This book contains the program and the abstracts submitted to the 7th International Weigl conference which will take place on September 26-29th, 2017 at the main conference hall of I. Franko Lviv National University.

Rudolf Weigl (1883 – 1957) is famous Polish microbiologist of Austrian descent who worked in Lviv, then Lwow in Poland, during period between the two World Wars. He was the first who proposed exploiting the laboratory animals as the source of microorganisms used for vaccine preparation. He deviced the first anti-typhus vaccine and developed technology of its production on the industrial scale. The work initiated by Prof. R. Weigl was further continued and developed in Lviv after the Second World War by his assistant Dr. Henryk Mosing and many Ukrainian microbiologists. In commemoration of great scientific achievements of Prof. Rudolf Weigl for development of Polish and Ukrainian microbiology, immunology and biotechnology, the group of scientists from both countries decided to organize regular bilateral Weigl conferences, which later have been transformed to international meetings. Previous Weigl conferences have been organized in Lviv (2003, Ukraine), Warsaw (2007, Poland), Odesa (2009, Ukraine), Wroclaw (2011, Poland), Chernivtsi (2013, Ukraine) and Gdansk (2015, Poland). This year, mictrobiologists from 12 countries will gather again in the native city of Prof. Rudolf Weigl, Lviv. Organizers of the 7th International Weigl conference offer participants an interesting scientific and social program.

Authors of oral presentation will have opportunity to publish their works in the Thematic Issue of the leading Ukrainian microbiological journal “Mikrobiolohichnyi Zhurnal” (Microbiological Journal, see; http://microbiolj.org.ua/en/) which is indexed by PubMed, Medline, Scopus, EBSCO, Google Scholar and other databases. The manuscripts will be peer-reviewed before publication. The deadline for manuscript submission is December 1st, 2017.

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Program of 7th International Weigl Conference, Lviv, Ukraine, September 26-29, 2017

September 26 (TUESDAY)

9.00 – 16.00 Registration, Poster Mounting

12.00 – 12.30 Opening Ceremony: Greetings from Lviv National University, Lviv city Council, Presidium of NAS of Ukraine, Ukrainian and Polish co-organizers

12.30 – 15.00 Plenary Session 1

Chairs, A. Sibirny, Ukraine; G. Wegrzyn, Poland

12.30 – 13.00 Bauke Oudega (Amsterdam, The Netherlands) FEMS: PRESENT, FUTURE AND STRATEGY

13.00 – 13.30 Serhiy Komisarenko (Kyiv, Ukraine) BIOSAFETY IN UKRAINE AND BIOTHREATS DUE TO THE PROGRESS IN MODERN BIOLOGY

13.30 – 14.00 Grzegorz Wegrzyn (Gdansk, Poland) LIGHT AND DARK SIDES OF THE (PHAGE) FORCE: 100 YEARS OF STUDIES ON BACTERIOPHAGES

14.00 – 14.30 Andrzej Gamian (Wroclaw, Poland) ENTEROBACTERIAL OmpC PROTEIN RECOGNIZED BY PROTECTIVE UMBILICAL CORD ANTIBODIES AS A POTENTIAL ANTIGEN FOR CONJUGATE VACCINE

14.30 – 15.00 Andriy Sibirny (Lviv, Ukraine) THE ROLE OF AUTHOPHAGY IN DEGRADATION OF THE CYTOSOLIC ENZYMES OF METHANOL METABOLISM IN THE YEAST *KOMAGATAELLA PHAFFII (PICHIA PASTORIS)*

15.00 – 15.30 Coffee Break

15.30 – 17.00 Session 1 Metabolism and Regulation

Chairs, O. Tehlivets, Austria, R. Daugelavicius, Lithuania

15.30 – 15.50 Oleh Stasyk (Lviv, Ukraine) *HANSENULA (OGATAEA) POLYMORPHA* HEXOSE TRANSPORTER Gcr1 IS A NON-CONVENTIONAL GLUCOSE RECEPTOR

15.50 – 16.10 Rimantas Daugelavičius (Kaunas, Lithuania) POSSIBILITIES TO CONTROL OF EFFLUX ACTIVITY IN MICROORGANISMS

16.10 – 16.30 Magdalena Kotowska (Wroclaw, Poland) REGULATORY TARGETS OF A ZINC BINDING GNTR-LIKE PROTEIN OF *STREPTOMYCES COELICOLOR* A3(2)

16.30 – 16.50 Dagmara Jakimowicz (Wroclaw, Poland) THE HIGHLY PROCCESIVE TOPOISOMERASE I – WHY DO THE ACTINOBACTERIA NEED ONE?

17.20 – 18.00 Concert

18.00 – 20.00 Get-Together Party

September 27 (WEDNESDAY)

8.30 – 10.00 Poster Session 1

10.00 – 11.30 Session 1 Metabolism and Regulation, continued

Chairs, O. Stasyk, Ukraine, D. Jakimowicz, Poland

10.00 – 10.20 Oksana Tehlivets (Graz, Austria) HOMOCYSTEINE DEREGULATES FATTY ACID SYNTHESIS VIA DIFFERENT MECHANISMS IN YEAST

10.20 – 10.40 Paweł Olszewski (Gdansk, Poland) RNAP CRASH TEST: DISSECTING ROLES OF PROMOTER SEQUENCE ELEMENTS IN TRANSCRIPTIONAL INTERFERENCE BETWEEN CONVERGENT PROMOTERS IN *ESCHERICHIA COLI*

10.40 – 11.00 Anna Pawlik (Lublin, Poland) LIGHT: AN IMPORTANT ENVIRONMENTAL FACTOR INFLUENCING METABOLIC PROPERTIES AND CHEMICAL SENSITIVITY OF WOOD DECAY FUNGUS *CERRENA UNICOLOR*

11.00 – 11.10 Volodymyr Antonyuk (Lviv, Ukraine) *LACTARIUS* FUNGI PROTECTION SYSTEM FROM INSECTS AND ANIMALS EATING AND MICROBIAL DAMAGE

11.10 – 11.20 Maciej Dylewski (Gdansk, Poland) THE AUTOREGULATION OF THE greA GENE EXPRESSION- THE IN CIS ROLE OF THE GRAL SMALL RNA

11.20 – 11.30 Michał Sobala (Gdansk, Poland) ENZYMATIC AND FUNCTIONAL ANALYSIS OF A NOVEL (p)ppGpp SYNTHETASE USING AN *IN VIVO/IN VITRO* APPROACH

11.30 – 12.00 Coffee Break

12.00 – 13.30 Session 2 Immunology

Chairs, S. Komisarenko, Ukraine, M. Czerwinski, Poland

12.00 – 12.20 Marcin Czerwinski (Wroclaw, Poland) ANTIGENS OF HUMAN BLOOD GROUP SYSTEM P1PK AS RECEPTORS FOR SHIGA TOXINS

12.20 – 12.40 Yuriy Kit (Lviv, Ukraine) APPLICATION OF THE MAGNETIC MICROSPHERES FOR DETERMINATION AND PURIFICATION THE ANTIBODIES POSSESSING A DIAGNOSTIC AND PROGNOSTIC VALUE

12.40 – 13.00 Rostyslav Stoika (Lviv, Ukraine) ANTIOXIDANTS IN CANCER TREATMENT AND IMMUNE RESPONSE

13.00 – 13.20 Joanna Koziel (Krakow, Poland) CORRUPTION OF NEUTROPHIL EXTRACELLULAR

TRAPS BY PATHOGENS

13.20 – 13.30 Maria Sabadashka (Lviv, Ukraine) EVALUATION OF NITRATIVE STRESS IN RATS` LEUKOCYTES UNDER EXPERIMENTAL DIABETES MELLITUS

13.30 – 14.30 Lunch

15.00 – 18.00 City tour

19.00 – 23.00 Banquet (optional)

September 28 (THURSDAY)

8.30 – 10.00 Poster Session 2

10.00 – 11.30 Session 3 Microbial Biotechnology

Chairs, V. Passoth, Sweden, A. Rapoport, Latvia

10.00 – 10.20 Mykhailo Gonchar (Lviv, Ukraine) $(HIS)_6$-TAGGED *CORYNEBACTERIUM GLUTAMICUM* CREATININE DEIMINASE AS A BIOSENSING ELEMENT FOR CREATININE ASSAY

10.20 – 10.40 Kostyantyn Dmytruk (Lviv, Ukraine) YEAST *CANDIDA FAMATA* AS A PRODUCER OF RIBOFLAVIN AND ITS DERIVATIVES

10.40 – 11.00 Dariya Fedorovych (Lviv, Ukraine) CHROMIUM: BIOLOGICAL IMPORTANCE, TOXICITY AND DETOXIFICATION STRATEGIES IN YEASTS

11.00 – 11.20 Rostyslav Panchuk (Lviv, Ukraine) *STREPTOMYCES* ANTIBIOTICS OF LANDOMYCIN FAMILY AS NOVEL ANTICANCER DRUGS

11.20 – 11.30 Oresta Vasyliv (Lviv, Ukraine) COMPARISON OF POWER DENSITY OF AN ONE-CHAMBER AND A TWO-CHAMBER MICROBIAL FUEL CELL

11.30 – 12.00 Coffee Break

12.00 – 13.30 Session 3 Microbial Biotechnology, continued

Chairs, P. Fickers, Belgium, M. Gonchar, Ukraine

12.00 – 12.20 Derya Önal Darilmaz (Aksaray, Turkey) EVALUATION OF PROBIOTIC POTENTIAL AND OXALATE DEGRADING ACTIVITY OF EXOPOLYSACCHARIDE PRODUCER LACTOBACILLUS STRAINS ISOLATED FROM TRADITIONAL TURKISH CHEESES

12.20 – 12.40 Marta Semkiv (Lviv, Ukraine) IMPROVEMENT OF GLYCEROL PRODUCTION DURING ANAEROBIC FERMENTATION BY THE YEAST *SACCHAROMYCES CEREVISIAE*

12.40 – 13.00 Olena Kurylenko (Lviv, Ukraine) ROLE OF PEROXISOMAL AND CYTOSOLIC TRANSKETOLASE AND TRANSALDOLASE IN XYLOSE ALCOHOLIC FERMENTATION IN THE METHYLOTROPHIC THERMOTOLERANT YEAST *OGATAEA (HANSENULA) POLYMORPHA*
13.00 – 13.20 Justyna Ruchala (Rzeszow, Poland) PEROXISOMES ARE INDISPENSABLE FOR XYLOSE ALCOHOLIC FERMENTATION IN THE METHYLOTROPHIC BUT NOT IN THE NON-METHYLOTROPHIC YEASTS
13.20 – 13.30 Joanna Żur (Katowice, Poland) BIODEGRADATION OF PARACETAMOL BY IMMOBILIZED *PSEUDOMONAS MOOREI* KB4 STRAIN

13.30 – 14.30 Lunch

14.30 – 16.00 Session 4 Medical Microbiology
Chairs, A. Gamian, Poland, J. Dziadek, Poland

14.30 – 14.50 Maxim Lootsik (Lviv, Ukraine) STUDIES ON THE ANTIBACTERIAL ACTIVITY OF HONEYBEE CHITOSAN AND ITS DERIVATIVES
14.50 – 15.10 Jolanta Zakrzewska-Czerwińska (Wroclaw, Poland) HUPB: A MYCOBACTERIAL NUCLEOID-ASSOCIATED PROTEIN WITH AN INDISPENSABLE EUKARYOTIC-LIKE TAIL
15.10 – 15.30 KatarzynaKosznik-Kwaśnicka (Gdansk, Poland) BACTERIOPHAGESAS A POTENTIAL MEAN FOR PREVENTION AGAINST *SALMONELLA SPP*. CONTAMINATION OF POULTRY
15.30 – 15.40 Volodymyr Shchodryi (Kyiv, Ukraine) SPECTRAL STUDY OF INTERACTION BETWEEN MANITOL AND RIBONUCLEOSIDES BY FLUORESCENT PROBE
15.40 – 15.50 Nataliia Melnichuk (Kyiv, Ukraine) CHANGE OF SOME GENESEXPRESSION IN MICE LUNG AFTER PREVENTION AND TREATMENT WITH OLIGORIBONUCLEOTIDES-D-MANNITOL COMPLEXESOF INFLUENZA A VIRUS H1N1 (A/FM/1/47) INFECTION
15.50 – 16.00 Sebastian Wawrocki (Lodz, Poland) IL-18 SECRETION IN RELATION TO IL-18 GENE POLYMORPHISM IN *MYCOBACTERIUM TUBERCULOSIS*-INFECTED AND UNINFECTED INDIVIDUALS

16.00 – 16.30 Coffee Break

16.30 – 17.20 Session 5 Pharmacology
Chairs, W. Barabasz, Poland, R. Stoika, Ukraine
16.30 – 16.50 Wiesław Barabasz (Krakow, Poland) MASKED MYCOTOXINS
16.50 – 17.00 Andrii Lozynskyi (Lviv, Ukraine) ANTIMICROBIAL ACTIVITY OF SOME THIOPYRANO[2,3-D]THIAZOLES AND THEIR STRUCTURE-RELATED ANALOGUES
17.00 – 17.10 Danylo Kaminskyy (Lviv, Ukraine) 5-ENE-THIAZOLIDINONES: MEDICINAL CEMISTRY ASPECTS

17.10 – 17.20 Anna Kryshchyshyn (Lviv, Ukraine) THIAZOLIDINONE CORE IN THE DESIGN AND DEVELOPMENT OF NEW ANTITRYPANOSOMALS

17.20 – 17.40 Sponsor presentations

18.00 – 21.30 Performance in Lviv Opera (optional)

September 29 (FRIDAY)

9.00 – 10.30 Session 6 Environmental Microbiology

Chairs, N. Kuisiene, Lithuania, J. Truu, Estonia

9.00 – 9.20 Esra Deniz Candan (Ankara, Turkey) MOLECULAR IDENTIFICATION OF BACTERIAL AND FUNGAL COMMUNITIES OF GREEN TURTLE (*CHELONIA MYDAS*) NESTS

9.20 – 9.40 Jarosław Grządziel (Pulawy, Poland) METAGENOMIC APPROACH TO INVESTIGATE THE MICROBIOTA OF DIFFERENT SOIL TYPES

10.40 – 11.00 Nomeda Kuisiene (Vilnius, Lithuania) EVALUATION OF BIOACTIVITY OF MICROORGANISMS ISOLATED FROM KRUBERA-VORONJA CAVE: CLASSICAL *VS*. MOLECULAR APPROACH

11.00 – 11.20 Jaak Truu (Tartu, Estonia) INTEGRATION OF MULTIPLE OMICS DATA FOR ASSESSMENT OF SPECIFIC MICROBIAL COMMUNITY RESPONSE TO ACCIDENTAL RELEASE OF OIL INTO THE MARINE ECOSYSTEM

11.20 – 11.30 Amanda Pacholak (Poznan, Poland) INTERACTIONS BETWEEN ENVIRONMENTAL MICROORGANISMS AND NATURAL CARBOHYDRATE SURFACTANTS

11.30 – 12.00 Coffee Break

12.00 – 14.00 Plenary Lectures 2

Chairs, A. Sibirny, Ukraine, G. Wegrzyn, Poland

12.00 – 12.30 Andrzej Dzugaj (Wroclaw, Poland) COLLABORATION BETWEEN POLISH AND UKRAINIAN BIOCHEMISTS HAVE BEGAN IN THIRTIES OF THE LAST CENTURY IN PARNAS LABORATORY

12.30 – 13.00 Volkmar Passoth (Uppsala, Sweden) YEAST LIPID PRODUCTION FROM SECOND GENERATION SUBSTRATES FOR BIODIESEL AND FISH FEED

13.00 – 13.30 Patrick Fickers (Gembloux, Belgium) METABOLIC ENGINEERING OF *YARROWIA LIPOLYTICA* FOR THE SYNTHESIS OF ADDED VALUE CHEMICALS

13.30 – 14.00 Alexander Rapoport (Riga, Latvia) DEVELOPMENT OF WASTE-LESS PROCESS FOR THE CONSECUTIVE PRODUCTION OF FURFURAL, BIOETHANOL AND OTHER VALUABLE COMPOUNDS FROM HEMICELLULOSE AND LIGNOCELLULOSE-CONTAINING RESOURCES

14.00 – 14.30 Jaroslaw Dziadek (Lodz, Poland) DNA REPAIR IN THE PATHOGENICITY OF TUBERCLE BACILLI

14.30 – 15.00 Best poster presentations

15.00 – 15.30 Awards for the best poster presentations. Closing Ceremony, information on the next Weigl conference

15.30 Optional tours and/or departure

Plenary Session 1

Chairs, A. Sibirny, Ukraine; G. Wegrzyn, Poland

FEMS: PRESENT, FUTURE AND STRATEGY

Bauke Oudega
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Email address: b.oudega@vu.nl

The beginning and history of The Federation of European Microbiology Societies will be presented. Furthermore, the present situation of FEMS and the strategy and future developments will be discussed. Special emphasis will be put on the benefits for member societies and their members.

BIOSAFETY IN UKRAINE AND BIOTHREATS DUE TO THE PROGRESS IN MODERN BIOLOGY

Serhiy Komisarenko
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Possible threats of biological origin in Ukraine: 1) biological warfare, 2) non intentional creation of possible biothreats – “Dual-use” research of concern, 3) infectious diseases (human, animal, avian, etc), 4) intentional (deliberate) development and/or use of biological agents = bioterrorism, 5) bad quality food products, pharmaceuticals, water and 6) GMOs as a threat to biodiversity.
Globall outbreaks of infectious disease present a growing threat to international security. Most dangerous infectious diseases are often tropical diseases that emerge in developing countries but move now to the North. Infectious diseases now spread across borders as never before. Natural outbreaks represent unpredictable sources of dangerous pathogens for terrorists. Preparedness to fight efficiently outbreaks of dangerous infectious diseases means nation’s preparedness to fight bioterrorists attacks.
Unprecedented progress in Life sciences is of utmost importance for modern medicine, agriculture, environment for the benefit of the society but it also creates options for the nonintentional and intentional misuse of the results of Life sciences. Artificial reconstruction of Poliovirus, A1H1 “Spanish” flue pandemic virus, mutant bird flu virus transmissible in animals, wide-spread DIY bioengineering are only some examples of Dual-use research of concern.
The ways how to fight possible Biothreats in Ukraine will be discussed.

LIGHT AND DARK SIDES OF THE (PHAGE) FORCE: 100 YEARS OF STUDIES ON BACTERIOPHAGES

Grzegorz Wegrzyn
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The scientist who introduced the name “bacteriophage” in 1917 was Felix d’Herelle, who investigated these bacteria-infecting viruses for many years, and opened new fields of research, including bacteriophage therapy. In fact, it is assumed that bacteriophages have been described for the first time in 1915 by Frederick Twort, however, this researcher did not recognize them as viruses. During next years, bacteriophages became important model organisms in genetics. Many basic discoveries have been made in studies on these viruses, with as spectacular examples as demonstrations that: DNA is a genetic material, viruses can encode enzymes, gene expression proceeds through mRNA molecules, genetic code is based on nucleotide triplets, gene expression can be regulated by transcription antitermination, specific genes encode heat shock proteins, there are specific mechanisms of the regulation of DNA replication initiation based on formation and rearrangements of protein-DNA complexes. Regulatory processes occurring in bacteriophage-infected cells have been considered as paradigms of the control of developmental pathways. On the other hand, the history of research on bacteriophages also passed through dark times, when at the end of 20th century there was a common impression that we knew almost everything about these simple viruses, and it was a time to investigate only more complex organisms. Nevertheless, subsequent discoveries indicated that such assumption was definitely false, and now the studies on molecular genetics and biotechnology of bacteriophages are extensive again. There are also light and dark sides of the (phage) force: from phage therapy to phage-encoded toxins.

ENTEROBACTERIAL OMPC PROTEIN RECOGNIZED BY PROTECTIVE UMBILICAL CORD ANTIBODIES AS A POTENTIAL ANTIGEN FOR CONJUGATE VACCINE

Andrzej Gamian[1], Piotr Naporowski[1], Anna Jarząb[1], Danuta Witkowska[1], Aleksandra Lewandowicz-Uszyńska[2]
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The OmpC is a major enterobacterial protein recognized by human immune system playing a protective role against enterobacterial infections. The reactivity of this protein from *Shigella flexneri* 3a with sera in ELISA correlated with significantly low total IgA and IgG levels in immunoglobulin-deficient children,[1] indicating the OmpC may serve as an immunodiagnostic marker. The epitope was identified as a peptide recognized by antibodies.[2]This protein is recognized by umbilical cord serum, what might indicate for its use as protective antigen and a safe carrier in conjugate vaccines. The aim of this work was to evaluate diagnostic potential of OmpC and its epitope as well as a candidate for vaccine. Sera of patients, bacterial strain and cultivation, isolation and purification of bacterial OmpC protein, enzyme-linked immunosorbent assay (ELISA) and synthesis of peptides as well as statistical analyses have been performed as previously.[1,2] The OmpC protein was isolated from cell mass of *S. flexnerii* 3a strain and analyzed with SDS-PAGE/immunoblotting assay to check its purity and immunoreactivity with human umbilical cord serum samples. The epitope peptide synthesized on solid phase was also recognized by umbilical cord serum. In order to evaluate reactivity of epitope peptide with samples of sera, the peptide has been conjugated to tetanus toxoid or bovine serum albumin as carriers. The OmpC and peptide conjugates were used in ELISA. The level of IgA and IgG antibody was determined in sera of child patients with IgA and/or IgG immunoglobulin deficiencies, as well as in children with recurrent respiratory tract inflammation, where the level was at the healthy controls. In conclusion, enterobacterial OmpC protein recognized by human umbilical cord antibody may be considered as a biomarker of humoral immunodeficiency in children and also as a potential antigen for conjugate vaccine.

[1] Witkowska D., Masłowska E., Staniszewska M., Szostko B., Jankowski A., Gamian A. Enterobacterial 38-kDa outer membrane protein is an age-dependent molecular marker of innate immunity and immunoglobulin deficiency as results from its reactivity with IgG and IgA antibody. FEMS Immunol. Med. Microbiol. 2006, 48, 205-214.
[2] Jarząb A, Witkowska D, Ziomek E, Dąbrowska A, Szewczuk Z, Gamian A. *Shigella flexneri* 3a outer membrane protein C epitope is recognized by human umbilical cord sera and associated with protective activity. PLoS One. 2013 Aug 5;8(8):e70539. doi: 10.1371/journal.pone.0070539.

THE ROLE OF AUTHOPHAGY IN DEGRADATION OF THE CYTOSOLIC ENZYMES OF METHANOL METABOLISM IN THE YEAST *KOMAGATAELLA PHAFFII (PICHIA PASTORIS)*

Nina V. Bulbotka[1], Kateryna O. Levkiv[1], Olena V. Dmytruk[1], Andriy A. Sibirny[1,2]
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Methanol metabolism in the methylotrophic yeast *Komagataella phaffii (Pichia pastoris)* occurs both in peroxisomes (alcohol oxidase, catalase, dihydroxyacetone synthase) and cytosol (formaldehyde and formate dehydrogenase, S-formylglutathione hydrolase, fructose-1,6-bisphosphatase). The shift of methanol-grown cells to glucose initiates inactivation of both peroxisomal and cytosolic enzymes. The mechanisms of inactivation of cytosolic enzymes involved in methanol metabolism are not known. Inactivation and degradation start from glucose sensing. Specific glucose sensor has been identified in *K. phaffii*, however, its functional groups responsible for glucose recognition have not been identified. The aim of this work was to study role of autophagy in inactivation of cytosolic enzymes of methanol metabolism in *P. pastoris*: formaldehyde and formate dehydrogenase and fructose-1,6-bisphosphatase. We also identified the sequences of glucose sensor Gss1 required for glucose-induced inactivation of these enzymes. It was found that during inactivation of fructose-1,6-bisphosphatase caused by shift of methanol-grown cells in glucose medium, degradation of the corresponding protein occurred. The proteasomal inhibitor MG-132 only slightly inhibited this inactivation. At the same time, degradation of fructose-1,6-bisphosphatase was defective in the mutants of *K. phaffii pep4 prb1* defective in vacuolar proteinases and *ccz1Δ, mon1Δ* or *ypt7Δ* whichproducts act in complex at the late stages of vacuole–endosome fusion. This suggests the role of vacuoles in degradation of cytosolic enzyme of methanol metabolism. Degradation of fructose-1,6-bisphosphatase was also strongly retarded in *gss1Δ* mutant defective in glucose sensor suggesting the role of glucose signaling in this process. Thus, specific autophagic degradation is involved in degradation of both fructose-1,6-bisphosphatase and alcohol oxidase. Glucose sensing and signaling is important for initiating this process. We suggest that other cytosolic enzymes of methanol metabolism (formaldehyde and formate dehydrogenase, S-formylglutathione hydrolase) are also inactivated using autophagy pathway.

Session 1 Metabolism and Regulation

Chairs, O. Tehlivets, Austria, R. Daugelavicius, Lithuania

HANSENULA (OGATAEA) POLYMORPHA HEXOSE TRANSPORTER Gcr1 IS ANON-CONVENTIONAL GLUCOSE RECEPTOR

Olena Stasyk[1, 2], Oleh Stasyk[2]
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Gcr1 hexose transporter-like protein from the methylotrophic yeast *Hansenula* (*Ogataea*) *polymorpha* was identified as a regulator of glucose catabolite repression and glucose transport (Stasyk et al., 2004). However, the functionality of *Hp*Gcr1 as glucose transporter or transceptor has not been experimentally established. Using *Saccharomyces cerevisiae hxt null* strain deleted in all hexose transporters, we observed that heterologous expression of *Hp*Gcr1was unable to restore glucose uptake. Similarly to *S. cerevisiae* non-transporting sensors Snf3 and Rgt2, substitution of the conserved arginine residue *Hp*Gcr1^{R165K} converted the protein into an aberrantly signaling form. Production of *Hp*Gcr1^{R165K} in *gcr1*Δ strongly impaired growth on various carbon sources and did not restore either glucose transport or repression in *gcr1*Δ mutant. On the other hand, *Hp*Gcr1 appeared to be essential for normal growth on methanol and induction of peroxisomal alcohol oxidase. Overexpression of HpHxt1, a functional *H. plymorpha* glucose transporter (Stasyk et al., 2008), in *gcr1*Δ mutant partially elevated growth on glucose, restored glucose repression but did not rescue impaired growth on methanol. Thus, HpGcr1 has apparent sensing function in the absence of glucose as an effector carbon source. Simultaneously, *Hp*Gcr1 overexpression led to increased sensititvity to extracellular toxic glucose analogue 2-deoxyglucose, thus supporting a functional glucose carrier status of HpGcr1.
The *Hp*Gcr1 orthologue is absent in the genomes of most yeasts, but a few closely phylogenetically related to *H. polymorpha* species, such as *Kuraishia capsulata.* Other closest homologues of *Hp*Gcr1 from the current database are fungal high affinity glucose transporters, e.g. high-affinity glucose H^+- symporter MstA from *Aspergillus niger*, indicating that *Hp*Gcr1 originates from either differential ancient gene retention or horizontal gene transfer from Eurotiales fungi.
Thus, the *Hp*Gcr1may represent a previously unknown type of yeast glucose-transporting transceptor, which function in *H. polymorpha* apparently involves both glucose transport and downstream signaling via yet unknown mechanism.

Stasyk O.V. et al. J. Biol. Chem. 2004, 279(9):8116-8125.
Stasyk O.G. et al. Eukar. Cell. 2008, 7 (4):735-746.

POSSIBILITIES TO CONTROL OF EFFLUX ACTIVITY IN MICROORGANISMS

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Efflux pumps are systems devoted to the extrusion of noxious compounds from prokaryotic and eukaryotic cells. The expression of these pumps can raise cell resistance by several orders of magnitude, rendering some antibiotics and cytostatic compounds clinically useless. Efflux systems play a particularly important role in multidrug resistance (MDR) and is a challenging direction of research in the fields of antimicrobials and chemotherapy.

In addition to low outer membrane permeability and high activity of periplasmic β-lactamases, efflux is one of the major causes for antibiotic resistance in opportunistic pathogens such as *Salmonella enterica, Escherichia coli* or *Pseudomonas aeruginosa.* In Gram-negative bacteria clinically the most important efflux pumps depend to Resistance-nodulation-division (RND) family. Prevention of the efflux of administered antibiotics using the pump inhibitors could increase efficiency of the treatment. Phenylalanyl-arginyl-β-naphthylamide (PAβN) or 1-(1-naphthylmethyl)-piperazine (NMP) are universal inhibitors of the RND family pumps. Activity of the pumps, efficiency of the inhibitors and permeability of the bacterial outer membrane can be monitored in real time using potentiometry and selective electrodes. Monitoring of tetraphenylphosphonium (TPP^+) or ethidium accumulation in bacterial cells in the absence and in the presence of the pump inhibitors allows to evaluate the total activity of efflux. Studies using PAβN and NMP-selective electrodes revealed that gram-negative bacteria bind high amount of PAβN because of 1) the affinity of this inhibitor to lipopolysaccharides (LPS) and 2) the membrane voltage-dependent accumulation this compound in bacterial cytosol. Our results suggest that the high affinity of PAβN to bacterial LPS is an important factor strengthening the action of this inhibitor.

A special intriguing feature of the efflux pumps is a lack of specificity. In experiments with the cancer cell lines we discovered that cultivation of cells in the presence of TPP^+ leads to the activation of anticancer drug doxorubicin efflux. Due to demethylation of the promotor region and amplification of the genes, in media with TPP^+ expression of ABCB1 efflux pump dramatically increases. Cultivation of *P. aeruginosa* in the presence of TPP^+ or ethidium also leads to activation of the efflux. In addition, an increased efficiency of biofilm formation was observed. Analogous experiment we performed with *Candida albicans* yeast cells.

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REGULATORY TARGETS OF A ZINC BINDING GNTR-LIKE PROTEIN OF *STREPTOMYCES COELICOLOR* A3(2)

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Bacteria from the genus *Streptomyces*, potent producers of antibiotics and other important natural compounds, undergo a developmental cycle including mycelium differentiation associated with a shift from primary to secondary metabolism. The processes are controlled by a complex regulatory network involving hundreds of regulatory proteins. GntR-like proteins form a broad superfamily of regulators which respond to environmental factors such as nutrient availability and whose activity is modulated by binding ligands. They include a number of pleiotropic regulators, which may act both as repressors and activators. They have similar N-terminal winged helix-turn-helix DNA binding domains and various C-terminal effector binding - oligomerization domains.
We describe here a protein from a model organism *Streptomyces coelicolor*A3(2) which belongs to FadR family of GntR-like regulators. The DNA sequence recognized by the protein in its native promoter region was identified by DNA footprinting. Binding of six DNA fragments containing similar motifs located in other promoter regions including a putative zinc metalloprotease and dihydropicolinate synthase was confirmed by the electrophoretic mobility shift assay. We found that DNA binding of the recombinant protein is prevented by the addition of divalent metal ions. Results of the tryptophan fluorescence assay show the strongest effect of zinc and suggest, that it is the metal preferentially recognized by the regulator. Comparison of luciferase reporter genes expression in wild type and deletion mutant strains revealed, that the GntR-like protein acts as a repressor.

THE HIGHLY PROCCESIVE TOPOISOMERASE I – WHY DO THE ACTINOBACTERIA NEED ONE?

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Topoisomerases are the enzymes that maintain chromosome supercoiling and therefore are required during all the processes that involve the changes of chromosome topology. The topoisomerases are divided into two subclasses depending on their structure, activity and function. Topoisomerases of type I are the enzymes that remove the excess of negative supercoils in the reaction that does not require ATP. The Actinobacteria, the class of bacteria that encompasses *Streptomyces* and *Mycobacteria*, encode topoisomerase I (TopA) that includes unusually long C-terminal domain with unique structure. In our model organism *S. coelicolor* TopA (similarly as in *M. tuberculosis*) is the only topoisomerase of type I and therefore it is essential. The single molecule approach that we used to study the TopA activity revealed unusually high processivity of the enzyme (1).
We addressed the question if the C-terminal domain of actinobacterial TopA homologues contributes to their high processivity. We explored the activity of the modified protein and biological consequences of TopA diminished processivity. On the basis of our results we propose the model that explains the mechanism of action of actinobacterial TopA homologues. We also suggest the explanation why the highly processive TopA is required during actinobacterial cell cycle. We believe that understanding of actinobacterial TopA function may be beneficial for optimization of industrial *Streptomyces* cultures or for its use as the antituberculosis drug target.

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Session 1 Metabolism and Regulation, continued

Chairs, O. Stasyk, Ukraine, D. Jakimowicz, Poland

HOMOCYSTEINE DEREGULATES FATTY ACID SYNTHESIS VIA DIFFERENT MECHANISMS IN YEAST

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Hyperhomocysteinemia (HHcy) is a common pathological condition characterized by elevated levels of homocysteine (Hcy) in the blood and associated with a number of diverse disorders of modern society including cardiovascular and neurological diseases, fatty liver, insulin resistance and cancer. Evidence suggests that *S*-adenosyl-*L*-homocysteine (AdoHcy), rather than Hcy, is the trigger of HHcy-associated disorders. AdoHcy is a strong product inhibitor of *S*-adenosyl-*L*-methionine (AdoMet)-dependent methyltransferases, which are involved in methylation of wide range of targets including nucleic acids, proteins and lipids. Previously we have shown that elevation of AdoHcy levels similarly as supplementation with Hcy is associated with triacylglycerol accumulation in yeast in the absence of lipid precursors, inositol and choline. Here using an alternative bacterial pathway for AdoHcy catabolism we show that AdoHcy is the key trigger involved in the deregulation of lipid metabolism in response to Hcy in yeast. In addition to triacylglycerol accumulation Hcy-challenged wild type yeast exhibit an elevation of total fatty acid levels suggesting an upregulation of fatty acid synthesis. Indeed, Hcy supplementation of wild type yeast results in resistance to cerulenin, a specific inhibitor of fatty acid synthase, as well as sensitivity to Soraphen A, a specific inhibitor of acetyl CoA carboxylate, Moreover, Hcy supplementation of wild type yeast results in alteration of protein levels and/or localization of Acc1-GFP, Elo2-GFP and Elo3-GFP fusion proteins. Our data suggest that the deregulation of fatty acid synthesis in response to Hcy include both AdoHcy-dependent and AdoHcy-independent mechanisms and go beyond AdoHcy-induced deficiency of phospholipid methylation.

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RNAP CRASH TEST: DISSECTING ROLES OF PROMOTER SEQUENCE ELEMENTS IN TRANSCRIPTIONAL INTERFERENCE BETWEEN CONVERGENT PROMOTERS IN *ESCHERICHIA COLI*

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Transcriptional interference (TI) is a phenomenon defined as a suppressive effect of a transcription from one promoter on a transcription from a nearby promoter (Shearwin et al. 2005). It manifests itself at genomic *loci* containing promoters in divergent, tandem, or convergent orientation, which are found in all organisms including humans, bacteria and viruses. Regulatory potential of TI on gene expression is known for decades. This phenomenon frequently occurs at toxin-antitoxin loci or in compact genomes of bacteriophages (Olszewski et al. 2014). Moreover, it is an important factor which can influence the efficiency of synthetic gene assemblies utilized in synthetic biology (Bordoy et al. 2016; Brophy and Vogit, 2016). Despite long-term research the mechanisms underlying regulation through TI and their outcomes are still obscure, limiting utilization of TI as another tool for gene expression regulation in synthetic biology.
The aim of our work was to provide a comprehensive experimental analysis of TI between convergent promoters. We constructed an experimental model allowing for efficient selection of interfering promoters generated by random mutagenesis of discriminator, -10 element, spacer, -35 element, or whole promoter sequence. In total we analyzed 576 clones, out of which we selected 294 promoters for further analysis. For each promoter we estimated its strength, potential for, analyzed sequence using promoter prediction software and calculated promoter energy scores. This detailed analysis revealed unexpected complexity of bacterial promoters and frequent occurrence of bidirectional promoters as well as promoter clusters in generated sequences. Nevertheless, analysis of interfering promoters suggests that mechanism of TI is more complicated than simple TI-promoter strength correlation. Taken together, results of this study provide a comprehensive analysis of TI and compose a data base of empirically tested promoter sequences that can be utilized for improved gene expression regulation.

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LIGHT: AN IMPORTANT ENVIRONMENTAL FACTOR INFLUENCING METABOLIC PROPERTIES AND CHEMICAL SENSITIVITY OF WOOD DECAY FUNGUS *CERRENA UNICOLOR*

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Sunlight is a very important signal for every living cell, serving either as a source of energy or information from the surrounding environment, and it can be considered crucial for successful competition and survival in nature. To sense light, only a few photoreceptor systems have developed during evolution. In fungi, light controls developmental decisions and physiological adaptations as well as the circadian clock.
Transcriptome analysis performed for *Cerrena unicolor*, a wood-degrading basidiomycete, revealed the presence of transcripts coding for photoreceptor proteins: white collar proteins, opsins, phytochromes, and cryptochromes, which suggests that fungal photoreceptors are synthetized constitutively in *C. unicolor* and this microorganism is capable to sense light from blue till far red.In this work the effect of different lightning conditions (D, darkness; W, white; B, blue; R, red; G, green light) on *Cerrena unicolor* metabolism was investigated. The application of BIOLOG FF MicroPlates and Phenotype MicroArrays chemical sensitivity panel (PM21-PM25) allowed to compare the metabolic capabilities of the *C. unicolor* and to assess chemical sensitivity of the fungus in various lightning conditions. *C. unicolor* substrate utilization profiles revealed a great variability, which was reflected by the index of overall metabolic activity (AWCD). Significant differences were also demonstrated in the substrate richness (R) values. The D lightning variant of *C. unicolor* showed the highest catabolic activities, which was indicated by the decomposition of 74/95 of the total number of the tested C-sources. In turn, the W variant appeared to assimilate only 38 substrates. The metabolic preferences of the *C. unicolor* to a particular group of substrates appeared to be strictly correlated with the lightning conditions. Interestingly, D variant of *C. unicolor* was not able to utilize D-glucose, D-xylose, and bromosuccinic acid, which were quite significantly metabolized in other lightning conditions. The chemical sensitivity profile analysis showed diversity between compared *C. unicolor* variants. In general, the fungus is the most sensitive to nitrogen compounds and antibiotics. While, it is more resistant to chelators and some cations, which occur naturally in the environment of *C. unicolor* and could be engaged in wood degradation process. The D variant of *C. unicolor* appeared to have increased capacity to utilize a wide spectrum of chemical agents, in contrast to the W variant, which appeared to be the most sensitive to each tested group of chemicals. Inhibitory effect of cinnamic acid (organic compound), berberine, and benzamidine (cyclic compounds) has also been demonstrated.
In summary, the results present the most comprehensive analysis of metabolic capabilities and chemical sensitivities of *C. unicolor* in different lightning conditions. In context of *C. unicolor* abilities to degrade of wood material and production of industrially relevant compounds of pharmacological and medical importance, better understanding of the effect of lightning conditions on selective substrate utilization can be exploited for improvement of biotechnological processes.

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LACTARIUS FUNGI PROTECTION SYSTEM FROM INSECTS AND ANIMALS EATING AND MICROBIAL DAMAGE

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Milk juice of fungi the *Lactarius* genus contains a complex of substances that effectively protect them from eating insects and animals and damage to microorganisms. The effectiveness of this protection is evidenced by the fact that at a young age these mushrooms are practically not eaten by worms and animals. These substances may be of interest to medicine, since they exhibit high antimicrobial, antifungal and antiproliferative activity.
We have done a number of research works on the chemical composition of fruiting bodies of fungi of the genus *Lactarius* (Antonyuk V.O.,2013; Panchak L.V. et al.2012; Tsivinska M. V. et al., 2015). The subjects of the study were *Lactarius pergamenus, L. quetus, L. volemus* and several others to identify substances that protect them from damage and may be of interest to medicine. Water, methanol and methylene chloride extracts of freshly collected, frozen and dried fungi were studied using gas chromatography methods – mass spectrometry, thin-layer chromatography and chromatography on silica gel columns were studied.
Milk juice of fungi the Lactarius genus is a complex emulsion, dominated by three groups of substances: 1) phthalates, 2) sesquiterverne and 3) higher fatty acids and their derivatives (esters or amides). Function of phthalates and sesquiterpenes are antiphidant and repellent; of fatty acids - stabilization of the emulsion. In the aqueous part of the milk juice, high activity of polyphenol oxidase was found, which also performs a protective function. It has been established that fresh Lactarius quetus fungus juice reduces the viability of transformed mice fibroblasts of the L929 line and causes the death of crustacean (Cyclops) plankton. Since the L. volemus mushroom contains azulene and its derivatives in a much smaller amount, the biological activity of its milk juice in mammalian cells and small crustaceans was less pronounced. From the dried fruit bodies of Lactarius pergamenus, a substance was found that exhibited high activity against yeast-like fungi of the genus Candida, which was used to create an ointment composition for external treatment of foot mycosis. This substance also showed antimicrobial action.
However, most of the substances of milky juice are very unstable and, after fulfilling their biological tasks, are rapidly destroyed. Thus, Nature in the process of evolution created a perfect system of protection of fungi of this genus. The time of maturation of the fruiting bodies lasts only 2-3 weeks, so for Nature there is no need to protect them longer. Therefore high-active substances of the system of protection of mushrooms of genus Lactarius are very labile. These substances may be of interest to medicine, but because of their lability, there are problems in creating effective and stable drugs.

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THE AUTOREGULATION OF THE *greA* GENE EXPRESSION- THE *IN CIS* ROLE OF THE GRAL SMALL RNA

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Transcription is one of the most important processes in the cell. As such, it is controlled at every step by many different factors. Some of them are Gre-factors (GreA and GreB). They exert anti-arrest and anti-pause effects, achieved by stimulating the intrinsic endonuclease activity of the RNA polymerase. Despite this, regulation of gene expression of these factors is still poorly understood.
Our research is focused on autoregulation of the *greA* gene. GreA is expressed from two overlapping promoters and it's known to inhibit its own expression. Furthermore, the *greA* leader region contains a terminator, which stops about 2/3 of transcripts before reaching their full length. This results in creation of small non-coding RNAs, named GraL. What is unique, termination of GraL is imprecise: it gives rise to an array of transcripts of different lengths (1).
A biological role for GraL is still unclear. Early microarray analyses detected only a few changes in gene expression in wild type *E.coli* cellsoverproducing GraL. However, similar experiments in a $ppGpp^0$ strain (a strain lacking ppGpp- an important cellular alarmone) noted that over 100 transcripts were affected, but no definitive regulatory pattern could be seen. There is a possibility that GraL acts both, *in cis* and *in trans*, which would be a unique characteristic for an sRNA.
Here we report data on *greA* autoregulation, with special focus on the role of GraL in this process. Our results suggest that the GraL sequence together with the termination region are crucial for autoregulation to occur, as opposed to the promoter sequence itself which seems to be irrelevant. In addition, we monitored GraL levels under different conditions and in different strains background and it seems that the sRNA GraL level is negatively correlated with the GreA protein level.
There is also a possibility that another, third factor could be involved in this autoregulation. To explore this option, we have constructed a random transposon library and we are currently investigating the effect of the various gene disruptions on the *greA* autoregulation.

