates were identified by multiplex PCR and MALDI-TOF Biotyper. There was 100% identity of the results in both methods. Minimum inhibitory concentration (MIC) of tested strains [n=45] was determined by broth microdilution susceptibility method. In order to investigate the genetic basis of resistance to erythromycin (mutation in position 2075 domain V 23S rRNA) and ciprofloxacin (mutation in *gyrA* gene) Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) were used. Polymerase chain reaction (PCR) was used to detect genes such as: *tetO* (tetracycline), *aadE* (streptomycin), *bla-OXA$_{61}$* (ampicillin) and *cmeB* (multi-drug efflux pump).

In this study all strains had mutation in *gyrA* gene and no mutation in position 2075 domain V 23S rRNA. Among 45 isolates *tetO* gene was detected in 36%, *bla-OXA$_{61}$* gene in 29% and *cmeB* gene in 7% strains. All strains were resistant to ciprofloxacin, 86.7% to nalidixic acid and 64.4% to tetracycline. All strains were susceptible to azithromycin, erythromycin, gentamycin, florfenicol, telithromycin and clindamycin. This study showed a frequent occurrence of fluoroquinolone resistance and the presence of selected resistance mutation in *Campylobacter jejuni* and *Campylobacter coli*.

Key words: *Campylobacter*, antimicrobials resistance, poultry

VI.P.57

The occurrence of killer activity in yeast strains isolated from natural environments

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Killer properties of yeasts were first described and investigated on the strain *Saccharomyces cerevisiae* in 1964. Since then, almost 100 species of yeast belonging to more than 20 genera (including *Hansenula, Candida, Torulopsis, Williopsis, Pichia, Debaromyces, Ustilago*) were known and their number is still growing. Killer yeast is capable of secretion of the protein or glycoprotein known as killer toxin. These proteins kill sensitive cells of yeast, bacteria or fungi. Currently, more than 11 different killer toxins are known. The mechanism, temperature and pH of action depend on the type of toxins and the sensitive strain. Synthesis of killer toxins is associated with the presence of intracellular particles of different virus groups. Depending on the capabilities of the strain to secrete toxins and sensitivity to toxins, strains are singled out as killer yeast, neutral and sensitive. Killer yeasts are usually obtained from various natural habitats, ranging from fermentation environments, soil, through various types of fruits, vegetables and flowers. Under natural conditions killer systems play a major role in the ecology of yeasts and constitute one of the most important mechanisms of competition between the strains in a particular niche. Potential use of killer yeast and / or toxins produced by them is mainly related to the neutralization of pathogenic or infectious microorganisms in given environment, inter alia, in brewing, winemaking or bakery. In a study yeast isolates from different natural habitats have been used. They were subjected to screening in terms of killer toxin secretion using the plate method. If the inoculated strain was surrounded by bluish coloured cells and a clear zone <1 mm or only surrounded by a blue zone, the reaction was recorded as "w" (weak killer reaction). If the inoculated strain was surrounded by bluish coloured cells and a clear zone ≥1 mm, it was designated as "+" (positive killer reaction).

Key words: killer yeast, toxin killer, natural environments

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Pseudomonas aeruginosa ability to create biofilms on polyvinyl chloride and styrene-acronitrile copolymer surfaces

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Biofilms are highly organized microbial communities expressing high resistance to disinfectants and other external environmental factors. Medical equipment such as stents and catheters can be colonized by a variety of bacteria including opportunistic pathogens dangerous for patients with lowered immune system. A variety of polymers and copolymers are developed for medical equipment to minimize a bacterial biofilm formation.

The aim of the research was to evaluate the biofilm formation on the suction catheters by environmental *Pseudomonas aeruginosa* strains.

Catheters made of polyvinyl chloride and styrene-acronitrile copolymer which were cut into 1 cm pieces and introduced into TSB liquid medium (Merck) were inoculated with four different environmental strains of *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa* ATTC 15542 reference strain (approximately 10^8 CFU/ml). After incubation without agitation in 30°C for 24 h catheters were rinsed with PBS buffer for removing the loosely adhered cells. Afterwards catheters were put in sterile tubs with saline solution and sonicated for 30 sec (60 Hz) to disperse the cells. Colony forming units considered as biofilm-creating were obtained by the count plate method on TSA medium (Merck). All the tested *Pseudomonas aeruginosa* strains expressed a strong ability to create the biofilm on the examined catheters. The biofilm formation by two environmental isolates and the reference strain were estimated at the level of 2.2–6.0×10^6 CFU/cm^2. The other isolates reached the level of biofilm production of 5.3–5.5×10^6 CFU/cm^2.

The research proved that both *Pseudomonas aeruginosa* environmental and reference strains are capable of colonizing polyvinyl chloride and styrene-acronitrile copolymer surfaces with a high efficiency.

Key words: biofilm, *Pseudomonas aeruginosa*, environmental strains

VI.P.59

Cellulolytic bacteria from digestive tract of Svalbard reindeer (*Rangifer tarandus platyrhynchus*)

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Reindeers have a circumpolar distribution, with seven subspecies occupying different boreal and arctic regions. The Svalbard reindeer is a small subspecies of *Rangifer tarandus* endemic to Svalbard archipelago. A majority of reindeers living today are domesticated or semi-domesticated. As ruminants, they depend on symbiotic rumen microorganisms for the digestion of fibrous plant material. To cope with a high variety of vascular plants, mosses and lichens, reindeer rumen consists of highly complex and unique symbiotic microbial community, mostly bacteria. The composition of bacterial communities appears to be influenced by the host age and condition as well as available plants and seasonal factors not yet understood.

The microbiome of the rumen is responsible for the breakdown of plant fiber. Fibrolytic bacteria degrade fibrous material, which allows nutrition by utilization of plant fiber. A wide variety of enzymes expressed by bacteria, belonging to the phyla *Firmicutes* and *Bacteroidetes* contribute to fibre digestion, metabolizing different carbohydrates including celluloses. Those two phyla are the most abundant bacteria among reported reindeer populations. *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* are presently recognized as the major cellulolytic bacterial species found in the rumen and are important in degradation of dietary fiber. Interestingly, Svalbard reindeers are known for their high rumen concentration of cellulolytic bacteria. In the faecal samples collected by us in Hornsund, *R. flavefaciens* and *F. succinogenes* were the most commonly isolated cellulolytic rumen bacteria, while in rumen samples from different Svalbard reindeer population and from Norwegian reindeers those bacteria were not reported. Unfortunately, those results cannot be directly compared as they were obtained with the use of different methodological approaches.

Digestion of plant fiber is the basis of the mutualism between specialised microbes and vertebrate herbivores, which in case of reindeers results in unique and complex bacterial flora. This kind of relationship merits deepen investigation.

Key words: faecal bacterial community, 16S rRNA New Generation Sequencing, Arctic, reindeer, fibrolytic bacteria

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