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ENZYMATIC AND FUNCTIONAL ANALYSIS OF A NOVEL (p)ppGpp SYNTHETASE USING AN *IN VIVO/IN VITRO* APPROACH

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(p)ppGpp is a small nucleotide alarmone that is involved in cellular stress response regulation in bacteria and also in plants. The hallmark of the (p)ppGpp-mediated response is inhibition of transcription initiating from ribosomal promoters, and thus limitation of the rRNA and tRNA in the cell. In *Escherichia coli*, (p)ppGpp is synthesized by two enzymes: RelA and SpoT. The latter one also possesses a (p)ppGpp hydrolyzing activity. However, many other bacterial strains possess only a single bifunctional enzyme (RSH), along with one or more small alarmone synthetase (SAS) that possess only the synthetase activity. Many of those enzymes are not well characterized and regulation of their activity is still elusive- often purification of those enzymes turned out to be futile (formation of inclusion bodies).
In order to investigate the activity of a putative (p)ppGpp synthetase, an RSH enzyme from *Methylobacterium extorquens* AM1, we have first set to overexpress and purify it to test its activity *in vitro.* After successful purification of the enzyme using a SUMO-tag translational fusion, we tested its activity *in vitro* and comfirmed its activity [(p)ppGpp synthetase]. For efficient alarmone synthesis, the purified RSH enzyme requires cobalt cations - in contrast, all of the known specific alarmone synthetases' requires magnesium for efficient (p)ppGpp synthesis. After biochemical characterization of the RSH enzyme activity, we decided to take an *in vivo* approach utilizing a ribosomal promoter *rrnB*P1-*lacZ* fusion in different strain backgrounds (wt, ΔrelA, ΔrelA ΔspoT), for fast scanning of a random mutant library. We are able to perform scans of the libraries for constructs that lead to upregulation [↓(p)ppGpp] or downregulation [↑(p)ppGpp] of the *rrnB*P1 promoter. By using this fast and reliable phenotype test it is possible to scan a large amount of random mutations leading do inactivation of our protein. Such obtained clones are then further exploited to elucidate their biological properties in *in vivo* and *in vivo* tests. In addition, we have discovered that this RSH enzyme also synthesizes another nucleotide derivative, whose identity we are currently trying to establish.

Session 2 Immunology

Chairs, S. Komisarenko, Ukraine, M. Czerwinski, Poland

ANTIGENS OF HUMAN BLOOD GROUP SYSTEM P1PK AS RECEPTORS FOR SHIGA TOXINS

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Shiga toxin-producing *Escherichia coli* (STEC) strains, similarly to *Shigella dysenteriae* serotype 1, pose a growing threat to public health. Shiga toxins: Stx1 (VT1) and Stx2 (VT2), may cause hemorrhagic colitis and hemolytic uremic syndrome, often leading to death. The toxins, after being released in the digestive tract by enterohemorrhagic strains of *E. coli* (EHEC), cross the intestinal epithelium by a yet unknown mechanism and bind to endothelial cells, which allows internalization and cytotoxicity. Shiga toxins and their variants consist of five *B subunits* that bind to the receptor and one A subunit with RNA N-glycosidase activity, which depurinates 28S ribosomal RNA resulting in translation arrest and cell death.
The main receptor for Shiga toxins is globotriaosylceramide, also called Gb3/CD77 or P^k (Galα1→4Galβ1→4Glc-Cer), a member of the human P1PK blood group system, which also consists of antigen P1 (Galα1→4Galβ1→4GlcNAcβ1→3Galβ1→4Glc-Cer) and rare NOR antigen (Galα1→4GalNAcβ1→3Galα1→4Galβ1→4Glc-Cer). The P^k antigen is expressed on human RBCs (except those of p phenotype), whereas P1 is present only in a fraction with ethnicity-specific frequency, thus underlying two common blood group phenotypes: P_1, if the P1 antigen is present, and P_2, if the P1 antigen is absent [Kaczmarek et al, 2014]. In addition to erythrocytes, P^k antigen is expressed on smooth muscle cells and endothelium of digestive, nervous and urinary systems.
It was shown before that P1 synthesis depends on two SNPs found downstream of Exon 1, which give rise to P^1 and P^2 alleles [Kaczmarek et al, 2014]. We found that Shiga toxin binds to P^k and P1 antigens, but not to NOR antigen. To evaluate the relationship between described SNPs and the capacity of human erythrocytes to bind Shiga toxins, we determined SNPs (rs8138197, rs5753148) in 40 individuals of known phenotypes (P_1, P_2, P_1NOR) and compared them with the number of Shiga toxins *B subunits* [Kato et al, 2015] bound to erythrocytes of different genotypes. We found that binding of Shiga toxins strongly depends on the genotype: in most cases, only P^1/P^1erythrocytes were bound by Shiga toxins, while P^1/P^2 and P^2/P^2 erythrocytes showed no binding. TLC analysis of erythrocyte-derived glycosphingolipids revealed that Shiga toxin binds to P^k and P1, but not to NOR. In summary, our results suggest that sensitivity to Shiga toxins may depend on P_1/P_2 status, making it a potential novel predictor of infection severity.

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APPLICATION OF THE MAGNETIC MICROSPHERES FOR DETERMINATION AND PURIFICATION THE ANTIBODIES POSSESSING A DIAGNOSTIC AND PROGNOSTIC VALUE

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Blood serum has been extensively explored as a source of bio-markers, as it may contain not only blood proteins *per se,* but also proteins originating from all tissues of the body. It was estimated that up to 10,000 different proteins (and/or their fragments) might be present in blood serum, and most of them are there in very low concentrations. A selection of protein preparation, and especially enrichment procedures, might aid in a successful search for the bio-markers. Systemic lupus erythematosus (SLE) is a heterogeneous, inflammatory and multisystem autoimmune disease at which antinuclear antibodies are present in blood, often years before clinical symptoms appear. SLE is a systemic autoimmune disease associated with production of the different autoantibodies. Some of these antibodies were shown to be diagnostically important. Anti-nucleosome or anti-chromatin antibodies (ANAs) play a key role among them since circulating ANAs are found in ≈90% of patients with SLE. Isolation of these antibodies is thus of crucial importance to confirm the diagnosis and prognosis of patients with some autoimmune diseases. Isolation can be performed advantageously using magnetic microspheres that offer easy and quick manipulations with a magnet and avoid sample dilution. We developed calf thymus histone-conjugated magnetic poly(2-oxoethyl methacrylate) (POEMA-His) microspheres using a multiple-stage swelling technique followed by histone immobilization.They were successfully used to identify the anti-histone immunoglobulins (IgGs) from blood serum of systemic lupus erythematosus patients [1].

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ANTIOXIDANTS IN CANCER TREATMENT AND IMMUNE RESPONSE

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The results of meta-analyses using 53 randomized trials on 241,883 human participants aged from 18 to 103 y.o. with 44.6% women were un-expected. It was revealed that Beta-carotene in a dose above 9.6 mg significantly increased mortality, Vitamin A in a dose above the RDA (Recommended daily allowances ттт> 800 μg) did not significantly influence mortality, and Vitamin E in a dose above the RDA (> 15 mg) significantly increased mortality [1]. Doses below the RDAs did not affect mortality, but data were sparse. The induction of ROS (reactive oxygen species) by the anticancer drugs differs significantly in type of ROS and time of their elevation. Inactivation of drug-induced ROS by the antioxidants depends significantly on type of inducer and type of the antioxidant. There is a balance of free radicals that immune macrophages use to work. Macrophages release their own free radical, nitrous oxide, to destroy bacteria, parasites, viruses, and other pathological (also tumor) cells. The Vitamin C can be involved in boosting the immune system: it increases the production of white blood cells and antibodies. It also increases interferon that coats cell surfaces, preventing the entry of viruses. Vitamin E boosts the immune system by increasing the production of natural killer cells - important for destroying germs and cancer cells. It also increases B cells responsible for making antibodies to fight specific germs. Beta-carotene increases production of natural killer cells and helper T-cells. The immune cells use free radicals in their functions, and the antioxidants preserve an adequate function of the immune cells against homeostatic disturbances caused by the oxidative stress. Antioxidants preserve an adequate function of the immune cells against homeostatic disturbances caused by the oxidative stress [2].Paradox exists in “Devil’s action” of the antioxidants that can decrease the effectiveness of the anticancer drugs, simultaneously increasing a resistance of tumor cells to the oxidative stress, and their “Angels’ action” via increasing the effectiveness of functioning of the immune system.
Here we present the data demonstrating a lack of positive effect of the antioxidants in treatment of tumor cells *in vitro*, while some of the antioxidants were effective in tumor treatment *in vivo* [3]. We suggest an indirect action of these antioxidants probably via modulation of homeostasis of the immune cells.

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CORRUPTION OF NEUTROPHIL EXTRACELLULAR TRAPS BY PATHOGENS

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Neutrophil extracellular traps (NETs) are networks composed of chromatin and neutrophil granule proteins with high bactericidal potential. A variety of different proinflammatory stimuli, including cytokines, pathogens, and some virulence factors, have been shown to activate the formation of NETs. NETs are thought to neutralize pathogens and create a barrier preventing the spread of bacteria, therefore are classified as an antimicrobial strategy of innate immunity. In this presentation, the diversity of mechanisms used by pathogens to elude neutrophil extracellular traps will be summarized. Moreover, I will present our recent data showing a double prong strategy of exploiting NETosis by *Porphyromonas gingivalis,* the main human periodontopathogen. Like many other bacteria, *P. gingivalis* is the potent inducer of traps formation. In the same time, however, it avoids the entrapment in NETs and subsequent killing. This may explain a paradox that despite the massive accumulation of neutrophils and NETs formation in periodontal pockets, periodontal pathogens thrive in this environment.

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EVALUATION OF NITRATIVE STRESS IN RATS` LEUKOCYTES UNDER EXPERIMENTAL DIABETES MELLITUS

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Type 1 diabetes mellitus is a chronic autoimmune disease caused by inappropriate immune responses to host antigens trigger an autoimmune attack against the pancreatic β-cell, leaving neighbouring islet α- and δ-cells intact. Activated by hyperglycemia immunocompetent cells produce a large amounts of pro-inflammatory cytokines and reactive oxygen and nitrogen species (ROS and RNS). Excessive amounts of $ONOO^-$ caused modification of proteins, lipids and nucleic acids, mitochondrial dysfunction, endoplasmic reticulum stress and apoptosis of all cell types. In particular, NO and $ONOO^-$ in combination with inflammatory responses can provoke β-cell dysfunction and death through JNK signaling pathway activation. The aim of the work was to investigate the state of the L-arginine / NO system and the level of 3`-nitrotyrosine modified proteins in peripheral blood leukocytes of rats under experimental diabetes mellitus, with and without cells pre-incubation with chemotactic factor fMLP for 30 seconds, 1 and 5 minutes.
It was shown the increasing of NO-synthase activity at 5.7 times in leukocytes under diabetes. We established the disarrangement of leukocytes activation after fMLP stimulation, because the reaction turns out by the figure increasing in 11 times only at 5 min compared to control under studied pathology. Imbalance of L-arginine / NO system was manifested also in the fact that trends of NO-synthase activity changes didn't alterated similar to the content of the substrate of reaction under diabetes mellitus. In particular, within 30 sec and 1 min stimulation the content of L-arginine was higher at 1.9 and 2.1 times, respectively, compared to diabetes. However, after 5 min of incubation the index was higher than the diabetic values only at 1.3 times.
The level of nitrogen oxide stable metabolites was studied. It was shown the reducing of nitrites and nitrates levels in blood leukocytes of rats with diabetes compared to control. Under leukocytes pre-incubation with bacterial tripeptide the level of nitrite was not changed compared to control, whereas the level of nitrate increased after 1 and 5 min of stimulation.
We investigated the level of 3-nitrotyrosine modified proteins and found the greatest increase of the index in leukocytes of diabetic animals after 1 min and the decrease after 5 min of stimulation. In leukocytes lysates the presence of a dominant protein with a molecular weight of about 40 kDa that undergoes nitration was observed. So the next step of the work was to separate this protein using affinity chromatography and to identificate using mass spectrometry. This analysis revealed that the protein in question was gamma-actin under the conditions of control and diabetes. Gamma-actin is present in mammalian nonmuscle cells and its main function is to maintain of the shape of the plasma membrane and mitochondrial membranes, and is essential for the functioning and survival of cells. Actin tyrosine residues nitration caused inhibition of actin polymerization, which is associated with the loss of biological functions of actin and immune cells. The development of diabetes mellitus was accompanied with increased nitrated actin level in immune cells. In the case of fMLP stimulation of leukocytes from diabetic rats elicited no significant changes in the actin nitration. We speculate that this could be explained by a decrease in the number of fMLP receptors or by abberant intracellular signal transduction under hyperglycemia and indicate the pre-activated state of leukocytes.

Session 3 Microbial Biotechnology

Chairs, V. Passoth, Sweden, A. Rapoport, Latvia

$(HIS)_6$-TAGGED *CORYNEBACTERIUM GLUTAMICUM* CREATININE DEIMINASE AS A BIOSENSING ELEMENT FOR CREATININE ASSAY

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Creatinine is one of the end products of nitrogen metabolism of vertebrates and man. It is formed from creatine in catabolic conversion of arginine, glycine, and methionine. Creatinine is excreted in the urine and the level of its content in urine and serum is conditioned mainly by muscle mass and excretory capacity of the kidneys. Therefore, an increase in serum creatinine usually indicates a decrease in functional activity of this tissue. Determination of the content of this metabolite in the blood and urine are used for calculation of glomerular filtration and renal function. Additionally, the content of creatinine in laboratory practice is a biochemical parameter for the diagnosis azotemia and hyperthyroidism.

A lot of methods are known for the determination of creatinine in biological fluids. However, most of them have significant drawbacks, including the need for preliminary processing of the samples and the requirement for expensive equipment, and, in some cases, they are characterized by insufficient selectivity. In addition, some modern techniques (such as HPLC) are used in conjunction with a method based on Jaffe reaction, which increases uncertainty in the determination of creatinine.

Creatinine deiminase (CDI, EC 3.5.4.21) is an enzyme that catalyzes the hydrolysis of creatinine to N-metyl hydantoin and ammonia. In microorganisms, this enzyme is used for catabolism of creatinine. CDI can serve as a bioanalytical tool for the determination of creatinine in biological fluids, especially, for the construction of creatinine-sensitive biosensor that can be used to control the process of hemodialysis.

In previous studies, we constructed a recombinant strain of *Escherichia coli*, capable of to over-produce *Corynebacterium glutamicum* $(His)_6$-tagged CDI (Gonchar et al., 2014). Also we proposed a simple and effective method to isolate and purify CDI from recombinant *E. coli* cell-free extracts using metal-affinity chromatography on Ni-NTA-agarose and studied the main physico-chemical and catalytic properties of the enzyme.

Electrophoretically homogeneous preparation of CDI with a specific activity of 10 micromol$\cdot min^{-1}\cdot mg^{-1}$ protein has been used to create a new amperometric biosensor based on ammonia-sensitive membrane consisting of hybrid electrodeposited Cu-containing nanoparticles. For CDI immobilization on the surface of the modified electrode, it was used monolayer electrodeposition of 4-amino phenylacetic acid, which after diazotization was condensed with the NTA derivative and bound with $(His)_6$-tagged CDI via Ni^{2+} ions. The constructed biosensor has a high selectivity to creatinine and reveals a wide range of linearity (0.02 - 15 mM), a fast response to the analyte (1 min) and satisfactory storage stability (50% output after 10 days).

Gonchar et al. FEBS J. 2014, 281(Suppl.1):595.

YEAST *CANDIDA FAMATA* AS A PRODUCER OF RIBOFLAVIN AND ITS DERIVATIVES

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Riboflavin (vitamin B_2) is one of the most important vitamins required for human and animals. This vitamin is metabolic precursor of flavin nucleotides, FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide) involved as coenzymes in numerous enzymatic reactions, mostly of oxidative metabolism. Riboflavin is necessary for normal cell function, growth, and energy production. This compound is manufactured for use as additive to animal feed premixes, colorant in beverages, component of vitamin mixtures and medicine. Flavocoenzymes (FMN and FAD) as the active form of riboflavin are used in medicine and also are important as reagents to be used in biochemistry. Aminoriboflavin is riboflavin derivative and biosynthetic precursor of antibiotic roseoflavin revealing a strong suppressive effect on Gram-positive bacteria.

The riboflavin overproducing mutants of the flavinogenic yeast *Candida famata* isolated by conventional selection methods belong to the best flavinogenic organisms known and have until recently been used for industrial riboflavin production. However, industrial strain dep8 is quite unstable rising spontaneous non-flavinogenic revertants. Studying the regulation of riboflavin synthesis and construction of more stable and active riboflavin producers as well as its derivatives are highly desirable.

Improvement of riboflavin synthesis by *C. famata* mutants may be achieved applying metabolic engineering approaches. Development of basic tools for metabolic engineering of *C. famata* including transformation system, selective markers, insertional mutagenesis, reporter system, CRISPR-Cas9 genome editing system and others is described. Developed tools let us to clone structural (*RIB1, RIB2, RIB3, RIB5, RIB6, RIB7*) and regulatory (*SEF1*) genes of riboflavin synthesis as well as genes involved in synthesis of riboflavin precursor GTP (*RPS3, ADE4*). The set of yeast strains based on industrial riboflavin producing strain dep8 and its non-reverting analog AF-4 expressing different combination of mentioned genes was constructed. Resulted strains revealed enhance of riboflavin production. Recombinant strains of *C. famata* able to FMN and FAD production by overexpressing the *FMN1* and *FAD1* genes encoding riboflavin kinase and FAD synthetase were constructed. The heterologous *rosB* gene from actinomycete *Streptomyces davawensis* was expressed on the background of FMN overproducing strain of the yeast *C. famata.* Constructed strains acquired the ability to produce putative aminoriboflavin.

CHROMIUM: BIOLOGICAL IMPORTANCE, TOXICITY AND DETOXIFICATION STRATEGIES IN YEASTS

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Chromium occurs in the environment in two most stable oxidation forms: the hexavalent [Cr(VI)] and trivalent [Cr(III)] ones. These oxidation states have different chemical features and affect organisms in different ways. Cr(III) is an essential trace element associated with carbohydrate, lipid and protein metabolism, and chromium deficiency causes an impaired glucose tolerance. Supplementing of diet with Cr(III) is widely has often been proposed for treating such metabolic disorders as insulin resistance, type 2 diabetes. It has also been used as a muscle development agent. At the same time, chromium is well known for its toxic, carcinogenic, and mutagenic effects on humans and other living organisms; hence it has been classified as a priority pollutant. Cr(III) affects DNA replication, causes mutagenesis, and alters enzyme structure and activity by reacting with the protein carboxyl and thiol groups. Chromate toxicity is linked to the process of Cr(VI) reduction, generation of Cr(V) and reactive oxygen species which may become major factors leading to cellular damage. Yeasts are a suitable model for studying mechanisms of chromium interactions with living cells. Some yeast species are able to grow at high concentrations of Cr compounds, tend to accumulate significant quantities of chromium within cells and can transform Cr compounds into less toxic forms. Extra-cellular reducing substances secreted by the yeast cells were found to play an important role Cr(VI)-detoxification [Ksheminska et al., 2006, 2008] and Cr(VI)-resistant mutants of the yeast *Pichia guilliermondii* have been selected, able to reduce 90% of chromate to Cr(III). As a product of chromate reduction, Cr(III) complexes with some specific components of culture liquid were generated, which were not adsorbed by the cells. The role of some extracellular agents, iron, riboflavin, and glutathione (GSH) in Cr(VI) detoxification in yeast was investigated. Medium supplementation with exogenous riboflavin resulted in a decreased toxicity of Cr(VI). It was found that riboflavin substantially promoted the Cr(VI) reduction. To evaluate the role of GSH in chromate detoxification we used recombinant strains of *Ogataea polymorpha* with overexpressed *GSH2* (coding for the first enzyme of GSH biosynthesis, gamma-glutamyl cysteine synthetase) and *MET4* (the central regulatory gene of sulfur metabolism) genes. High level of GSH in the mutant *O. polymorpha* cells slightly changed the sensitivity/tolerance to chromate, but increased the rate of reduction of Cr(VI) and reduced the amount of chromium accumulated in the cells. Future perspectives for the use of yeast for the management of chromium-contaminated wastes as well as for obtaining chromium-enriched biomass containing Cr(III) bio-complexes with a potential pharmaceutical and nutritional importance have been discussed.

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STREPTOMYCES ANTIBIOTICS OF LANDOMYCIN FAMILY AS NOVEL ANTICANCER DRUGS

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Landomycins (Ls) are angucycline antibiotics produced by *Streptomyces*, that possess antineoplastic potential. All identified natural landomycins share the same aglycon (landomycinone) moiety distinguishes them from other aromatic polyketides, and differ only in the of carbohydrate structure - a linear glycosidic chain containing only di- and trideoxysugars (β-D-olivose and α-L-rhodinose). Ls demonstrate broad activity against many cancer cell lines with a tendency that compounds possessing longer saccharide chains show higher activity. Recently, we found that landomycin E (LE) strongly relies on ROS production and subsequent early activation of effector caspase-7 upstream of mitochondria (Panchuk et al, 2017). This specific mechanism of landomycin action may contribute to their unique feature – circumvention of cancer drug resistance (Korynevska et al, 2007).

The main aim of current study was to dissect structure-functional relationships underlying ROS production by various members of landomycin family, identify *in silico* their potential molecular targets, and verify them *in vitro*, as well as evaluate their therapeutic potential in animal tumor models.

Chemical structure of landomycin core closely resembles menadione – vitamin K_3 known for its pro-oxidant activity and specific targeting of NADPH dehydrogenases of NQO family. *In silico* docking study of NQO1 and NQO2 enzymes revealed that menadione possesses the highest affinity to both these enzymes, effectively binding to their active site. Landomycinone possessed similar properties while LE's connection to both NQO1 and NQO2 was unstable due to short glycosidic tail (3 sugars) which prevented its tight binding. In contrast, aglycon of LA completely filled in active site of NQO1 while its long oligosaccharide chain (6 sugar residues) was tightly fixed in specific extra pocket on enzyme surface. NQO2 lacks this pocket, thus LA's binding to it was weak, as in case of LE. The results of ROS assay demonstrate that LA caused 2-fold higher ROS production compared to LE and landomycinone, while NQO1 inhibitor dicoumarol decreased by 25-30% both LA toxicity and ROS production. Surprisingly, menadione in low, non-toxic concentrations (5 μM) reduced both ROS production and cytotoxicity of LA (but not of LE and landomycinone) more significantly (2-fold) than dicoumarol. These data suggest the inhibition of LA binding to NQO1 by menadione due to its higher affinity to this enzyme. Thus, NQO1 seems to be primary target of LA that is responsible for ROS burst in tumor cells.

Therapeutic activity of LA was studied *in vivo* using murine NK/Ly lymphoma and B16 melanoma. LA caused a complete tumor remission in ~30% of NK/Ly lymphoma-bearing animals, and significantly (>2 fold) lengthened lifespan of animals compared to control. Similar tendencies were observed for B16 melanoma where LA also caused 2-fold increase of lifespan of tumor-bearing animals as well drastic tumor growth inhibition, while doxorubicin possessed much weaker effects. Concluding, these results suggest high therapeutic potential of LA which could be proposed for further introduction in clinical practice.

COMPARISON OF POWER DENSITY OF AN ONE-CHAMBER AND A TWO-CHAMBER MICROBIAL FUEL CELL

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Microbial fuel cell (MFC) is the bioelectrochemical devise that allow to convert organic compounds into electric current because of microorganisms activity. One of the main challenges of MFC technology is finding the most efficient bacterial strain in terms of electricity generation while biooxidation of organic matter. It was determined that current recovery by *D. acetoxidans* is high, with 82%-84% Coulumbic efficiency while acetate oxidation (Logan, 2007).
The aim of work was to compare the power generation by *Desulfuromonas acetoxidans* IMV B-7384 while its application as the anode biocatalyst in the constructed one- and two-chamber MFC.
Bacteria were cultivated in the modified Postgaite C medium under the anaerobic conditions at the temperature T = 25-28 ^{0}Cduring twenty days in the anode chamber of the MFCs. Sodium citrate in concentration 42 mM served as the sole Carbon source. 0.1% $KMnO_4$ and bacterial suspension with 0.200±0.050 g/l initial biomass were used as catholyte and anolyte respectively in both constructions.
Two-chamber MFC contained graphite rods thatserved as the electrodes. Anode total surface area was 130 cm^2. Anode and cathode chamber with volume 0.3 l each were separated by a proton-exchange membrane (PEM) (Millipore, pore size: 0.20 μm, 2.5 cm^2). Application of *D. acetoxidans* IMV B-7384 in the two-chamber MFC caused stabile power generation during twenty days. The highest power density, which equaled 7.44±0.28 mW/m^2, was obtained on the 208th hour of bacteria cultivation. Increase of incubation time caused insignificant decrease of power density. It equaled 5.69±0.14 mW/m^2 on the 480th hour of *D. acetoxidans* IMV B-7384 growth. Other research showed that different construction of two-chamber air-cathode MFC produced maximal power density 14 mW/m^2 at the application of pure culture of *D. acetoxidans* (Bond, 2002).
One-chamber MFC consisted of the anode chamber (50 ml) that was inserted into the cathode chamber (250 ml) and separated by PEM (Millipore, pore size: 0,20 μm, 44 cm^2). Graphite rods have been used as the electrodes. Anode surface area equaled 16 cm^2. Maximal power density has been observed through the period from 200 to 306 hours of investigated bacteria cultivation. Its maximal value was 1620.00±57.07 mW/m^2 on the 250th hour of bacteria growth. Power density of constructed MFC equaled 562.50±65.71 mW/m^2 at the end of investigated time (480th hour).
Hence maximal power density of the one-chamber MFC was higher by 218 times comparing to its value obtained in the two-chamber MFC while bacterial oxidation of sodium citrate (42 mM). Therefore one-chamber MFC is more efficient in terms of electricity generation while application of *D. acetoxidans* IMV B-7384 in comparison with the two-chamber MFC. Since internal resistance of the one-chamber MFC is lower in comparison with the two-chamber MFC, presumably it causes increase of an efficiency of power generation because of bacterial exoelectrogenesis.

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Session 3 Microbial Biotechnology, continued

Chairs, P. Fickers, Belgium, M. Gonchar, Ukraine

EVALUATION OF PROBIOTIC POTENTIAL AND OXALATE DEGRADING ACTIVITY OF EXOPOLYSACCHARIDE PRODUCER LACTOBACILLUS STRAINS ISOLATED FROM TRADITIONAL TURKISH CHEESES

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Oxalic acid is found in dietary sources (such as coffee, tea, and chocolate) or is produced by the intestinal microflora from metabolic precursors, like ascorbic acid. In the human intestine, oxalate may combine with calcium, sodium, magnesium, or potassium to form less soluble salts, which can cause pathological disorders such as hyperoxaluria, urolithiasis, and renal failure in humans. More recent studies have demonstrated the presence of several oxalate-degrading bacteria in human gut and their ability to control oxalate levels by influencing intestinal absorption of dietary oxalate. Lactobacilli are common inhabitants of the human gut and are widely included in fermented dairy products as probiotic adjuncts. In the present study 20 Lactobacillus strains were isolated from the fourteen different traditional home-made Turkish cheese samples and were screened exopolysaccharide (EPS) production, resistance to low pH and tolerance to bile salts. Four strains, which displayed the lowest (*Lactobacillus fermentum* BP5), and the highest (strains *L. rhamnosus MP1*, *L. fermentum* IP5, *L. brevis* YG7) resistance to gastrointestinal conditions, EPS production and resistance to high oxalate concentration, were selected from 20 Lactobacillus strains for oxalate degrading analysis. Different amounts of EPS were produced by different strains: however, EPS levels ranged from 12.74 to 45.70 mg/l in MRS medium and from 8.05 to 54.19 mg/l in 20 mM MRS-ox media. There was no evidence about the EPS production ability of Lactobacillus strains in oxalate containing medium and its correlation with oxalate degradation activity. We hypothesized that EPS play role in oxalate degradation activity and protect strains from toxic effect of oxalate. The high EPS-producing *L. fermentum IP5* and *L. brevis* YG7 strains showed high oxalate degrading activity (29 and 36%), whereas the low EPS-producing strain *L. fermentum* BP5 showed low oxalate degrading activity in 10 (2%) and 20 mM (9%) oxalate concentrations. All strains grew in the presence of 20 mM sodium oxalate illustrating that oxalate at this concentration is not toxic to lactobacillus strain. The interaction between the oxalate degrading ability and the EPS-producing capacity of lactobacilli strains has not been examined until now. On the basis of these results, EPS production could play a protective role in oxalate degrading metabolism.

IMPROVEMENT OF GLYCEROL PRODUCTION DURING ANAEROBIC FERMENTATION BY THE YEAST *SACCHAROMYCES CEREVISIAE*

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Glycerol is widely used in cosmetic, paint, automotive, food, tobacco, pharmaceutical industries. Glycerol can be produced by microbial fermentation from sustainable carbohydrate feedstocks. Yeast *Saccharomyces cerevisiae* could be used for cost-effective glycerol production under anaerobic conditions. In *S. cerevisiae* glycerol synthesis occurs from dihydroxyacetone phosphate by subsequent action of glycerol-3-phosphate dehydrogenase (Gpd1) and glycerol-3-phosphate phosphatase (Gpp2). Synthesized glycerol is exported via channel formed by aquaglyceroporin Fps1, which can be in opened or closed form. In physiological conditions the major part of intracellular dihydroxyacetone phosphate is isomerized to glyceraldehyde-3-phosphate by triose phosphate isomerase (Tpi1) and subsequently converted to pyruvate and eventually to ethanol. Enzyme acetolactate synthase (Ilv2) can convert pyruvate to acetolactate and CO_2 thus decreasing amount of pyruvate available for alcohol dehydrogenase reaction which competes for NADH with Gpd-reaction.
The *S. cerevisiae* strain BY4742 was subjected to the following genetic modifications: homologous recombination was used for partial substitution of *TPI1* gene promoter region with selective marker; multicopy integration module was used for expression of hybrid fused *GPD1-GPP2* ORF under the control of strong promoter; modified gene *FPS1* (with eliminated part encoding amino acid residues 76-230, that form transporter's "cap") and truncated gene *ILV2* were expressed under the control of *ADH1* promoter. The obtained recombinant strains with corresponding modifications possessed reduced Tpi1 activity and/or increased Gpd1 and Gpp2 activities. Strain with combination of increased Gpd1 activity and constantly active form of Fps1 channel had moderate growth defect.All obtained recombinant strains were able to grow in anaerobic conditions in contrast to, for example, *tpi1Δ* strain. Addition of every new modification led to the increase in glycerol accumulation during alcoholic fermentation. The best strain – BY/tpi25/gpd1gpp2f/fps1m/ilv2 – accumulated up to 9 times more glycerol under micro-aerobic conditions and up to 4.7 times more glycerol under anaerobic conditions in comparison to the parental strain BY4742.

ROLE OF PEROXISOMAL AND CYTOSOLIC TRANSKETOLASE AND TRANSALDOLASE IN XYLOSE ALCOHOLIC FERMENTATION IN THE METHYLOTROPHIC THERMOTOLERANT YEAST *OGATAEA (HANSENULA) POLYMORPHA*

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Xylose is the major component of hemicellulosic moiety in lignocellulose, which represents important renewable feedstock that can be converted to ethanol or other chemicals. *Ogataea (Hansenula) polymorpha* is methylotrophic yeast with ability to ferment glucose, cellobiose and xylose and apparently is the most thermotolerant yeast known with maximal growth temperature of 50 °C which is compatible with the process of simultaneous saccharification and fermentation. However, the efficiency of xylose fermentation in the*O. polymorpha* wild-type strains is very low. Using metabolic engineering approaches the advanced ethanol producers from xylose were constructed. Further improvement of high-temperature alcoholic fermentation is however necessary, which depends on identification of bottlenecks in xylose conversion pathway to ethanol in *O. polymorpha*.
The methylotrophic yeast *O. polymorpha* contains both cytosolic transaldolase (gene *TAL1*) and transketolase (gene *TKL1*) and their peroxisomal counterparts (genes *DAS1* and *TAL2*, respectively). We found that peroxisomal transketolase (known also as dihydroxyacetone synthase) and transaldolase in *O. polymorpha* are required for xylose alcoholic fermentation but not for growth on this pentose. Mutants with knock out of *DAS1* and *TAL2* normally grew on xylose though were defective in its conversion to ethanol. The mutant of *O. polymorpha* with knock out of *TAL1* normally grew on glucose and did not grow on xylose; this defect was restored by overexpression of *TAL2*. Conditional mutant *pYNR1-TKL1* that express cytosolic transketolase gene under control of ammonium repressible nitrate reductase promoter *YNR1* did not grow on xylose and poorly grew on glucose media supplemented with ammonium. Overexpression of *DAS1* only partially restored defects of *pYNR1-TKL1* mutant. Separate overexpression or co-overexpression of *DAS1* and *TAL2* in the wild-type strain increased ethanol synthesis from xylose 2-4 times with no effect on glucose alcoholic fermentation. Overexpression of *TKL1* and *TAL1* also elevated ethanol production from xylose. Finally, co-overexpression of *DAS1* and *TAL2* in the best isolated *O. polymorpha* ethanol producer from xylose led to elevated level of accumulated ethanol up to 16.5 g/L at 45 °C or 30-40 times more than is produced by the wild-type strain.

PEROXISOMES ARE INDISPENSABLE FOR XYLOSE ALCOHOLIC FERMENTATION IN THE METHYLOTROPHIC BUT NOT IN THE NON-METHYLOTROPHIC YEASTS

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The role of peroxisomes in metabolism and alcoholic fermentation of xylose has not yet been investigated. Methylotrophic yeast *Ogataea polymorpha* possesses well-developed peroxisomes which could occupy up to 80% of cellular volume during growth on methanol (Klei et al., 2006). The main hypothesis of this research is that xylose metabolism and alcoholic fermentation in the yeast *O. polymorpha* and possibly in other, non-methylotrophic yeasts, depends on ubiquitous eukaryotic organelles, peroxisomes. This suggestion is based on our investigations, which showed that in *O. polymorpha* peroxisomal enzymes of pentose phosphate pathway, transketolase and transaldolase, are indispensable for xylose utilization and alcoholic fermentation (Kurylenko et al., in press) To test our hypothesis, we decided to study the effects of *pex* mutations which block peroxisome biogenesis on xylose and glucose alcoholic fermentation. The peroxin Pex3 has been identified as an integral peroxisomal membrane protein whereas peroxin Pex6 makes up the peroxisomal AAA ATPase complex (AAA-complex) in yeast. We tested alcoholic fermentation of *pex3*∆ and *pex6*∆ mutants in yeast *O. polymorpha* and compared to that of the *pex3Δ* mutants of the non-methylotrophic yeasts *Schffersomyces stipitis* and *Saccharomyces cerevisiae*.
We have found that the growth of *pex3Δ* and *pex6Δ* mutants of *O. polymorpha* on xylose was unimpaired whereas xylose fermentation was totally abolished. However, ethanol production from glucose was close to that in the wild-type strain. Inability of the mutants defective in peroxisome biogenesis to ferment xylose could be a consequence of mislocalization of peroxisomal transketolase and transaldolase into cytosol; alternatively, it could be an intrinsic property of peroxisome requirements for xylose fermentation due to other reasons. To evaluate these possibilities, we decided to isolate peroxisome-deficient mutants in non-methylotrophic xylose-fermenting yeast *S. stipitis* and recombinant xylose-fermenting yeast *S. cerevisiae,* which genomes do not contain genes for presumably peroxisomal transketolase and transaldolase. Mutants with knock out of *PEX3* gene of both species have been isolated. They were unable to grow on oleic acid as a sole carbon source. It was found that *pex3Δ* mutants of *S. stipitis* and *S. cerevisiae* did not differ from the wild-type strain regarding growth and fermentation of glucose and xylose. Thus, in the non-methylotrophic yeasts *S. stipitis* and *S. cerevisiae* peroxisomes are not required for xylose fermentation, in contrast to that of *O. polymorpha*. We suggest that in *O. polymorpha pex3Δ* or *pex6Δ* mutants, these enzymes are mislocalized in cytosol where possibly cannot fulfill their functions, whereas in *S. stipitis* and *S. cerevisiase*, which do not contain peroxisomal transketolase and transaldolase, xylose fermentation does not require any peroxisomal enzymes. It is interesting to note that overexpression of *AOX1* gene coding for the key peroxisomal enzyme alcohol oxidase activated ethanol production from xylose in *O. polymorpha* which further prove significance of peroxisomes for xylose alcoholic fermentation in this species.

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BIODEGRADATION OF PARACETAMOL BY IMMOBILIZED *PSEUDOMONAS MOOREI* KB4 STRAIN

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A wide range of unique emerging pollutants, including pharmaceuticals, are continuously introduced into aquatic environment mainly from hospital and municipal wastewater or manufactures. One of the most frequently identified group of xenobiotics are nonsteroidal anti-inflammatory drugs (NSAIDs) including e.g. ibuprofen, diclofenac, naproxen, ketoprofen or paracetamol, also usually classified as NSAIDs, but lacking the anti-inflammatory component. Even though the detected concentrations of NSAIDs ranging from nanograms per liter in sewage effluents to micrograms per liter in natural waters a variety of potential negative effects for these low levels have been described, e.g. reproductive or DNA damage, accumulation in tissues, oxidative stress, lipid peroxidation and behavioral changes observed in algae, microcrustaceans or fish. Currently, the wastewaters which contain pharmaceuticals are mainly treat with Advanced Oxidation Processes (AOP) including Fenton and photo-Fenton processes, photocatalysis with TiO_2, UV photolysis/H_2O_2 or ozonation/H_2O_2. Despite the high efficiency of chemical treatment methods high operational costs, harsh reaction conditions and frequent formation of secondary metabolites with high or indefinite toxicity often limit their use. Therefore, biodegradation and/or biotransformation of pharmaceuticals by bacterial strains with increased degradation capabilities is considered as a promising environmentally and economically sustainable tool in wastewater treatment. In this study *Pseudomonas moorei* KB4 strain, immobilized on a natural sponge and an activated carbon was used to paracetamol degradation under cometabolic conditions. The immobilization was confirmed by fluorescence (DAPI, SYBR Green/propidium iodide) and scanning electron microscopy. The metabolic activity of immobilized bacteria was evaluated using the method of 2,3,5-triphenyltetrazollium chloride (TTC) to 1,3,5-triphenylformazan (TPF) reduction. Moreover, degradation of paracetamol was also performed by modified KB4 strain (transformed with EGPF plasmid) closed in alginate-chitosan core-shell microcapsules. In both experiments, the concentration of drug was determined with the reversed phase high-performance liquid chromatography.

Session 4 Medical Microbiology

Chairs, A. Gamian, Poland, J. Dziadek, Poland

STUDIES ON THE ANTIBACTERIAL ACTIVITY OF HONEYBEE CHITOSAN AND ITS DERIVATIVES

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Chitosan (Cts) has found a wide application in medicine due to its antibacterial effect. It is used as component of materials for wound dressing, healing of wounds and burns. Detailed investigation of this effect was conducted mostly with Cts from marine crustacean organisms, which are presently used as a principal source for industrial production of Cts. However, some insects (honeybees, locust and silkworms) can be also a perspective alternative source for Cts production on a large scale. It is possible that it differs from Cts of marine organisms, specifically in antibacterial activity, however such information is scarce. The aim of this work was to investigate the antibacterial activity of honeybee Cts and its derivatives towards selected microorganisms with regard to methods of preparation of Cts and its medicinal forms.

Honeybee Cts was prepared from insect corpses purchased from local apiculture farms. Two ways of purification were used: 1) according to the method developed earlier (Lootsik et al., 2016); 2) the same way but excluding chitin decoloration step, thus, producing chitosan-melanin complex. Cts from crab shells was prepared in our laboratory (Lootsik et al. 2015) and it was used for comparison. *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* were used for evaluating the antibacterial activity since they showed high sensitivity to marine Cts (Muzzarelli et al.,1990). The main problem with Cts is poor solubility of this polymer in aqueous media at pH > 7. Thus, traditional methods of testing antibacterial activity based on a diffusion of the agent in agar gel are not effective. After trying several approaches, the following method was considered to be the most suitable for determination of antibacterial effect of Cts specimens. 0.2-1 mg of Cts particles (size <0.2 mm) were suspended in 0.2 ml of Saburo medium, then 5 µl of culture of microorganisms were introduced and mixture was incubated at 37 °C for appropriate time (2-16 hrs, determined experimentally). Thereafter, 5 µl of mixture were sequentially diluted 5, 25, 125 or more fold and 3-5 µl of each dilution were applied onto Saburo agar plate and incubated at 37 °C for 24 hrs. The inhibition of bacteria growth was evaluated by counting the number of colonies in subsequent dilution of control and experimental tubes. It was revealed that honeybee Cts-melanin complex exhibited the most potent antibacterial effect. After 2 hrs of preincubation, no colonies of *C. albicans* or *St. aureus* could be detected in undiluted mixture on Saburo agar. The efficiency of a short time of preincubation supports the idea that binding of the bacterial cells with Cts particles/molecules is principally important in mechanism of antibacterial activity. Cts decolorized by hypochlorite treatment retained about 4% of active chlorine and showed moderate antibacterial effect, while a complete elimination of chlorine abolished this effect. Cts from crab shells displayed a distinct antibacterial effect. Cts-melanin complex retained its antibacterial activity after partial acid hydrolysis and after treatment of its powder or solution in a boiling water bath for 15 min.

In conclusion, honeybee Cts-melanin complex exhibits a distinct antibacterial effect and it is suggested to be a perspective material for local application in treatment of wounds and burns. Studies of antibacterial action of Cts -melanin complex towards a wider panel of bacteria are in progress.

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HupB: A MYCOBACTERIAL NUCLEOID-ASSOCIATED PROTEIN WITH AN INDISPENSABLE EUKARYOTIC-LIKE TAIL

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Bacterial chromosomal DNA must be efficiently compacted (in *E. coli*, ~1000 times) to fit inside the small cell compartment, but it must also be available for the protein machineries involved in various cellular processes, such as DNA replication, chromosome segregation, transcription and translation. We currently know little about the organization of the mycobacterial chromosome and its dynamics during the cell cycle. Among the mycobacterial nucleoid-associated proteins (NAPs) responsible for chromosome organization and dynamics, HupB is one of the most intriguing. It is composed of two domains: an N-terminal domain that resembles bacterial HU; and a long and distinctive C-terminal domain that contains several PAKK/KAAK motifs, which are characteristic of the H1/H5 family of eukaryotic histones. The HupB protein is also known to be crucial for the survival of tubercle bacilli during infection.

We analyze the *in vivo* binding of HupBon the chromosome scale. Using PALM (photoactivated localization microscopy) and ChIP-Seq, we show that the C-terminal domain is indispensable for the association of HupB with the nucleoid. Strikingly, the *in vivo* binding of HupB displays a bias from origin (*oriC*) to terminus (*ter*) of the mycobacterial chromosome (numbers of binding sites decrease towards *ter*). We hypothesize that this binding mode might reflect a role of HupB in organizing newly replicated *oriC* regions. Thus, HupB may be involved in coordinating replication with chromosome segregation.

Given that tuberculosis remains a serious worldwide health problem (10.4 million new TB cases were diagnosed in 2015 according to WHO) and new multidrug-resistant *M. tuberculosis* strains are continually emerging, further studies of the biological function of HupB are needed to determine if this protein could be a prospect for novel antimicrobial drug development.

BACTERIOPHAGES AS A POTENTIAL MEAN FOR PREVENTION AGAINST *SALMONELLA SPP*. CONTAMINATION OF POULTRY

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Salmonellosis is one of most common foodborne diseases. Every year more than 100 million people suffers from infections caused by *Salmonella* rods all around the world, and about 90 thousand cases ends with patient's death. *Salmonella* rods are often present in healthy chicken's gut and the main source of human infection are contaminated chicken meat and eggs. Due to rapid development of farms and industrialization of food production in 20th century, it was made a priority to counteract *Salmonella* outbreaks on poultry farms. To this day antibiotics are used as main mean of prevention against *Salmonella* contamination. However, a rapid emergence of drug-resistant strains is observed in recent years. This raises the need to find an alternative strategy of preventing *Salmonella* outbreaks. One of such strategies may be the use of bacteriophages – viruses that infect bacteria.
In our work we focused on isolating and characterising bacteriophages that infect various *Salmonella* strains, including those responsible for poultry meat contamination. Phages were isolated from wastewater sewerage and chicken stool samples. We have studied phage ability to infect various *Salmonella* strains in different growth conditions of bacterial culture and we have tested the effectivity of phage "cocktails" against given strains .We hope that our research will arouse the interest in phages as a potential mean of prevention agianst *Salmonella* outbreaks.

SPECTRAL STUDY OF INTERACTION BETWEEN MANITOL AND RIBONUCLEOSIDES BY FLUORESCENT PROBE

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Therapeutic drugs which are based on ribonucleic acid are highly effective, non-toxic and with a wide range of biological effects. In particular, RNA-containing drugs can increase immune reactivity, have antiviral and anti-inflammatory activity and can regulate main metabolic ways in various pathological conditions.
Linear absorption spectra were obtained with a Specord 210 Plus UV−visible. The steady-state fluorescence and excitation anisotropy spectra were measured with a Jasco FP-8200.
During the research we have observed the decrease of fluorescence intensity of the dye water solution with ribonucleosides; this point to stack interaction between dye molecule and ribonucleosides base. Increasing of concentration of nucleosides is accompanied by regular decreasing of fluorescence intensity to saturation of nucleosides molecules. The saturated decreasing of fluorescence intensity depends on nucleosides type and hence are different for solutions of A,C, U and G. Maximum effect was obtained for fluorescent sensor solution with riboadenosine.
Additional decrease of intensity was observed in complexes with D – mannitol. The largest difference in fluorescence intensity was observed in riboadenosine and his complex with D-mannitol. The spectral effect may indicate the interaction between oligonucleotsides and mannitol. Basing on quantum-chemical calculations, it is established that the hydrogen bonds between manitol molecule and two centers can be generated. Also, the calculations show appreciable changes in charge distribution in base molecules, especially at that atoms which can generate hydrogen bonds. This can be accompanied by change in stack interaction between sensor and ribonucleoside molecules.
The spectral study of water solutions of mixture of dye probe with ribonuleosides has shown about stack interaction between studying molecules. Introducing of the manitol in investigated solutions causes the additional spectral effect which point to interaction between ribonucleosides and mannitol by generating of two hydrogen bonds.

CHANGE OF SOME GENESEXPRESSION IN MICE LUNG AFTER PREVENTION AND TREATMENT WITH OLIGORIBONUCLEOTIDES-D-MANNITOL COMPLEXES OF INFLUENZA A VIRUS H1N1 (A/FM/1/47) INFECTION

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The influenza (flu) virus infection causes activation of NO synthase II (NOS2), arginase II (Arg2), xanthinoxidase (XO) enzymes involved in the production of free radicals, such as $NO^{\cdot}$, $O_2^{\cdot-}$, $ONOO^{-}$. Free radicals involved in signal transduction pathways, activate transcription factors such as NFκB and may lead to lung tissue damage. Previously, it was studied that the oligoribonucleotides-D-mannitol complexes (ORNs-D-mannitol) possess antiviral activity against the flu A (H1N1) virus *in vitro* and *in vivo*. However, the mechanisms of the ORNs-D-mannitol anti - flu activity are still not clear. Current research was aimed at study of the ORNs-D-mannitol effects on expression of the *nos2, arg2, xdh, nfkbia, nfnb1* genes in mice lung under flu infection. To achieve this goal we applied a two-step RT-PCR assay. In the flu virus-infected mice were detected the overexpression of all investigated genes compared to the healthy ones. The ORNs-D-mannitol injection for prevention reduced the mRNA level of *arg2,nos2, xdh, nfkbia*, *nfkb1* expressionby 56, 48, 32, 70, 63 % respectively vs. the virus-infected mice. And the ORNs-D-mannitol injection for treatment reduced the mRNA level of *arg2,nos2, xdh, nfkbia*, *nfkb1* expressionby 26, 41, 38, 40, 52 % respectively vs. the virus-infected mice.
Our results show thattheexpression of all investigated genes is modulated by the ORNs-D-mannitol after injection for prevention and treatment of the flu A virus infection *in vivo*. It allows us to assume that by modulating the expression of *nos2, arg2, xdh, nfkbia*, *nfkb1* genes, these complexes can inhibit flu virus replication and protect lung from free radical damage under the flu infection.

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IL-18 SECRETION IN RELATION TO IL-18 GENE POLYMORPHISM IN *MYCOBACTERIUM TUBERCULOSIS*-INFECTED AND UNINFECTED INDIVIDUALS

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The interaction between *Mycobacterium tuberculosis* (*M.tb*) and the host at the molecular and cellular levels remains poorly understood. According to the estimates of the WHO, over 1/3 of the human population is infected with *M.tb*. Most of the infections are latent, however in approximately 10% of the cases they can progress into active tuberculosis (TB). The development of active TB is based on immune-driven mechanisms involving multiple cytokines including interleukin-18 (IL-18), acting as an interferon (IFN)-γ-inducing factor.
The aim of the study was to compare the concentrations of IL-18 in cultures of whole blood from *M.tb*-infected and *M.tb*-uninfected individuals and answer the question as to whether the level of produced cytokine could be associated with IL-18 gene polymorphisms (IL18-607/C/A and IL18-137G/C).
The study group comprised of 179 adult BCG-vaccinated Polish volunteers with or without with *M.tb* infection. The *M.tb* infection was evaluated on the basis of IFN-γ production in the QuantiFERON TB Gold In Tube test (IGRA). IL-18 concentrations were assessed immunoenzymatically using DuoSet®ELISA Development Kits (R&D) in plasma samples obtained after 24h stimulation of whole blood with mycobacterial antigens in the IGRA assay. The polymorphisms of IL-18 gene (-607C/A, -137G/C) were determined by allelospecific PCR.
Sixty five out of 179 (36%) individuals were infected with *M.tb* (IGRA(+)). There was no difference in the frequency of IL-18 production in cultures of blood in studied groups. The production of IL-18 was found in 29/65 (45%) cultures of blood from IGRA(+) subjects and 55/114 (48%) plasma samples from IGRA(-) volunteers. The mean level of IL-18 produced in *M.tb*-stimulated blood cultures was higher in IGRA(+) group compared with IGRA(-) individuals. However, statistical analysis did not show any association between IFN-γ production and IL-18 secretion in such cultures. The frequencies of C/C, C/A, and A/A IL-18-607C/A genotypes were 37%, 55% and 8% among IGRA(-) volunteers and 35%, 55% and 10% in IGRA(-) group. Similarly, no difference was noticed in the percentage of IL18-137G/C genotypes. G/G or C/C IL18-137G/C homozygotes were 51% or 4% IGRA(-) subjects and 52% or 11% IGRA(+) individuals. There was no correlation between the IL-18 polymorphism and the level of produced cytokine either in IGRA(-) or in IGRA(+) group.
The results suggest that the level of IL-18 secreted in the response to *M.tb* antigens is not determined by studied IL-18 polymorphisms. Higher IL-18 levels observed in the cultures of blood from IGRA(+) individuals confirm the involvement of the cytokine in the development of TB-driven immune response.

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Session 5 Pharmacology

Chairs, W. Barabasz, Poland, R. Stoika, Ukraine

MASKED MYCOTOXINS

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Toxins from molds (so called fungal toxins) are known as "mycotoxins". Mycotoxins are secondary metabolites of mold fungi, toxic to humans, animals, plants and microorganisms. Molds that produce them as a by-product of metabolic processes or as defense products may have strong toxic effects, mutagenic or teratogenic properties, can arise in a wide range of agricultural products and under a range of environmental conditions. These toxins are insensitive to many technological processes (cooking, frying, baking, distillation, fermentation), so they can survive in products obtained from contaminated raw materials. Mycotoxins can also be found in plant tissues during growth, especially when dealing with damp vegetative periods and plant pests primarily with fungi from genera of *Fusarium*. Plants recognize mycotoxins and then process them with glucosylation (binding to a glucose molecule) or coupling to a sulfur molecule, which effectively neutralizes mycotoxin. Such transformed mycotoxins become substances that are called "masked mycotoxins". Most often masked mycotoxins are toxins produced by *Fusarium* genus. They are compounds that do not harm the plant in which they occur. However, these processes do not protect animals and humans from poisoning. After ingestion of grain feeds containing inactive "masked mycotoxins", glycosidic linkages are digested, resulting in the toxin recovering its activity. It has also been shown that the human gut bacteria in the colon attach to DONG and ZENG and modify them by releasing original forms of these toxins. Masked mycotoxins are not covered by current food safety regulations because of insufficient data on what happens in humans and animals after consumption and are not detectable by conventional methods, liquid chromatography or ELISA.

ANTIMICROBIAL ACTIVITY OF SOME THIOPYRANO[2,3-*D*]THIAZOLES AND THEIR STRUCTURE-RELATED ANALOGUES

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Thiazolidinone-based molecules are attaractive targets in the rational design of «drug-like» compounds which possess wide range of biological activities such anti-inflammatory, antioxidant, antitumor, choleretic, antiviral and other. In addition, 4-thiazolidinones are known as potent inhibitors of PRL-3 and JSP-1 phosphatases, HCV NS3 protease, Ras farnesyl transferase (Lesyk et al., 2004). Interestingly, the prominent interest among thiazolidinone derivatives belong also to their condensed analogues, especially thiopyrano[2,3-*d*]thiazoles and their structure-related derivatives. In addition, it was found that fixation of a highly active 4-thiazolidinone moiety in a structure-related thiopyrano[2,3-d]thiazole core allows to store of this biophore fragment in a rigid fused system without Michael accepting functionalities that are important in the modern drug discovery process. Thus, in our previous studies we reported about thiopyrano[2,3-*d*]thiazoles as potential antitripanosomal, antioxidant, anticancer and anti-inflammatory agents. In addition some structure-related derivatives to thiopyrano[2,3-*d*]thiazoles a considerable attention and interest have drawn to thiazolo[4,5-*b*]pyridines due to their diverse biological activity and clinical applications. Thiazolopyridine derivatives have shown a broad range of interesting biological activities, such as analgesic, antioxidant, anti-inflammatory, anticancer, antifungal and herbicidal properties (Hegde et al., 1993).
In the present work, we described our ongoing research effort in the synthesis of a series of novel thiopyrano[2,3-*d*]thiazoles and their structure-related derivatives and characterization of these compounds for antimicrobial activity. Thus, the synthesized compounds were screened for their *in vitro* antibacterial and antifungal activities using the agar diffusion method against gram-negative and gram-positive bacterial strains: *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli* and *Candida albicans*. The obtained data reveal that some of the tested compounds especially thiopyrano[2,3-*d*]thiazole-5-carboxylic acids, thiazolo[4,5-*b*]pyridine-5-carboxylic acids and their amides possess good activity toward tested microbial strains at a dose of 20 μg per well as compared to Streptomycin and Amphotericin-B as standard drugs. The minimum inhibitory concentrations for the most active compounds were calculated using the microdilution susceptibility method. In summary, we have successfully developed an efficient method for the construction of thiopyrano[2,3-*d*]thiazoles and their structure-related derivatives. The synthesized compounds were assessed for their antimicrobial capacities and preliminary results allowed to identify the most active compounds with effect level comparable to standard drugs.

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5-ENE-THIAZOLIDINONES: MEDICINAL CEMISTRY ASPECTS

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4-Thiazolidinones are recognized as privileged heterocycles in medicinal chemistry. Main achievements are related to the introduction of approved drugs into medical practice (hypoglycemic glitazones and aldoso reductase inhibitor). Among variety of 4-thiazolidinone subtypes and thiazolidinone-bearing compounds 5 substituted thiazolidinones, namely 5-ene derivatives, are of special interest due to their chemical characteristics and pharmacological profiles. Compounds of mentioned sub-type are high-affinity ligands to a number of biological targets, lead-compounds, and drug-candidates with antidiabetic, antimicrobial, antiviral and anticancer activities.

The main directions for 5-ene-thiazolidinones synthesis and modification, mainly within structure-based approach, can be compiled into the next groups: *i*) complication of C5 fragment (following the thesis about crucial impact of the C5 moiety);*ii*) introduction of the substituents in the N3 position (especially fragments with carboxylic/hydroxylic groups);*iii*) synthesis of isosteric heterocycles; *iv*) combination with other pharmacologically attractive fragments withinhybrid pharmacophoreapproach; *v*) annealing in complex heterocyclic systems; *vi*) utilization of 5-ene-thiazolidinones for the synthesis of other heterocycles.

The conjugation of C5 double bound with C4 carbonyl group of main core allows to assign the 5-ene-4-thiazolidinones (especially 5-ene-rhordanines) as Michael acceptors. Two opposite meanings of this functionality are discussed. 5-Ene-4-thiazolidinones are often treated as frequent hitters or pan-assay interference compounds, which are useless in modern medicinal chemistry because of compounds interaction with thiolic groups of proteins, and apparently their low selectivity. Michael acceptors currently are considered as “new old tool” for new drug creation, especially anticancer agents (Michael acceptors are one of the most effective activators of Nrf2, efficient covalent inhibitors of set of approved biotargets; structural similarity to endogenous Michael acceptors leads to modulation of ROS-dependent regulatory pathways, etc).

One of the possible solutions is the fixation of 5-ene-4-thiazolidinone fragment in the fused heterocycles within privileged substructure-based diversity oriented synthesis. The prime examples are thiopyrano[2,3-d]thiazoles obtained from 5-ene-thiazolidinones. Following the study of biological activities, thiopyrano[2,3-d]thiazole based compounds are considered as cyclic mimetics (with the same pharmacological profiles) of pharmacologically active 5-ene-4-thiazolidinones but without Michael acceptor functionality.

Ref. and details: *https://goo.gl/Ak3Z9F*

THIAZOLIDINONE CORE IN THE DESIGN AND DEVELOPMENT OF NEW ANTITRYPANOSOMALS

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Human african trypanosomiasis or sleeping sickness and Chagas disease belong to the serious regional neglected tropical diseases which have now spread to other continents and require new effective and non-toxic drugs. Despite the advances in understanding the biology of protozoa, global intensification of the antitrypanosomal agents creation (e.g. within Special Programme for Research and Training in Tropical Diseases) no new drugs were marketed since eflornithine was approved in 90^{s}. Currently the main directions in the new antitrypanosomal agents' discovery are design of new high affinity ligands and search for new indications of existing drugs. Limitations of highly active ligands application are due to low effectiveness *in vivo* or toxicity. Following the current trends, pentamidine analogs, derivatives of benzofuran and benzoxaborol, thiosemicarbazones and their metal complexes as well as thiazoles are of special interest. 4-Thiazolidinones can be treated as cyclic analogs and biomimetics of thioureas/thiosemicarbazones and are used for the design of new antitrypanosomals mainly within structure-based design and hybrid-pharmacophore approach.
The presented project is an extension of our ongoing efforts towards search for new 4-thiazolidinone-based antiparasitic agents. The design of the project involved: primary screening of antitrypanosomal activity (*T. brucei*, *T. cruzi*) of the diversity in house thiazolidinones library; SAR analysis, design and synthesis of focused sub-libraries within combinatorial and privileged-substructure-based diversity oriented synthesis strategies and molecular hybridization; sub-libraries screening, hits and leads identification; (Q)SAR(P) analysis and formation of the direction for structure optimization; in depths study of the identified hit-compounds mode of action, toxicity evaluation. Additionally the row of thiazolidinone-based compounds with anticancer activity was involved into the study following the new findings about the simultaneous anticancer and antitrypanosomal activities.
Screening results led to the formation of focused 4-thiazolidinone based compounds sub-libraries and identification of hit- and lead-compounds. Project outcomes allow us to summarize some findings: 2-amino(imino)substituted-4-thiazolidinones and 5-ylidene-rhodanine-3-carboxylic acids subtypes are the most prominent; anticancer thiazolidinones are the good sources for antitrypanosomal agents design; thiopyrano[2,3-*d*]thiazole core is perspective scaffold for further optimization. Hybrid-pharmacophore approach allowed identification of the highly active 5-aminomethylene-4-thiazolidinones that inhibited growth of *Trypanosoma brucei ssp.* at sub-micromolar concentrations in the *in vivo* assay. Moreover, this series of compounds possessed low toxicity levels towards human fibroblasts and showed excellent selectivity indices.

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Session 6 Environmental Microbiology

Chairs, N. Kuisiene, Lithuania, J. Truu, Estonia

MOLECULAR IDENTIFICATION OF BACTERIAL AND FUNGAL COMMUNITIES OF GREEN TURTLE (*CHELONIA MYDAS*) NESTS

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Green turtles (*Chelonia mydas*), which are spread in tropical and subtropical waters, are endangered species. Also, green turtles are susceptible to environmental pollution. Bacterial and fungal communities are found in the egg content, eggshell, and sand from sea turtle nests. Therefore, the presence of some communities in sea turtle nests, acts as an indicator of pollution levels in the surrounding area. The objective of this study is to determine the potential bioindicators by isolating the bacterial and fungal communities in nests. A total of 31 different nests were studied during the nesting season of 2014 at Sugözü Beaches where under the pressure of intense human activity; maritime traffic, industry and agriculture. 31 sand samples and 18 failed eggs which contains dead embryo were taken from the nests randomly and stored sterile plastic bags for further analysis. The isolates were classified by using 16S and 18S RNA screening by Real Time PCR. The sequences can be compared to the GenBank database by using the NCBI BLAST serve. The phylogenetic relationships were using the Neighbor-Joining method. Fungal communities were found in the sand and eggshell while bacterial communities were detected in the egg content as well. For the fungal communities *Aspergillus* spp. were dominating the diversity in nests. As for the bacterial communities, *Pseudomonas* spp. and *Bacillus* spp. were the most abundant among the flora. Most of the isolated bacteria and fungi species are known to degrade polycyclic aromatic hydrocarbons or petroleum hydrocarbons and could be potential bioindicator of petroleum pollution in sea turtle nests.

METAGENOMIC APPROACH TO INVESTIGATE THE MICROBIOTA OF DIFFERENT SOIL TYPES

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Soil health is most simply defined as 'the capacity of the soil to function'. It's consists of the chemical, physical, and biological components of a soil and their interactions. Biological component of soil is extremely important in the maintenance of soil health, because this soil dynamic system is mediated by a diversity of living organisms, especially the soil microorganisms. Different soils are assumed to harbour specific microbial communities.
Microbial taxonomic diversity in eight different types of soils were determined by DGGE-PCR and high throughput sequencing (MiseQ, Illumina) in the micro-plot experiment, where the collected soils were under the same agricultural management for over 130 years. This research aimed to discover the differences affected directly by the soil type omitting any other factors. The soil core microbiomes were calculated, for five soils considered as good quality and three considered as acidic, poor quality. Some of genera (Conexibacter, Bacillus, Saccharopolyspora, Cohnella) were present in an almost equal number in all soils, it could be the most widespread bacteria, characteristic for most typical soils.
The research provided the basis for the search of soil marker bacteria and a better understanding of the relationships between soil physiochemical properties and the microorganisms inhabiting them.

The research was partially founded by the Ministry of Science and Higher Education, research task: Development of molecular standards for the rapid identification of dominant bacteria inhabiting different soil environments, using denaturing gradient gel electrophoresis technique (DGGE) /2017.

EVALUATION OF BIOACTIVITY OF MICROORGANISMS ISOLATED FROM KRUBERA-VORONJA CAVE: CLASSICAL *VS*. MOLECULAR APPROACH

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Caves are considered to be an under-exploited environment to screen for novel antimicrobials. But the scientists searching for novel drug producers in caves are faced with several challenges: the culture media composition can affect the yield of antimicrobial compounds; variations in the culture media composition can induce expression of biosynthetic pathways leading to the production of different antimicrobials; the antimicrobials predicted from genome analysis are not produced under standard laboratory conditions (Ghosh et al., 2017). Therefore, the aim of our study was to evaluate bioactivity of microorganisms isolated from Krubera-Voronja cave using both classical and molecular approaches.
It is known that a depth of the caves directly correlates with the production of antimicrobial substances. Krubera-Voronja cave is the deepest known cave in the world. Therefore, the chances to discover novel antimicrobials producing bacteria in this cave were supposed to be high. Unexpectedly, the classical approach revealed a low number of bioactive bacterial strains. Despite this, both volatile and proteinaceous bioactive compounds were detected and identified in these cave microorganisms. In order to analyse the silent potential of bioactivity in the cave microorganisms, the search for polyketide synthase and nonribosomal peptide synthetase gene was performed. These genes were successfully identified in the strains without any identifiable phenotypic bioactivity. Taxonomic position of the tested strains was also determined.

Ghosh S. et al. Biochem. Pharmacol. 2017, *in press*, published online 17 November 2016.

INTEGRATION OF MULTIPLE OMICS DATA FOR ASSESSMENT OF SPECIFIC MICROBIAL COMMUNITY RESPONSE TO ACCIDENTAL RELEASE OF OIL INTO THE MARINE ECOSYSTEM

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The aim of the current study is to determine key bacterial species and metabolic pathways responsible for the degradation of different oil fractions in different compartments (aerobic and anaerobic water and sediments) of the Baltic Sea and the Northern Atlantic. We are assessing biodegradation rates of different oil fractions in seawater and sediments, and relate this data to environmental parameters and dispersant application. In order to maximize output from obtained data sets we apply integrative knowledge discovery from multiple omics sources. For this purpose high-throughput sequencing datasets obtained during current project and relevant public domain data are integrated. Following sub-tasks are performed. (1) Creation of database of functional genes relevant to biochemical pathways of oil degradation in marine environment. This database is utilised to screen and functionally annotate obtained metagenomics datasets. (2) Recovery of individual genomes of oil degrading bacteria from obtained metagenomics datasets using binning of assembled contigs to species-level groups both from single metagenomes and related multiple metagenomes. This approach enables to better understand the role of uncultivated microbial species in oil biodegradation. (3) Creation of metagenomic prediction platform for inferring oil biodegradation activity in marine environment. Information about microbial community taxonomic composition and metabolic markers together with abiotic factors is related to oil biodegradation kinetic parameters and oil remediation strategies using different modelling approaches (structural equation modelling, network analysis, random forest, in silico biodegradation network reconstruction).
The main results of the study are characterization of oil-degrading microbial community structure in geographically different marine environments and impact of dispersant, and environmental parameters on natural oil biodegradation capacity. Based on results of meta-analysis of obtained omics data sets and modeling metagenomics prediction platform for inferring oil biodegradation capacity in marine environment is established.

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INTERACTIONS BETWEEN ENVIRONMENTAL MICROORGANISMS AND NATURAL CARBOHYDRATE SURFACTANTS

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Surfactants are commonly used in households as cosmetics and detergents and in many fields of industry as foaming, emulsifying or adhesive agents. Surface active agents have also found the environ-mental applications, for example in the bioremediation of hydrophobic pollutants and heavy metals from contaminated soil. However, the synthetic surfactants may have negative effect on the environment, the main concerns are related to their low biodegradability and toxicity to environmental organisms. Therefore, a valuable alternative for synthetic surfactants might be the natural ones, which are of bacterial or plant origin. They are described to be non-toxic, biodegradable and biocompatible. Moreover, they can potentially be as effective as synthetic surfactants (Mulligan et al. 2001; Urum et al. 2004). Due to the fact, that in the near future, natural surfactants may replace their synthetic equivalents, the questions arise: What is their impact on environmental microorganisms? Are they toxic to bacterial cells?
The aim of the present study was the assessment the cytotoxicity of surfactants on three bacterial strains isolated from an activated sludge: *Pseudomonas putida*, *Rahnella aquatilis* and *Raoutella planticola*. The surfactants used in the experiments include: saponins – plant surfactants isolated from *Saponaria officinalis* L., rhamnolipids – surface active agents of bacterial origin and Triton X-100 that isa nonionic, synthetic compound. The scope of research comprises spectrophotometric tests: MTT assay that assess the metabolic activity of bacterial cells and ONPG assay which was performed for detection of β-galactosidase activity that is an indirect measurement of inner membrane permeability.
The results of the experiments indicate that Triton X-100 has shown the highest cytotoxicity towards selected bacterial strains. The proliferation of *Rahnella aquatilis* cells was equal to 116%, 46% and 10% in cultures with saponins, rhamnolipids and Triton X-100, respectively. The proliferation was calculated in the relation to the control sample that did not contain any surfactant. Moreover, for two of the three strains, the cytotoxicity of surfactants was increasing with the increase of their concentration. Considering the results obtained for ONPG assay, among tested strains, the cells of *Rahnella aquatilis* were characterized by the lowest inner membrane permeability which was equal to 0.3 – 0.6 μM ONPG/min. However, the highest inner membrane permeability (1.75 μM ONPG/min) was noticed for *Pseudomonas putida* cells in culture with Triton X-100. The correlation between inner membrane permeability and the cells proliferation was equivocal and depended on the bacterial species. In general, it should be noticed that bacterial cells were more sensitive when exposed to synthetic surfactant than to natural ones.

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Plenary Lectures 2

Chairs, A. Sibirny, Ukraine, G. Wegrzyn, Poland

COLLABORATION BETWEEN POLISH AND UKRAINIAN BIOCHEMISTS HAVE BEGAN IN THIRTIES OF THE LAST CENTURY IN PARNAS LABORATORY

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The city of Lviv in the thirties of the last century was beautiful, but it was not a city of peace. Ukrainians fought for independent Ukraine and believed that Lviv should be a Ukrainian city, the Jews fought a growing wave of anti-Semitism, and the Poles sought to maintain the status quo. In this city lived and worked outstanding scholar biochemist Jakub Karol Parnas, head of the Department of Physiological Chemistry at the University of John Casimir. His contribution in resolving of glucose metabolism was appreciated, glycolysis was known as Emden, Meyerh, Parnas pathway. Parnas was a man devoid of prejudice. In his laboratory, Poles, Jews and Ukrainians worked in a friendly atmosphere. It is quite symbolic that the authors of one of the greatest discoveries in Parnas' lab (discovery of PFK) were three young scholars: Ostern-Jew, Guthke-Polish and Terszakovec-Ukrainian. Good time in Parnas' lab ended with the outbreak of World War II. In September 1939 the Red Army entered Lviv, which was incorporated into the Ukrainian Republic of Soviet Union. Jan Kazimierz University was renamed Ivan Franko University, but Parnas and his staff retained their positions. Nevertheless the deficiencies in the supply of reagents virtually prevented research. Further dramatic events took place in June 1941, when the Nazis invaded the USSR. People of Jewish origin had to flee the Nazis. Father of prof. Komisarenko took care of Parnas and his family and allowed them to travel to Ufa. In 1943 Parnas with his family were transferred to Moscow. Parnas was initially cherished by the Soviet authorities, he was nominated as the Director of the Department of Chemistry of the Institute of Experimental Medicine. and even became the member of the Soviet Union Academy of Sciences. The situation changed dramatically on January 29, 1949, when Parnas was arrested and imprisoned in Lubianka. He died in a jail in so far unexplained circumstances. Parnas name was banned even could not be mentioned. It was not until 1960 that the name Parnas began to reappear in the press and in some publications. Parnas name kept in mind his students and associates: Baranowski, Meybaum-Katzenelenbogen, Heller, Mozołowski, and Skarżyński in Poland and Sobchuk in Lviv. After transformation time when Poland and Ukraine became independent countries, the idea appeared of establishing cooperation between Polish and Ukrainian biochemists The first Polish-Ukrainian conference commemorating Parnas was held in Lviv in 1996. Since that time Parnas Conferences were organized either in Poland or in Ukraine. In 2009 Polish and Ukrainian Biochemical Societies decided to extend the Parnas Conference formulae for biochemists from Israel. In 2016 the tenth Polish Ukrainian and the third Polish, Ukrainian, Israeli Parnas Conference was held in Wrocław.

YEAST LIPIDS FROM SECOND GENERATION SUBSTRATES FOR BIODIESEL AND FISH FEED

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First generation biodiesel provides a low per-hectare energy yield, is competing with food production and can promote rain-forest cutting. Aquaculture is utilising large resources of either wild caught fish or food-grade plants to cover the demands for oil and protein in the feed. Oleaginous yeasts can accumulate lipids to more than 50% of their biomass, utilising waste raw materials such as crude glycerol from biodiesel production or lignocellulose (including hemicellulose). These lipids can be converted both to second generation biodiesel production and ingredient in fish feed. We have tested a variety of ascomycetous and basidiomycetous oleaginous yeast strains for growth and lipid production on hemicellulose hydrolysate and crude glycerol. We tested novel methods for *in situ* determination of lipid content and –composition. Basidiomyceteous yeasts (*Rhodotorula* and *Rhodosporidium* spec.) usually produced lipids more rapidly and to higher concentrations from lignocellulose and crude glycerol than ascomycetes (*Lipomyces* spec.). Fermentation techniques were introduced to test lipid production under reproducible conditions (e.g. Brandenburg et al. 2016). In an analysis of a biorefinery approach, i.e. biogas and electricity production from fermentation residues, the energy output from biolipid production from lignocellulose had an energy balance similar to ethanol production (41% of the total energy in the biomass) (Karlsson et al. 2016). We have also tested lignocellulose-based yeast oil as ingredient in fish feed, and did not find any negative impact on the cultivated fish.

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METABOLIC ENGINEERING OF *YARROWIA LIPOLYTICA* FOR THE SYNTHESIS OF ADDED VALUE CHEMICALS

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Y. lipolytica is a non-conventional yeast, well-known for its unusual metabolic properties. Based on its ability to secrete high amounts of proteins and metabolites of biotechnological interest, *Y. lipolytica* has several industrial applications, including heterologous protein synthesis or citric acid production. We will report on strain development for the synthesis of two added value chemicals: erythritol and erythrulose. Erythritol is a four-carbon sugar alcohol with application as food additive due to its sweetening properties. Erythrulose is a derivative of erythritol and is an intermediate of its catabolism. It has application as sunless tanning agent and as precursor for the synthesis of different drugs. By overexpressing gene *GUT1* and *TKL1*, which encode a glycerol kinase and a transketolase, respectively, strain-overproducing erythritol were obtained. Erythrulose producing strain was obtained by deleting gene YALI0F01606g than code for an erythrulose kinase.

DEVELOPMENT OF WASTE-LESS PROCESS FOR THE CONSECUTIVE PRODUCTION OF FURFURAL, BIOETHANOL AND OTHER VALUABLE COMPOUNDS FROM HEMICELLULOSE AND LIGNOCELLULOSE-CONTAINING RESOURCES

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One of the most important tasks of modern biotechnology is the development of the processes directed to the substitution of oil which according to forecasts of experts will be completely exhausted within the next 30-40 years. The main compounds which have to be produced in these processes are bioethanol (necessary as a fuel) and furfural (as the most important intermediate for chemical industry). Both these compounds are produced by industry already now from the renewable agricultural resources in the individual processes which till the moment could not be connected together. The main problem is linked with very close kinetic parameters of furfural formation and cellulose destruction. As the result significant amount of the hemicellulose and cellulose-containing substrates was irreversibly damaged and it was not more possible to use it for the production of bioethanol. In our new process we succeeded to develop the technology for the consecutive production of furfural, bioethanol and other valuable compounds from the crop residues and waste of wood-processing industry. It was reached on the basis of new unique patented pre-treatment of the raw material in the presence of catalyst solution with steam. The destruction of lignocellulose in this process was prevented and it became possible to use it for the further production of bioethanol. This pre-treatment necessary for the furfural obtaining process decreased also the degree of cellulose polymerization in the lignocellulose residue to 300. Such additional effect of furfural production process facilitated the next stage – cellulose enzymatic hydrolysis. At this stage commercial cellulase Accellerase 1500 was used with the addition of laccase-containing enzymes complex obtained at the last stage of our process – during the growth of medicinal mushrooms. At the stage of microbiological production of bioethanol traditional yeast *Saccharomyces cerevisiae* was used in the immobilised form. Yeast immobilisation was reached using our new technology with application of special dehydration stage. Lignin residue after enzymatic hydrolysis stage was used as the additive for the improvement of the growth of medicinal mushrooms which can be necessary as the additive at the treatment of various diseases and in the pharmaceutical industry. Our new process was developed for hardwood waste, wheat straw and colza straw.

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DNA REPAIR IN THE PATHOGENICITY OF TUBERCLE BACILLI

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Mycobacterium tuberculosis (Mtb) remains the most dangerous bacterial pathogen, being responsible for 1.5 million deaths each year. This serious pathogen has greatly spread worldwide and over the recent years we have observed an increase in the number of multi-drug resistant and totally drug resistant *M. tuberculosis* strains. The danger of tuberculosis becoming an incurable disease has emphasized the need for discovery of a new generation of antimicrobial agents. The development of novel alternative medical strategies, new drugs and the search for optimal drug targets are top priority areas of tuberculosis research. The tuberculosis disease is an airborne respiratory tract infection, with infected individuals serving as a source of disease spread. During the course of an infection bacteria reside primarily within the macrophages and at later stages of infection, inside inflammatory structures called lung granulomas. *Mtb* is exposed to oxygen and nitrogen radicals, generated by the immune response cells and these radicals are known to cause oxidative and nitrosative DNA damage to the bacterial genome. Mycobacteria can also be exposed to desiccation, during transport from one individual to another, which is proposed as an equivalent to ionizing radiation (IR). Both desiccation and IR are known to cause double strand breaks (DSBs) in DNA, which can also be generated by a number of other exogenous and endogenous factors. The integrity of bacterial DNA can also be altered via causing damage to the DNA processing proteins. Mycobacteria, similarly to other bacterial species, possess various mechanisms of DNA repair, which can be alternatively activated in response to various types of damage. Major DNA damaging events require a well-coordinated and multiprotein response to avoid replication arrest and promote cell's survival.
The lecture will summarize the recent progress made by our group and our co-workers in the understanding of DSBs repair processes in respect to the pathogenicity of tubercle bacilli.

The study was supported by grant of National Science Centre, Poland 2014/15/B/NZ7/01002 and 2015/19/B/NZ6/02978.

POSTER SESSION 1

Poster # 1

PREVALENCE OF *KLEBSIELLA PNEUMONIA* IN RENAL FAILURE PATIENTS USING PRIMER SPECIFIC GENES

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The current study was done to identify the bacteria isolated associated with renal failure patients specifically hemodialysis.The study included 102 blood samples (61 males (59.8%) and 41 females (40.1%)), 150 urine samples for patients undergoing hemodialysis at industrial kidney unit in Marjan Teaching Hospital from November 2013 to June 2014. Sample were cultured on different media for full bacteriological identification. In addition, to applied of specific primers for detection of bacteria. Bacteriological investigations revealed positive culture was 89.3%. the total number of isolates was 150. Gram negative bacteria were predominant and represented 84.3%. While gram positive group were 15.67%.

The most common gram negative bacterial isolates were *Escherichiae coli* (38.8%), followed *Klebsiella pneumoniae* (34.32%), *Enterobacter spp* (6.71%) and *Pseudomonas aerogenosa* (4.47%).The gram positive bacteria were *Staphylococcus aureus* (13.43%) and *Staphylococcus epidermidis* (2.24%).

PCR assay were performed to identify the presence of some genes of *Klebsiella pneumoniae* isolates, and some of virulence ,included(*cps, phoE*), the study revealed that cps gene give positive result in 11 isolates out of 28 (39.28%), while in the case of gene phoE only 8 isolates out of 28(28.57%) give positive result.

Key words*: K. pneumonia, cps, phoE*, PCR, dialysis.

Poster # 2

SINGLE NUCLEOTIDE POLYMORPHISM OF TNF ALPHA RELATIONSHIP WITH KIDNEY INJURY PATIENTS

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Acute and chronic kidney injury represent a great health problem worldwide.The current study aimed to investigate the correlation between TNF alpha levels and its single nucleotide polymorphism for kidney injury patients. Eighty blood samples were collected from kidney failure patients who admitted to hemodialysis unit at marjan hospital, Hilla – Iraq. serum concentrations of TNF a show highly increased in patients compared with healthy persons but there is no significant differences appear between patients before and after hemodialysis. Molecular investigations for TNF a -380 A/C single nucleotide polymorphism show increase in homozygous genotype AA than heterozygous AC and homozygous CC with a percentage of 60, 30 and 10% respectively, sodium and potassium concentrations also reveals a noticeable variations after hemodialysis processes.
Keywords: kidney, TNF a, polymorphism, electrolytes, hemodialysis.

Poster # 3

SERO-EPIDEMIOLOGICAL AND MOLECULAR STUDY OF TOXOPLASMOSIS IN THE BLOOD DONORS AND APPLICANTS FOR MARRIAGE PEOPLES IN THE BABYLON PROVINCE, IRAQ

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Identification and sero-epidemiological and molecular study were conducted for blood donors and applicants for marriage to investigate the infected person with Toxoplasmosis in the Babylon province for two sexes during the period from November 2014 till February 2015, ELISA technique for 140 serum samples for blood donors and 166 serum samples for Applicants for marriage and by polymerase chain reaction (PCR) technique for 66 blood samples (where positive with ELISA technique), according to epidemiological criterions (area region , Sex ,age group). The results showed that the total toxoplasmosis samples positive using the ELISA antibody IgG (34%) and antibody IgM (3%) for blood donors and antibody IgG (24%) and antibody IgM (%5).polymerase chain reaction technique was 8 (21.6%) for blood donors while was 12(41.37%) for Applicants for marriage the results showed that Prevalence of toxoplasmosis was slightly higher in rural area than compared to cities when using the three detecting methods, infection in male highly frequency when using ELISA and PCR technique compared with females . all age groups and ages generally more than 35 years more vulnerable to infection.
Keywords: Toxoplasmosis, ELISA technique, PCR technique.

Poster # 4

APPLICATION OF CRISPR-CAS9 SYSTEM FOR SITE-SPECIFIC MUTAGENESIS IN YEAST *CANDIDA FAMATA* AND *HANSENULA POLYMORPHA*

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Recently developed CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system has been successfully applied for modification of genomes from bacteria to human as quick method of rational genome engineering. CRISPR system contains bacterial Cas9 nuclease and a synthetic guide RNA (gRNA) that directs Cas9 to cleave regions in the genome. Homologous genome region (up to 20 bp) hybridizes to guide from the synthetic guide RNA when it is followed by the specific sequence NGG (known as protospacer-associated motif (PAM)). Variations of CRISPR-Cas9 have been developed for numerous groups of organisms; however, the system still was not applied and adapted for biotechnologically important yeast species *Candida famata* and *Hansenula polymorpha*.The aim of this work is development CRISPR/Cas system as a genome editing tool for yeast species *Candida famata* and *Hansenula polymorpha.* Gene *ADE2* was used as a target locus to assess the efficiency of the system for these yeasts. Disruption of the gene leads to accumulation of red pigment to confer easily detectible phenotype of the *ade2* mutants.
It was found that CRISPR-Cas9 system optimized for *Candida albicans*, revealed low efficiency in flavinogenic yeast *C. famata*. Only 5% of selected transformants showed the mutant red colonies phenotype. Obtained result could be explained by inefficient expression of genes encoding components of the system. In *H. polymorpha*, an endogenous gene promoter *GAP1*, encoding glyceraldehyde-3-phosphate dehydrogenase was cloned in front of cas9 gene to ensure effective expression Cas9 nuclease. Increased gene expression *CAS9* led to an increase in the frequency of mutations in the gene *ADE2*. Among the selected transformants ≈70% colonies accumulated red pigment.

Poster # 5

ASSESMENT OF AGRO-INDUSTRAIL WASTES AS A GROWTH MEDIUM FOR LIPASE PRODUCTION BY *RHIZOMUCOR VARIABILIS* IN SUBMERGED FERMENTATION

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Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are important industrial enzymes due to their versatile applications. Lipase produced by microorganisms can be used in many industrial applications, such as detergent formulation, oil/fat degradation, pharmaceutical synthesis, biodiesel and cosmetics production. Due to the increase in demand for lipase, increasing attention has been paid to how to produce it efficiently and economically. This can be achieved through the use of low cost culture media especially residues from agro-industry (Godoy et al., 2011). The present study investigated the valorization of oilcake by its use as a possible growth medium for the microbial production of extra-cellular lipase. The production of lipase by *Rhizomucor variabilis* using submerged fermentation and oilcake as substrate was investigated. Various process parameters like initial pH of basal media, incubation time, agitation and aeration rate and rapeseed oilcake concentration were optimized for lipase production. The highest lipase activity (192 U/ml) was achieved after 48 h of reaction in the fermentation medium with initial pH 5.5; 2% of rapeseed oil cake at 28 °C. Enzyme had an optimal activity at 40°C and pH 8.0, The characteristics of isolated lipase give this enzyme great potential for use in the field of biocatalysis.

Godoy M.G. et al. J. Ind. Microbiol. Biotechnol. 2011, 38(8):945-953.

Poster # 6

LIPASE FROM *FUSARIUM OXYSPORUM* AS A USEFUL BIOCATALYST FOR THE ESTER SYNTHESIS

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Lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) catalyzes a wide range of bioconversions, including hydrolysis, esterification, transesterification, and aminolysis. Thus, lipases are used to modify lipids, pharmaceutical agents, agricultural chemicals, perfumes, and to synthesize functional materials. In this study, we report characterization and application of organic solvent tolerant lipase from *Fusarium oxysporum* for ester synthesis. The main highlight of our study is the application of this lipase in the synthesis of food and biodiesel esters. Various organic solvents with different log P value from –2,0 to 4,0 were tested. Dioxan, cyclohexane and n-hexane significantly enhanced activity of lipase from *Fusarium oxysporum*. The lipase was stable in chloroform and acetone with activity of 105% and 100%, respectively. The esterification activity of the enzyme was found to be optimum at 40^{o}C and n-hexane was the best solvent for esterification of 1-ethyl oleate and 1-butyl caprylate. The highest conversion rate (80%) for ethyl oleate was observed after 24 h with 440 U of free *F. oxysporum* lipase. In the case of butyl caprylate, the optimum conditions, giving 89% of conversion after 24 h, were obtained with molar ratio caprylic acid to butanol of 1:1 and 380 U of free enzyme.

Poster # 7

ENHANCEMENT OF ESTER PRODUCTIVITY BY *RHIZOMUCOR VARIABILIS* LIPASE IN THE PRESENCE OF MICROBIAL EPS

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The lipase-catalyzed esterification reactions attracted research interest during the past decade, due to an increased use of organic esters in biotechnology and the chemical industry. In the esterification reaction, the water molecule is released as a side product which has a potential to revert the forward reaction (reversible). Hence the removal of water from the reaction mixture becomes a crucial factor. Various approaches such as the use of dehydrating agents, molecular sieves, specialized membranes, and zeolites have been reported in the literature (Erika et.al, 2009). The *Rhizomucor variabilis* lipase was used for synthesis of the flavor ester butyl caprylate in organic solvent. The influence of various reaction parameters such as molar ratio, time, temperature, enzyme concentration, effect of various microbial EPS were studied. The rate of catalyzed synthesis of esters was very dependent on the solvent medium, and maximum activity was found when n-hexane was used as the solvent. The optimal temperature for the catalyzed synthesis ranged from 35 to 45°C and the maximal temperature was 40°C for the synthesis of butyl caprylate. Addition of naturally obtained EPS significantly enhanced the ester synthesis. The highest conversion rate (88.3%) for butyl caprylate was observed in the presence of EPS from *Bradyrhizobium elkani* USDA76 after 12 h with 500 U of free *R. variabilis* lipase. In the case of butyl caprylate synthesis without EPS, low conversions (42%) were observed after 12 h reaction.

Erika F. et al. Desalination 2009, 241(8):56-62.

Poster # 8

TMI1 GENE IS INVOLVED IN THE REGULATION OF ALCOHOLIC FERMENTATION OF GLUCOSE AND XYLOSE IN *SCHEFFERSOMYCES STIPITIS*

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Fuel ethanolfrom renewable lignocellulosicbiomass has a great economic and ecological significance. However, the feasible technology for the bioethanol production from non-starch lignocellulosic materials has not been developed yet. The main reason is the absence of a robust microorganism capable of efficient alcoholic fermentation of all the main sugars of lignocellulose, most importantly, xylose. One of the microorganisms that are capable of xylose utilization is the yeast *Scheffersomyces (Pichia) stipitis*. Among the xylose-fermenting yeasts, *S. stipitis* seems to be the most promising for industrial application, because it ferments xylose with a high ethanol yield and low xylitol production.

The aim of this work is to investigate the role of the gene *TMI1* (Transport into Mitochondria) in the alcoholic fermentation of glucose and xylose by the yeast *S. stipitis*. The possibility of the *TMI1* gene's influence upon the efficiency of alcoholic fermentation was revealed using the insertional mutagenesis method combined with the positive selection of ethanol overproducers, based on the usage of 3-bromopyruvate as a selection agent. 3-bromopyruvate specifically inhibits key enzymes of glycolysis: hexokinase, pyruvate kinase and pyruvate decarboxylase, therefore the yeast cells resistant to 3-bromopyruvate should have intensified glycolysis, and this may stimulate ethanol production during the alcoholic fermentation.

Among the selected 3-bromopyruvate resistant insertional mutants, strain *#4.6* revealed reproducible increase of ethanol accumulation during glucose or xylose fermentation. In this strain, the insertion was found within the ORF of a gene homologous to *Saccharomyces cerevisiae* gene *YDL119C*, encoding mitochondrial transporter. Confirmation that the observed increased glucose/xylose fermentation performance of strain *#4.6* is a result of insertion cassette integration, rather than the secondary mutation occurring elsewhere in the genome, is an essential part of the study. Wild-type phenotype was restored via complementation of the insertional mutation by the wild allele of *TMI1* gene, however, deletion of *TMI1* on the background of *Ku80* strain (Maassen et al, 2008) did not improve ethanol production on glucose/xylose containing media.

Obtained results revealed that the gene *TMI1* is involved in the regulation of alcoholic fermentation of glucose and xylose. The mechanism of the enhanced ethanol production in the insertional mutant strain will be further studied.

Poster # 9

EXTRACELLULAR CAPACITY OF YEAST *OGATAEA POLYMORPHA* TO REDUCE TOXIC CHROMATE AND FORMATION OF CHROMIUM(III) BIOCOMPLEXES

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Bioconversion of toxic substances is the current issue of modern biotechnology, toxicology, microbiology, and practical enzymology. Among these dangerous compounds, there are industrial products of human activity (xenobiotics). Chromate is an example of such substances. For microbial bioremediation of chromate, recombinant yeast *Ogataea (Hansenula) polymorpha* tr1, overexpressing a mitochondrial flavoprotein, flavocytochrome b_2 was used. The enzyme contributes to a higher bioremediation activity of the recombinant yeast cells, compared to the wild type cells. This strain is able for efficient (100%) reductive bioremediation of 1 mM chromate.
It was also shown that the yeast *O. polymorpha* tr1 has the extracellular capacity to reduce toxic chromate and to chelate chromium(III), resulting in the formation of Cr(III) biocomplexes in the culture medium. The physico-chemical analysis shows that isolated biocomplexes Cr(III) consist of at least two components, including protein. The complexes have strong absorbance peaks at 275 and 325 nm, and weaker - at 580 and 975 nm. The isolated Cr(III) biocomplexes are also able for a green fluorescence. They reveal an antioxidant activity (9.9 ÷ 21.9 J g^{-1} of chromium) in the tests based on using ABTS and iron(III).
The isolated Cr(III) bio-complexes show a positive biological activity in physiological studies using rats as the test model: induce a decrease of blood glucose level without any toxic effect (approved by ALT and AST transaminases' assay test).

Smutok O. et al. Chemosphere.2011, 83:449-454.

Poster # 10

OPPOSING REGULATION BY ppGpp AND pppApp AT THE *rrnB*P1 PROMOTER

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Stringent response allows bacteria to adapt to changing environmental conditions. In this process (p)ppGpp, guanosine 5'triphoshphate - 3'-diphosphate (pppGpp) and guanosine 5'diphoshphate - 3'-diphosphate (ppGpp) play a major role leading to reprograming transcription and metabolism in bacteria. These alarmones directly bind to the RNA polymerase (RNAP) leading to inhibition of transcription initiating from the ribosomal *rrnB*P1 promoter. In addition, the effect of (p)ppGpp is enhanced in the presence of DksA, RNA polymerase secondary channel binding factor.
It is interesting whether, apart from (p)ppGpp there are other nucleotides that could influence the process of regulation of RNA synthesis in microorganisms. The goal of this study is analysis of the potential impact of (p)ppGpp analogs on transcription initiating from the *rrnB*P1 promoter. By using *in vitro* transcription, we have established that both, ppApp and pppApp have a stimulatory effect on transcription initiating from the ribosomal promoter. Analysis of the *rrnB*P1 transcription regulation in the presence of (p)ppApp together with additional factor, DksA had shown that the activatory effect of (p)ppApp is suppressed. Furthemore, (p)ppApp stabilizes the RNAP – DNA open complexes formed at the *rrnB*P1 promoter. Also, pppApp can compete with ppGpp for binding to RNAP and this effect depends on the order of addition of each alarmone.
These results may enhance our knowledge about the impact of binding of modified nucleotides to polymerase and thus modulating regulation of transcription.

Poster # 11

KOMAGATAELLA PHAFFII (PICHIA PASTORIS) STRAINS EXPRESSING β-GALACTOSIDASE AS A TOOL TO STUDY THE PROTEOLYTIC DEGRADATION OF CYTOSOLIC PROTEINS

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The methylotrophic yeasts have become important host organisms for recombinant protein production. This is highly linked to the presence of very strong and tightly regulated promoters of some genes of the methanol utilization pathway such as the alcohol oxidase, formaldehyde dehydrogenase etc. Proteolytic degradation has been a perpetual problem when yeasts are employed to express recombinant proteins. Since the β-galactosidase assay is simple and well visualized method, we decided to use it for investigation of the mechanisms involved in inactivation and proteolytic degradation of cytosolic proteins in the methylotrophic yeast *Komagataella phaffii* (former name *Pichia pastoris*). For this purpose, we constructed vectors containing β-galactosidase gene *LAC4* from the yeast *Kluyveromyces lactis* under control of regulated promotors of the formaldehyde dehydrogenase (P_{FlDH}), formate dehydrogenase (P_{FDH}) and fructose-1,6-bisphosphatase (P_{FBP}) genes. We also obtained strain of *K. phaffii*which synthesizes β-galactosidase under control of strong regulated promotor of the formaldehyde dehydrogenase gene.

The β-galactosidase gene *LAC4* from *Kluyveromyces lactis* strain CBS 2359 was amplified using the High-Fidelity DNA polymerase and the primers N3 and N4. This fragment was cloned into the multiple cloning site of the plasmid pIB1. The promoters of *FlDH, FDH* and *FBP* were obtained by the PCR-amplification of following sequences from the *K. phaffii* genomic DNA. Promotor sequences were cloned into the plasmid pIB1-*LAC4* upstream to the *LAC4* ORF resulting in the constructs pIB1-P_{FlDH}-*LAC4*, pIB1- P_{FlDH} -*LAC4* and pIB1- P_{FBP}-*LAC4* respectively. The wild type strain of *K. phaffii* GS200 *his4 arg4* and the protease-deficient strain SMD1163 (*pep4, prb1*) were transformed with plasmid DNA pIB1-P_{FlDH}-*LAC4*. Colonies of the *K. phaffii* transformants were selected on the minimal medium without histidine and analyzed by PCR. β-galactosidase activity assay was held in verified mutants upon short- or long-term duration of glucose starvation. The cells pregrown on the medium with methanol were shifted to the glucose containing medium either with the nitrogen source or without it. The changes of the β-galactosidase activity were compared in the obtained *K. phaffii* pIB1-P_{FlDH}-*LAC4* transformants and in the *Saccharomyces cerevisiae* strain expressing the β-galactosidase under control of the constitutive promotor of the alcohol dehydrogenase (*ADH*) gene. The obtained*K. phaffii* strains synthesizing β-galactosidase under control of strong regulated promotor of the formaldehyde dehydrogenase gene will be used for further screening characteristics of the degradation of cytosolic proteins.

Poster # 12

PHAGE REPLICONS PROPAGATION IN *ESCHERICHIA COLI* CELLS DEVOID OF HFQ PROTEIN

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The Hfq protein is one of the most important regulatory factors, acting in cells at the RNA level. It belongs to the family of thermostable bacterial proteins and the occurrence of its orthologue genes has been reported in nearly half of the sequenced bacterial genomes. In bacterial cells, it is present at high abundance in the cytoplasmatic fraction associated with ribosomes, and it is also located close to the bacterial membrane. Moreover, recent studies showed that the Hfq protein is one of the Nucleoid Organization Proteins, which may indicate still poorly described role in DNA-related processes. On the other hand, many well-known functions of this protein is associated with RNA metabolism. It has been reported that Hfq plays a role as RNA chaperone which facilitates sRNA base pairing with their target mRNA. It is also involved in mRNA decay. Nevertheless, recent studies, which indicated that Hfq is a DNA-binding protein, opens new chapter in discussion about the biological properties of this protein in bacterial cells. Since Hfq protein affects many cellular processes, effects of deletion of the gene encoding this protein are pleiotropic.

The DNA replication is one of the fundamental metabolic processes of all living organisms. In our studies we found that in *Escherichia coli* cells devoid of the Hfq riboregulatory protein, the synthesis of selected replicons is stimulated. However, some plasmids revealed unusual replication patterns. Those plasmids originated from bacteriophage P1and lambdoid phages, some of them were derivates of Shiga toxin encoding phages.Those replication patterns suggest complex and multilevel involvement of Hfq protein in the DNA replication regulation.

The results of this work indicate that Hfq protein plays a role in DNA replication regulation, despite the fact that the full mechanism of this regulation remains not fully revealed.

Poster # 13

THE SOLUBILITY OF EXOPOLYMERS FROM *RHODOCOCCUS* STRAINS

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Extracellular polymeric substances (EPS) are macromolecules with ability to form flocs and biofilms, aggregate bacterial cells and adhere to different surfaces. The main components of such polymers are usually polysaccharides and proteins, nevertheless their composition and properties depend on type of microorganisms which synthesise EPS (Aljuboori et al., 2013). Apart from the wide application in industry, exopolymers can be also applied in medicine as anticoagulant, antithrombotic, immunomodulatory, anticancer and drug delivery factors.
The solubility of polymers plays significant role in their applications and an understanding of this process allows not only for the selection of a suitable solvent but also matching optimal process conditions. For example, controlled drug delivery system requires substances, that provides possibly optimal dose of the drug to proper place in the organism. The system is composed of a solute/drug dispersed within a polymer matrix. The selection of the adequate solvent for the specific polymer can increase the drug mobility, which is released into surrounding fluid (Miller-Chou et al., 2003).
In the present study, the optimal solvents for *Rhodococcus* exopolymers solubilisation were chosen. The analyses were carried out with exopolymers solutions at concentration 1 mg/mL, using polar (alcohols, acids, buffers) and non-polar solvents (e.g. chloroform). The solubility was studied by absorbance measurements at 560 nm and meandiameter of particles in range from 0.6 nanometres to 6 micrometers (Zetasizer Nano ZS). Additionally, the EPS solutions with solvents were centrifuged and the obtained precipitates were dialysed against distilled water and freeze-dried using a lyophilisator (Labconco, USA). The lyophilisates were weight to establish the actual amount of solubilised exopolymers.

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Aljuboori A. H. R. et al. Bioresour. Technol. 2013, 127:489-493.
Miller-Chou B. A. et al. Prog. Polym. Sci. 2003, 28:1223-1270.

Poster # 14

ACQUISITION OF BACTERIAL EXOPOLYMER R-202

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Actinomycetes are the gram-positive filamentous bacteria able to produce two types of mycelium: substrate (formed on the agar surface or into the agar) and aerial (filaments extended into the air). The extension of filaments depends on the strain, the temperature of incubation, and the growth media (Varghese et al., 2012). Actinomycetes commonly occur in different environmental conditions. Most of them are able to survive in wide spectrum of temperature, pH and atmospheric pressure. This kind of bacteria is known as a rich source of biologically active compounds like antitumor agents, enzymes, antibiotics, pesticides and alkaloids. It is assumed that almost half of microbial metabolites are obtained from Actinomycetes and about 75% of antibiotics applied in medicine are synthesized by this class of bacteria (Radhakrishnan et al., 2011).
Obtaining the highly molecular compounds from natural sources is relatively problematic, due to the selection of optimal conditions on almost every stage of isolation. The crucial element, which is exopolymer precipitation method from the culture broth, inappropriately chosen leads to losses in quantity of product (More et al., 2014). Currently applied precipitation methods can be divided into two groups: physical (e.g. centrifugation, high temperature) and chemical (e.g. the addition of ethanol, methanol, NaOH, enzymes, H_2SO_4, TCA and EDTA); the more efficient method is the chemical precipitation.
In the present study, the process of exopolymer receiving from culture broth of *Rhodococcusrhodochrous* strain was optimised due to the type and the volume of precipitation factors, the duration of the precipitation, and the stirring impact on the amount of obtained exopolymer. The precipitates from all variants were dialysed against distilled water and lyophilized to receive products. Based on the weight of obtained extracellular polymers, the optimal conditions of precipitation were established.

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More T. et al. J. Environ. Manage. 2014, 144:1-25.
Radhakrishnan M. et al. Indian J. Mar. Sci. 2011, 40:407-410.
Varghese R. et al. IJID 2012, 1(3):142-144.

Poster # 15

CHEMICAL COMPOSITION ANALYSIS OF THE BACTERIAL EXOPOLYMER R_S-202 BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Polymers received from natural sources such as plants, bacteria, fungi called biopolymers, have established essential part of studies over organisms' resistance. Therefore, the adaptation to different environmental conditions (wide range of temperatures, different pH values, presence of heavy metals) of these highly molecular compounds is possible. Additionally, biopolymers are well-known from their antioxidant, antibacterial, and antifungal activities (Salehizadeh et al., 2014). The significant element of investigations over natural polymers is their purification. Only highly purified products can be analysed by their chemical structure, which includes chemical bonds and functional groups. Fully characterised physicochemical properties allow to recognise the biopolymers mechanisms as well as to select factors responsible for their biological activity.
The studies of chemical composition were conducted on the exopolymer received from culture broth of *Rhodococcusrhodochrous* strain. The acidic hydrolysis of exopolymer was carried out with the addition of 8 M trifluoroacetic acid. The hydrolysed samples of exopolymer were dried using a vacuum rotary concentrator, flushed a few times with methanol, and dissolved in Milli-Q water (1 mg/mL). The HPLC analysis were conducted on the Shimadzu chromatograph system with refractive index detector (RID-10A), using the REZEX RPM-Monosaccharide Pb^{2+} column.

This project was financed by The National Science Centre based on the decision number DEC-2012/07/B/ST5/01799.

Salehizadeh H. et al. Biotechnol. Adv. 2014, 32:1506-1522.

Poster # 16

CONSTRUCTION OF *SACCHAROMYCES CEREVISIAE* RECOMBINANT STRAINS CAPABLE OF CONVERTING XYLOSE INTO ETHANOL

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Plant biomass is a renewable energy source and therefore has great potential as a feedstock to produce fuel ethanol. Yeast *S. cerevisiae* is unable to catabolize and ferment xylose, the second main sugar of lignocellulosic hydrolysates, due to lack of enzymes catalyzing initial stages of the pentose catabolism.

Therefore, for efficient lignocellulose conversion to ethanol the development of *Saccharomyces cerevisiae* strains capable of both glucose and xylose fermentation is of great interest. *Spathaspora passalidarum* belongs to the natural xylose-fermenting yeast species with higher ethanol production rate on xylose that on glucose under low-oxygen conditions. *S. passalidarum XYL1, XYL2* and *XYL3* genes, encoding xylose reductase, xylitol dehydrogenase and xylulokinase, respectively, were cloned under control of *S. cerevisiae ADH1* gene promoter and introduced into the genome of *S.cerevisiae* industrial strains, namely AS400; Y563; PE2. Selected transformants were unable to grow on xylose, despite the increased specific activity of xylose reductase. Moreover, the increased specific activity of xylitol dehydrogenase was confirmed for transformants derived from Y563 and PE2 strains. The adaptation of constructed recombinant strains to xylose as carbon source is under the progress.

Poster # 17

EXPRESSION OF *MYCOPLASMA HOMINIS* SOLUBLE ARGININE DEIMINASE IN *ESCHERICHIA COLI*

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Our studies aimed on evaluating arginine-degrading enzyme arginine deiminase (ADI, catalyzes the imine hydrolysis of arginine to produce citrulline and ammonia) as a promising anticancer drug and a key element for arginine biosensor construction. ADI anticancer activity is based on the previous findings that demonstrated the positive effect of arginine depletion on remission of the certain types of cancer (mainly hepatocarcinomas and melanomas) in vitro and in vivo. The reported procedure for production of *Mycoplasma hominis* ADI expressed in *Escherichia coli* is cost consuming and therefore needs to be improved.

Recombinant strain of *E. coli* which overexpresses *M. hominis* gene encoding ADI was constructed. The protocol for high-level production of ADI in a cheap mineral medium was developed. Previously, it was shown that generally rADI of *M. hominis* was accumulated as the inclusion bodies in *E. coli*, however, in this study we evaluated the expression of soluble ADI in *E. coli*.

Bacterial cells expressing ADI were grown in mineral or LB medium with the addition of 50, 100 and 200 mM of phosphate buffer (pH 7.2) at different temperatures ranging (from 10 to 32°C) and different rotation speed of flasks (from 130 to 250 rpm). The activity of soluble ADI of *M. hominis* in *E. coli* cell-free extracts was determined. The results revealed that in general the activity of soluble ADI was higher in the cells grown at low temperatures (18°C)with the increased concentration of phosphate buffer in culture medium (200 mM of phosphate buffer pH 7.2) relative to the cells grown at elevated temperatures (32°C). Lower aeration additionally increased the activity of soluble ADI by 2.3 and 5.1 times in cells grown in LB and mineral medium, respectively. Thus, the highest activities of soluble ADI in *E. coli* cell-free extracts were achieved, namely,0.072 and 0.054 U/mg of protein, when cells were grown in LB or mineral medium with the addition of 200 mM phosphate buffer at 18°C and 130 rpm, respectively.

The enzyme was purified by the anion-exchange chromatography using Q-Sepharose column and the linear gradient of 0-1M NaCl in 20 mM sodium phosphate buffer. The active fractions were pooled and NaCl concentrationwas adjusted to 2M. The enzyme was applied to the Phenyl-Sepharose column, washed with 20 mM sodium phosphate buffer containing 2M NaCl and eluted with the buffer containing 1 M NaCl. As a result, stable enzyme preparations with the specific activity of 15-20 U/mg of protein were obtained. The affinity of soluble ADI for arginine (K_m) was examined in phosphate buffer, pH 6.5 at 37°C. The K_m ($S_{0.5}$) values were determined from the initial velocity data measured as the function of substrate concentration. All assays were performed in triplicate and the means of 3 separate experiments were presented in Lineweaver-Burk plot.The value of V_{max} for soluble ADI was 0.019 μM/min·ml and the value of K_m was 100±20 μM that is 3-fold lower ascompared to rADI of *M. hominis* accumulated as the inclusion bodies in *E. coli* (Fayura, Biotechnol. 2013).

The possible application of obtained ADI preparations will be discussed.

Poster # 18

METABOLIC ENGINEERING OF *YARROWIA LIPOLYTICA* FOR THE SYNTHESIS OF ADDED VALUE CHEMICALS

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Y. lipolytica is a non-conventional yeast, well-known for its unusual metabolic properties. Based on its ability to secrete high amounts of proteins and metabolites of biotechnological interest, *Y. lipolytica* has several industrial applications, including heterologous protein synthesis or citric acid production. We will report on strain development for the synthesis of two added value chemicals: erythritol and erythrulose. Erythritol is a four-carbon sugar alcohol with application as food additive due to its sweetening properties. Erythrulose is a derivative of erythritol and is an intermediate of its catabolism. It has application as sunless tanning agent and as precursor for the synthesis of different drugs. By overexpressing gene *GUT1* and *TKL1*, which encode a glycerol kinase and a transketolase, respectively, strain-overproducing erythritol were obtained. Erythrulose producing strain was obtained by deleting gene YALI0F01606g than code for an erythrulose kinase.

Poster # 19

NEW FILAMENTOUS MUTANT OF *YARROWIA LIPOLYTICA* AND ITS USE IN BIOFILM BIOREACTORS

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The non-conventional yeast *Yarrowia lipolytica* is widely investigated for its unusual metabolic properties. Among them is the ability of *Y. lipolytica* to adopt an ovoid or hyphal morphology according to environmental conditions. The mechanism of dimorphic transition involves numerous genes, which have been poorly documented to date. Here, we report on the isolation of a filamentous mutant from an insertion mutagenesis library, the subsequent identification of the mutated gene, and the use of this filamentous mutant in biofilm bioreactors. The gene YALI0E06519g is similar to the *S. cerevisiae* gene *HSL1* encoding a septin-binding kinase involved in the synchronization of cell cycle progression and bud formation. Due to its filamentous morphology, strain ΔYALI0E06519 grows attached on steel packings in flasks and bioreactors. Such a feature could greatly simplify the production of recombinant proteins or biomolecules with biotechnological or pharmaceutical applications in *Y. lipolytica*.

Poster # 20

ASSESSMENT OF METABOLIC ACTIVITY OF BACTERIA IN SOIL UNDER DIFFERENT TILLAGE INTESITY SYSTEMS

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Biolog® EcoPlatemethod enables the evaluation of microorganisms ability to utilization a different carbon sources (CLPP – *Community Level Physiological Profiling*). EcoPlates are used to determine metabolic profiles of microbial communities inhabiting in different environments. Each EcoPlate® include 31 different carbon sources, in three replications each. The results showed the higher metabolic diversity and activity of microbial communities in soil under lower-intensity tillage as compared with intense tillage.

The aim of the research was to determine the effects of different tillage intensity systems on changes in diversity and metabolic activity of microorganisms in the soil under winter wheat. The research was conducted in the years 2014-2016 on the long-term field experiment with different soil tillage systems at the ES of IUNG-PIB in Grabów (Mazovian voivodeship), Poland. Diversity of the metabolic activity in soil microorganisms was analyzed using the Biolog® EcoPlateSystem (Biolog, Hayward, CA, USA).

The obtained results szowed higher metabolic diversity and activity of microbial communities in soil under less intense tillage as compared to high-intensity tillage.

The research was conducted within the frames of Task 1.4 Multi-Annual Programme IUNG-PIB 2016-20120 and Research Statute Program 2.26.

Poster # 21

ENZYMATIC ACTIVITY AS AN INDICATOR OF QUALITY OF SOIL ENVIRONMENT AFFECTED BY DIFFERENT FARMING SYSTEMS

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Soil enzymes are very sensitive to environmental changes. Relatively easy procedure of estimation of enzymes activity allows to use them as a good indicator of ongoing changes in soil environment. The subject of the study was to determine changes in enzymatic activity in soil under winter wheat grow in different farming systems. The research was conducted in the years 2014 - 2016 on fields under four cultivation systems at the ES of IUNG-PIB in Osinach (Lublin voivodeship), Poland. Each farming system differs in crop rotation system and whole agrotechnics, which have been adapted to its specificity. Determination of dehydrogenase activity was performed using Casida et al. (1964), expressing their activity in milligrams three-phenyloformazan (TF) per 100 g of soil within 24 hours. Activity of alkaline and acid phosphatases was determined by Tabatabai and Bremmer (1969) method, indicating their activity in micrograms sodium 4-nitrophenylphosphate (PNP) per 1 g of soil within 1 hours.
The results showed that farming system based on crop rotation which enhance the ability of soil to store organic matter and simplified tillage influenced beneficially soil environment, which was confirmed in higher enzymatic activity as compared to the soil under monoculture of winter wheat, where enzymatic activity was relatively low.

Casida L.E., Klein D.A., Santoro T., Soil Sci., 1964, 98: 371-376.
Tabatabai M.A., Bremner J.M., Soil Biol. Biochem., 1969, 1: 301-307.

The research was conducted within the frames of Task 1.4 Multi-Annual Programme IUNG-PIB 2016-20120 and Research Statute Program 2.26.

Poster # 22

COMPARISON OF MICROBIAL ACTIVITY OF SOIL UNDER MAIZE (*ZEA MAYS*) AND WINTER WHEAT

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The biological activity of the soil is very important for maintaining soil fertility and high yields. The tillage system and the type of crop can have a significant impact on the microbial diversity in the soil, and thus on its quality.
The aim of the study was to compare the activity and functional biodiversity in soil under the cultivation of two different cereals: common corn (*Zea mays*) and winter wheat (*Triticum aestivum*), both grown in the same pattern of two different tillage systems. The research was carried out in the years 2013 - 2016 on the basis of long-term field experiment at the ES of IUNG-PIB in Grabów (Mazowieckie Province), Poland. Soil samples for comparative analysis were collected in July 2016 at the fields under: simplified tillage (depth up to 10 cm) and conventional tillage (depth up to 25 cm). The soil dehydrogenases activity with Casida et al. (1964) method, microbial biomass carbon and nitrogen content with fumigation-extraction method and functional diversity of soil microorganisms using the Biolog® EcoPlate™ System were determined.
The results of the research showed that, in general, higher dehydrogenases activity, biomass carbon and nitrogen content and metabolic activity were found in soil from winter wheat as compared to soil under maize.

Casida L.E., Klein D.A., Santoro T., Soil Sci., 1964, 98: 371-376.

The research was conducted within the frames of Task 1.4 Multi-Annual Programme IUNG-PIB 2016-2020 and Research Statute Program 2.26.

Poster # 23

INCREASED SUSCEPTIBILITY OF GASTRIC EPITHELIAL CELLS TO CYTOTOXIC EFFECT OF *HELICOBACTER PYLORI* IN THE MILIEU OF ACETYLSALICYLIC ACID AND 7-KETOCHOLESTEROL IN A DIFFERENT TIME POINTS

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Helicobacter pylori colonize gastric epithelium and initiate cell damage. This barrier can be also destroyed by some anti-inflammatory drugs such as acetylsalicylic acid (ASA), used in patients with ischemic heart disease. Gastric epithelium of *H. pylori* infected persons potentially is more susceptible to harmful effects of ASA. On the other hand cytotoxicity of ASA may promote *H. pylori* colonization.

In *H. pylori* infected patients increases a concentration of cholesterol – atherosclerosis risk factor. It is unknown whether the proinflammatory activity of *H. pylori* can be modulated by cholesterol.

The aim of this study was to assess the cytotoxicity to gastric epithelial AGS cells of *H. pylori* components: glycine acid extract (GE), CagA protein, urease subunit (UreA), lipopolysaccharide (LPS) [Prof. A.P. Moran] and 7-ketocholesterol (7-kCh) or ASA using MTT reduction assay.

Metabolic activity of AGS cells was diminished by *H. pylori* LPS but not *E. coli* LPS [25 ng/ml]; GE [10 µg/ml], CagA [1 µg/ml], UreA [5 µg/ml]. ASA at 5 mM showed significant cytotoxic activity and such tendency at 1 mM. Similarly 7-kCh at 20 µg/ml but not at 2.5 µg/ml diminished metabolic activity of the cells. The cytotoxic effect of *H. pylori* antigens was increased in the milieu of 5 mM ASA and 20 µg/ml 7-kCh alone or both ASA and 7-kCh. By comparison 7-kCh at 2.5 µg/ml but not ASA at 1 mM neutralized the cytotoxic effect of 5 mM ASA and *H. pylori* antigens, especially after 24 and 48 hours after cell exposure.

In conclusion, gastric epithelial AGS cells were susceptible for deleterious effect of different *H. pylori* components and 7-kCh as well as ASA on dose dependent manner. The synergistic effect of *H. pylori* components with ASA and 7-kCh in downregulation of metabolic activity of the cells has been shown.

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Poster # 24

PRODUCTION OF IL-33 AND MMP-9 IN RESPONSE TO *H. PYLORI* INFECTION ON THE PRIMARY GUINEA PIG GASTRIC CELLS

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Helicobacter pylori-(Hp) causes in humans gastritis/gastric and duodenal ulcers and cancers. The Hp induced inflammation can be upregulated by some soluble host metaloproteinases-(MMP) and monitored by interleukins-(IL), including IL-33, which is an alarmin in the immune system.

To determine the production of IL-33/MMP-9 by primary cell derived from guinea pig gastric tissue exposed to Hp antigens *in vitro*.

Guinea pigs were euthanized (decision of Ethical Committee:58/ŁB45/2016).Stomachs were isolated and divided into smaller fragments, homogenized, incubated with collagenase for 20 min, and then centrifuged (1400 rpm/15min). Supernatant was removed and the cell pellet was suspended in modified F12 culture medium (Sigma). The cell suspension at the density of $2x10^6$ cell/well was distributed to 6-well culture plates and incubated for 24 h (37°C, 5% CO_2) in the culture medium alone or in the milieu of Hp antigens: glycine acid extract-(EG)-10µg/ml; CagA protein-1µg/ml; UreA-5µg/ml; Hp/*E.coli* lipopolysaccharide-(LPS)-25ng/ml. The ability of the cells to heal an experimental cell damage was assessed in the “scratch assay” on the basis of cell migration and viability (MTT reduction). The levels of IL-33 and MMP-9 were determined in the cell supernatants by commercial ELISA.

Hp antigens used in experimental concentration did not diminish the ability of primary gastric cells to reduce MTT. However, both Hp LPS and *E.coli* LPS inhibited cells migration, which was associated with increased production by the cells of MMP-9 but not IL-33. By contrast Hp GE/UreA /CagA did not impair cell migration and in such culture the level of IL-33 was increased.

Hp antigens might modulate differentially cell migration and the production of IL-33 and MMP-9. *In vivo* Hp LPS in the inflammatory milieu can delay tissue regeneration in conjunction with increased production of MMP-9.

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Poster # 25

THE COMPARISON OF BIOCHEMICAL AND MICROBIOLOGICAL INDICATORS OF SOIL QUALITY ON EXAMPLE OF REPRESENTATIVE SOILS OF POLAND

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The aim of the study was to compare the most commonly used indicators of soil quality on the example of the representative soil of Poland. Soil samples from Monitoring of arable soils in Poland were taken for analysis. The monitoring includes 216 points of sampling on arable land across Poland. They represent areas of quite high degree of intensive tillage located in areas of other than agriculture influence of human activity. Database that characterizes location of soil sampling includes: pH, salinity, content of metals, and organic compounds, carbon content, macronutrients content, texture and others. Biological diversity was determined in addition to total number of bacteria and fungi and enzymatic activity. The functional diversity and biodiversity indexes were determined using the Biolog EcoPlates method.

The research was conducted within the frames of Tasks 1.3, 1.4. Multi – Annual Programme IUNG – PIB 2016 – 2020.

Poster # 26

ASSESSMENT OF THE METABOILC DIVERSITY OF MICROORGANISMS AND GLOMALIN CONTENTS AS A SOIL ENVIRONMENTAL QUALITY INDICATOR

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Soil microbial community and their diversity under maize growth in different cultivation techniques were determined using Biolog EcoPlates and other microbial and biochemical methods used for the determination of soil properties. Comparisons of the patterns of substrate utilization and the diversity indexes showed differences in community composition of microorganisms related to different cultivation techniques and seasons. The soil samples collected in spring were characterized by statistically significant lower indexes of biological activity in comparison to the soil collected from the flowering stage of maize. The soils collected in spring from the plots with full tillage as the cultivation technique were showing similarly high biological activity as soils obtained from maize flowering season. The principal component of PCA analysis, showed the strong correlation between the parameters of soil quality and biodiversity indicators. Selected indicators of soil microbial diversity explained 71.51% of biological variability in soils. Based on the PCA analysis two major groups of soils have been indicated. The season was the main differentiating factor (spring and summer). The yield of grain was 22% higher in the rotation tillage system than of direct sowing tillage system.

The research was conducted within the frames of Tasks 1.2, 1.4. Multi – Annual Programme IUNG – PIB 2016 – 2020.

Poster # 27

ENZYMATIC ACTIVITY AS THE INDICATOR OF SOIL QUALITY

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Soil quality and its fertility is based on intense microbial activity, including enzymatic activity. The choice and elaboration of indicators for evaluation and formation of microbial diversity in soils and soil microorganisms activity in various habitats and management systems is of great importance. Soil enzymes take part in metabolism and catalyze processes connected to matter and energy processing in soil. Any changes in soil properties can lead to the changes in number and activity of soil organisms including their species composition and biodiversity.
The aim of the study was to use the methodology of determination of enzymatic activity of soil as an indicator for evaluation of quality in arable soil monitoring programme in Poland.
Soil samples from Monitoring of arable soils in Poland were taken for analysis. The monitoring includes 216 points of sampling on arable land across Poland. They represent areas of quite high degree of intensive tillage located in areas of other than agriculture influence of human activity. Database that characterizes location of soil sampling includes: pH, salinity, content of metals, and organic compounds, carbon content, macronutrients content, texture and others. Enzymatic activity of soils (dehydrogenase activity, acidic and alkaline phosphatase activity) was determined in soil samples and correlated with data regarding physical and chemical properties of soils.
Key words: soil, enzymatic activity, dehydrogenase, phosphatase, microbial indicator.

The research was conducted within the frames of Tasks 1.3 and 1.4. Multi – Annual Programme IUNG – PIB 2016 – 2020.

Poster # 28

THE DIFFERENTIATION OF THE *RHIZOBIUM LEGUMINOSARUM* BACTERIA STRAINS METABOLIC ACTIVITY

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Bacteria of the *Rhizobum* genus form a group of microorganisms existing in the environment in two forms: symbiotic- in the root nodules of *Fabaceae* plants and free-living , saprophytic in the soil environment. The basic function of *Rhizobum* in a symbiosis is to reduce nitrogen to ammonia directly assimilated by the plant. The subject of the study was to evaluate the ability of strains of *Rhizobium leguminosarum* bacteria to metabolize three groups of compounds: carbohydrates, amino acids, carboxylic acids and fatty acids. The study used 16 strains of *Rhizobium leguminosarum* bacteria from the collection of Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation in Puławy. Based on the sequencing of PCR products, we found that all strains belong to one species- *Rhizobium leguminosarum.* The study of metabolic activity was performed using both the BIOLOG system and the GEN III plates. Based on results the heat maps were made and the cluster analysis according to Ward's method conducted thus illustrating the diversity of strains in terms of the intensity and pace of the individual compounds consumption.

The research was conducted within the frames of Task 1.4. Evaluation and formation of biodiversity of soil and microbial activity of soil with regard to habitat conditions and management system. Multi- Annual Programme IUNG- PIB 2016- 2020.

Poster # 29

INFLUENCE OF *RHIZOBIUM LEGUMINOSARUM* AND *AZOSPIRILLUM BRASILENCE* ON THE RED CLOVER (*TRIFOLIUM PRATENSE*) GROWTH AND NODULATION UNDER CONDITIONS OF PAHs CONTAMINATION

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The bacteria of the *Rhizobium* genus create symbiotic systems with the *Fabaceae* plants. These bacteria form the nodules on the plant roots, in which the suitable conditions to reduce nitrogen to ammonia further absorbed by the plant. This symbiosis results is a better growth and development of the plants, regardless of the nitrogen content in the soil. The additional plant inoculation with the associative nitrogen-fixing bacteria of the *Azospirillum* genus can enhance the effect of symbiosis between the plants and bacteria of the *Rhizobium* genus. The subject of the study was to evaluate the impact of the red clover (*Trifolium pratense*) inoculation with the *Azospirillumbrasilence* bacteria on the growth and root nodulation of the plants contaminated with phenanthrene, anthracene and pyrene. The study used 17 strains of *Rhizobium leguminosarum* and 3 strains of *Azospirillum brasilence* bacteria from the collection of Department of Agricultural Microbiology IUNG- PIB in Puławy. The result of the research was to evaluate the synergy of two groups of soil microorganisms (associative nitrogen-fixing *Azospirillum spp*. and symbiotic nitrogen fixing *Rhizobium* spp.)in the process of phytoremediation of the soils contaminated with PAHs with the use of red clover.

The research was conducted within the frames of Task 1.4. Evaluation and formation of biodiversity of soil and microbial activity of soil with regard to habitat conditions and management system. Multi-Annual Programme IUNG- PIB 2016- 2020.

Poster # 30

THE DIFFERENTATION OF SOIL ENZYMATIC ACTIVITY EXEMPLIFIED BY THE SOILS TYPICAL OF LUBLIN VOIVODSHIP

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Lublin Voivodship is located in southeastern Poland. The borders of the region are determined by the valleys of two rivers: Vistula and Bug. In this region are beneficial conditions for crop production due to the presence of good quality soils and a large share of agricultural land. Fertility and productivity of ecosystems depend on the intensity of the biochemical processes in the soil which are catalyzed by the enzymes. The aim of the study was to compare the biological indicators of soil quality (dehydrogenase activity, acid phosphatase and alkaline phosphatase) on the example of agricultural soils. The study used 18 soil samples representing 11 types of soils characteristic of the Lublin region. The soil enzymes activity was determined by the methods described by Tabatabai et al. (1969) and Caside and al. (1964). The soil enzymatic activity was significantly dependent on type of soil.

Caside L., Klein D., Santoro T. (1964) *Soil dehydrogenase activity*. Soil Sci., 98: 371-376.
Tabatabai M. A., Bremner J. M. (1969) *Use of p-nitrophenyl phosphate for assay of soil phosphatase activity.*Soil Biol. Biochem. 1: 301 – 307.

The research was conducted within the frames of Task 1.4. Evaluation and formation of biodiversity of soil and microbial activity of soil with regard to habitat conditions and management system. Multi-Annual Programme IUNG- PIB 2016- 2020.

Poster # 31

PEROXIDASE-LIKE NANOZYMES AS EFFICIENT PLATFORMS FOR OXIDASE-BASED AMPEROMETRIC BIOSENSORS

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Catalytically active nanomaterials (nanozymes) as artificial enzymes have several advantages over natural analogues, namely, a high stability and low-cost. Enzyme-like nanozymes, including metallic nanocomposites, are promising catalysts for biosensing applications. Peroxidase-liked nanozymes may be as efficient chemosensors on hydrogen peroxide, final product of oxidase-catalized hydrolysis of oxidase's substrate. Therefore, selection and development of artificial enzymes are actual tasks of nanotechnology.

The aim of the current research is to construct and study the cheap effective oxidase-based bioelectrodes with the usage of efficient nanozymes as peroxidase mimetic catalysts. The number of mono- and bi-metallic nanoparticles were synthesized on the surface of carbon electrode by electrochemical polymerization and characterized as electro-active chemosensors on hydrogen peroxide for further biosensing applications. The most effective bi-metallic chemo-sensor on H_2O_2 was chosen for construction of biosensors, being coupled with different microbial oxidases: yeast alcohol oxidase (AO) and recombinant methylaminooxidase (MAO), fungal glucose oxidase (GO). Each of the developed biosensors exhibits a high sensitivity, a broad linear range, a good selectivity toward natural substrate and satisfactory storage stability. The constructed biosensors (BS) were tested on real samples of food products: AO-based BS – for ethanol assay in wines and yoghurts; MAO-based sBS – for methyl amine assay in fish tissue extracts. A high correlation of the obtained results and the declared by manufacturer values was demonstrated for the contents of the target analytes.

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Poster # 32

QANTITATIVE AND QUALITATIVE ASSESSMENT OF AIRBORNE MICROORANISMS IN THE HISTORICAL AND MODERN REPOSITORIES IN THE REGIONAL MUSEUM IN RZESZÓW (POLAND). A CASE STUDY

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A museum is an institution established to collect, study and deal with the restoration of cultural heritage objects that have some historical or artistic value. Museum artefacts are kept for a long time in depositories and are prone to microbiological colonization. The goal of this research was to assess the risk of microbiological colonization of cultural heritage objects stored in the Regional Museum in Rzeszow (Poland). The museum buildings consists of the historic (former 17-th monastery complex, in the early 50s intended for the museum) and newly built (in the 1960's) parts. The aim was to verify the hypothesis about higher airborne concentrations of microorganisms in the historic part.
The research was planned in such way that the same type of exhibit (e.g. paintings, books) was warehoused in the repositories located both in the historic and modern part of the museum. Air samples were collected in winter using portable equipment - AESAP 1075 Saimpl'air Lite. Bacterial colonies were identified using the automated system – VITEK 2 Compact. Fungal isolates were identified on the base macro- and micro-morphological features.
In all repositories, the number of bacterial colonies was higher than those of fungi. The most polluted depots were those where different types of artefacts were stored. There were no differences in the number of fungal colonies in the repositories located in the historic and modern parts of the museum. The differences concerned only bacteria. The most common fungi were *Penicillium expansum* and *Penicillium spp*., while among bacteria *Micrococcus luteus/lylae* and *Staphylococcus warneri*. The highest microorganism biodiversity was in the historical depots and the lowest in the room with a book collection in the modern part of the museum. It has been found that the microbial biota is not specific for either building type or the kind of artefact.
We can conclude that in the Regional Museum in Rzeszow the cultural heritage objects are not exposed to too high concentrations of bacteria and fungi. Regarding bacteria we detected the effect of '*historical building air*'.

Grabek-Lejko D., Tekiela A., Kasprzyk I. Risk of biodeterioration of cultural heritage objects stored in the historical and modern repositories in the Regional Museum in Rzeszow (Poland). A case study. Int. Biodeter. Biodegrad. (2017-accepted).

Poster # 33

CHANGES IN *ABORTIPORUS BIENNIS* METABOLISM IN RESPONSE TO OXALIC ACID ADDITION

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Abortiporus biennis is a saprophytic fungus causing the white rot of wood in nature. The white rot fungi belong to the most effective organisms able to degradation of all wood components. In lignocellulose degradation both high molecular weight (HMW) and low molecular weight (LMW) compounds are involved. Among HMW compounds numerous hydrolytic and oxidative enzymes are reported. The most important oxidative enzymes are extracellular laccases (EC 1.10.3.2) and peroxidases e.g. manganese-dependent peroxidase (MnP, EC 1.11.1.13), lignin peroxidase (LiP, EC 1.11.1.14), versatile peroxidase (VP, EC 1.11.1.16) and dye-decolourising peroxidase (DyP, EC 1.11.1.19) (Janusz et al., 2013, Polak and Jarosz-Wilkołazka, 2012). Fungal hydrolytic enzymes consist of numerous exo- and endo-acting cellulases and hemicellulases. The enzymatic stage of ligninocelulose decomposition is preceded by the action of LMW compounds (e.g. organic acids, metal ions, phenolic chelators, reactive oxygen species) and their main function is to make the structure of the cell wall more available to the enzymes, which are too large to start catalysis on the intact cell wall. Oxalic acid is the main organic acid and important LMW compound secreted by wood-degrading fungal species. This acid is involved indirectly in the degradation of the lignocellulose complex and its concentration is under active regulation by mycelium. The concentration of oxalate can also influence laccases or peroxidases synthesis by fungi, and the effectiveness of the Fenton reaction (Grąz and Jarosz-Wilkołazka, 2011).
In presented study changes in *Abortiporus biennis* metabolism were monitored at transcriptomic level. The obtained results showed that 18 genes were significantly up-regulated and 64 down-regulated by oxalic acid. Among genes for wood degrading enzymes expression of oxidative enzymes like VP, MnP, chlorperoxidase, Lac were down-regulated but in different extent. Genes for hydrolytic enzymes like xylanase as well as exo- and endoglucanase were in contrast up-regulated under the influence of oxalic acid. Increase expression of oxalic acid decomposing enzymes were also observed but among them the genes for formate dehydrogenase (FDH) was up-regulated in the greatest extent. Three transcripts for FDH and one annotated for oxalate degrading enzymes were found.

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Poster # 34

IDENTIFICATION OF DOMINANT BACTERIA IN DIFFERENT TYPES OF SOIL USING PCR-DGGE MOLECULAR STANDARDS

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Most studies apply PCR-DGGE method for the estimation of microbiological diversity based on the operational taxonomic units (OTUs) without knowing the detailed species composition. The development of molecular standards in this project allowed for a better understanding of the major species involved in the life of the soil. In addition, DGGE analysis using molecular standards can be a routine method for rapid monitoring of changes occurring under different factors such as agrotechnical treatments or changing atmospheric conditions. This will be an extremely valuable method for quick results (few days after environmental sampling).
Standards were prepared by cutting out the most distinctive and clear bands from DGGE gel. Afterwards the bands were reamplified and sequenced. PCR products were subsequently cloned into chemically competent *E.coli* cells and stored in -80°C as a DGGE-PCR molecular standards bank.
A similar composition of bacteria was found for soils with similar physicochemical parameters and types. The greatest differences in population composition were observed between good quality and acidic soils.

The research was partially founded by the Ministry of Science and Higher Education, research task: Development of molecular standards for the rapid identification of dominant bacteria inhabiting different soil environments, using denaturing gradient gel electrophoresis technique (DGGE) /2017.

Poster # 35

COMPARATIVE PROTEOME ANALYSIS OF THE WOOD DECAY FUNGUS *CERRENA UNICOLOR*

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Cerrena unicolor, a white-rot fungus, is a parasite/saprophyte of several hardwood trees and even capable to degrade dead logs of softwood. *C. unicolor* was proved to produce cellulases, xylanases, manganese peroxidase, lignin peroxidase and laccase, the most studied enzyme of this microorganism. The genome of this fungus has recently been sequenced, thus providing a better insight into its wood degrading enzyme inventory. However, little is known about *C. unicolor* strategies of lignocellulose decomposition. To elucidate the number of enzymes involved in wood degradation, the white-rot fungus *Cerrena unicolor* FCL139 growing on maple, birch and mineral medium (as control) was submitted for whole secretome analysis using peptide sequencing by LCMS/MS. The output list of precursor and product ions was compared with the dedicated protein database of *Cerrena unicolor* (12978 sequences; 5371935 residues) using the MASCOT (version 2.4.1) local server. The *C. unicolor* v1.1 (Cerun2) genome assembly, downloaded from the U.S. Department of Energy Joint Genome Institute (DOE JGI, http://jgi.doe.gov), was used as the reference for the mapping. The obtained results indicated that the composition of extracellular proteome differed considerably depending on the carbon source. One hundred and fifty-eight proteins were found when *C. unicolor* was grown on mineral medium, whereas only 138 and 136 when on maple and birch, respectively. Up to 97 proteins were the same when comparing birch and maple medium, whereas sawdust medium compared to mineral medium showed only 41 (birch and control) and 39 (maple and control) common proteins. When growing on any sawdust medium, *C. unicolor* produced the same set of wood degrading enzymes: laccase, pyranose dehydrogenase, endo-β-1,4-xylanase, alcohol oxidase, endoglucanase, exoglucanase, cellobiohydrolase, exo-β-1,3-glucanase, cellobiose dehydrogenase, glyoxal oxidase, lytic polysaccharide monooxygenase, mannanase, β-glucuronidase, xyloglucanase.

Keywords: *Cerrena unicolor*, secretome, LCMS/MS.

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Poster # 36

RNASEQ BASED ANALYSIS OF *CERRENA UNICOLOR* DIFFERENTIAL GENE EXPRESSION IN RESPONSE TO CHANGING LIGHTING CONDITION

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Cerrena unicolor belongs to the group of white-rot fungi and is growing mainly on live or dead logs of many hardwood trees: *Fraxinus excelsior*, *Betula* sp., *Salix* sp., *Acer* sp., *Malus sylvestris*, *Sorbus* sp., *Aesculus hippocastanum*. To elucidate the number of genes involved in response to changing lighting conditions, the *Cerrena unicolor* FCL139 growing in the white, blue, green, and red light as well as the darkness as control was submitted for whole transcriptomes analysis (RNAseq) using next generation sequencing (NGS) approach. From 81.5 to 142.3 million pair-end reads were obtained for each of the tested culture variant, of which 34.3 to 48.6 million were mapped to 12,966 gene model predicted for *C. unicolor* genome, providing good coverage of putative coding sequences and allowing for analysis of differential gene expression. Transcripts of 5487 genes were present in transcriptomes of fungus growing in all light variants. Amongst detected gene transcripts, 289 were at least two times more abundant during growth in any of applied light when compared to darkness, while transcription of 382 genes was downregulated. However, each of the lightning conditions revealed different number of unique genes engaged in various metabolic pathways, whose expression was at least two fold upregulated, namely: white vs. dark (210); green vs. dark (3); red vs. dark (41) and blue vs. dark (35). Noteworthy, among differentially expressed genes several encoding for enzymes engaged in wood degradation were found. White light affected expression of laccase, cytochrome P-450, β-glucuronidase and manganese peroxidase Blue light influenced only transcription of β-glucosidase.Whereas, red light had significant impact on aryl-alcohol dehydrogenase, manganese peroxidase, and cytochrome P-450.

Keywords: Cerrena unicolor, light, whole transcriptome analysis, NGS.

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Poster # 37

OXALATE METABOLISM OF *ABORTIPORUS BIENNIS* IN THE PRESENCE OF HEAVY METALS

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Oxalic acid, the simplest dicarboxylic organic acid, plays a significant role in biological and geochemical processes in natural environment. Better understanding the oxalic acid fungal metabolism is very important due to fact, that fungi are known accumulators of oxalates which among others can be used as the chelator of metal ions such as calcium, manganese, cadmium, and lead (Jarosz-Wilkołazka and Grąz, 2006; Grąz et al., 2009). Heavy metals have a detrimental effect on many microbial processes. Fungal strain *Abortiporus biennis* was reported as an oxalic acid producer, that can potentially facilitate metal ions mobilization and thus the bioremediation processes (Jarosz-Wilkołazka and Grąz, 2006). Enzymatic regulation of oxalic acid in *A. biennis* cultures is catalysed by enzyme oxalate oxidase (EC 1.2.3.4) (Grąz et al., 2016). Described in our studies the oxidative way of oxalic acid decomposition is untypical for fungi, which expressed typically activity of oxalate decarboxylase (EC 4.1.1.2). The degradation of oxalate *via* action of oxalate oxidase was found predominantly in higher plants and only three oxalate oxidases of basidiomycete fungi have been described - the enzyme from *Tilletia contraversa*, the best characterised so far enzyme from *Ceriporiopsis subvermispora*, and exactly the enzyme produced by *A. biennis* (Grąz et al., 2009).
Abortiporus biennis was selected during our earlier study as the strain which has a tendency toward intracellular accumulation of cadmium ions inside the hyphae rather than biosorption by the cell wall components. We also reveal that Cd presence affects morphology of whole mycelium of *A. biennis*. This strain also was reported as oxalic acid producer which can potentially facilitate metal ions mobilization (Jarosz-Wilkołazka and Grąz 2006). In the present study the oxalic acid metabolism was monitored in *A. biennis* cultures amended with different heavy metal ions (Cu^{2+}, Pb^{2+}, Mn^{2+}, Cd^{2+}). The activity of oxalate degrading enzyme was monitored as well as concentration of oxalic and formic acids in the medium and the concentration of particular heavy metals in mycelium.

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Poster # 38

CONVERSION OF GLYCEROL TO ETHANOL USING ENGINEERED YEAST *OGATAEA (HANSENULA) POLYMORPHA*

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Biodiesel is produced by a transesterification reaction using vegetable oils or animal fats, and methanol being the preferred one. This process generates large amounts of glycerol as a by-product. Excess of contaminated glycerol is a big environmental problem. The availability of crude glycerol is predicted to increase during the next years due to tremendous growth in the production of biodiesel worldwide. To maximize the full potential of the biodiesel process, it is important to convert waste glycerol into useful chemicals, e.g. ethanol by microorganisms. Thermotolerant methylotrophic yeast *Ogataea (Hansenula) polymorpha* is able to convert glycerol to ethanol, moreover, it could metabolize residual methanol. However, the yield of ethanol from glycerol by wild-type strain is extremely low and needs to be substantially improved before industrial implementation. Therefore, the tools of metabolic engineering along with classical selection should be used for construction of the recombinant strains with improved ethanol production.
The aim of this work was to improve production of ethanol from glycerol using thermotolerant methylotrophic yeast *O. polymorpha.* Consequently, we decided to overexpress key genes encoding enzymes involved in glycerol metabolism. For this, the vectors for multicopy integration of both *PDC1* (encodes pyruvate decarboxylase) and *ADH1* (encodes alcohol dehydrogenase) genes under control of strong constitutive promoter of glyeraldehyde-3-phosphate dehydrogenase gene have been constructed. Obtained recombinant strains of *O. polymorpha* with overexpression of both *PDC1* and *ADH1* revealed increased specific activities of both Pdc1 and Adh1 and ethanol production in glycerol medium. In addition, optimization of fermentation conditions resulted in further increase of ethanol accumulation from glycerol (up to 5 g/L). The next steps of our work was aimed in overexpression of genes encoding enzymes of the initial reactions of glycerol catabolism. *O. polymorpha* uses two pathways of glycerol catabolism. First one involves the *GCY1* (encoding glycerol dehydrogenase) and *DAK1* (encoding dihydroxyacetone kinase), while the alternative pathway is consists of *GUT1* (encoding the glycerol kinase), and *GPD1* (dehydrogenase of glycerol-3-phosphate). The corresponding vectors pUC19/GAPpr-GPD1-GAPtr/NTC and pUC19/GAPpr-GCY1-GAPtr/NTC were constructed and transformed into yeast cells. As a host strain NCYC/PDC1/ADH1 (with overexpressed of *PDC1* and *ADH1*) was used. The next step was to analyze the expression level of target genes through RT-PCR. Transformants with the highest levels of *GUT1, DAK1, GPD1* and *GCY1* gene expression were selected. The selected strains were analyzed for ethanol production and consumption of glycerol. The best constructed strain produced up to 11.6 g/L of ethanol under fermentation at 45ºC and 15% of glycerol as a sole carbon source.

Poster # 39

TIME LAPSE MICROSCOPY AS A TOOL TO INVESTIGATE CHROMOSOME TOPOLOGY AND CELL DIVISION IN STREPTOMYCES VENEZUELAE

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The size of bacterial cell requires significant compaction of the chromosomal DNA. Bacterial DNA chromosome is organized by DNA binding proteins: a high molecular – weight SMC (in *E. coli* MukB functional homolog of SMC) and number of small nucleoid associated proteins (NAPs). SMC proteins affect the global chromosome topology by the long-range interactions while NAPs affect chromosome organization locally. NAPs are abundant proteins that dimerise or oligomerise and bind DNA with a low sequence specificity. There is a great variation in NAPs in different bacteria (Wang et al., 2013).

Streptomyces are soil bacteria appreciated as the producers of broad range of secondary metabolites (i.e. antibiotics, immunosuppressants). Main characteristic of *Streptomyces* is their mycelial growth and complex life cycle involving two distinct phases: vegetative growth and sporulation. During vegetative growth elongated compartments of branched hyphae contain several uncondensed chromosomes. During sporulation multigenomic aerial hyphae, subsequently develops into chain of unigenomic spores. Conversion of the aerial hyphae to spores requires the condensation and segregation of multiple chromosomes accompanied by synchronous placement of regulatory spaced Z-rings (Flardh &Buttner, 2009).

Although the *Streptomyces* life cycle has been extensively investigated not much is known about changes chromosome topology during sporulation. Here, we present the real time single cell microscope analysis of differentiations and nucleoid dynamics during sporulation, in a novel model species, *Streptomycesvenezuelae*. Visualization of *S. venezuelae* chromosome was performed by tagging with EGFP one of nucleoid associated proteins –HupS (HU homolog from *E. coli*). Moreover, to verify a role of NAPs protein (Lsr2, HupS) and SMC in chromosome architecture we analyzed sporulation of single and double mutants. Our time-lapse microscopy results indicate that changes in DNA topology may affect the cell division during *Streptomyces* differentiation.

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Poster # 40

CONSTRUCTION, EXPRESSION AND ANALYSIS OF HYBRID PLASMID PARTITIONING PROTEINS REPB OF *RHIZOBIUMLEGUMINOSARUM*

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Rhizobia are soil bacteria, which establish symbiosis with legumes providing them with fixed nitrogen and enabling their growth on nitrogen-limited soils. Rhizobialgenomes usually comprise circular chromosome and large plasmids equipped with *repABC* cassettes that control both their replication and partition process. The genome of model strain *Rhizobium leguminosarum* bv. *trifolii* TA1 (RtTA1) consists of a chromosome and four plasmids (pRleTA1a-d) with *repABC* operons. Individual RepB partitioning proteins bound specifically to centromere-like *parS* elements of the parental plasmids, which is the crucial step towards the proper segregation of plasmids within multipartite bacterial genome (Koper et. al 2016). Although particular RepB proteins share only limited homology, they have common domain structure, with N-terminal domain engaged in interaction with RepA, centrally localized HTH motif responsible for *parS* recognition and binding as well as dimerization domain in C-terminus.

The issue we wanted to address with this study was whether the centrally localized HTH motif is the sole element responsible for binding specificity between RepB proteins and *parS* elements. To answer this question using Overlapping Extension PCR (OE PCR), a gene encoding hybrid RepB/b protein was constructed, with HTH motif derived from RepB/c protein (responsible for pRleTA1c plasmid segregation) and rest of polypeptide originating from RepB/b. Obtained hybrid recombinant protein named RepB/bHTHc, was used in series of non-radioactive EMSA experiments with DNA probes (annealed 30 bp oligonucleotides), comprising different *parS* sequences derived from both pRleTA1b and pRleTA1c plasmids. It was demonstrated that hybrid protein was able to shift *parS* elements originating only from pRleTA1b, suggesting that HTH motif is not the only determinant of RepB-*parS* binding specificity.

Additionally RepB/bHTHc was assayed for its self-association pattern. Cross-linking experiments with dimethyl pimelidinate (DMP) showed that protein form at least stable dimers and trimers in the solution and is fully functional in terms of oligomerization. Currently recombinant RepB/b protein deleted for HTH element was constructed and will be submitted for further analysis with respect to *parS* binding and oligomerization abilities.

Koper P. et al. Mol Microbiol 2016, 102(3):365-544.

Poster # 41

REPA AND REPB PROTEINS INTERACTION SPECIFICITY CONTRIBUTE TO FINE-TUNED COEXISTENCE OF SEVERAL PLASMIDS WITH SIMILAR SEGREGATION SYSTEMS IN THE MULTIPARTITE BACTERIAL GENOME

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RepA and RepB are members of the ParA and ParB family of partitioning proteins. Genome of model strain *Rhizobium leguminosarum* bv. *trifolii* TA1 (RtTA1) comprises four large plasmids (ranging in size from 476 kb – 808 kb), each equipped with *repAB* genes. In the partitioning (*par*) systems of enterobacterial strains harboring singleplasmids like episomal prophages P1, ParA and ParB interact with each other for proper plasmid partition. While *parAB* equipped plasmids usually propagate as the sole extrachromosomal replicon in the cell, large rhizobial plasmids with similar RepAB/*parS* partitioning systems coexist in one bacterial cell. This raises the risk of heterologous interaction between multiple RepA and RepB proteins within same bacterium, which may lead to impairment in expression control or segregational dysfunction resulting in unstable plasmids maintenance.
The aim of this study was to analyze potential heterotypic interactions between the RepA and RepB proteins originating from two RtTA1 plasmids (pRleTA1c and pRleTA1d). Bacterial two-hybrid system (BACTH) (Karimova et al. 1998) was employed for examination of RepA and RepB interplays. The *repA* and *repB* encoded on respective plasmids were each cloned into "bait" and "prey" vectors and all relevant combinations were co-transformed into the reporter strain *E. coli* DHM1. No positive clones with significant reporter activity were obtained for all tested combinations (RepA/c-RepA/d and RepB/c-RepB/d). Our studies demonstrated that no cross-reactivity between similar segregational elements originating from different plasmids occurs *in vivo.* Obtained results clearly support the model of spatial and temporal separation of individual replicons within complex, multipartite bacterial genome.

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Poster # 42

GAME ANIMALS' MICROFLORA ASSESSED BY MALDI-TOF MASS SPECTROMETRY

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In the last decade the population of wild boars (*Sus scrofa*) has increased in Europe including territory of Poland. In order to get insight into their microbial diversity and to estimate the role of game animals as a reservoir of potential pathogenic bacterialspecies, especially *Yersinia enterocolitica*, as well as the potential risk of game animals' meat for consumers, we investigated isolates from the swabs of the tonsils of 605 wild boars (*Sus scrofa*) and 93 roe deer (*Capreolus capreolus*) hunted in west Poland during 2016/2017 hunting season. The ICT/CIN culturing for 48h/30h at 30°C has provided 444 isolates from *Sus scrofa* and 82 isolates from *Capreolus capreolus.* MALDI-TOF mass spectrometry enabled proteomic identification of the isolates. The most common species isolated from *Sus scrofa* and *Capreolus capreolus* were *Rahnella aquatilis* (129/444; 27/82), *Serratia liquefaciens* (85/444; 15/82) *and Ewingella americana* (44/444; 5/82).The most uniquebacterial species from *Sus scrofa* were *Serratia plymuthica, S. quinivorans, S. ureilytica, Pseudomonas chlororaphsis, P. extremorientalis, P. libanensis, P. monteilii, P. proteolytica, Yersinia enterocolitica, Y. bercovieri, Y. frederiksenii* and *Y. kristensenii.* The most uncommon bacterial species from *Capreolus capreolus* were *S. entomophila, S. quinivorans, P. antarctica, P. fragi, P. gessardii, P. grimontii, P. libanensis, Shewanella baltica* and *Y. enterocolitica.* Some of the isolates (35/444 from *Sus scrofa* and 13/82 from *Capreolus capreolus*) were not identified, due to the lack of bacterial reference spectra in the MALDI Biotyper database. *S. entomophila* is used in the fight against a larvae (*Costelytra zealandica*) causing grass grub disease in New Zealand [1]. *S. baltica* is a causative agent of seafood spoilage [2]. *Y. enterocolitica* is a major foodborne pathogen causing yersiniosis [3]. *P. monteilii* is correlated with environmental isolates and clinically important oppurtunistic strains as well. According to the results, game animals can be considered the source of environmental, clinical relevant and also spoilage bacteria. Moreover, there is a need to extend and update the library of MALDI Biotyper spectra, to get more accurate identification of isolates.

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Poster # 43

ANALYSIS OF ISOTHIOCYANATES ACTION AGAINST *VIBRIO CHOLERAE*

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Cholera is an acute epidemic infectious disease still common in developing countries. The WHO reports about 1.3 to 4.0 million cases of cholera and 21000 to 143000 deaths worldwide due to this disease each year. *Vibrio cholerae,* the causative agent of infections is usually associated with contaminated water and food. The primary virulent factor which is produced by *V. cholerae* is enterotoxin encoded on CTXphi phage genome. The aim of this study is to evaluate the antimicrobial activity of plant secondary metabolites, isothiocyanates (ITCs) against pathogenic bacteria. There are limited evidence about molecular mechanism of antimicrobial properties of ITCs. Previously, we showed that ITC are promising agents against enterohemorrhagic *Escherichia coli* (EHEC) strains due to impairment of Shiga-toxin harboring bacteriophage development.

The mechanism of ITC antimicrobial activities were tested according to CLSI standard methodology. Bacteria were grown in MH medium at 37°C with aeration achieved by shaking. MIC (minimal inhibitory concentration) were assessed by the twofold broth microdilution methods. Growth inhibition kinetics or time-kill curve was determined spectrophotometrically or by plating on MH agar, respectively, in the presence of relevant concentration of ITCs. The assessment of nucleic acid synthesis was performed using radioactive precursor of DNA and RNA synthesis. The activity of ITCs on biofilm formation by *Vibrio cholerae* was evaluated in three different variant of experiments to measured biofilm preventions activity of ITCs, cell adaptation to ITCs and biofilm prevention in MIC and 5xMIC concentration. The biofilm mass was quantified using crystal violet and the cell metabolic activity was quantified using the MTT assay.

We determined MIC and MBC (Minimal Bactericidal Concentration) for phenethyl isothiocyanate (PEITC), sulforaphane (SFN) and benzyl isothiocyanate (BITC). We observed the growth inhibition by ITCs, varying for different compounds. Also, we found that ITCs showed bactericidal time-dependent properties. Higher concentration of BITC and PEITC showed bactericidal effects, lower concentration of ITCs were bacteriostatic as they fully inhibited the growth but did not kill the bacteria. We showed that SFN, BITC and PEITC caused inhibition of DNA and RNA synthesis. The preventive effects were found for the biofilm formation of cell grown of every ITCs, but ITCs seemed to prevent biofilm formation to a greater extent than to mass reduction of the already formed biological layer.

Keywords:*Vibrio cholerae*, isothiocyanate, cholera, antibiotic, toxin, biofilm, MBC, MIC, ITC, SFN, PEITC, BITC.

Poster # 44

HOST RANGE AND GENOME ANALYSIS OF PHAGE DN1 – A VIABLE DERIVATIVE OF THE DE3 PROPHAGE

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A gene expression system based on *Escherichia coli* BL21(DE3) holds one of the leading positions in industrial genetic engineering. However, the question of the prophage from DE3possessing the ability to produce viable progeny and lead to massive cell lysis is still open. The aim of the present research was to propagate and identify a *Siphoviridae* bacteriophage that had been found in an isolate from lysed batch culture of recombinant *E. coli* BL21 (DE3).

Previously, we reported that a *Myoviridae* phage Lw1 was isolated as a contaminant of the industrial recombinant *E. coli* BL21 (DE3) strain (Kushkina at al., 2013). At the same time, a siphoviral phage was found in a minor amount in the studied isolate. Several pure lines of this phage were isolated and propagated on *E. coli* c1a (called DN-phages). Analysis of complete genome sequence of phage DN1 showed that it is a bio-transducing derivative of the DE3 prophage that lacks red-gam genes. The transduced fragment is about 10 kb in length. Fine genetic structure of the transduced locus is identical to *E. coli* BL21 derivatives. It consists of eight full bacterial genes (*ybhB, bioA, bioB, bioF, bioC, bioD, uvrB, ubhK*) and the last one that was partially deleted (*moaA*). The fragments of the bacterial *moaA* gene and phage imm21 region combined in a fused ORF which is expected to be translated into a hypothetical product of 166 aa in length. Phage DN1 harbors 75% of the original DE3 sequence with 100% identity.

Comparative restriction analysis of other DN-isolates demonstrated that four out of five phages (DN1-DN4) have similar fragments corresponding to the phage DN1 and different in the transduced bacterial fragment.

Host range and Spi-phenotype examination showed that DN-phages have high plating efficiency on r-m+ cells such as *E. coli* c1a and BL21, as well on *E. coli* NapIV. They did not produce any plaques on *E. coli* B^E and were restricted to 10^{-4} PFU by *E. coli* C600 strains. *E. coli* c1a(P2) did not restrict the propagation of DN1-likes, except for DN5. All obtained phages did not reproduce on the parent *E. coli* BL21 (DE3) strain.

Conclusions. During phage induction, an assortment of viable transducing derivatives of DE3 prophage originated from BL21 (DE3). They can be isolated on in r-m+ host bacteria. Most of the obtained DN-isolates are assumed to carry fragment of the bio-transducing locus of different length in their genomes and express Spi^--phenotype, because of the red-gam genes deletion. The latter was confirmed for phage DN1 by genome sequence analysis.

Poster # 45

MODIFIED FLUORAPATITE, A NOVEL BIOCIDE MATERIAL WITHOUT CYTOTOXICITY

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Calcium phosphate ceramics, owing to their resemblance to bone structure, are widely used for different biomedical applications. However, such a specific bone-structure pattern is making those materials susceptible to colonisation by pathogenic bacteria. In this topic, an attempt was made to synthesize new apatites incorporated with silver nanoparticles (AgNPs) and to test their bactericidal/fungicidal activity and cytotoxicity toward mammalian cells. Among many strategies used to successfully synthesize nanoparticles/doped nanomaterials, those based on silver NPs are particularly promising due to their antibacterial activity. However, the impact of this nanometal on human cells is still unclear. Here, we report on the physical characterization and biological properties of modified man-made fluor- and hydroxyapatites. The structures and physical properties were characterized by means a diffuse reflectance spectra, FTIR, Raman spectroscopy, TEM and Zeta-potential analyses. The antimicrobial properties of AgNPs were assessed on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*, the main pathogenic species responsible for implant-associated infections. It was found that manufactured materials present typical structures of biological (bone) apatites. Doping with silver led to an increased biocidal activity, despite not cytotoxic effect could be observed on mammalian cells. Therefore, Ag-doped fluorapatite is a promising new resistant biomaterial with great bactericidal effect that could be applied in tissue engineering, or dentistry.

Wojnarowska-Nowak R. et. al., Adv. Appl. Ceram. 2017, Vol. 116, No. 2, 108–117.

Poster # 46

IDENTIFICATION OF THE REGIONS OF GSS1 PROTEIN INVOLVED IN GLUCOSE SENSINGIN THE YEAST *KOMAGATAELLA PHAFFII (PICHIA PASTORIS)*

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Investigation of proteins involved in glucose sensing and/or signalling in the methylotrophic yeast *K. phaffii (P. pastoris)* is necessary for further understanding of the molecular mechanisms of glucose-dependant pexophagy induction. Nowadays these processes are not fully investigated despite the identification of more than 40 *ATG* genes.

Previously it wasshown that *K. phaffii Gss1* (homolog of both *Sc*Snf3 and *Sc*Rgt2)is required for normal growth on high and low glucose concentrations, micropexophagy (occurred in the case of shift of methanol grown cells into glucose-containing medium) and glucose catabolite repression. We also studied the role of the of cytoplasmic C-tail of this protein and it was shown that complete removal of its cytoplasmic tail leads to the severe growth defect in the media with mannose,fructose and glucose (both high and low glucose concentrations) as sole carbon source, caused significant retardation of alcohol oxidase inactivation during adaptation for substrates more favorable than methanol (glucose, fructose or mannose) and has damaged catabolite repression, in contrast to the partial C-tail removal (ΔC_{50}, ΔC_{100}). We also suppose that cytoplasmic tail of the Gss1 protein is necessary not only for glucose signaling but also for hexose uptake in *K. phaffii*.

To identify regions of *KpGss1* involved in glucose recognition we decided to make error prone mutagenesis of the ORF *PpGSS1.* For this purpose, we used PCR-mixture with additional Mn^{2+}, increased concentrations of Mg^{2+}, dCTP, dTTP and with decreased dGTP, dATP. Vectors with mutant forms of *KpGSS*1 were constructed, linearized and used for electrotransformation of either *Δgss1* or GS200. Then we checked phenotype of the transfomants which were able to growth in mineral media without histidine. We search for 2-DOG-resistant strains with damaged micropexophagy and normal growth on glucose. Unfortunately we didn't reveal mutants with such phenotype characteristics among approximately 400 His^+-mutants, despite the majority of them possessed some *KpGSS1*-mutant form (verified by PCR). Our attempts to obtain transformants directly on mineral media with methanol and 2-DOG also were unsuccessful, so we decided to change selective marker of the vector. For this purpose we used zeocin containing plasmid. We are going to use obtained vectors for electrotransformation of GS200 strain and for further identification of regions of *GSS1* involved in glucose recognition.

Poster # 47

IMMUNOMODULATORY EFFECT OF RAT FETAL BRAIN NEUROGENIC CELLS CONDITIONED MEDIUM

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The current state of development of the problem of brain neurogenic stem and progenitor cells (NSC / NPC) immunobiological properties is focused on the mechanism of their regenerative action in neurotransplantation, the so-called "*bystander*" effect, which justifies the concept of "*functional or therapeutic plasticity*" [Martino G. et al., 2011; Gutierrez-Fernandez F. et al., 2014] and is realized by the expression and production of NSC / NPC immunoregulatory cytokines and growth factors. The aim of this work was to investigate the effect of rat fetal brain neurogenic cells conditioned medium (NCCM) on the functional indices of immune system cells after intraperitoneal administration in experimental animals, namely cytotoxic activity (CA) of immune cells in model systems (allogeneic and xenogeneic) with tumor cells.

NCCM was received from suspension of rat fetal brain neurogenic cells on 14th day of gestation.

Action of NCCM in xenogeneic system was examined at intraperitoneal administration in intact CBA mice in the following groups: 1) 1x administration of NCCM (0.02 mg of protein concentration, n = 15); 2) 5x administration of NCCM (daily for 5 days, the total amount 0.10 mg of protein concentration, n = 15); 3) intact animals (control, n = 5). The animals were examined after 24 hours, 5 and 10 days after administration of NCCM (group 1) and after 24 hours, 5 and 10 days after the first administration of NCCM (group 2). To determine the CA the immune cells (effector cells) of the experimental animals were studied in MTT-test with rat glioma cells 101.8 (target cells) in a ratio of 5:1.

Intraperitoneal administration of NCCM in mice modulated the CA of immune cells in the MTT-test with xenogeneic rat glioma cells 101.8. The CA of immune cells of mice (group 1) revealed the trend to increase through 5 and 10 days after the 1x administration of NCCM ($p = 0.15$, U-Mann-Whitney criterion). After 5x administration of NCCM (group 2) there was a a marked enhancement in CA of immune cells ($p = 0.003$, ANOVA Kraskell-Wallis test). Thus, augmentation of the NCCM amount (up to 0.10 mg) and multiplicity of its administration led to a significant increase of CA of immune cells of mice in cytotoxic test with xenogeneic rat glioma tumor cells 101.8.

Action of NCCM in allogeneic system was studied at intraperitoneal administration in intact white rats after 3x administration of NCCM (3 times with a 1-day gap, the total amount 0.12 mg of protein concentration, n = 12) and compared with intact control rats (n = 12). The animals were examined one week after the last injection of NCCM. After 3x intraperitoneal administration of NCCM there was statistically significant increasing of CA of immunocytes against allogeneic rat glioma tumor cells 101.8 ($p = 0.016$, U-Mann-Whitney criterion).

Thus, we have shown a stimulating effect of NCCM on the functional indicator of immune cells - CA against allogeneic and xenogeneic tumor cells. We applied modified cytotoxic MTT-test which allows to determine the total CA of effector immune cells - both cytotoxic lymphocytes and natural killers. Presumably, provided impact of NCCM on immune effector cells triggers signaling cascades that lead to increased expression of MHC class I antigens and components of their processing mechanism, contributing for better recognition of allogeneic or xenogeneic tumor cells and, therefore, increases the CA of immune cells.

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Gutierrez-Fernandez F. et al. J.Stem Cells. 2014, 9(1):53-64.

Poster # 48

IMMUNOMODULATORY IMPACT OF FETAL RAT BRAIN NEUROGENIC CELLS ON THE EXPERIMENTAL BRAIN TUMOR

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Relevance of research of immunobiological characteristics of fetal brain neurogenic cells is determined by intensive development of cell treatment technologies of various CNS diseases using neurogenic stem and progenitor cells (NSC/NPC), which have significant potential for migration to sites of lesions in the CNS and are able to express and produce variety of imunomodulatory molecules [Ulrich H. et al., 2015]. NSC/NPC can migrate to the glioblastoma, induce the death of tumor cells, prolong survival of animals or almost completely inhibit the growth of glioma [Aboody K.S. et al., 2013; Bagó J.R. et al., 2016], but mechanisms of antitumor properties of NSC/NPC remains unclear.

The aim of this work was to study the imunomodulatory and antitumor properties of neurogenic cells (NC) of fetal rat brain (14th day of gestation). Study was carried out in rats with brain glioma (strain 101.8) in the following groups: 1) rats with glioma (n = 14); 2) rats with 2x intraperitoneally (i/p) NC injection (1 x 105 cells at 9th and 13th days after glioma inoculating, n = 14); 3) rats with 3x i/p NC conditioned medium (NCCM) injection from 5 th to 10 th day after glioma inoculating (n = 12); 4) intact rats (control, n = 12). At the peak of clinical manifestations (the 17th day of glioma inoculating) the cytotoxic activity (CA) of splenocytes (effector cells) of experimental rats were evaluated in colorimetric MTT-test with allogeneic glioma 101.8 cells (target cells) in a ratio of 5:1.

In animals with glioma 101.8 (group 1) the immune cells CA was significantly higher than the indices of intact animals ($p = 0,003$, U-Mann-Whitney criterion), but the anti-tumor immunity in these animals was ineffective. Survival analysis of rats using the method of Kaplan-Mayer multiple assessments and Wilcoxon-Gehan two-sample criterion found no statistically significant differences between the experimental groups 1 and 2. The immune cells CA of rats of group 2 decreased compared with the indices of tumor-bearing animals of group 1. Injected NC probably migrated and after contact with immune cells of tumor-bearing recipients caused clonal anergy / apoptosis of the immune cells, reducing the pool of available alocytotoxic clones; or realised their suppressive effect by inhibiting the CA of immune cells on tumor cells.

In rats of group 3 with NCCM administration the life expectancy and median survival were statistically significantly increased compared to animals of groups 1 and 2 (criterion $\chi 2$, $p = 0.0004$), and CA of immune cells grew, compared with the rate of animals of group 1 ($p = 0.24$, U-Mann-Whitney criterion). Presumably, under the impact of NCCM in effector immune cells signaling cascades are triggered that lead to increased expression of MHC class I antigens and the components of their processing mechanism, which leads to better recognition of allogeneic tumor cells and, therefore, increases the CA of immune cells, ensuring the implementation of anti-tumor NC properties and allowing to extend the life expectancy of tumor-bearing animals.

Thus,intraperitoneal NCCM administration increases the immune cells cytotoxic function in rats with glioma and prolong their life expectancy and median survival. Intraperitoneal NC administration in the applied mode has no effect on the survival of rats with glioma and reduces effector function of cytotoxic immune cells.

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Poster # 49

TRANSCRIPTION START SITE – ASSOCIATED RNAS AS A RESULT OF RNA POLYMERASES COLLISION AT CONVERGENT PROMOTERS IN *ESCHERICHIA COLI*

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Recently plethora of novel transcripts, including short RNAs associated with transcription start sites (TSS) have been discovered (Thomason et al., 2015). In comparison to the most of previously discovered small RNA transcripts (sRNA), both the biological function and biogenesis of TSS – associated RNAs still remain unknown. However, the detailed analysis of RNA Seq data from experiments focused on small RNAs revealed that short TSS – associated RNAs are abundant at convergent promoters' *loci* regions and therefore may be a residue after the transcriptional interference caused by collision of two RNA polymerases.

The aim of this project was to verify the hypothesis whether short TSS – associated RNAs are a product of collision between RNA polymerases transcribing convergent genes. Here, we present the results of our research where we used available RNA Seq data from experiments concentrated on small RNA analyses for further study of convergent promoters' *loci* and selection of candidate regions. Subsequently, we examined promoters' activity at selected regions using fluorescent reporters. Moreover, we tried to develop a method for efficient enrichment of small RNA fraction which allowed us to detect and quantify short TSS – associated transcripts.

Genomic *loci* containing convergent promoters are a source of small TSS – associated RNAs. These transcripts are detected in RNA Seq experiments and can be descried with other experimental methods for RNA detection. What is more, the application of labelled adapter's ligation methods (SplintRligation(Jin et al., 2016) and splinted ligation(Maroney et al., 2008) methods) reveals more information about their 5' and 3' ends. Furthermore, our results suggest that transcriptional interference at these *loci* is a major source of short TSS – associated RNAs in *Escherichia coli.*

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Poster # 50

PHYLOGENETIC ANALYSIS OF 16S RDNA OF *LEMBOTROPIS NIGRICANS* SYMBIONTS SUPPORTS BACTERIA CLASSIFICATION BY NUMERICAL ANALYSIS OF PHENOTYPIC PROPERTIES

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Rhizobia are soil bacteria that form nitrogen-fixing symbiosis with fabacean plants. In the last four decades, the taxonomy of these bacteria has been investigated extensively and many new species have been described.
In this study, we examined the genus position and evolutionary history of diazotrophic symbionts of *L. nigricans* (black broom) using numerical analysis of phenotypic properties and comparative sequences analysis of 16S rDNA.
33*L. nigricans* symbionts and 35 reference strains representing different species of the genera: *Bradyrhizobium*, *Ensifer*, *Mesorhizobium, Neorhizobium*, and *Rhizobium* were analyzed for 86 phenotypic properties and subjected to numerical analysis by the NT-SYS program. On the basis of a cluster analysis, *L. nigricans* nodulators grouped on a dendrogram together with slow-growing bacteria of the genus *Bradyrhizobium.* These results suggest that black broom rhizobia belong to the genus *Bradyrhizobium*.
Currently, comparative analysis of 16S rDNA sequences is widely used in the study of the taxonomic position of bacteria at the genus level. In order to clarify the genus position of *L. nigricans* symbionts, the 1312 bp long fragments of 16S rRNA genes of studied symbionts were amplified, sequenced, and compared with those of other rhizobia available in the GenBank database. The level of sequence similarity between the 16S rDNA of black broom rhizobia and those of *Bradyrhizobium, Ensifer, Mesorhizobium, Rhizobium, Neorhziobium,* and *Azorhizobium* were 96-99, 87-88, 86-88, 86-88, 87-88, and 87-89%, respectively. In the 16S rDNA tree, *L. nigricans* symbionts clustered together with the *Bradyrhizobium* species with high confidence (97%). The results from comparative 16S rDNA sequence analysis supported that studied black broom rhizobia are members of the genus *Bradyrhizobium* according to the rule that bacteria with at least 95% 16S rDNA nucleotide identity to each other belong to the same genus.

Poster # 51

A NOVEL TWO-COMPONENT SYSTEM AFFECTING EXOPOLYSACCHARIDE SYNTHESIS AND MOTILITY IN *RHIZOBIUMLEGUMINOSARUM* BV. *TRIFOLII*?

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Exopolysaccharide (EPS) produced by rhizobia plays several important physiological roles, both in the saprophytic lifestyle and as determinant of a successful symbiotic interaction with legume host plant. Heteropolymeric exopolysaccharide of *Rhizobiumleguminosarum*bv. *trifolii* (*Rlt*) is composed of octasaccharide units and synthesised in a pathway known as Wzx/Wzy-dependent. Several proteins involved in EPS synthesis were characterised and most of them are encoded by genes clustered in a 30-kb Pss-I region of *Rlt* TA1 strain chromosome (1). Biosynthesis of EPS is achieved through a complex network of interactions and several two-component systems (TCS) were shown to be involved in regulation of the process.

Genes encoding hypothetical proteins resembling components of the two-component systems were identified in the vicinity of the Pss-I region. *mgl2* encodes a hypothetical methyltransferase. Recently, it was shown that Mgl2 protein plays a role in EPS production, stress survival, motility, and ability to form nitrogen-fixing symbiosis with clover (unpublished results). *mgl2* is adjacent to a divergently transcribed *pssV*, encoding a hypothetical histidine kinase, and *regA* gene, encoding a hypothetical DNA-binding protein with a HTH-domain.

Here, a detailed study concerning a role of *mgl2* in motility/chemotaxis of *Rlt* TA1 and influence of Mgl2 protein on electrophoretic properties of the PssO protein indispensable in EPS synthesis (2) is presented. *mgl2* mutation affected bacterial motility in a complex manner depending on the used medium (e.g. complete vs. minimal) and resembled vastly the phenotypic outcome of the deletion of *pssO* gene. Unknown relation between Mgl2 and PssO was further reinforced by the unusual behaviour of PssO protein when analysed through Western immunoblotting of whole cell proteins from the wild-type and the *mgl2* mutant. Mgl2 might play a role in modification of PssO; however, the nature and the mechanism are still to be discovered. Although no interactions of Mgl2 with previously characterized Pss proteins, PssV or RegA were revealed in the two-hybrid analysis, it remains an open question if *regA*, *mgl2*, and *pssV* are components of a two-component signalling system. Mutagenesis of the remainder genes is expected to reveal any potential relationships. An overview of the bioinformatic analyses of hypothetical PssV and RegA proteins sequences will also be presented.

1. Król, J. E., Mazur, A., Marczak, M., Skorupska, A. 2007. Genomics 89:237–247.
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Poster # 52

THE INFLUENCE OF SELECTED INHIBITORS OF HOST IMMUNE RESPONSE ON THE COURSE OF EXPERIMENTAL RABIES INFECTION IN MICE

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Rabies is known as a disease since around 4000 years. Despite the development of knowledge rabies virus causes over 60 000 human death yearly [1]. It is likely, the pathogenesis of rabies encephalitis is multifactorial with both virus and host factors being involved similar to many others neurotropic virus infections. Understanding the mechanism of rabies virus pathogenesis and the host immune response are crucial to identify potentially therapeutic molecules used for the treatment of rabies encephalitis.

The main goal of the study was to investigate the effect of selected immune response inhibitors on the course of rabies virus infection in mice. Eight weeks old mice were infected with SHBRV-18 strain. Since fifth day after virus infection mice were treated with MAPKs, IL – 6 and TNF-α inhibitors respectively. Mitogen-activated protein kinases plays role in regulation of immune response and have effects on gene expression and cell apoptosis. IL-6 and TNF-α play an important role in the inflammatory and immune response. Animals were monitored twice a day and euthanized when hind quarter and paralysis were established.

After animal experiment several analyzes were done. Survival curves comparison was carried to find if treatment with above – mentioned inhibitors has an impact on rabies infection. Virus titre analysis and analysis of number of copies of gene for rabies nucleoprotein were done to investigate the effect on virus life cycle and administration of the virus in nervous system. To find the impact of selected drugs on immune response analysis of expression of selected genes were carried. Results from infected/ treated groups were compared with virus control group. All the results were analyzed using appropriate statistical methods.

This work was performed under the international project ASKLEPIOS [2].

[1] Cleaveland S., Fevre E.M., Kaare M., Coleman P.G. (2002) Estimating human rabies mortality in the United Republic of Tanzania from dog bite injuries, Bull. World Health Organisation, 80:304-310
[2] http://asklepiosfp7.eu/

Poster # 53

FIRST CASE OF BBLV INFECTION IN BAT IN POLAND

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Bats are known as carriers of the rabies virus since 100 years. However, their role as a reservoir of this virus is not exactly known. Moreover, bats can be a reservoir for another viral diseases that can potentially be transmitted to humans and animals.

Lyssaviruses cause progressive encephalitis known as rabies. Each year almost 70 000 human dies on rabies is registered, 95% of which is caused by rabid dogs. Unregistered cases can be even more. Until the second half of the 20th century there were only one known virus representing genus *Lyssavirus*. Today we can distinguish 14 species within genus *Lyssavirus* [1]. Three species still waiting for classification are: *Lleida bat lyssavirus* (LLEV), *Gannoruwa bat lyssavirus* (GBLV) and *Taiwan bat lyssavirus* (TWBLV). All of the Lyssaviruses (excluding *Makola lyssavirus* and *Ikoma lyssavirus*) were isolated from bats.

Most of the viruses isolated from rabid bats in Europe belongs to EBLV – 1 (*European bat 1 lyssavirus*) and EBLV – 2 (*European bat 2 lyssavirus*) species. First isolation of *Bokeloh bat lyssavirus* from bat (*Myotis nattereri*) took place in 2010 in Germany. The same species was later isolated in 2012 in France and Germany. The first case of rabies in bats (family *Vespertilionidae*) in Poland was reported in 1972. Until 1998 there were only 3 more rabies cases in bats in Poland (1985, 1990, 1995). After this time number of bats examined for rabies increased. Until last year, 81 cases of bat rabies in Poland were registered, mostly caused by *European bat 1 lyssavirus.*

The first case of BBLV infection in bat in Poland was registered on the 26th October 2016 in Greater Poland. The brain from bat was examined with immunofluorescence test. After receiving a positive result molecular analyzes were done to define the species of the virus. NGS analysis confirmed BBLV genotype.

[1] https://talk.ictvonline.org/taxonomy/

Poster # 54

TRANSCRIPTIONAL ANALYSIS OF *GMS* GENES INVOLVED IN THE SYNTHESIS OF GLUCOMANNAN IN *RHIZOBIUM LEGUMINOSARUM* BV. *TRIFOLII*

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Soil bacteria belonging to the family Rhizobiaceae, collectively called rhizobia, possess a unique ability to establish symbiosis with legumes (Fabaceae). These bacteria infect host plant roots and induce the formation of special structures called nodules, within which they convert atmospheric dinitrogen to ammonia, a plant-accessible form of nitrogen. This type of symbiotic interactions is highly important for the functioning of the biosphere, from both ecological and economic perspectives, since the amount of reduced of nitrogen introduced into the environment from symbiosis is similar to that introduced into the soil in the form of artificial nitrogen fertilizers.
The establishment of symbiosis is a complex process involving a coordinated exchange of signals between legumes and their microsymbionts, among them flavonoids secreted by host plant roots and bacterial lipo-chito-oligosaccharides play key roles (Gibson et al. 2008). In addition, both bacterial and plant cell surface components participate in this plant-microbe interaction.
Rhizobia produce various types of surface polysaccharides, such as exopolysaccharide, lipopolysaccharide, capsular polysaccharide, gel-forming polysaccharide and glucomannan. The last of these polysaccharides plays a significant role during the first stage of host plant infection (Williams et al. 2008). Glucomannan is mainly composed of glucose and mannose (95%), with small amounts of galactose and rhamnose (5%). A mutant strain of *Rhizobium leguminosarum* unable to synthesize glucomannan was shown to be totally defective in attachment and biofilm formation on root hairs in acidic pH.
In this study, we performed a transcriptional analysis of *gms* genes involved in glucomannan synthesis of *R. leguminosarum*bv. *trifolii*. We determined *gms* expression in the wild-type strain Rt24.2 and the *rosR* mutant Rt2472. In addition, the influence of various environmental factors on the transcription of *gms* genes was established. For these analyses, a *gms-lacZ* transcriptional fusion, containing a promoter region of *gms* genes cloned upstream of promoterless *lacZ,* was constructed on the pMP220 plasmid and introduced into the Rt24.2 and Rt2472 strains. It was established that *gms* operon contained a promoter sequence of a moderate strength (653 Miller units). Moreover, the level of *gms-lacZ* transcription was four times lower in the *rosR* mutant than in the wild-type strain, confirming the important role of the global regulatory protein RosR in positive regulation of the expression of the genes involved in glucomannan production. In addition, it was established that transcription of *gms* genes was dependent on the type of carbon source used, with the highest expression level observed in a culture medium containing mannose.

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Poster # 55

THE INFLUENCE OF VARIOUS NITROGEN SOURCES ON TRANSCRIPTION OF *PSS* GENES INVOLVED IN THE SYNTHESIS OF EXOPOLYSACCHARIDE IN *RHIZOBIUM LEGUMINOSARUM* BV. *TRIFOLII*

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Rhizobium leguminosarum bv.*trifolii* belongs to a unique group of soil bacteria which are able to reduce atmospheric dinitrogen to ammonia inside specialized organs formed on legume roots, called nodules, thus providing available nitrogen forms to host plants and making them independent of the external input of this nutrient. Owing to their nitrogen-fixing properties, rhizobia play a significant role in the environment from both the economic and ecological point of view (Olivares et al. 2013). *R. leguminosarum* bv. *trifolii* is a microsymbiont of clover plants (*Trifolium* spp.), which are commonly cultivated in temperate climates. This microorganismproduces large amounts ofacidic exopolysaccharide (EPS), which plays an important role in bacterial protection against various stress factors as well as in symbiotic interactions with host plants. As reported recently, the low-molecular-weight fraction of EPS secreted by rhizobia is also a signal molecule involved in early stages of symbiosis (Kawaharada et al. 2015). The structure of the EPS produced by *R. leguminosarum* has been established in detail. This polymer contains octasaccharide repeating subunits composed of D-glucose, D-glucuronic acid, and D-galactose in a molar ratio 5:2:1. These subunits are additionally substituted with pyruvyland *O*-acetyl groups. EPS production by *R. leguminosarum* depends on soil conditions such as availability of some nutrients (nitrogen, phosphate, and carbon sources) and legume root exudates (Downie 2010).
In this study, we established the influence of various nitrogen sources on the transcription levels of *pss* genes involved in EPS synthesis of *R. leguminosarum* bv*. trifolii*. The experiments were performed using *pss-lacZ* transcriptional fusions for *pssD, pssJ, pssP, pssT, pssV, plyA* and *exoB* genes. It was established that among the *pss* genes tested, the limitation of nitrogen sources (NH_4Cl and KNO_3) positively affected the expression of the *plyA-lacZ, pssD-lacZ, pssP-lacZ* and *pssV-lacZ* fusions. In contrast, the expression of *exoB-lacZ* was decreased when the nitrogen source was limited. The transcription of the remaining *pss* genes was independent of the kind of nitrogen source used and their concentration in the culture medium. Our data indicate that this environmental factor plays an important role in the regulation of the expression of the *pss* genes involved in EPS synthesis in *R. leguminosarum* bv. *trifolii.*

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Poster # 56

THE ROLE OF STRINGENT RESPONSE FACTORS ON TRANSCRIPTION FROM *ESCHERICHIA COLI* TRNA PROMOTERS

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One of the most important processes taking place in bacterial cells is gene expression. Because of the importance of this process, it is a subject to very strict control and one of the global mechanisms involved in its regulation in *E. coli* is stringent response. This mechanism is mediated by specific nucleotides – guanosine tetraphosphate and pentaphosphate (ppGpp and pppGpp) which are commonly called stringent alarmons. Level of these specific nucleotides increases during stress conditions, e.g. in various physical conditions. The most spectacular effect of the stringent response is down-regulation of the energy-consuming processes such as ribosomal promoter activity. Several tRNA promoters are also under negative control of ppGpp. In addition to ppGpp, DksA protein (belonging to group of proteins that can bind to RNA polymerase (RNAP)) can also regulate gene expression during adverse environmental conditions. Very important role in transcription regulation is also played by the discriminator. A specific sequence located in a promoter region between -10 box and +1.

Our RT-PCR analysis indicates that the regulation by ppGpp and DksA molecules can differ for each of the tRNA promoters. The main aim of this work is to investigate the mechanism of regulation of transcription from tRNA promoters by this three factors: discriminator sequence, ppGpp and DksA. Here, we report differences in *in vitro* transcription efficiency from tRNA promoters with different sequence of discriminator, in the presence of increasing concentrations of ppGpp and DksA. It is known that ppGpp has negative influence on transcription from several tRNA promoters. Our results from RT-PCR analysis confirmed this also for the remaining promoters. These results indicated that the most important place in discriminator sequence, for ppGpp and DksA regulation, is -5 position. It was reported that tRNA promoters are inhibited by ppGpp in stationary phase. DksA also affects tRNA transcription initiation. Depending on the tRNA promoter, the effect of ppGpp and DksA, can vary, from synergistic to antagonistic. Using *in vitro* transcription analysis, we confirmed that both ppGpp and DksA can activate or inhibit transcription from tRNA promoters. We also showed that both of the stringent response factors may have influence on open complex stability of some tRNA promoters.

Poster # 57

COMPARISON OF DIFFERENT METHODS IN IDENTIFICATION OF *SALMONELLA* SP. USING PCR ASSAY, REAL-TIME PCR, MADI-TOF AND REFERENCE METHOD ACCORDING PN-EN 6579:2003

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Pathogenic bacteria of *Salmonella* genus are the most common infections in food industry. They might to contaminate wide range of products, protein food e.g. meat, milk food, eggs and egg foods, plants and its preserves, fodder and feeds. Detection and identification of *Salmonella* sp. are made using traditional methods corresponding with standard PN-EN 6579:2003. This time consuming and laborious method is commonly used in the microbiological laboratories.
The aim of the work was to evaluate the usefulness of a classic PCR, real-time PCR method and MALDI TOF analysis to detect the bacteria *Salmonella* in foodstuff samples. The results were confirmed by a classic method (PN-EN ISO 6579:2003) and verified by many interlaboratory tests.
PCR with all the *Salmonella* strains produced a 343 bp DNA fragmentthat was absent from all the non-*Salmonella* strains tested. Interlaboratory proficiency studies also included foodstuff samples from outside suppliers and the analyses were considered tests confirming the results achieved with a classic method and proteomic fingerprint identification with MALDI Biotyper method
Strains:*Salmonella* sp. KKP 1008, KKP 1039, KKP 1040, KKP 1169 from Culture Collection of Industrial Microorganisms, Institute of Agricultural and Food Biotechnology, Warsaw.
Using a modern molecular biology and proteomic techniques allows to confirm the *Salmonella* identification in 24-hours without enrichment and isolation steps. The development of MALDI-TOF MS technology for bacterial strains identification is a simple, low-cost and rapid method.

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POSTER SESSION 2

Poster # 1

SOLVING THE PUZZLE OF MOLECULAR MECHANISMS OF ISOTHIOCYANATES EFFECT ON SHIGA-TOXIN PRODUCING STRAINS

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Since penicillin development, bacterial infections are still a serious problem worldwide. Phytochemicals are a great source of bioactive compounds with unique properties which may be used to combat bacterial pathogens. Isothiocyanates (ITCs) are a most promising group well known from their biological activity in chemoprevention, anti-inflammatory effect and antibiotic properties. Shiga toxin (Stx) is one of the most potent bacterial toxins. Stx is found in *Shigella dysenteriae* 1 and in some serogroups of *Escherichia coli* STEC (called Stx1 in *E. coli*). In STEC, these toxins are often encoded on lambdoid bacteriophages and are major virulence factors for these organisms. Although the bacteriophage-encoded *stx* genes of STEC are highly mobile, the *stx* genes in *S. dysenteriae* 1 have been suggested to be chromosomally encoded and not transmissible. In this report we evaluated a possibility of using natural phytochemicals - isothiocyanates against pathogenic bacteria producing Shiga toxins: *Shigella dysenteriae* as well as STEC group members. The main aim of this work was to investigate the molecular mechanism of ITCs action and analysis of their impact on *stx* genes expression and toxin synthesis.
Here, we present evidence that isothiocyanates treatment leads to bacterial growth inhibition together with significant impairment of main cellular processes such as DNA and RNA synthesis. Moreover, these compounds provoke strong induction of the host global regulatory mechanism, the stringent response, comparable to this observed under amino acid starvation. The elevated level of stringent control alarmone, (p)ppGpp, in host cell can effectively inhibit Stx bacteriophages development and impair toxins production, abolishing STEC virulence. We conclude that isothiocyanates are potent phytochemicals, and their activity may be considered to be used in fight against Shiga toxin producing bacteria.
Keywords: Enterohaemorrhagic *Escherichia coli*; *Shigella dysenteriae*; *stx*; Shiga toxin; isothiocyanate; sulforaphane; antibiotic; phytochemicals.

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Poster # 2

THE IMMUNIZATION EFFECT OF THE BCG OR RECOMBINANT BCG WITH THE EXPRESSION OF IL-18 (RBCGMIL-18) ON SELECTED IMMUNE PARAMETERS IN IMMUNOSUPPRESSED AND IMMUNOCOMPETENT MICE C57BL/6

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According to the WHO, 1/3 of the world population is latently infected with *Mycobacterium tuberculosis*, The reactivation of TB infection can occurred under specific circumstances e.g. during immunosuppressive drug administration, during autoimmunological diseases and cancer therapy or post-transplantation procedures.
The aim of the study was to compare the effect of BCG and rBCG immunization on selected immune parameters in immunosuppressed and immunocompetent mice C57BL/6. The histological analysis of lungs and the axillary lymph nodes was performed, as well as, the levels of IFN-ϒ and IL-10 in the culture supernatants of lymph nodes cells after their 3-day stimulation with mycobacterial antigens (PPD, lysate, BCG, rBCG) were measured.
The mice were injected with BCG or rBCG at the dose of 1×10^6. 6 weeks post the immunization, mice were treated intraperitoneally with CTX (50 μg/per body weight) or NaCl for 7 consecutive days. The isolated tissues were assessed with paraffin embedded sections. The level of the cytokines was determined by ELISA.
There were no significant differences in microscopic view of lung tissue in all experimental groups. The lymph nodes from BCG/NaCl and rBCG/NaCl groups were clearly larger than the lymph nodes from the CTX groups. Moreover, the lymph nodes from BCG/CTX and rBCG/CTX groups showed no follicles with germinal center development. The highest production of IFN-ϒ was observed in BCG/NaCl group independently of the stimulator which was used. In case of rBCG/NaCl group the level of IFN-ϒ was dramatically lower in comparison to BCG/NaCl group after PPD, Mtb lysate and BCG stimulation. Thus, the CTX administration were accompaniedwith the lymph nodes size reduction and the decrease in the number of follicles. Generally, the CTX administration sharply decreased IFN-ϒ production by lymph node cells in BCG immunized mice whereas this effect was not observed when mice were treated with rBCG.

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Poster # 3

DAIRY PROPIONIBACTERIUM STRAINS WITH POTENTIAL OXALATE DEGRADING ACTIVITY AND THE EFFECT OF EXOPOLYSACCHARIDE PRODUCTION

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Hypercalciuria and hyperoxaluria are among the most important pathophysiologic causes of kidney stone formation. An increased intestinal absorption of oxalate is known to lead to hyperoxaluria with a significantly enhanced risk of urinary stone formation. Propionic acid bacteria (PAB) have widely been used as probiotic and starter cultures in dairy products. In the present study, five strains of *Propionibacterium*, which were identified and selected from 29 strains isolated from traditional homemade Turkish cheeses, exhibited gastrointestinal system tolerance, antimicrobial activity, resistance to different antibiotics, autoaggregation and coaggregation abilities (Onal Darilmaz and Beyatlı, 2012a and 2012b; Onal Darilmaz 2013). All strains were examined for their abilities to survive at 10 and 20 mM oxalate concentrations, and exopolysaccharide (EPS) production in these conditions as well as oxalate degrading activities. There was no evidence about the EPS production ability of *Propionibacterium* strains in oxalate containing medium and its correlation with oxalate degradation activity. To determine the toxic effect of oxalate on EPS production ability and viability, EPS production of Propionibacterium strains was determined in YEL, 10 and 20 mM YEL-ox media. All strains grew in the presence of 10 and 20 mM sodium oxalate illustrating that oxalate at these concentrations are not toxic to *Propionibacterium* strains. No major loss of viability was observed at YEL-ox media. In this study, different amount of EPS were produced by different strains; however, EPS levels ranged 89.82 to 109.07 mg/L in YEL media and from 67.04 to 143.79 mg/L in 10 mM YEL-ox and from 55.00 to 119.14 in 20 mM YEL-ox media. A high variability in the oxalate-degrading capacity was found in the different species and oxalate concentrations. The oxalate degradation behavior shown by the two *P. freudenreichii* subsp. *freudenreichii* strains tested was heterogeneous. *P. freudenreichii* subsp. *freudenreichii* DO8 strain showed 10.57-42.45% of oxalate consumption in both media respectively and *P. freudenreichii* subsp. *freudenreichii* DO6 strain exhibited 9.56-11.96% degrading activity. The high EPS-producing *P. jensenii* BDP6 and *P. freudenreichii* subsp. *freudenreichii* DO8 strains showed high oxalate degrading activity, whereas the low EPS-producing *P. jensenii* DO6 showed low oxalate degrading activity in both oxalate concentrations. A better understanding of the mechanisms related to EPS production and oxalate degrading activity can be used for preliminary screening in order to determine potentially probiotic bacteria applications for human or animal use.

Poster # 4

EVALUATION OF AGGREGATION, HYDROPHOBICITY PROPERTIES AND EXOPOLYSACCHARIDE PRODUCTION OF *ENTEROCOCCUS FAECIUM* STRAINS ISOLATED FROM TRADITIONAL NATURALLY FERMENTED CHEESE PRODUCTS IN DIFFERENT REGIONS OF IRAN AND TURKEY

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The purpose of this study was to evaluate the some probiotic properties of *Enterococcus faecium* RI41, RI56, RT81 strains isolated from traditional naturally fermented cheese produced in different regions of Iran and Turkey. All isolates and E. faecium DSMZ 20447 reference strains were examined with regard to their hydrophobic characteristics and their autoaggregation and coaggregation abilities since these traits have been shown to be indicative of adherence in other microorganism or epithelial surfaces. Therefore, in the current study, we assessed the effect of exopolysaccharides (EPSs) produced by *E. faecium* strains on the aggregation and hydrophobicity properties. All of the tested strains showed the strong adhesion to ethyl acetate, a basic solvent, in comparison with microbial adhesion to chloroform, an acidic solvent, which demonstrated the particularity of *E. faecium* to have an important electron donor and acidic character. Also, these strains simultaneously showed affinity to 3 hydrocarbons, suggesting a high complexity of the cell surface. *E. faecium* strains tested showed autoaggregation and coaggregation ability with the *Salmonella enteritidis* ATCC 13076, but the results were strain-specific dependent on EPS production and incubation conditions. These observations suggested that the strain *E. faecium* RI41 may be used in the future as a good candidate probiotic bacteria suitable for human or animal use.

Poster # 5

CHARACTERISTIC OF A HALOPHENOL-DEGRADING SOIL STRAIN – *MICROBACTERIUM RESISTENS* MCHC

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Halophenols belong to the group of the aromatic compounds of wide use. They find applications in production of pesticides and antiseptics as well. Moreover, the synthesis of several pharmaceuticals bases on halophenols. It should be noticed, that the halogenated derivatives of aromatic compounds are considered as persistent organic pollutants of toxic impact on environmental organisms (Juretic et al., 2014). The crucial role in biodegradation of halophenols in ecosystems play bacteria. However, only few strains are capable of effective degradation of this group of pollutants. The fission of the aromatic ring and dehalogenation are considered as the most important part of metabolic pathways involved in bacterial degradation of halophenols. Nevertheless, the bioavailability of pollutants for the cells of microorganisms can be a major factor limiting the efficiency of the biodegradation (Parales et al., 2008). Hence, the cell adaptation processes occurring in the cell structure and influencing on bioavailability are important research areas.

The aim of the study was to evaluate the biodegradation potential of *Microbacterium resistens* MChC strain, isolated from the soil sample. The genetic identification of the strain as well as its biochemical profile were investigated. Moreover, the membrane fatty acid profile was studied. The further stage of research included the biodegradation tests with three phenol derivatives (4-fluorophenol, 4-chlorophenol and 4-bromophenol). Additionally, the changes in cell surface properties of the strain, accompanying the biodegradation process, were analysed. In that purpose the measurements of zeta potential and cell surface hydrophobicity were realized.

The experiments indicated that in the cell membrane dominate the anteiso fatty acids with 15 and 17 carbon atoms, representing respectively 40% and 30% of total membrane fatty acids. Moreover, in the presence of the degraded halophenols the noticeable changes in cell surface properties were observed. The studied bacterial strain shows significant ability to biodegrade 4-fluoro- and 4-chlorophenol. In the 21-days cultures there was observed decay of more than 43% and 23% of the initial amount of these two compounds, respectively. It indicates the considerable potential of the isolated strain for application in bioremediation of halophenols that contaminate wastewaters and soil.

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Poster # 6

AROMATIC AND ALIPHATIC HYDROCARBONS BIODEGRADATION BY HYDROCARBONS-EXPOSED MICROORGANISMS

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Organic compounds entering the environment from anthropogenic sources, such as hydrocarbon pollutants, are generally considered to be toxic and hazardous to health (Dąbrowska et al., 2005). Their presence in the soil affects the properties of environmental microorganisms such as surface properties and proteins, as well as factors responsible for transport and metabolism of hydrocarbons (Segura et al., 1999). Moreover, some of the microbes exposed on hydrocarbons-stress are capable to remove the pollutants (Kaczorek et al., 2013).
In this studies, we compared hydrocarbons degradation properties of three environmental strains kept on glucose and exposed on selected hydrocarbons for twelve months. For this purpose, microorganisms were isolated and identified biochemically and genetically. Then, bacteria capable to grow in the presence of hydrocarbon were subjected to a 12-month exposure to aliphatic and aromatic hydrocarbons. At the end of the exposure period, changes in surface properties, membrane composition and fatty acid profiles in cells were defined and compared to those obtained for control strains. After that, comparative analyzes of the efficiency of biodegradation of selected hydrocarbons by bacteria exposed to hydrocarbons and cultured on solid media were performed.
The results of the studies showed significant changes in the properties of cells exposed to hydrocarbon contaminants compared to control samples. We noticed the decrease of surface zeta potential and changes of surface properties to more hydrophobic. Furthermore, hydrocarbons-exposed cells significantly modify the composition of the cell membrane fatty acids. All the observed changes allowed them to more effective transfer and biodegradation of the hydrocarbon substrates, as the biodegradation rates of selected hydrocarbons increased by 15-50% comparing to control, reaching the final efficiency around 70% after 14 days for all analyzed compounds.

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Poster # 7

THERMOPHILIC ACTINOBACTERIA AS A CAUSE OF MUSHROOM COMPOST WORKER'S LUNG

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Thermophilic bacteria, often found in composting plant material, represent trigger to the occupant respiratory health. For the first time, an occupational respiratory disease in the workers employed at farms growing white button mushrooms (*Agaricus bisporus*) was described in the USA in 1959 [1]. Next cases of the disease defined as the "Mushroom Compost Worker's Lung" (MCWL) indicated thermophilic actinobacteria as possible etiologic factors of the disease.

In the years 2009-2017, 10 workers employed at the production of compost destined for cultivation of the spawn of white button mushroom and/or at cultivation of spawn itself, presented at the Clinic of Pneumonology of the Medical University in Lublin, reporting acute respiratory and general symptoms after the inhalation exposure to dust from compost. In all of them the diagnosis of hypersensitivity pneumonitis (HP) was established. To test the suspected role of thermophilic actinobacteria as causative agents of disease, two samples of compost used for cultivation of mushroom spawn were subjected to microbiological analysis. The concentrations of bacteria and fungi in the compost samples were determined by dilution plating. In compost besides of more abundant *Bacillus* spp. two types of thermophilic actinobacteria were isolated. Actinobacteria of type 1 (X-1) appeared macroscopically as hard, light colonies covered with snow-white "aerial mycelium", and that of type 2 (X-2) were in the form of hard, brown colonies with white-yellowish aerial mycelium. These two isolates, (X-1) and (X-2), were selected for the phenotypic and genotyping identification tests and for production of antigens used for serologic examination.

Both strains had distinct chemotaxonomic profile. Strain X-1 was characterized by presence of phospholipid type I, one major glycolipids, branched chain fatty acids from C14:0 to C18:0 (with major *iso* C16:0), and no DAP isomer in peptidoglycan. In contrast, strain X-2 possessed phospholipid type II, two major glycolipids, branched chain fatty acids (major fatty acid *iso* C15:0), and *meso* DAP isomer in peptidoglycan. Both strains were analyzed by MALDI-TOF mass spectrometry Biotyper system and revealed diverse MALDI-TOF mass spectra. However, regardless of the different preparation methods used [2], the identification in Biotyper database (6903 entries) was unreliable. The 16S rRNA gene sequence (1200 bp) of X-1 strain showed the highest similarity (97.04%) to *Ureibacillus thermosphaericus* DSM 10633^T and strain X-2 revealed 100% similarity to *Laceyella sacchari* KCTC 9790^T. The chemotaxonomic characteristics were consisted with genotyping results.

The agar-gel precipitation test was performed by Ouchterlony double diffusion method with the antigens of X-1 and X-2 isolated from compost and 11 other antigens used in the routine diagnostic testing for HP. The one serum of mushroom compost worker showed a strong reaction to the antigen of X-1 isolate. Further studies, aiming to determine, with genomic methods, the identity of the actinobacterial species suspected to be a causative agent of MCWL, and other thermophilic strains are currently in progress.

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Poster # 8

ORAL MICROBIOME OF PATIENT WITH MOUTH ULCERS AFTER STEROID THERAPY

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Treatment of respiratory tract diseases by inhalation is a form of therapy which has been used for many years. Although the topical manner shows lower potency of side effects, nevertheless secondary immunodeficiency and sinopulmonary infections are underestimated. Patient with severe oral mucositis was admitted to the Department of Clinical Immunology in Wroclaw because of suspicion of Sjögren's syndrome. Immunological analysis excluded primary immunodeficiency, especially deficiency of IgA that was at normal level in blood, but quantitative deficit of saliva was observed. His past history comprise hypersensitivity with rhinitis and asthma treated by topical steroids (fluticasone furoate or propionate) with episodes of overdosing. The aim of the studies was identification of cultivable microorganisms from patient with oral mucositis after steroids therapy.

The study material included two swabs from the mouth ulceration. The isolates were cultured under aerobic and anaerobic conditions at 37° C on blood agar, tryptic soy agar or BHI agar from 24 hours to 7 days. Identification of the isolates was carried out using the MALDI- TOF Biotyper 3.1, according to the manufacturer. MALDI-TOF Biotyper analysis demonstrated the presence of a number of oral bacteria of the genera *Neisseria* and *Streptococcus*, alongside with the potentially pathogenic species *Actinomyces* spp. and *Rothia* spp. Three different *Rothia* species with distinct MALDI-TOF mass spectra were identified: *R. aeria*, *R. dentocariosa* and *R. mucilaginosa,* and were analysed also by chemical markers. The *Rothia* spp. studied possessed one major glycolipid of the same TLC mobility (Pasciak et al. 2004). The analysis of whole cells fatty acids showed similar profile of branched chain fatty acids C15:0, C16:0 and C17:0. Contrary to other isolates *R. dentocariosa* strain had additional monounsaturated C16:0 and C18:0 acids. *Rothia* spp. are part of human oral microbiome and are considered as opportunistic species, but isolation of these strains from patient with mouth ulcers after steroids therapy may suggest their participation in infectious process. Although IgA concentration in blood and saliva were normal, the serious infection with salivary gland defect prompt low excretion of all humoral component of saliva: IgA as well as lysozyme, and amylase. The local oropharyngeal deposits of steroids impair innate immune response: epithelial barrier, lysozyme. Weak digestion of sugars by amylase, proapoptotic concentration of steroids in oral cavity shows hot-spot of inhalation therapy. Ideally, particles should be approximately under 5 μm (respirable range) for inhalation into the lungs. Particles greatly exceeding 5μm diameter are readily deposited in the oropharynx and those particles which reach the lungs are unlikely to penetrate to the periphery. The disadvantages of aerosol steroid therapy could be inappropriate deposition in upper respiratory tract causing microbiome modification. Changes in oral microbiome after the use of inhalation therapy needs further studies.

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Poster # 9

AN SCO6288 SARP PROTEIN IS AN ACTIVATOR OF A COELIMYCIN PRODUCTION BY STREPTOMYCES COELICOLOR A3(2)

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Antibiotics and other bioactive compounds production by streptomycetes coincides with morphological differentiation. *Streptomyces coelicolor* A3(2) is a model organism for investigation of the regulatory network which coordinates these processes. One of the products of *Streptomyces coelicolor A3(2)* is coelimycin (CPK) – yellow polyketide of unknown properties. This substance is synthesized by a type I polyketide synthase, which is coded by *cpk* gene cluster, whose expression is tightly controlled. CpkN is a regulatory protein homologous to SARPs (*Streptomyces Antibiotic Regulator Proteins*) coded by SCO6288 gene in the cpk cluster.

The aim of the study was to investigate the influence of CpkN protein on coelimycin production and expression of Cpk polyketide synthase gene cluster. The CpkN function was analyzed in connection with the second cluster situated SARP protein –CpkO.

The study involved construction of *S. coelicolor* mutant strains with induced over-expression of *cpkN* gene and luminescent promotor probes monitoring activity of cluster promotors.

Experiments have shown that the level of coelimycin synthesis correlates with the level of CpkN protein expression. Induced CpkN overexpression may over-take control of some of the factors necessary for activating coelimycin synthesis, while other conditions such as growth phase have to be met. We have found that CpkN protein is an activator of coelimycin production, but other factors and activators are required to activate the synthesis process.

Poster # 10

THE USE OF PHENOTYPE MICROARRAY TO DEMONSTRATE THE RELATIONSHIP BETWEEN *CERRENA UNICOLOR* METABOLIC PROFILE AND SAWDUST SUBSTRATE

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Cerrena unicolor, commonly known as a mossy maze polypore, is an aggressive decay organism which also attacks living trees causing extensive white rot. This wood-degrading basidiomycete commonly lives on hardwoods such as *Aesculus hippocastanum*, *Fraxinus excelsior*, *Acer* sp., *Betula* sp., *Fagus* sp. or *Quercus* sp., very rarely reported on conifers. Strains of *C. unicolor* are also well described as producers of enzymes and other bioactive compounds of pharmacological and medical importance. Although genomic and transcriptomic analysis has provided new insights into*C. unicolor* ligninocellulose degradation, the knowledge related to its phenotypic characterization and the effect of environmental conditions on fungal metabolism has not been studied.

Here we report a comparison of the metabolic profiles of *C. unicolor* FCL139 cultivated under SSF (solid-state fermentation) conditions on a different sawdust substrates. Biolog PM1 and PM2 Phenotype Microarrays (PMs) were applied to obtain data on utilization of 190 different carbon sources and mitochondrial activity of *C. unicolor* cultivated on a maple, ash, and birch sawdust substrates. The analysis revealed a broad variability of substrate utilization profiles and the substrate richness (R) values demonstrated a significant differences. The highest catabolic activities were exhibited when the birch sawdust was used. There is a clear correlation in metabolic preferences to a particular group of substrates.*C. unicolor* cultivated on the ash and maple sawdust exhibited preferences for fatty acids utilization. Whereas, polymers and fatty acids/carbohydrates constitute a groups of the most easily metabolized carbon sources when *C. unicolor* was grown on a birch sawdust and in a control medium (LH), respectively. In general, mannose, xylose, arabinose, dextrin, pectin, and dihydroxyacetone were used most universally by all *C. unicolor* sawdust variants.

Summarizing, with the development of Biolog Phenotype MicroArray technology, high throughput determination of microbial nutritional requirements and global phenotypic characterization is now possible. In context of *C. unicolor* abilities to degrade of wood material and production of biotechnologically significant compounds, better understanding of selective substrate utilization may be extremely valuable. Furthermore, it can contribute to explaining the parasitic lifestyle of the fungus allowing it to decompose several kinds of wood.

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Poster # 11

TAXONOMY AND ECOLOGY OF LIGNIN DEGRADING FUNGI

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Lignin is a complex oxyphenyl propanoid polymer that occurs in plant cell walls. The complex is found in all vascular plants including herbaceous species, which provides rigidity, support, and protection to plants. Lignin encloses the bundle cells, such as wood fibres and sclerenchyma cells. The lignin in wood is relatively difficult to decompose by most biochemical mechanisms, while cellulose in wood is oxidized and depolymerized rapidly.

Microbiological lignin degradation is a complex biochemical process, in which bacteria, fungi, and insects are involved. However, fungi are the most efficient lignin degraders in nature and for this reason play a key role in C cycling. Fungal species that degrade lignin are often grouped into white rot (WRF), brown rot (BRF), and soft rot (SRF), based on the colour of the decayed substrate. Fungi breaking down wood mostly live as saprotrophs or weak parasites in natural and anthropogenic ecosystems. Lignin degradation by fungi has been most extensively studied in Basidiomycota, Ascomycota, and their anamorphic stages, in which a number of enzymes and mechanisms involved in lignin attack have been elucidated. The most efficient lignin degraders in nature are basidiomycete fungi causing white rot of wood. Wood decay species in Basidiomycota mostly belong to the Polyporales, Agaricales, Atheliales, Boletales, Gloeophyllales, Hymenochaetales, and Russulales orders. Ecologically, they represent white and brown rot fungi, litter-decomposing, plant-pathogenic, and ectomycorrhizal fungi (ECM). Representatives from Ascomycota (mainly members of the Xylariales order) and their anamorphic stages mostly cause soft rot decay.

The fungal community changes during the time of litter and wood decomposition. Fungal succession of woody substrates, i.e. the source of organic matter, shows dominance of Basidiomycota in the earlier stages of wood decomposition and Ascomycota in the later stages. Additionally, studies on some fungal species and their relationship with plant species have shown specialization with reference to the substrate type (i.e., hardwood, softwood, sapwood, heartwood).

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Poster # 12

DANYLO ZABOLOTNY, THE SCIENTIST AND THE CITIZEN TRIBUTE TO THE 150TH ANNIVERSARY OF ACADEMICIAN D.K. ZABOLOTNY

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Glorious names of the scientists, who contributed not only to certain scientific discoveries but also to the course of human civilization for decades and centuries, will be always remembered by generations. The merits of the prominent scientist, one of the founders of modern epidemiology and microbiology Danylo Kyrylovych Zabolotny, whose 150th anniversary we celebrate now, are outstanding. His research of plague, cholera, malaria, diphtheria, typhoid fever and epidemic “jail fever” typhus, syphilis, gas-gangrene and other infections is well known all over the world.

After the high school graduation, having bright propensity to life sciences Danylo Zabolotny unhesitatingly choose to study at the natural science department of Physics and Mathematics faculty of Novorossiysk University in Odessa.

In 1886 I.I. Mechnikov organized the first in Russia bacteriological station in Odessa. Zabolotny joined this laboratory after his release from detention and became very interested in bacteriology. There he studied the microflora of snow and hydrogen sulfide bacteria. Simultaneously, continued experiments on Odessa estuaries phosphorescence. In 1890 Danylo Kyrylovych started to develop experimental model to research the causative agent of cholera. His experiments with gophers convincingly proved etiological role of Vibrio cholerae in the disease development as well as the possibility of enteral immunization of these rodents against cholera.

His investigation on cholera agents influence are of great importance. The scientist has proved that these animals are very sensible to cholera vibrios, but it was possible to make them immune with the help of oral vaccination.

The alarming news came from India about the plague outbreak in Bombay in the autumn of 1897. This caused a big concern in many countries. The head of the research team studying the plague in Bombay, Kyiv Professor V.K. Vysokovych invited Zabolotny as his research assistant. Zabolotny happily agreed to take part in this expedition. Vysokovych and Zabolotny managed to do a great job there. They studied in details the changes that occurred in the organisms affected by plague, discovered that the blood serums of the plague survivors were able to stick together the plague bacteria, and established the monkeys perception to plague.

In 1897–1900 he also had expeditions to Arabia, Eastern Mongolia, Mesopotamia, Scotland, Kirghiz steppes, the Volga region and other places to research and fight plague outbreaks.

Zabolotny managed to reveal the main “secret of plague". It was discovered that the source of plague and its carriers are rodents abundantly inhabiting Mongolian plains, namely: the tarbahans.

Apart from his main research work on plague, Zabolotny studied many other serious diseases: cholera, syphilis, typhus, etc., and received extremely important theoretical and practical results.

In 1928 D.K. Zabolotny was elected as the President of All-Ukrainian Academy of Sciences. Holding the presidential position, he enthusiastically implemented the re-organization of the Ukrainian academy of sciences based on the perspective directions of the development of the economy of Ukraine, taking into account the achievements of the world science.

In 1928 Zabolotny organized the Institute of Microbiology and Epidemiology in Kyiv (now the Institute of Microbiology and Virology, National Academy of Sciences of Ukraine named after D.K. Zabolotny). Being the first director of the Institute, Danylo Kyrylovych took a great care of setting the scientific research and its equipping.

In November 1929 while commuting between Leningrad and Kyiv by train, Zabolotny suddenly got seriously ill with severe flu, which was later complicated by pneumonia and general sepsis. Zabolotny passed away on December 15, 1929.

Poster # 13

ANTIBIOTIC RESISTANCE PLASMIDS IN MUNINCIPAL WASTEWATER TREATMENT PLANT

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The importance to public health of investigating the dissemination of the antibiotic resistance genes (resistome) and mobile genetic elements (MGE) that harbor these resistance genes (mobilome) in non-clinical environments has been highlighted recently (1). Plasmids are ubiquitous among all prokaryotes in various environments, as well as in wastewater treatment plants (WWTP) environment and play a significant role in the horizontal gene transfer (HGT) and interspecies dissemination of resistance and virulence determinants. The vast majority of plasmids carry a number of different determinants such as antibiotic and metal resistance genes (R-plasmids) or virulence factors. Furthermore, numerous R-plasmids contain multidrug-resistance (MDR) to three or more antimicrobial classes. Of particular concern is the fact that many of isolated plasmids are broad-host-range (BHR), capable of conjugative transfer. The current state of knowledge on the R-plasmids among bacteria in WWTP is incomplete. However, research results of metagenome demonstrate that WWTP bacteria are a reservoir for various clinically relevant ARG (2), diverse MGE and consist of the different bacterial phylum. The aim of this study was the bioinformatics analysis of antibiotic resistance plasmids that have been isolated from Warsaw WWTP. Studied R-plasmids were identified in culture-depended method from *Aeromonas* spp. and *Enterobacteriaceae* antibiotic resistant strains. R-plasmids mostly belong to IncP, IncQ, IncU and IncL/M incompatibility groups and differ in sizes (~70kb – 7kb). Plasmid-located resistance genes encode resistance to the majority of the main antibiotic groups: aminoglycosides (*aac*, *aadA*, *strA*, *strB*), β-lactams (bla_{GES}, bla_{OXA}, bla_{FOX}, bla_{CTX-M}), fluoroquinolones (*qnrS2*, *aac(6')Ib-cr*), tetracyclines (*tetA*), sulphonamides (*sul1*), trimethoprim (*dfrB3*, *dfrA17*), macrolides (*mph(A)*), phenicols (*catB3*) and rifampicin (*ARR-3*). Besides small size studied plasmids carry some interesting modules e.g. mercury operon genes (*mer*), partition systems genes (*par*) or mobilization genes (*mob*). What we found interesting, some of the plasmids were identified among different strains or even genera what highlights the possibility of their horizontal transfer in WWTP.

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Poster # 14

SCREENING TEST OF ORGANIC SOLVENTS FOR LACCASE-MEDIATED TRANSFORMATION OF SUBSTRATES INTO COLOURFUL PRODUCTS

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Fungal laccase was find as universal biocatalyst for the synthesis of wide range of bioproducts, biochemicals, pharmaceuticals and dyes. The oxidation of substrates into novel products required only oxygen, without using of harmful oxidants and harsh conditions (Polak and Wilkolazka, 2012). During the laccase-mediated transformation of substrates reactive radicals are formed, which undergo spontaneous and non-enzymatic coupling reaction forming new chemical structures about new and unique properties, including colourful dimers, oligomers, and polymers, whose colour can be used as dyes (Polak and Wilkolazka, 2012). To increase the number of compounds as potential laccase's substrates the addition of small amounts of organic solvents are required. Additionally a low concentration of organic solvents can influence the number of reactive radicals and consequently their specific interactions between them during the formation of products. The aim of this work was the screening test of organic solvents which can be used during the transformation of several heterogenic mixtures of simple organic precursors belonging to the benzene and naphthalene derivatives. During these transformations different coloured products were formed. The influence of pH value of transformation buffer and a type and a concentration of different organic solvents on the colour and the number of transformation products was checked using UV–Vis spectroscopy and TLC method.

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Poster # 15

INFLUENCE OF THE TRANSFORMATION PARAMETERS ON THE PYRIDINE DERIVATIVE OXIDATION USING FUNGAL LACCASE – PRELIMINARY RESULTS

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Laccase is an enzyme classified as oxidoreductase, found in many plants, fungi, and microorganisms. It oxidizes a variety of aromatic compounds using oxygen as the electron acceptor and producing water as by-product. Among broad range of laccase substrates amino- and hydroxy-derivatives of pyridine can be found. The aim of this work was an optimization of transformation parameters such as a pH value of transformation buffer, a concentration and a molar ratio of the precursors and a type and a concentration of different organic solvents. Extracellular laccase from the fungal strain *Cerrena unicolor* was used as biocatalyst for homo- and heteromolecular transformations of pyridine derivative in the presence of aminomethoxybenzoic acid used as co-substrate to obtain coloured product. Obtained coloured products were characterized using UV–Vis spectroscopy, TLC and HPLC and fluorescence exhibiting product was observed in specific reaction condition. Therefore the type and the composition of the reaction tube on the character of transformation products was checked as an important parameter affecting the presence of the yellow fluorescent product.

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Poster # 16

D-LACTATE-SELECTIVE AMPEROMETRIC BIOSENSOR BASED ON THE MITOCHONDRIAL FRACTION OF *OGATAEA* (*HANSENULA) POLYMORPHA* RECOMBINANT CELLS FOR DAIRY PRODUCTS CONTROL

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During the recent decades, a lot of data about the significance of D-lactate determination in food technology and quality control has been accumulated. Nowadays, the development of new methods for the determination of D-lactate is very relevant, especially with regards to biosensors.
To construct a D-lactate-selective biosensor, we suggest using the mitochondria of recombinant yeast cells of *Ogataea (Hansenula) polymorpha* "tr6" (*gcr1 catX/Δcyb2, prAOX_DLDH*) overproducing D-lactate: cytochrome *c*-oxidoreductase (DLDH, EC 1.1.2.4) and lacking an L-lactate-specific enzyme (flavocytochrome b_2, E.C. 1.1.2.3). The usage of the pure enzyme is problematic due to the complexity of its isolation and stabilization because of the intramembranous localization of DLDH. The enzyme catalyzes D-lactate oxidation to pyruvate coupled with ferricytochrome *c* reduction to ferrocytochrome *c*.
The constructed biosensor is characterized by high sensitivity (18.5 $A \cdot M^{-1} \cdot m^{-2}$), a low detection limit (3 μM of D-lactate), wide linear ranges, good selectivity and sufficient stability. The analysis of D-lactate in the fermented samples of dairy products was performed, and high correlation of the obtained results with the reference approach ($0.7 < R < 1$) and literature data was demonstrated.

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Poster # 17

HOW MARINE BACTERIA CHANGE THEIR CELL ENVELOPE PROTEIN COMPOSITION TO SURVIVE

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Baltic Sea environment is extremely exposed to crude oil spills and other toxic waste contamination. My research aims to examine the influence of selected polycyclic aromatic hydrocarbons: benzo(a)anthracene (B*a*A), benzo(a)pyrene (B*a*P), benzo(b)fluoranthene (B*b*F) and chrysene (CHR) to protein composition and structure in the cell envelope. These PAHs are classified as priority pollutants by the U.S. Environmental Protection Agency and B*a*P is included as one of target compounds for controlling persistent, bioaccumulative, and toxic pollutants. Because of stability and hydrophobicity, PAH molecules are toxic for cells and persistent in the environment. For this study three marine bacteria were chosen: *Flavobacterium* sp, *Paracoccus* sp. and *Shewanella baltica* M1, which were already studied in our previous project and their genomes were sequenced by us. It is worth to notice that in the Baltic Sea known toxic level of B*b*f exceeds HELCOM organization designated norms thirtyfold. Study on marinebacteriacultured in medium containing PAHs shown modifications in protein composition in cell envelope.
This work shows changes occurring in cell envelope protein composition in three marine bacteria as a response to aromatic hydrocarbones. We suggest that these modifications facilitate bacterial survival in crude oil spills conditions.

Poster # 18

AN ANALYSIS OF TRANSCRIPTION OF ANTI0946 CODING A SMALL ANTISENSE RNA OF *LISTERIA MONOCYTOGENES*

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Antisense RNAs (asRNAs) are post-transcriptional regulators of gene expression in bacteria which are *cis*-encoded in relation to their target genes. Bacterial asRNAs regulate expression of their target genes by several mechanisms including alteration of target RNA stability, modulation of translation, transcription termination and transcriptional interference (1). Anti0946 was identified as asRNA encoded within *lmo0946* and *lhrC5* genes of Gram-positive human pathogen *Listeria monocytogenes* (2). The genes *lmo0946* and *lhrC5*,which are the target of action of Anti0946, belong to ferritin operon and are involved in stress adaptation of *L. monocytogenes.* The aim of the study was identification of the promoterof *anti0946*andinvestigation of the level of transcription of this gene in various stress conditions. *In silico* analysis of promoter region of *anti0946* allowed identification of consensus sequence characteristic for the alternative transcriptional factor δ^B of *L. monocytogenes*. Northern blot analysis revealed that deletion of chromosomal DNA region of *L. monocytogenes* containing the predicted promoter of *anti0946* resulted in absence of the analyzed asRNA. This result strongly suggests that transcription of *anti0946* proceeds from the predicted δ^B-dependent promoter. Northern blot analysis of RNA isolated from wild-type *L. monocytogenes* strain subjected to osmotic and cold stress revealed that expression of *anti0946* is induced exclusively under osmotic pressure therefore suggesting that Anti0946 is involved in regulation of *lmo0946* and *lhrC5* in this specific stress condition.

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Poster # 19

IDENTIFICATION OF NEW GENES OF *LISTERIA MONOCYTOGENES* INVOLVED IN HEME MATABOLISM

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Listeria monocytogenes is an opportunistic intracellular human pathogen. Heme is one of the primary sources of iron for *L. monocytogenes* during infection. The ability to acquire and effectively manage of heme is crucial to the pathogenicity of this bacterium (1). So far, only a few factors have been identified that play an important role in the transport and intracellular metabolism of heme in *L. monocytogenes*. Ferritin-like protein, encoded by *fri*gene, is responsible for iron storage in cells of *L. monocytogenes* (2). The *fri* gene belongs to a recently identified five-gene operon. The role of ferritin operon genes in heme iron metabolism is unknown. The aim of study was investigation of the effect of inactivation of ferritin operon genes on the ability of *L. monocytogenes* to use heme iron in various growth conditions. Deletion mutants were constructed in the genes of ferritin operon of *L. monocytogenes*using classical methods of molecular biology. The obtained mutant strains were tested by disc diffusion method for growth ability in rich and minimal medium supplemented with heme or hemoglobin as sole source of iron. These studies indicate that the mutant strain in *lmo0944* gene is unable to grow in a rich medium supplemented with heme or hemoglobin as the sole source of iron, while during growth in minimal medium mutants in genes *fri* and *lmo0946* are unable to use these sources of iron. Therefore, 3 new genes belonging to ferritin operon were identified as factors indispensable for heme iron metabolism in pathogenic bacterium *L. monocytogenes.*

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Poster # 20

INVOLVEMENT OF HFQ CHAPERONE OF *LISTERIA MONOCYTOGENES* IN REGULATION OF EXPRESSION OF FERRITIN OPERON GENES UNDER PENICILLIN G PRESSURE

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Listeria monocytogenes is the Gram-positive human pathogen, which shows ability to survive in wide range of stress conditions (1). Hfq-dependent regulation of gene expression of many species of pathogenic bacteria is crucial for adaptation to a variety of stress conditions, including those associated with virulence and antibiotic resistance (2). While the role of chaperone Hfq in Gram-negative bacteria is well known, the function of this protein in Gram-positive bacteria remains unclear. Ferritin-like protein (Fri) plays crucial role in adaptation of *L. monocytogenes* to multiple stresses (3). The *fri* is the first gene of a five-gene operon which consists of poorly characterized genes *lmo0944*, *lmo0945*, *lmo0946* but also *lhrC5* - small non-coding RNA interacting with Hfq. The aim of the study was defining the profile of transcripts produced in ferritin operon and investigation the involvement of Hfq chaperone in regulation of expression of ferritin operon genes under penicillin G pressure. Comparative analysis of RNA isolated from wild-type and Δ*hfq* mutant performed by northern blot revealed the presence of monocistronic transcripts of *fri*, *lmo0944* and *LhrC5*, a bicistronic transcript of *fri* and *lmo0944*, bicistronic transcript of *lmo0945* and *lmo0946*, tricistronic transcript of *lmo0945*, *lmo0946* and *lhrC5*, but also a polycistronic transcript of all the genes of the ferritin operon in both strains. Decreased amounts of all analyzed transcripts except for LhrC5 was observed in the Δ*hfq* strain background under penicillin G pressure. Furthermore, the examination of the ability of Hfq protein binding with the transcripts from the ferritin operon by coimmunoprecipitation procedure revealed that LhrC5 and also transcript of *fri* and *lmo0944*, interact with Hfq chaperone *in vivo*. These results indicate that chaperon Hfq participates in regulation of expression of ferritin operon genes of *L. monocytogenes* under β-lactam antibiotic pressure.

Acknowledgments:This work was supported by a grant 2015/18/E/NZ6/00643 founded by the National Center of Science, Poland.

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Poster # 21

EFFECT OF AGMATINE ON ACTIN REORGANIZATION IN WGA-STIMULATED LEUKOCYTES UNDER EXPERIMENTAL DIABETES MELLITUS

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Lectin WGA influences on leukocyte through interaction with complementary glioconjugates on the cell surface. This induces signal transduction and results in cytoskeleton activation. Cytoskeleton reorganization determines the avidity of adhesion molecules interaction with their complementary ligands and in that way regulates leukocyte activation and adhesion (Lin *et al*., 2016). Previously it was found (Brodyak *et al*., 2014) that total content of actin was reduced, but actin polymerization in short filaments intensified in leukocytes of animals with experimental diabetes (EDM). Agmatine administration to animals with EDM increases total content of actin and polymerized actin at a fraction of cytoskeletal filaments. It was caused by short actin filaments of cytoskeleton depolymerization.

The aim of the study was to investigate the effect of agmatine on redistribution of actin in factions of cytoskeleton filaments tightly associated with membrane, short actin filaments and actin monomers in leukocytes of rats with EDM. The dynamics of actin filaments polymerization-depolymerization evaluated after 30 seconds, 1 and 3 minutes of leukocytes preincubation with wheat germ lectin (WGA).

It was established that under EDM conditions a changes of number and structural organization of sialic acid containing glycoconjugates on the surface of white blood cells leading to disruption of WGA-induced transmembrane and intracellular signaling, resulting in enhanced process of depolymerization of short actin filaments and increases the content of G-actin in leukocytes under EDM. While in leukocytes of animals with EDM in the case of agmatine introduction an actin polymerization increased after 30 s of stimulation with WGA, was maximally after 1 min and decreased after 3 min of stimulation to initial state. Under these experimental conditions affinity of sialoglycoconjugates to bind with complementary ligands and signal transduction resulted in actin cytoskeleton reorganization, almost reaching to control level.

The results indicate that agmatine positively effect on the functional state of leukocyte of animals under EDM. Probably the normalization of blood glucose level in animals with EDM after agmatine injection (Ferents *et al*., 2012) is one of the mechanisms that mediate leukocytes functional changes in EDM conditions.

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Poster # 22

FUNCTIONAL ACTIVITY OF DIABETIC RATS' NEUTROPHILS IMPROVES OVER THE ADMINISTRATION OF YACON EXTRACTS

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Impaired leukocytes functions are the factors that cause the susceptibility to infections in patients with diabetes mellitus (DM). Hence, characterization of the immunological profile and changes therein during the disease course is of outmost importance for the understanding of immunological processes involved in DM pathogenesis. In addition, promising area of study is the search for effective drugs that would ensure the silence and/or would modulate the immune response, preferably without negative effects. Several studies have shown increased immune system efficiency after the consumption of functional foods such as fructans. To fructans belong fructooligosaccharides (FOS) including inulin that are found in plant foods. The yacon plant contains high levels of these compounds in the roots, whereas the leaves contains high amounts of flavonoids, phenolic acids, and tryptophan. These components are able to stimulate immune defense by exercising antioxidant, anti-inflammatory, antimicrobial, and anticancer effects.

According to phagocytic activity assay were calculated the next phagocytosis indexes: PI – phagocytic index – the percentage of cells started the phagocytosis of the total number of cells (PI_{30} and PI_{120} after 30 min and 120 min of incubation, respectively); PN – phagocytic number – the average number of yeasts cells that were inside phagocytes; IPC – index of phagocytosis completeness – the division quotient of PN_{30} on PN_{120}.

After 30 min of incubation 13.17±0.73% of neutrophils of control animals were in process of phagocytosis, compared to 11.00±3.5% neutrophils of animals with DM. In addition, in the case of DM we have established a decrease in the capacity of neutrophils to consume yeasts cells by 19.3% compared to control. A slightly different picture was observed in the case of a longer incubation of neutrophils with yeast cells. It was established the growth of PI_{120} at 28.8% compared to control. It was also shown the increase of yeasts cells average number that was inside phagocytes from 1.53±0.09 in control animals to 1.84±0.08 in animals with DM. These data may indicate a decrease of neutrophils phagocytic capacity under studied pathology condition. In particular, it was established that neutrophils require more time for an adequate response to foreign body penetration. This was confirmed by the decrease in the IPC under conditions of DM by 19.7% compared to control value. Yacons` leaves extract administration to animals with DM results in 2.1 fold increases of PI_{30} (compared to DM) and PI_{120} growth in animals with DM at 75.1% compared to control.

Two-weeks yacons leaves extract administration course to animals with DM led to increasedyeast cells consume intensity, as indicated by growth of PN_{30} (to 39.4%) and PN_{120} (to 8.2%) compared with diabetes. Established changes point to the intensification of the phagocytosis process under the condition of yacons leaves extract administration.

In animals with DM the administration of yacons root tubers extract causes the PI_{30}increase at 50.0%, comparing DM. The tested extract in animals with DM caused PN_{30} rate increase to 1.52±0,04 (compared to 1.42±0.08 in animals with DM). In later stages of the phagocytosis process, the amount of consumed yeast cells were reduced almost to control level. Thus, the usage of yacons root tubers extract in animals with diabetes leads to normalization of neutrophils phagocytic activity that was indicated by the growth of IPC to 28.5%, comparing to DM.

Normalization of neutrophil functional competence by yacons extracts under the condition of DM can improve course of the disease and next to their hypoglycemic action may prevent the development and progression of diabetes complications.

Poster # 23

CONTENT OF HEAVY METALS IN GLYCEROL SAMPLES OF BIODIESEL PRODUCTION

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The work presents the results of measurement of harmful substances including heavy metals in 6 selected glycerol samples from the biodiesel production process. One of the samples came from Ukraine, five more from two different Polish plants. Samples from Polish refineries contained glycerol for testing at various stages of purification. Glycerol from the first Polish plant was in purity form with purity successively 80%, 96.5% and 99.5%. The second Polish plant provided technical glycerol and glycerol purified as a result of the distillation process.

The samples (0.05 grams) were subjected to mineralization in the Millstone Ethos One high-pressure mineralizer in the presence of nitric acid HNO_3 (65%). The samples were then diluted with demineralized water to perform spectral measurements on ICP-OES Dual Dual iCAP 6500 Thermo Scientific using an internal standard (Y, Yb), 3 point calibration curve and measurement line identification and exclusion of measurement interference by standard addition and A blank sample.

The obtained results showed that the highest content of the total amount of measured minerals was in the sample of Ukrainian glycerol and amounted to about 90 g/kg of glycerol. In Polish samples not subjected to purification processes, the results of the total amount of minerals were close to each other, they contained about 56 g/kg for 80% glycerol from the first production plant and about 58 g/kg for the second plant glycerol. This result significantly decreased after the purification process and for the purest glycerin from the first plant was about 200 mg/kg, and for glycerol distilled from the second plant only 80 mg/kg.

We found relatively high cadmium content in analyzed glycerol, for a Ukrainian sample it was about 0.2 mg/kg, for samples from Poland from the first plant 0.4 mg/kg, while from the second plant 0.2 mg/kg. The purification process of each Polish glycerin analyzed did not remove this heavy metal from glycerol in the slightest. For glycerol from Ukraine also significant amounts of lead were found at the level of 2 mg/kg, Polish glycerol was characterized by a significantly lower level of this element

There were significant differences in the sulfur content of the analyzed samples. For glycerol from Ukraine it was 25 mg/kg, whereas in the Polish glycerol the result of sulfur content ranged from 2 to 7000 mg/kg, which was probably due to various contents of this element in the starting esterified material.

Poster # 24

INFLUENCE OF SYNBIOTICS ON INTESTINAL MICROBIOTA OF FINISHER PIGS

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Synbiotics are combination of probiotics and prebiotics with the synergetic benefits for a host health via intestinal microbiota, where probiotics are defined as living microorganisms which have an advantageous effect if administrated in the right amount, whereas prebiotics are nondigestible food ingredients that selectively stimulate growth or/and activity of bacteria in colon of the host.
The aim of this research was to designate an impact of newly designed synbiotics on intestinal microbiota of finishers (pigs for slaughter).
The trial was conducted on finishers divided into six study groups. Animals belonging to groups A, B and C were fed forage with newly designed synbiotics. Feed for animals assigned to groups D and E included commercially available probiotic products, which was BioPlus and Cylactin®. Control group were accounted for reference animals for which forage wasn't enriched witch any additives. Fininsher pigs were bred in the farm "P" in Wielkopolska region. Faeces samples from selected animals belonging to each group were collected once a week by Department of Swine Diseases of National Veterinary Research Institute placed in Pulawy. Determinaton of changes in gastrointestinal microbiota in finishers faeces samples took place at Institute of Fermentation Technology and Microbiology, Lodz University of Technology, where they were analysed for 9 different groups of microorganisms, including the total number of anaerobic bacteria, *Bifidobacterium*, *Lactobacillus*, *Clostridium*, *Enterobacteriaceae*, coliform group, *Bacteroides*, *Enterococcus* and Yeasts. Intestinal microbiota was analysed by the plate method, using selective microbiological media.
Studies have showed that synbiotics have beneficial influence on intestinal microbiota of finishers. The decrease of number of potentially pathogenic microorganisms was observed with simultaneous increase number of beneficial species of bacteria. Usage of synbiotics resulted in more significant changes than in case of using probiotics as an additive to forage. Usage of synbiotcs as an additive to a forage for finishers may have a beneficial influence on their intestinal microbiota and by that on finishers' health.

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Poster # 25

SYNBIOTICS EFFECT ON INTESTINAL MICROBIOTA OF CHICKENS FED WITH FORAGE CONTAMINATED BY *SALMONELLA* TYPHIMURIUM

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Salmonella enterica serovar Typhimurium (ST) bacteria is a common intestinal pathogen of animals and causes zoonoses in humans. One of methods of reducing infections is usage of synbiotics as feed additives. Synbiotics are a mixture of probiotics and prebiotics with synergistic activity. Probiotics are defined as living microorganisms which have an advantageous effect if administrated in the right amount, whereas prebiotics are nondigestible food ingredients that selectively stimulate growth or/and activity of bacteria in colon of the host.
The aim of the research was to evaluate the effect of elaborated synbiotics on intestinal microorganisms of broilers chickens fed with forage contaminated by *Salmonella* Typhimurium. Experimental work was performed on 140 chickens between 1st to 42nd days of their lives. Animals randomly divided into 5 groups of 28 animals each. Animal studies were conducted in Department of Pathology and Veterinary Diagnostics, SGGW. At chick rearing, forage contaminated by *Salmonella* Typhimurium was administered ad libitum alone and with the addition of a suitable synbiotic (A, B and C). Animals belonging to the control group were fed with uncontaminated forage without additives. Tested symbiotic preparations contained *Lactobacillus* sp. bacteria, *Saccharomyces cerevisiae* yeast and inulin as a prebiotic. During the experiment, dominant microflora in contents of intestines (the small intestine and caecum) and faeces samples from chickens were analysed. Intestinal microbiota was determined by the standard Koch's plate method, using selective microbiological media. During experimental work total number of anaerobic bacteria (Plate Count Agar), *Lactobacillus* (MRS agar), *Bifidobacterium* (RCA Agar), *Clostridium* (TSC agar), *Enterobacteriaceae* (VRBD agar), coliform bacteria (TBX agar), *Salmonella* (SS Agar), *Enterococcus* (BAA agar), *Bacteroides* (VL agar) and yeasts (SDA agar) were examined in samples.
It was observed that feeding chickens forage contaminated by *Salmonella* Typhimurium contribute to reduced number of beneficial bacteria and an increase in number of potential pathogens. The total number of anaerobic bacteria was at a comparable level in all animal groups.
Conducted experiment showed that *Salmonella* Typhimurium bacteria have adverse effect on the intestinal microflora of chickens. This pathogen inhibits the growth of beneficial bacteria. Feed supplementation with three used synbiotics allows to reduce number of *Salmonella* Typhimurium bacteria in intestinal microbiota of broilers chickens.

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Poster # 26

A NOVEL MEDIATORLESS BIOSENSOR FOR NON-INVASIVE *L*-LACTATE ANALYSIS OF HUMAN LIQUIDS

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Lactate is a key metabolite of the anaerobic glycolytic pathway which plays an important role in human health. The level of *L*-lactate content in human blood is an important clinical indicator of hypoxia, acidosis, heart attack, drug toxicity, and serves as a marker for the evaluation of the optimal sportsmen's training. *L*-lactate is also an important biomarker for different types of cancer due to Warburg phenomenon. Moreover, as a lactate threshold can be increased greatly along with training, the monitoring of the athletic performance with the purpose of evaluating the best training equipment and regimes is quite relevant. Therefore, reliable determination of *L*-lactate is important in clinical diagnostics and sports medicine.

The measurement of metabolites in fluids other than blood is becoming increasingly significant because of major advantages of non-invasive analysis (safety, rapidity, and accuracy). In sensor technology, the most efficient means for the non-invasive analysis of human fluids seems to be the third generation (mediatorless) amperometric biosensors, as they do not require any exogenous cofactors, toxic electron transfer mediators or the use of high working potential.

The aim of this work is the construction of a novel non-invasive electrochemical biosensor of the third generation for *L*-lactate based on yeast *L*-lactate:cytochrome*c* oxidoreductase (EC 1.1.2.3; flavocytochrome b_2, FC b_2) immobilized onto gold nanoclusters (nAu).FC b_2 is a tetramer with four identical subunits, each consisting of FMN- and heme-binding domains. The main properties of FC b_2 of thermotolerant yeast *Ogataea (Hansenula) polymorpha* are the selectivity for *L*-lactate, its high stability and ability to direct electron transfer. The developed FC b_2-nAu modified bioelectrodes are characterized by improved parameters compared to the enzyme electrodes, obtained without the use of nAu. The constructed bioelectrodes were adapted for direct non-invasive analysis of human saliva and sweat samples using the amperometric microcomputer-based analyzer system. The obtained results of the real samples analysis have a high correlation ($0.7 < R < 1$) with the reference approaches.

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Poster # 27

INFLUENCE OF ECTROMELIA VIRUS ON THE NONCANONICAL NF-κB ACTIVATION IN JAWSII MURINE DENDRITIC CELL LINE

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NF-κB is a pleiotropic transcription factor that is crucial for antiviral response. Canonical (classical) NF-κB activation pathway regulates innate immunity and inflammation,whereas noncanonical (alternative) NF-κB signaling is involved in lymphoid organogenesis and immune cell maturation. Manipulation of noncanonical NF-κB activation pathway by oncogenic viruses has been studied extensively (Sun, 2012). It has also been shown that certain nononcogenic RNA viruses influence noncanonical NF-κB activation (Yang and Sun, 2015). At the same time, the canonical NF-κB signaling may be modulated by numerous viral pathogens, including ectromelia virus (ECTV), as well as other members of the *Poxviridae* family of large dsDNA viruses (Brady and Bowie, 2014). Since there is a crosstalk between canonical and noncanonical NF-κB signaling, the aim of our studies was to investigate the impact of ECTV on noncanonical NF-κB activation components. Fluorescence microsopy analysis of subcellular localization of RelB subunit of NF-κB in mock-, UV-inactivated (uvi)-ECTV- and ECTV-infected JAWSII cell line has revealed that in unstimulated cells RelB was localized predominantly in the cytoplasm. At 4 and 18 hours post infection (hpi) the highest percentage of cells with slightly elevated nuclear level of RelB manifested by the discrete increase in the fluorescence intensity was observed in ECTV-infected cells in comparison with mock- and uvi-ECTV-infected cells. At 12 hpi, in turn, this effect was diminished. However, in most of ECTV-infected cells the level of nuclear RelB remained unchanged. Additionally, after cell treatment with PMA/ionomycin, which induces RelB nuclear translocation, twofold decrease in the percentage of stimulated cells was observed for ECTV-infected cells, which displayed the presence of regular viral factories. Fourfold decrease in the percentage of cells with RelB nuclear translocation, in turn, was observed after the release of numerous progeny virions to the cell cytoplasm. Immunoblot analysis of mock-, uvi-ECTV- and ECTV-infected JAWSII cells at early (4 hpi) and late (18 hpi) stages of ECTV replication cycle has shown no dramatic change in the intracellular level of NIK and IKKα kinases, the key components of noncanonical NF-κB signaling pathway. Taken together, our data suggest that ECTV may actively influence the noncanonical NF-κB signaling, but uncovering novel mechanisms of such viral manipulation requires more detailed study.

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Poster # 28

CHARACTERIZATION OF LONG CELLULAR EXTENSIONS FORMED BY DENDRITIC CELLS PRODUCTIVELY INFECTED WITH ECTROMELIA VIRUS

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Ectromelia virus (ECTV) is a natural pathogen of mouse and causes the mousepox, a lethal disease of certain strains that present similar clinical features to smallpox in humans, caused by variola virus (VARV). ECTV, like VARV, coevolved with and is restricted in replication to its natural host. Therefore, ECTV can adapt and replicate even in terminally differentiated cells, such as dendritic cells (DCs). Our previous study has shown that ECTV is able to productively infect conventional DCs (cDCs) and induces formation of long cellular extensions at later stages of infection (24 hpi) (Szulc-Dąbrowska et al., 2017). This phenomenon has been shown for the first time, since the life cycle of other orthopoxivruses, such as vaccinia virus (VACV) and cowpox virus (CPXV) is aborted in DCs before formation of viral replication centers (Chahroudi et al., 2006). The aim of this study was to characterize the structure of ECTV-induced long cellular extensions formed *in* vitro by infected cDCs. Using fluorescence microscopy analysis we documented that such extensions were composed of filamentous (F-actin) bundle and microtubules (MTs). Mitochondria together with progeny virions were also found within cellular extensions, especially in the area of “cytoplasmic packets” – the convex structures resembling “bubbles”, formed at certain lengths of long cellular extensions. Moreover, ECTV infection of cDCs was accompanied by increased acetylation of α-tubulin, what probably stabilized MTs, since treatment of infected cells with nocodazole (a microtubule-depolymerizing agent) for 5 and 30 min. did not induce complete degradation of MTs. Acetylated α-tubulin was also found in actin-based long cellular extensions. Additionally, these extensions exhibited higher focal adhesion compared to those formed by uninfected cells. Taken together, our data indicate that ECTV has high adaptation capabilities to its natural host immune cells, in which it induces formation of stable and durable actin-based cellular extensions, presumably for efficient dissemination.

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Poster # 29

INFLUENCE OF ECTROMELIA VIRUS INFECTION ON CATHEPSINS AND CYSTATINS GENE EXPRESSION IN JAWS II DENDRITIC CELL LINE

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Dendritic cells (DCs) are involved in both innate and adaptive immune responses and play an essential role as potent antigen-presenting cells (APCs). Cathepsins are group of proteases found in endosomes of APCs and are responsible for extracellular proteins degradation. The role of cathepsins B, D, E, F, K, L and S is crucial during infections since they participate in antigen processing before presentation in the context of major histocompatibility complex (MHC) class I and II molecules. Certain viruses cause perturbations in the APCs by inhibiting antigen processing mechanisms. Poxviruses are known for evolving numerous mechanisms of immune evasion and many such mechanisms have not been elucidated. Although the most notorious poxvirus, variola virus, has been eliminated from the earth, there is still need to understand the poxvirus-host interaction. Particularly, other poxviruses such as monkey poxvirus have recently caused illness in humans and may pose a health hazard to humans previously not vaccinated. Understanding the mechanisms that inhibit immune response after infection with poxviruses may yield information valuable in the rational design of vaccines or therapeutic approaches. The aim of this study was to determine whether cathepsins are targeted by ectromelia virus, a poxvirus, by measuring the mRNA expression profiles of cathepsins B (*Ctsb*), L (*Ctsl*) and S (*Ctss*) and cystatins A (*Csta*), B (*Cstb*) and C (*Cts3*) following infection of JAWS II cells, a dendritic cell line. JAWS II mock- and ECTV-infected cells were harvested after 4, 12 and 24 hours post infection (hpi). The mRNA level of *Ctsb*, *Ctsl* and *Ctss* and *Csta*, *Cstb* and *Cst3* was evaluated by quantitative real-time PCR. Results showed profound down-regulation of gene expression for *Ctsb*, *Ctsl* and *Ctss* at 24 hpi with ECTV. Similarly, *Cstb* and *Cst3* mRNA levels were down-regulated in infected JAWS II cells. The level of mRNA expression for *Csta* was undetectable in uninfected and infected cells. The results show that ECTV may impairs the function of cathepsins and cystatins in DCs, serving as a viral strategy to escape immune responses enabling the virus to replicate effectively in infected cells.

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Poster # 30

IMMOBILIZATION OF CELLOBIOSE DEHYDROGENASE FROM *PHANEROCHAETE CHRYSOSPORIUM* IN CALCIUM ALGINATE GEL

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Cellobiose dehydrogenase (CDH, EC 1.1.99.18, CAZy AA 3.1) is an extracellular oxidoreductase secreted by wood-degradingfungi when cellulose is used as a carbon source (Cameron and Aust, 2001). CDH is composed of a large catalytic flavin domain containing non-covalently bound FAD and a b-type heme-containing cytochrome domain connected by a flexible linker region. Cellobiose dehydrogenase oxidizes several β-1,4-linked disaccharides including lactose and cellobiose specifically at position C-1 of the reducing sugar moiety to the lactones which hydrolyze spontaneously to the corresponding aldonic acids (Zamocky et al., 2006). Due to its versatile properties CDH has been applied in enzymatic biofuel cells as anode catalyst, in biosensors for the detection of lactose, for the production of lactobionic acid, as well as in biodegradation and bioremediation (Sygmund et al., 2013).

Current advancements in biotechnology have promoted the usage of immobilized enzymes for a wide range of applications not only in the field of biotechnology but also in the pharmaceutical, environmental and food industries. Therefore, developing a CDH immobilization strategies is very important. Entrapment within insoluble calcium alginate gel is recognized as a rapid, non-toxic, inexpensive and versatile method for immobilization of enzymes.

In this work, partially purified cellobiose dehydrogenase from *Phanerochaete chrysosporium* was immobilized in calcium alginate beads by entrapment method and characterized. CDH was entrapped with or without substrates (cellobiose and lactose). The catalytic properties of immobilized CDH or CDH/substrate system were investigated. Also the influence of pH, temperature, and operational stability of the immobilized enzyme was studied.

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Poster # 31

ANTIOXIDANT AND ANTIMICROBIAL SYSTEMS BASED ON FUNGAL CELLOBIOSE DEHYDROGENASE ENTRAPMENT IN ALGINATE MATRICES

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The cellobiose dehydrogenase (CDH) is oxidoreductase produced by lignocellulolytic fungi belonging to phyla Basidiomycota and Ascomycota. It is a monomeric enzyme that belongs to the glucose-methanol-choline (GMC) family of oxidoreductases containing a C-terminal flavin adenine dinucleotide (FAD) domain and the N-terminal heme domain connected by a flexible linker region (Zamocky et al., 2006). CDH catalyzes the oxidation of β-1,4-linked di- and oligosaccharides such as cellobiose, cellodextrins, and lactose to corresponding lactones and H_2O_2. The antioxidant properties seem to be associated withhydrolysis of lactones to the aldonic acids such as lactobionic or cellobionic acid enzymatically produced from lactose or cellobiose as substrate.The antimicrobial activity of CDH is attributed to the toxicity of formed hydrogen peroxide (H_2O_2), a well-known antimicrobial agent (Thallinger et al., 2013) The ability of fungal cellobiose dehydrogenase to simultaneously produce antioxidant and antimicrobial components is highly interesting for biotechnological, biomedical and industrial applications.
The present research related to the immobilization of CDH from selected fungi or CDH/substrate system by entrapment in calcium alginate gel. The activity of immobilized CDH under various conditions (pH, temperature), the stability of the enzyme binding to the matrices, antioxidant properties and antimicrobial efficiency of prepared alginate beads against selected indicator microorganisms have been evaluated. New antioxidant and antimicrobial system (CDH/substrate) immobilized in a semipermeable support material such as alginate gel, can be used in various areas such as food, medicine and cosmetics.

The research project founded by the National Science Centre in Poland (grant no. 2015/17/D/NZ9/02066).

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Poster # 32

EXOPOLYSACCHARIDES FROM *RHIZOBIACEAE* FAMILY AS A PROMISING COMPOUNDS WITH BINDING CAPACITY

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Rhizobiaceae bacteria produce polysaccharides which may be accumulated inside the cells as a reserve of energy, or be secreted outside the cells. The physiological role of extracellular polysaccharides (EPS) depends on the ecosystem exists in which the organism produces them. Exopolysaccharides secreted outside the cell by *Rhizobiaceae* bacteria, can act as an important signal molecule in the initiation of symbiosis with leguminous plants.Furthermore, exopolysaccharides by creating an appropriate coating on the outside also allow microorganisms to accumulate an appropriate amount of water and protect the organism against excessive drying. In addition, due to the anionic nature of exopolysaccharides, it is possible to capture the appropriate nutrients from the medium and protecting the organism against harmful heavy metal ions (Janczarek et al., 2007; Donot et al., 2012). One of the major civilization diseases of the last and present century is atherosclerosis with the deposition of cholesterol and cholesterol esters in the connective tissue of the arterial walls, leading to stenosis and occlusion of the blood vessel. Given their ability to reduce the cholesterol and lipid content in blood, bacterial polysaccharides may be one of the potential hypolipidemic agents. To date, is not known to use of chemically unmodified exopolysaccharides produced by *Rhizobia* to it simultaneously sorption of cholesterol, triglycerides and glucose. The aim of this work was to study the adsorption properties of the bacterial exopolysaccharides extracted from *Rhizobium leguminosarum* bv. *trifolii* Rt24.2, *Sinorhizobium melilotii* Rm1021, *Bradyrhizobium japonicum* USDA110 and *Bradyrhizobium elkanii* USDA76. Additionally, we examined the ability of exopolysaccharides to bind magnesium and iron ions. The chemical composition and microstructure of investigated EPS preparations were also determined.

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Poster # 33

THE INFLUENCE OF TOPOISOMERASE I (TOPA) INHIBITORS ON CHROMOSOME REPLICATION AT SINGLE CELL LEVEL IN *MYCOBACTERIUM SMEGMATIS*

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One of the major goals of modern microbiology is to find novel antibacterial agents. Chromosome replication, being a key stage in bacterial cell cycle, makes proteins involved in this process a potential targets for new antibiotic drugs. Our research conducted on model organism *Mycobacterium smegmatis* shows that normal replication process is dependent on the activity of proteins associated with DNA synthesis as well as proteins maintaining chromosome topology such as bacterial Topoisomerase I (TopA). By cutting and religating DNA strands TopA removes the excess of negative supercoils. Amsacrine – an anticancer drug - was reported to stop mycobacteria growth by inhibition of TopA activity. To assess the influence of amsacrine and its derivatives on replication we used *M. smegmatis* strains expressing DnaN (a subunit of DNA polymerase III) fused with EGFP to monitor single cell replication process in real time under microfluidic conditions. Our studies give strong evidence that inhibition of TopA by amsacrine and its derivatives have a significant effect on the chromosome replication, morphology and cell growth.

Poster # 34

MEDICAL BIOMATERIALS MODIFIED WITH PROTEOLYTIC ENZYME INHIBITORS AND THEIR SELECTED BIOLOGICAL PROPERTIES

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Implantology and material engineering belong to a group of sciences developing very intensively over recent years. Every year, the medical market is enriched with new biomaterials comprising whole or part of a living structure or biomedical device which performs, augments, or replaces a natural function. Such functions may be relatively passive (e.g. a heart valve), or may be bioactive with a more interactive functionality (e.g. hydroxy-apatite coated hip implants). Biomaterials are also used every day in dental applications, surgery, and drug delivery, for example a construct with impregnated pharmaceutical products which permits the prolonged release of a drug over an extended period of time. However, despite many positive aspects of implantation of prostheses and implants into the human body, such serious surgical intervention can cause many complications. One of the most dangerous infection of implanted medical biomaterials are these which are caused by bacteria of the genus *Escherichia* sp., *Staphylococcus* sp., *Streptococcus* sp., *Pseudomonas* sp., *Klebsiella* sp. and by yeast *Candida albicans*. Lack of proper treatment or wrong diagnosis may result in serious deterioration of the patient's health and even his death.
To eliminate infections within implanted prostheses, materials characterized by increased resistance to pathogenic microorganisms are desirabled. However, despite their relatively high effectiveness, biomedical market is still looking for new and better solutions. In the present work, serine protease inhibitors are proposed as novel molecules that can reduce amount of infections in implanted prostheses. As a result of conducted experiments, we obtained modified prostheses, which are stable in various conditions of reaction environment and can limit the growth and development of *Staphylococcus aureus* and *Candida albicans*.

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Poster # 35

EXPRESSION OF RECOMBINANT TICK-BORNE ENCEPHALITIS VIRUS-LIKE PARTICLES IN INSECT CELLS

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Tick-borne encephalitis (TBE) is a seasonal disorder of the central nervous system which may lead to serious medical complications, including meningitis and meningoencephalitis. TBE occurs widely across Europe, Russia and Far-Eastern Asia and more than 10 000 cases of this disease are reported annually. The causative agent of the zoonosis is tick-borne encephalitis virus (TBEV), which is transmitted by ticks. The incidence of TBE has increased over 400% times during the past 20 years in Europe, which makes TBE, after Lyme disease, the second most serious disease transmitted by ticks. Despite numerous strategies of research, there are currently no licensed therapeutics for the treatment of TBEV infections. Four vaccines against TBE based on inactivated virus are available in the market. Currently, vaccinations are not mandatory, but recommended only for residents and tourists traveling in endemic areas. Due to the fact that vaccine failures even after complete series of vaccine doses have been reported, research aimed at production of new types of vaccines are fully justified.

The aim of this study was to produce TBEV virus-like particles (VLPs) using baculovirus expression system in insect cells. Recombinant baculoviruses expressing full forms of prM-E proteins as well as M-E proteins were used to produce recombinant particles in infected cells. Expression of TBEV VLPs was determined by Western blotting, indirect immunofluorescence assay as well as sucrose gradient sedimentation. This study suggests that TBEV VLPs produced in insect cells offer a promising approach for vaccine and diagnostic purposes.

Poster # 36

LYOPHILIZED LEUCOCONCENTRATE OF HUMAN CORD BLOOD IN TREATMENT OF EXPERIMENTAL ATOPIC DERMATITIS

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Currently, one of the most urgent health problems is the wide spread of pathological conditions associated with impaired human immunity functions. In this regard, modern pharmacology has an important task is the search for new biologically active substances and development of the drugs based on them, normalizing the function of immunity and / or preventing its disorders. Scientific researches in the field of creation of new effective and safe immunotropic agents for immunocorrection led to the development the preparations based on organs and tissues of animal origin: thymus gland, bone marrow cells, embryonic tissue, placenta, skin, cord blood. Assuming all the above, it is important to develop and study new national immunomodulating drugs to treat of autoimmune pathologies, in particular, atopic dermatitis (AD).

The objective was to experimentally substantiate the possibility of using the lyophilized leukoconcentrate of human cord blood (LLHCB) to restore the IS indices when treating AD.

Materials and methods. The experiments were performed in male Wistar rats at the age of 6 months (1965). Dinitrochlorobenzene in 0.5 ml of 5% alcohol-acetone solution was rubbed into the skin of the back (3x4 cm^2) of animals daily through 21 days. Leucoconcentrate of human cord blood (LHCB) was lyophilized by the method of Goltsev AN et al. The subpopulation composition of spleen cells was studied by flow cytometry using Mab to CD3, CD4, CD8, CD25 molecules (BD, USA), level of IFN-γ, IL-4, IL-10, IL-17 cytokines and concentration of CIC in blood serum adhesive and phagocytic activity on cells from the peritoneal cavity were investigated. The analysis was carried out on the 3rd, 7th, 14th and 28th days before and after treatment. Statistical processing of the results was carried out using the software Statistica 7.0.

Results. In treatment of AD by the administration of LHCB to rats on the 7th day it was recovery of T-lymphocytes, IRI, phagocytic and adhesive activity, reduction of CIC indices, concentration of IgE and T-reg.

Conclusions. It has been experimentally established that the lyophilized leukoconcentrate of human cord blood (lLHCB) has an immunotropic property and can be used for immunocorrection in the treatment of atopic dermatitis.

Poster # 37

MODIFICATION OF YEAST *PICHIA PASTORIS* FOR IMPROVEMENT OF ETHANOL PRODUCTION FROM CRUDE GLYCEROL FRACTION OF BIODISEL INDUSTRY

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The rapid development of biodiesel production has led to the formation of huge amounts of glycerol-containing waste as the by-product of the process. This glycerol fraction, which is also called crude or raw glycerol, contains a large amount of impurities, such as methanol, heavy metal salts, soaps, and free fatty acids, that prevent it from further use. Therefore, an important task is the development of strains of microorganisms capable of effective crude glycerol conversion into other, value-added products, such as fuel ethanol. The use of the crude glycerol fraction in the biotechnological industry can be a good alternative way to disposal of such industrial waste. Methylotrophic yeast *Pichia pastoris* is considered to be a perspective host for this process, as it can tolerate high concentrations of methanol, which often accompany crude glycerol.
In order to increase the flow of glycerol into the cells, we decided to overexpress the potential glycerol transporter genes *FPS1*, *GUP1*, and *GT1* under the control of strong constitutive promoter of the *GAP* gene (encodes glyceraldehyde-3-phosphate dehydrogenase) in *P. pastoris* cells. Fps1 is an aquaglyceroporin that mediates controlled glycerol export during osmoregulation in *Saccharomyces cerevisiae*, but in *P. pastoris* it rather provides glycerol import. Gup1 is the polytopic integral membrane protein. Wild-type strain of *P. pastoris* produces 1.4 g/L of ethanol in the medium with 10% crude glycerol. Overexpression of *FPS1*, *GUP1* and *GT1* genes enhanced ethanol production till 2.63 g/L, 2.48 g/L and 2.74 g/L, respectively. The growth of recombinant strains and wild-type strain on different glycerol- or glucose-containing media was compared. We determined that recombinant strains have the same intensity of growth as the wild type strain. We performed also adaptation of *P. pastoris* to crude glycerol by continuous cultivation and gradual increase of glycerol concentration in the medium. Obtained adapted strain revealed better growth on crude glycerol-containing medium and produced 5.94 g/L of ethanol during fermentation in the medium containing 10% raw glycerol.

Poster # 38

METAGENOMIC ANALYSIS OF NITROGEN CYCLING MICROBIAL COMMUNITY STRUCTURE IN A PRISTINE AND DRAINED TROPICAL PEATLAND SOIL

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Tropical peatlands that play a crucial role in the maintenance of different ecosystem services are drained for agriculture, forestry, peat extraction and human settlement purposes. As the changes in soil nitrogen cycle are economically, ecologically and environmentally important there is a need to explore the ecology of the microbes involved in the nitrogen cycle in tropical ecosystems. This study provides the first detailed overview about nitrogen cycling microbial community structure in a tropical peatland, and assessed the effect of drainage on the peat soil microbial community structure and abundance using a shotgun metagenomics approach. The studied peatland situated in northern part of French Guiana. Our results show that drainage of tropical peatland resulted in structural changes in soil bacterial and archaeal community, and caused alteration in *nirK*, *nirS*, *nosZ*, *nifH* and archaeal *amoA* gene possessing microbial communities. The drainage decreased denitrification and N_2-fixing potential in the tropical peatland. In undisturbed peatland soil, the N_2O emissionwasmainly related to *nirS*-type denitrifiers and DNRA, while the conversion of N_2O to N_2 was controlled by *nosZ* clade I genes. The drainage induced changes in the soil denitrifying microbial community, andthe main reducers of N_2O weremicrobes harbouring *nosZ* clade II genes. The results of this study confirm that modifications in the water regime of tropical peatlands may cause substantial shifts in the microbially mediated nitrogen cycle.

Poster # 39

ENGINEERING OF THE HEXOSE TRANSPORTER HXT1 FOR IMPROVED UTILIZATION OF XYLOSE DURING HIGH-TEMPERATURE XYLOSE ALCOHOLIC FERMENTATION IN THE YEAST *OGATAEA (HANSENULA) POLYMORPHA*

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For the last three decades biofuels produced from renewable feedstocks have received much attention because of their potential to replace conventional fossil fuels. A major issue in the conversion of saccharified lignocellulosic biomass into biofuel is the utilization of xylose, since lignocellulosic feedstocks contain a significant amount of this pentose sugar.

The ability of the thermotolerant methylotrophic yeast *Ogataea polymorpha* to ferment xylose has made this yeast species a promising organism for high-temperature alcoholic fermentation. Although *O. polymorpha* recombinant strains metabolize xylose more efficiently, uptake, and therefore consumption of xylose, is strongly inhibited by glucose, due higher glucose affinity to the transporter proteins. The low-affinity transport systems are shared between glucose and xylose for sugar transport in *O. polymorpha*.

Recently, the first functional hexose transporter Hxt1 was identified in *O. polymorpha*. To increase the specific xylose uptake rate, the modified Hxt1 was engineered by substitution of asparagine to alanine residues at position 358. Furthermore, N-terminal lysine residues of Hxt1 predicted to be the target for ubiquitination were replaced for arginine residues. The modified versions of Hxt1 were overexpressed in *hxt1Δ* mutant and the efficiency of xylose and glucose co-utilization during high-temperature fermentation was studied. The made by us protein engineering of Hxt1 resulted in simultaneous utilization of both sugars during fermentation in the obtained recombinant strains.

Poster # 40

OBTAINING AND RESEARCHING THE EFFECTIVENESS OF SPINDLE TREE (*EUONYMUS EUROPAEA L.*) OIL ON NON-ALLERGIC CONTACT DERMATITIS

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Contact dermatitis is an inflammatory reaction of the skin in response to the effects of various chemical stimuli. Non allergic contact dermatitis, unlike allergic dermatitis, occurs exclusively in the place of action irritating factor. A compulsory component of complex treatment of contact dermatitis is local therapy. Very often, various ointments containing steroidal anti-inflammatory drugs, which should be used with caution to the elderly, children and pregnant women, are used for this purpose. An alternative to synthetic anti-inflammatory agents is phytopreparations that exhibit a soft and Integrated action.

Our attention was drawn to the oil of Spindle tree (*Euonymus europaea L.*), which, according to folk medicine, is effective in dermatomycosis and eczema.

The fatty oil was obtained from arriluse of Spindle tree seeds by extraction with petroleum ether ($t_{boiling}$ = 40 - 70 ° C) followed by removal of the solvent. In the obtained oil, numerical indices were determined according to the procedures of the State Pharmacopoeia of Ukraine, the determination of fatty acids was carried out by gas chromatography on “Chrom-5”, and by chemical reactions determined the presence of possible poisonous substances (alkaloids and cardiac glycosides).

The determination of the acute toxicity of the resulting oil was carried out on white, non pure-bred rats weighing 180-200 g by administering the oil orally at a dose of 10 g / kg of weight daily for 14 days. Investigation of anti-inflammatory effect of oil was performed on a model of non-allergic contact dermatitis on 24 white non-breeding rats. This pathology was created by rubbing into the cut out area of skin within 10 days of the measured dose of terpentine. As drug for comparison used buckthorn oil.

The purified oil is a viscous liquid of bright orange color with a specific odor. The yield of oil was 20-28% of the mass of the seed ariluse. In the composition of Spindle tree oil, high content of unsaturated fatty acids, in particular, oleic (47.15%), linoleic (32.40%) and linoleic (6.34%) and high iodine (182.1), ethereal (194.9) and the number of sapling (224.6). The oil, when injected into the stomach at a dose of 10 g / kg, did not cause death and intoxication of animals, indicating its non-toxicity.

In all animals, on the 10th day after the reproduction of non-allergic contact dermatitis, there was a pronounced swelling of the tissues, a clear erythema with seals and peptic ulcers, and the presence of ulcers with hemorrhagic crust, which, according to the intensity of skin lesions, corresponded to 3.0-3.3 bala on a 4-point scale. After 5 days of treatment with Spindle tree oil and buckthorn oil observed a marked therapeutic effect, which was confirmed by a decrease in the intensity of skin lesions and the disappearance of swollenness and hyperemia. After 5 days of treatment with Spindle tree oil and buckthorn oil almost all biochemical parameters were restored. It is established that on the effectiveness of anti-inflammatory action, the oil of spindle tree is slightly inferior to sea buckthorn oil. The conclusion is made of the possibility of using butter oil for its use in ointments in the treatment of non-allergic contact dermatitis.

Poster # 41

THE INFLUENCE OF STRUCTURE OF POROUS CARRIES ON ADSORPTION OF LACCASE FROM *PLEUROTUS OSTREATUS*

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Laccase ability to transform compounds such as synthetic dyes, drugs or endocrine disruptors, has made it one of the most intensively studied enzymes as a potential biocatalyst in xenobiotics degradation. Although many results seem to be very promising, the application of native laccase in industrially scale is still very limited due to high costs of the biocatalyst production. Moreover, conditions of reaction like pH value or the presence of organic pollutions may be too drastic and quickly inactivate the enzyme. An useful technique to overcome those limitations is the immobilisation of laccase, provides its better storage and operational stability as well as the recurrence of the degradation process. In addition to the technique, an important parameter of immobilisation is the structure of carrier, which may be varied in terms of the particle size, pores diameter or functional groups on its surface.
In the current study, the fungal laccase from *Pleurotus ostreatus* was immobilised through adsorption on six porous carriers from Purolite® about different functional groups, porosity and particles size. As a result, seven laccase-support preparations characterised by different immobilisation efficiency were obtained. Based on those results, we analysed the influence of carriers structure on immobilisation efficiency, operational stability and storage stability. In conclusion, we determined the optimal carrier size, porosity and functional group for the adsorption of laccase, which will be used as biocatalyst for transformation of different aromatic compounds in further studies.

This work was financially supported by National Science Centre (2015/17/N/NZ9/03647).

Poster # 42

ENHANCEMENTOF GLUTATHIONE SYNTHESIS IN THE METHYLOTROPHIC YEAST *OGATAEA (HANSENULA) POLYMORPHA*

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Glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH) is abundant conserved tripeptide present in most living organisms, predominantly, in eukaryotes. GSH is an important redox buffer, detoxifier, co-factor of several enzymes and a key source of nitrogen and sulfur under conditions of their depletion in the environment. Due to the antioxidative properties of GSH there is an increasing interest for application of this tripeptide in several industrial areas, including cosmetics, pharmaceutical and foodindustries. As an active ingredient of food, drugs and cosmetic products, GSH could alleviate harmful oxidative processes, scavenge toxic compounds at different kinds of human intoxications, and strengthen whitening and skin repair antiaging effect. Although GSH can be produced by chemical or enzymatic synthesis, microbiological production using natural or engineered microorganisms (yeasts *Saccharomyces cerevisiae* and *Candida utilis*, bacteria *Escherichia coli* and *Lactococcus lactis*, etc.) is currently the most common method for the commercial production of GSH. At present, GSH is produced mainly by fermentation using yeasts. Thermotolerant methylotrophic yeast with GRAS status *O. polymorpha* which naturally possesses high content of GSH is considered as a promising organism for design of competitive GSH producer.

The plasmid for overexpression of *GSH2* and *MET4* genes, encoding γ-glutamylcysteine synthetase and transcriptional activator of sulfur metabolism, respectively, under control of strong constitutive promoter of glyceraldehyde-3-phosphate dehydrogenase was constructed. This plasmid was introduced into genome of *O. polymorpha* wild-type strain by electroporation method. Selection of transformants was performed on YPD medium supplemented with nourseothricin. The presence of *GSH2* and *MET4* genes in the obtained transformants was confirmed by PCR. Selected transformants were analyzed for their GSH accumulation. Co-overexpression of *GSH2* and *MET4* genes led to improvement of GSH synthesis in the constructed recombinant strains and the intracellular level of GSH was increased for eight times as compared with wild-type strain.

Poster # 43

THE SIGNIFICANCE OF MUTATIONS ASSOCIATED WITH RESISTANCE OF *MYCOBACTERIUM TUBERCULOSIS* TO ISONIAZID

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INH resistance is most frequently associated with a specific mutation in *katG* gene which encodes the catalase-peroxidase enzyme in *M. tuberculosis*. However, mutations connected to INH resistance were also identificated in *inhA, ndh, ahpC* genes.
In this study, *M. tuberculosis* H_{37}Ra carrying internal deletion within *katG* gene, hightly resistant to isoniazid was engeenered by homologous recombination. The *M. tuberculosis ΔkatG* strain was constructed to allow verification of relationship between presence of a given mutation in *katG* gene and level of INH resistance. Next, *katG* gene from *M. tuberculosis* INH-susceptible laboratory strain and from clinical strains resistant to isoniazid (carrying Ser315Thr in *katG*) were introduced to *M. tuberculosis ΔkatG* host. The introduction of functional *katG* gene into INH-resistant strain led to recover susceptibility of complemented strains. The introduction of mutated *katG* (Ser315Thr) did not recover susceptibility of complemented strains, confirming that investigated mutation is responsible for INH resistance.
Suggested system of objective verification of currently known mutations in the *katG* genes will allow to select mutations that should be analyzed for the resistance of mycobacteria to INH. Limitation of the number of mutations associated with drug resistance will allow in future to propose a rapid and reliable tests that identify drug-resistant strains directly in clinical specimens from patients with tuberculosis.

Poster # 44

TRANSCRIPTIONAL ORGANIZATION OF THE *ACPP-MSBB* REGION RESPONSIBLE FOR BIOSYNTHESIS OF VERY LONG CHAIN FATTY ACIDS OF *AGROBACTERIUM FABRUM* C58 LIPOPOLYSACCHARIDE

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Bacteria belonging to *Rhizobium* and *Agrobacterium* genera can establish close relationships with plants, but the consequences of such interactions are very diverse: members of the genus *Rhizobium* are nitrogen-fixing legume symbionts, whereas most *Agrobacterium* species are phytopathogens. Bacterial cell surface polysaccharides, such as lipopolysaccharides (LPS), capsular polysaccharides (CPS), β-1, 2-glucans, and extracellular polysaccharides (EPS), are crucial for proper interaction between the *Rhizobiaceae* and their host plants.
LPSs are anchored in the outer membrane of almost all Gram-negative bacteria and are constituted by three parts: lipid A, a core oligosaccharide and an O-antigen polysaccharide. Rhizobial lipids contain unusual (ω-1) hydroxylated very long chain fatty acids (VLCFA), which, besides rhizobia, are synthesized by a very limited group of bacteria. All rhizobia having lipid A molecules modified with VLCFAs contain a highly conserved gene cluster located between the *acpXL* and *msbB* genes within their chromosome, which demonstrates substantial conservation of the gene content and organization. In the case of *A. fabrum* C58, this region comprise six ORFs, which are oriented in the same direction and could constitute at least one operon.
The aim of this work was to determine transcriptional organization of the *A. fabrum* C58 *acpP-msbB* gene cluster. Research methods comprised amplification of DNA fragments embracing potential predicted promoters and cloning into pMP220Km reporter vector with promoterless *lacZ* gene. Then, β-galactosidase assay in plasmid-cured *Agrobacterium tumefaciens* GMI9023 strain was conducted.
The obtained results allowed to demonstrate that the *A. fabrum* C58 *acpP-msbB* region is organized into two operons: *acpP*– *fabZ* and *fabF*–*fabF*–Atu1595–*msbB*, with promoters located upstream of *acpP* and *fabF*. It was showed that *acpP* gene promoter was the stronger one.

Poster # 45

IDENTIFICATION OF CHROMOSOMAL ORIGIN OF REPLICATION (*ORIC*) OF THE *RHIZOBIUM LEGUMINOSARUM* BV. *TRIFOLII* TA1

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Rhizobia are soil bacteria able to recognize and establish symbiosis with legumes, providing them with fixed nitrogen and enabling their growth on nitrogen-limited soils. Bacteria belonging to *Rhizobiaceae* family usually have large genomes partitioned into chromosome and several megaplasmids belonging to the *repABC* family.

Bidirectional replication of the bacterial chromosome begins with the specific interaction of the DnaA protein at a precise region on the replicon – the chromosomal origin of replication (*oriC*). *oriC* sequence is the site of initial unwinding of DNA and the location of replisome assembly. The characteristic features of bacterial chromosome origins are: (i) presence of the relatively conserved (amongst bacteria) binding sites for the replication initiator DnaA (DnaA boxes), and (ii) occurrence of an exceptionally AT-rich region adjacent to the DnaA boxes, which is the site of initial strand separation.

The aim of this work was to identify the origin of replication (*oriC*) of the *Rhizobium leguminosarum* bv. *trifolii* TA1 (RtTA1) chromosome. To achieve this goal, we amplified putative *in silico* predicted *oriC* site and cloned into pDK vector (suicidal in *Rhizobium* and *Agrobacterium* species). Constructed minireplicons were then introduced into plasmid-cured *Agrobacterium tumefaciens* GMI9023 and checked for autonomous replication.

The obtained results allowed to show that RtTA1 origin of replication is located within 1100 bp DNA fragment containing numerous putative DnaA-boxes. Minireplicons encompassing this region were maintained in *A. tumefaciens* GMI9023, hence it conferred autonomous replication to a non-replicating plasmid. RtTA1 *oriC* was localized adjacent to *hemE* gene and this corresponds to the same location as the well-studied chromosome origins from *Caulobacter crescentus* and *Sinorhizobiummeliloti*.

Subsequently, an attempt to overproduce recombinant RtTA1 DnaA protein was undertaken to demonstrate binding ability of DnaA to identified DNA fragment *in vitro* with electrophoretic mobility shift assay (EMSA).

Poster # 46

BIOTRANSFORMATION OF NAPROXEN BY IMMOBILIZED *PLANOCOCCUS* SP. S5 ON *LUFFA AEGYPTIACA*

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Non-steroidal anti-inflammatory drugs (NSAIDs) used on a wide scale have an anti-inflammatory and analgesic activity. Most of them are released into the environment in unmodified or slightly modified form. Their accumulation in the environment may have a negative impact on living organisms. Naproxen is one of the commonly used NSAIDs worldwide. Its utilization usually is carried out by physicochemical processes, but they are expensive and generate free radicals and subsequent contaminations. For this reason, utilization of naproxen by biological methods appears to be most favorable.

The aim of the study was to increase the cometabolic biodegradation of naproxen by *Planococcus* sp. S5 strain, by immobilizing it on a natural sponge derived from the *Luffa aegyptiaca*. During the experiment, naproxen biotransformation by free and immobilized bacterial cells was monitored by High Performance Liquid Chromatography (HPLC).

Isolated from activated sludge, free cells of bacterial strain *Planococcus* sp. S5, were able to degrade naproxen (6 mg/L) with additional source of carbon in 48 days. Immobilized cells of strain S5 on organic sponge from a plant *Luffa aegyptiaca*, were able to cometabolic biotransformation of two doses of naproxen (6 mg/L) in 36 days.

Obtained results show the beneficial effect of immobilization on the efficiency of naproxen degradation by *Planococcus* sp. S5 strain. Formed on a luffa sponge biofilm provided greater resistance to the negative affection of naproxen and its metabolites.

Poster # 47

HABITAT-RELATED GENETIC DIFFERENCES BETWEEN ENDOPHYTES, RHIZOSPHERIC AND SOIL BACTERIA

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Soil is the major environmental source of plant associated bacteria. Most of endophytes that live within plants without causing disease, originate from the rhizosphere. However, there is still too little information on what turns a rhizospheric bacterium into a plant endophyte. Identification of genes responsible for endophytic behavior will increase our knowledge about the genetic aspects of plant-endophyte interactions. The aim of the study was to predict some of the key features that distinguish endophytic from rhizospheric and soil bacteria. Genes encoding proteins involved in plant colonization, plant-endophyte interactions and plant growth promotion were identified with Comparative Pathway Tool implemented in PATRIC database and through direct blasting. As a result a difference in the occurrence of genes between groups of microorganisms with different life style was observed. It was shown that from 58 genes that were analyzed, in endophytes, genes encoding proteins responsible for protecting cells from the damaging effects of ROS occurred more frequently than others. The genes involved in hydrogen cyanide biosynthesis and genes that encode both subunits of nitrogenase occurred more frequently in rhizospheric bacteria than in endophytes while in the soil bacteria genes encoding tartaric acid dehydratese and agmatinase were more common. It was also shown that enzymes involved in degradation of compounds commonly found in the interior of plants are more common in endophytes and rhizospheric bacteria than in soil bacteria. It may suggest that their broad degradation capacity of the compounds produced by plants can promote colonization and provide an advantage for bacteria when living inside the plant.

Poster # 48

MULTIFUNCTIONAL NANOMATERIALS FOR DRUG DELIVERY

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Introduction. The actual problem of anticancer pharmacology is in non-addressed action causing side effects in the organism. Only0.01% of drugs applied intravenously can reach their biological targets in the organism, and during less than one year over 50% of cancer (also tuberculosis) patients gain resistance to applied chemotherapeutic drugs. Here we describe the achievements of three teams in the development of two types of drug delivery nano-systems – polymeric (poly(VEP-GMA)-graft-PEG additionally functionalized with phospholipid) and mineral (Fulleren C60).

Aims. Different anti-cancer drugs (Doxorubicin, Cisplatin, Ruthenium-containing drug KP-1019, Landomycin A, novel 4-tiazolodininone derivatives) were used in native and nanocarrier-immobilized form to treat mammalian tumor cells of various tissue origin and with different mechanisms of resistance to anticancer drugs, including multidrug resistance (MDR).

Results. A new polymeric-phospholipidic hybrid delivery system distinctly enhanced the accumulation and activity of Doxorubicin and KP-1019 in all tested cancer cell lines including several MDR cell models.The resistance levels against Doxorubicin were reduced from 6- to 22-fold. The new nanocarriers were shown to rapidly (within 10 min) and effectively transport Doxorubicin into drug-resistant as well as drug-sensitive cancer cells. The treatment with the new Doxorubicin-containing nanocarriers resulted in effective cell cycle arrest in G2/M phase and ROS-induced cell death. In both *in vivo* tumor models –murine NK/Ly lymphoma and murine L1210 leukemia –Doxorubicin delivery by the new nanoformulation resulted in 100% cured animals already at low concentrations (0.1 mg/kg), while the native Dox solely extended a survival time. Thus, polymeric nanocarriers functionalized with phospholipids and PEG can enhance the efficacy and reduce the toxicity of Doxorubicin.

In another set of experiments, Doxorubicin or Cisplatin (CDDP) were immobilized on Fullerene C60 that enhanced an ability of these anticancer drugs to circumvent resistance of tumor cells to chemotherapy *in vitro.* Cytotoxic activity of CDDP-C60 nano-complex towards different lines of drug-resistant tumor cells was 1.5-2.0 times higher than that of native CDDP. That effect was accompanied by an enhanced uptake of this drug and double induction of apoptosis in target tumor cells.

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Poster # 49

BIODIVERSITY OF BACTERIOPHAGES ISOLATED FROM LYSED BATCH CULTURE OF RECOMBINANT *ESCHERICHIA COLI* BL21(DE3)

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The biodiversity of bacteriophages isolated from different sources has been under scrutiny from the moment of bacteriophage discovery until today. Current techniques allow the identification of even uncultured phages. But, the relationships between viral species and strains, as well as mechanisms of phage infection in a particular isolate often remain unclear. Thus, the search for a usable and integral model for bacteriophage population biology investigations is the topic of current interest. And, applied methodological approaches for the population analysis are not of the least importance.

Heterogeneity of viral isolates may be observed, at least, at two levels – structural and functional, which could describe complexity as mono-species populations, as well as mixed isolates. This concept was used to study a case of sporadic phage infection of *E. coli* BL21(DE3) in a large-scale bioreactor. Being exceptionally undesirable, sporadic phage lysis in microbial production appears as a case of semi-natural environment, based on which it is possible to build an integral simulated model of phage infection in a lab.

The aim of the present research was to study structural heterogeneity and biodiversity of a phage isolate from completely lysed batch culture of the recombinant *E. coli* BL21(DE3). The principal method for the study consisted of phage separation by ion exchange chromatography on fibrous DEAE-cellulose (IEC).

Results. The studied system revealed two phage populations – a major population of *Myoviridae* T4-like phage, and minor one of a *Siphoviridae* phage. This fact was examined by restriction analysis, electron microscopy, pulse-field gel electrophoresis of the primary isolate and comparative analysis of the phage pure lines. A pure line of the *Siphoviridae* phage was sequenced and annotated as phage DN1, a derivative of prophage DE3. The *Myoviridae* phage was described earlier as Lw1 [Kushkina et al., 2013]. Statistic analysis of particle measurements enabled to determine that the population of the *Siphoviridae* phage was morphologically homogenous. Whereas, the *Myoviridae* phage population consisted of structurally distinct virions, having different negative surface charge. Quantitative ratios for the observed morphological forms, as well as for viable phages were established.

Conclusions. IEC gives high advantages in phage population biology research allowing mixed phage populations to be fractionated by virion surface charge with high fidelity. The studied phage isolate had low level of species biodiversity. The massive infection was initiated by the exogenous lytic *Myoviridae* phage Lw1, while the isolate included a population of the endogenous DE3 prophage derivatives. Despite the fact, that BL21(DE3) cells possess lysogenic immunity against DE3 phage superinfection, there is a possibility, that virulent mutants of viable DE3-derivatives could form and affect the microbial production.

Poster # 50

LECTINS IN THE PURPOSEFUL DELIVERY OF DRUGS TO BODY TISSUES AND INDIVIDUAL CELLS

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Lectins are proteins or glycoproteins of vegetable, microbial or animal origin that specifically bind the carbohydrate structure (simple carbohydrates or glycoconjugates).Lectins are a powerful tool for studying carbohydrates and their derivatives in solutions, as well as on the cell surface(on the microbial and animal organisms). In addition, the promising direction of studies is the using of lectins for targeted delivery of drugs to specific cell types and tissues or specifically to microorganisms. This application of drug delivery system provides the advantages over the using of non-target drugs. This approach is important for preventing a local action of drugs on the healthy tissues and enhancing their action towards target cells.

The main advantage of lectins over the synthetic polymers is their biodegradability, as the proteins are easily hydrolyzed by the lysosomal enzymes of the cells. The major disadvantage of lectins is their relatively high molecular mass, and fact, that their administration in blood stream can cause various side effects (allergic reactions, antibodies formation). However, they can be successfully applied to for the drug delivery to tissues of the intestinal tract, rectum, oral and nasal cavities, lung, eye and skin.

The purpose of this study - the conjugation of low-molecular compounds with antitumor activity to lectin and evaluation of the biological activity of the obtained conjugate. We used synthesized thiopyrano[2,3-d]thiazole derivative (Les-1351K*)* as a low-molecular weight ligand for the conjugation to the *Pisum sativum* lectin. This target compound was covalently attached to the lectin. Conjugation was carried out through the interaction of the aldehyde groups with the amino groups of lectin in the alkaline medium (pH 9.0). The compound (0.4 mg) was immobilized to the lectin (10 mg). Conjugation of pea lectin with the antineoplastic agent led to approximately 2.5 times amplification of the inhibiting action towards the mouse leukemia cells L1210 line. The neoplastic effect of the studied conjugate is fully manifested only on the third day after the beginning of experiment, possibly after penetration of the conjugate into cells and its desintegration (Antonyuk V. O. et al., 2016).

Administration of such conjugate to the ointment or suppository basis could be promising for the treatment of rectal cancer. For example, in our previous investigation it was found that the lectin from carp roe and *Phaseolus vulgaris* erythroagglutinin were not bound by normal colon tissue, however, higher affinity of these lectins to well differentiated colon adenocarcinomas was demonstrated (Antonyuk R. V. et al., 2016). The conjugation of these lectins with Les-1351K can be performed by using the described method.

The obtained results revealed that the lectins application could be a perspective model for developing ligand-targeted drugs. Thus, the creation of more effective drugs with a higher concentration of active ingredients in some histological structures is an important approach in medicinal chemistry.

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Poster # 51

ESCHERICHIA COLI: CHARACTERISTICS OF CARBAPENEM RESISTANCE AND VIRULENCE FACTORS

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Escherichia coli, which causes urinary tract infections, neonatal meningitis, sepsis and intestinal infections, is the most common factor of nosocomial and community acquired infections. In this study, 50 *Escherichia coli* clinic specimens isolated from abscess, bronchial, urea, blood, plevral efusion, tracheal, wound, catheter, body and ear fluid cultures were collected. These isolates were identified via automated system, conventional phenotyping methods and CHROMagar Orientation. Susceptibility to carbapenem was determined by CHROMagarKPC. These isolates were analyzed by multiplex polymerase chain reaction for the genes expressed carbapenemase and virulence factors in order to determine the presence of carbapenemase (*blaIMP, blaVIM, blaOXA, blaNDM* and *blaKPC*) and nine virulence factors and investigate the association between these two characteristics. When carbapenemase susceptibility was taken into consideration, OXA-48 type of carbapenemase was determined for 22% of the total strains. Also, virulence gene regions that encountered among E.coli isolates were 92% *iutA*, 90% *aer* and 84% *fimA,* related with ferric aerobactin, aerobactin and type I fimbria, respectively. Based on the combination of carbapenemase and virulence factor genes, 24 different gene profiles were determined for all strains. Virulence factors in carbapenem-resistant and susceptible isolates were found to be more diverse. The results of the study appear to indicate that *fimA* and *afa* genes correlate with carbapenem susceptibility, the relations of *fimA* with urinary tract infections and pap with complicated urinary tract infections. It also indicates that *sfa* and *afa* genes correlate with other infections except urinary tract infections. This study is important that both to prevent spread of carbapenem resistant infections and to plan for developing effective treatments.

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Poster # 52
SCREENING OF FUNGAL PRODUCERS OF EXTRACELLULAR LACCASE

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Laccase is one of a small group of enzymes called the large blue copper proteins or blue copper oxidases. Due to their broad substrate specificity, they are widely used in many industrial processes and environmental bioremediations for removal of a large number of pollutants.
During last decades, laccases attracted scientific interest also as highly promising enzymes to be used in Bioanalytics. The aim of this study is to select an effective fungal producer of extracellular thermotolerant laccase, purify, and characterize this enzyme for using in future to assay bisphenol A (BPA), a potent endocrine disrupting chemical.
To select the most effective microbial source of the enzyme, a screening of various fungal strains was carried out. Among 17 tested strains, the best producers of extracellular enzyme were identified as *Stachybotris chartarum* and *Monilinia fructicola*. For the fungus *S. chartarum*, the optimal conditions for cultivation were selected, analytical quantity of laccase was obtained, and molecular mass of the enzyme's subunit was determined (50 κDa). As a producer of laccase, the other fungus, *M. fructicola*, was chosen, the optimal cultivation conditions for a higher extra-cellular production of laccase were studied. The enzyme was purified from cultural liquid and molecular mass of its subunit was determined (app. 35 kDa). The optimal pH range for the isolated laccase is about 4.5-5.0, the optimal pH range for the highest storage stability is 3.0-5.0. The optimal temperature for laccase activity is 30 ºC.
On the base of the isolated laccase, amperometric biosensors have been suggested for the determination of phenolic compounds, including BPA. This enzyme will be also used for construction of enzymatic reactor for removal of toxic BPA, diclofenac.
Acknowledgments. This work was financially supported by the MES of Ukraine (project 0116U004737) and by FEMS grant FEMS-RG-2016-0097.

DISTANT PARTICIPATION

TUMOR NECROSIS FACTOR-ALFA AND SOLUBLE RECEPTORS R1 SERUM LEVEL IN PATIENTS WITH GANGRENOUS AND PHLEGMONOUS APPENDICITIS

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Appendicitis is one of the most common acute abdominal inflammatory illness. The study of immune regulation of the inflammatory process is still actual because of variety of mixed laboratory data. It is known that phlegmonous appendicitis may resolve spontaneously and gangrenous can progress to perforation and peritonitis, therefore the differential diagnosis of these forms of apendicitis is important. This study investigated the inflammatory response pattern of TNFα and its soluble receptor in patients with phlegmonous (n=41), gangrenous appendicitis (n=17) and acute mesenteric lymphadenitis (AML) (n=27). Periferal blood serum were analyzed with ELISA for concentration of TNFα and soluble form of TNF receptor (sTNF-R1) preoperatively.
The serum level of TNFα was significantly higher in the gangrenous appendicitis group (39.49±1.90 pg/ml) than the AML group (5.6±0.16 pg/ml) and the phlegmonous appendicitis (19.62±1.0 pg/ml) ($P<0.001$). The serum level of sTNF-R1 had the same character: the highest level was in gangrenous appendicitis group (8.25±0.35 ng/ml) than in the AML (2.99±0.21 ng/ml) and phlegmonous appendicitis (6.23±0.05 ng/ml) ($P<0.001$). The sTNF-R1/ TNFα ratio was highest in AML group (530±2.6) than in acute gangrenous appendicitis (208±1.2). The increase sTNF-R1 serum level in AML may be compensatory to bind serum TNFα to prevent destructive action of it. In gangrenous form of acute appendicitis TNFα is prevailing over the sTNF-R1 secretion, and this may play role in necrotic changes pathogenesis. Conclusion: Serum TNFα and sTNF-R1 level can be usefully in differentional diagnosis of acute appendicitis.

IMMUNOLOGICAL REACTIVITY OF WHITE RATS WITH DIMETHYLHYDRAZINE CARCINOGENESIS

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According to World Health Organization's data, each year about 10 million patients worldwide are diagnosed with "cancer", while oncological formations of the gastrointestinal tract take third place in this ranking.
Organism implements natural protective factors, forms specific immunity to combat malignant tumors. On the other hand, the neoplastic cells develop immunosuppressive effect on the immune system. We know that in the time between birth and death, 1 million mutant cells arise per day in the body. Among them cancerous cells are present. Mechanisms aimed at the destruction of cells and cause immune surveillance function, which is implemented through immune and non-immune reactions.
The aim of the above study was to determine changes in the functioning of cellular and humoral elements of the immune systems and cytokine profile changes under simulated conditions of carcinogenesis.
Research was conducted on 50 mature outbred white male rats with body weight (190±5) g, kept in standard vivarium conditions. Experimental animals were divided in 2 groups: control (20 animals) and a group of animals with modeled cancerogenesis. Cancerogenesis was induced by subcutaneous interscapular injection of 1,2-dimethylhydrazine dihydrochloride (DMH) once a week during 30 weeks. Serum immunoglobulins (IgA, Ig M, Ig G) levels were determined using a semi-automatic biochemical analyzer «Humalyser 2000" and reagent kits manufactured by "Human Gesellschaft für Biochemica und Diagnostica mbH" (Germany). The concentration of circulating immune complexes (CIC) in serum was determined (Hrynevych Yu, 1981). The functional activity phagocytic system was determined in whole blood (Chernushenko E., 1978). Determining the concentration Tumor Necrosis Factor Alpha (TNF-α), Interleukin 1 Beta (IL-1β), Interleukin 6 (IL-6), Interleukin 2 (IL-2), Interleukin 4 (IL-4), Interleukin 10 (IL-10) carried out by ELISA using kits of reagents «Enzyme-linked Immunosorbent Assay; Kits for Rat».
A statistically significant increase in concentrations of all studied classes of serum immunoglobulins in animals with simulated neoplastic intoxication were detected: Ig A by 41.7%; Ig M by 70.0%; Ig G by 51.7% compared with the similar indicators in control group of animals. Under conditions of chronic DMH-lesion, a significant increase in CIC concentration in blood of experimental animals was 3.0 times compared to the same index in control group of animals. A study of phagocytic activity of leukocytes in animals with simulated neoplastic toxicity revealed a significant decrease in two main indicators of this system: the number of phagocytic leukocytes (% PhL), significantly decreased by 30.6%, and the phagocytic number (PhN), decreased by 29.3% comparing with similar indicators in group of intact animals.
The IL-1β content in group of animals with DMH-lesion increased significantly by 38.1% compared to the same index in the group of unaffected animals. Concentrations of IL-6 and TNFα significantly increased by 21.5% and 166.9%, respectively, compared with control group. Simultaneously, a significant decrease in concentration of IL-2 in group of animals with induced cancerogenesis was established. Under conditions of simulated lesion, IL-2 content was (7.28±0.07) pg/ml, namely, significantly lower than in group of unaffected animals by 13.8%. Analysis of obtained digital material revealed a significant increase in content of anti-inflammatory cytokines IL-10 and IL-4 in the group of animals with DMH-carcinogenesis. The concentration of IL-10 was (17.12±1.49) pg/ml, namely, exceeded similar indexes in group of healthy animals by 2.2 times. The IL-4 content in group of affected animals increased by 98.7% compared to control index and was (2.98±0.11) pg/ml ($p < 0.001$).
Thus, significant disturbances in functioning of cellular and humoral elements of the immune systems, and significant changes in cytokine profile were found to occur in simulated carcinogenesis.

CORRECTION OF OXIDATIVE PROCESSES BY SELENIUM – CHROMIUM –LIPID SUBSTANCE WITH *CHLORELLA VULGARIS* IN RATS WITH EXPERIMENTAL DIABETES

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Among the biologically active additives (BAA) that are commonly used for the prevention of metabolic disorders are native dried microalgae and substances based on them in complexes with essential micronutrients. We already know about the high saturation of algae cells by lipids of different classes that formed the idea of possible removal of separate lipid fractions and their usage in the biotechnology of production of some products with nutritional, pharmaceutical and cosmetic purposes. We used the ability of chlorella cells to absorb and accumulate inorganic compounds of metals and non-metals against the concentration gradient from the culture medium and incorporate them into macromolecules. From C*hlorella vulgaris* we received a biologically active lipid substance that contained selenium and chromium, and tested its biological effects in healthy rats and in animals with streptozotocin-induced diabetes that was complicated by obesity.
The culture of *Chlorella vulgaris Beij.* microalgae CCAP-211/11b was grown on nutrient medium Zehnder and Gorham №11, to which we added aqueous sodium selenite (Na_2SeO_3) per Se (IV) – 10.0 mg/dm^3, chromium chloride ($CrCl_3 \cdot 6H_2O$) – 5.0 Cr^{3+} mg/dm^3. The biomass of alive cells was selected after seven days of culturing and lipids were extracted using the Folch method. Experimental diabetes mellitus (DM) was caused by a single injection of streptozotocin at the rate of 65 mg/kg in rats with obesity, which was later modeled by giving rats some high-calorie foods with sodium glutamate. Starch solution of selenium-chromium-lipid complex, 1 ml of which contained 1.85 mcg of selenium, 1.1 mcg of chromium in 0.5 mg of lipid was injected to white mongrel male rats weighing 160-180 g once daily for 14 days after the development of diabetes.
At the physiologically normal conditions, the level of free radicals formation and antioxidant reserve capacity of the system is balanced. During diabetes, as one of the most complex metabolic disorders, can be observed the activation of free radical oxidation. Under these conditions in animals were seen significant metabolic changes in the body comparing to the control animals: increased indicators of oxidative stress - the contents of ROS (reactive oxygen species) (in 1,7 times), TBA-active products (1.9 times in the blood, 1.8 times in liver), diene conjugates (in 2.0 times in blood, 1.4 times in liver), while decreased catalase activity (1.3 times in blood, but increased by 20% in the liver) and superoxide dismutase (2.1 times in the blood, 21% in the liver) and reduced glutathione content (1.2 times in the blood, by 35% in the liver). Regarding glutathione peroxidase, its value was within normal limits, both in blood and in the liver.
When giving rats food with added selenium-chromium-lipid complex on the background of diabetes, we found that indicators of oxidative status of their organism, compared with those with diabetes, improved, but remained lower than in animals of the control group. Thus, we observed the decrease of TBA-active products content in the blood on 16% and 10% in the liver, diene conjugates on 12% in the blood and 7% in liver, ROC – on 40%. In the blood, compared with diabetic animals, was the increased catalase activity (on 31%), superoxide dismutase (on 27%), glutathione peroxidase (on 13%). Thus, the introduction of Se-Cr-lipid complex from chlorella during 14 days after the development of diabetes with the background obesity, promotes the normalization of different indicators of metabolism or the reduction of background intoxication that accompanies this pathology.

ANTI-TUMOR AND IMMUNE MODULATORY EFFECT OF CRYOPRESERVED FETAL LIVER CELLS INTRODUCED AT BREAST CANCER PRE-CLINICAL SYMPTOMS STAGE

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Wide range of biologically active substances with antineoplastic and immunomodulating activity produced by fetal liver cells (FLCs) is the justification for their use when treating breast cancer (BC). Successful experimental model to study the state of immune system (IS), expansion rate of cancer stem cells (CSCs) in tumor carriers, as well as assessing the efficiency of anticancer therapy are C3H mice predisposed to the development at month 13 of breast cancer (breast cancer) caused by MMTV virus (mammary tumour virus).
The purpose of research was to assess the impact of introduction of cryopreserved fetal liver cells (FLCs) on an immune status, content of CSCs in mammary gland of C3H/He mice susceptible to the BC development.
Methods. Six-month-aged C3H/He mice were injected with either cryopreserved according to a specific protocol (cFLCs) or native fetal liver cells (nFLCs) of 14 days' gestation in a dose of either 1×10^6 cells or $5x10^6$. The control was CBA/H mice and C3H/He without treatment and after introduction of the liver cells of adult mice (LCAM) at the same terms. One month later introduction of the cells to mice the content of CSCs and their progenitors in mammary gland were evaluated. In thymus, subpopulation structure was evaluated, the concentration of the glucocorticoid-sensitive cells, cell expression bcl-2 molecule.
Results. The presence in mammary gland of untreated mice C3H/He of the cells with $CD44^{hi}$ phenotype, belonging to the most invasive CSCs; increase in the concentration of cells with $CD44^+/24$ phenotype $^-$ have been shown. In thymus there were observed the reduction of $CD3^+$ and $CD25^+$ cells and strengthening of spontaneous glyucorticoid-induced apoptosis of thymocytes at lowering of $bcl\text{-}2^+$ cells. After treatment with cFLCs as well as with nFLCs, there were marked the reduced number of CSCs in mammary gland, restored content of $CD3^+$, $CD25^+$ populations, and increase in $bcl\text{-}2^+$ cells in thymus. More pronounced anti-tumor and immune modulatory effect was observed when the cFLCs were administered in a dose of $5x10^6$ and nFLCs in a dose of $1x10^6$.
Conclusions: In experimental model of breast cancer there was established an antitumor activity of both native and cryopreserved FLCs. It has been proven that the FLCs can act as immunocorrector by activating the immune system to implement an anti-tumor protection.
Significance: These results justify the use of the FLCs for the treatment of breast cancer, and reveal some of the mechanisms of antitumor action.

DEPENDENCE OF PRESERVATION OF CRYOPRESERVED *STREPTOCOCCUS PNEUMONIAE* BACTERIA ON INITIAL CELL CONCENTRATION

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Streptococcus pneumoniae bacteria, comprising 84 serovars, are among the main pathogens of non-hospital pneumonias. To develop and produce diagnosticums and drugs for a specific prevention of *S. pneumoniae* infections, technological and effective methods of storage of clinical isolates derived in various geographical areas are necessary. Low-temperature preservation in media with cryoprotective agents is applied most frequently to store microorganisms.

The research aim was to study the possibility of increasing the preservation of *S. pneumoniae* bacteria during cryopreservation due to a rise in initial cell concentration in the frozen samples.

The subject of the study was a clinical isolate of *Streptococcus pneumoniae* derived from the patient's sputum. Bacteria were grown on 5% blood agar at 37˚C for 20-24 hrs in an atmosphere containing 8-10% CO_2 and washed with distilled water, saline or meat-peptone broth (MPB). The cells were then resuspended in the mentioned above suspension media to concentrations of 10^9, 10^{10} and 10^{11} CFU/ml. Some of the cells were frozen in cryolysates of *E. faecalis, S. aureus, E. coli B and K. pneumoniae* bacteria.

To obtain the latter, the bacteria were washed with distilled water from the growth media to a concentration of 10^9 CFU/ml and frozen three times down to -196˚C. After thawing the samples were centrifuged at 1500g for 15 minutes and the supernatant was filtered through a millipore filter. The cells of *S. pneumoniae* were suspended in the resulting lysates also to the concentrations of 10^9, 10^{10} and 10^{11} CFU/ml. All the cell samples were introduced into 2.0 ml cryovials and frozen by immersion into liquid nitrogen. The samples were warmed in a water bath at 37˚C.

It has been found that after freezing the number of viable cells suspended in saline in the samples with an initial concentration of 10^9, 10^{10} and 10^{11} CFU/ml was, respectively, 0.05; 0,2 and 67,1%, after freezing in distilled water the number was 2.8; 15.7 and 91.2% and after freezing in the MPB it was 11.8; 37.5 and 94.6%. In all the cell samples frozen in bacterial lysates, the number of viable *S. pneumoniae* cells remained at baseline.

Thus, to cryopreserve bacteria, in particular *S. pneumoniae*, it is expedient to use high initial cell concentrations, i.e. 10^{11} CFU/ml and higher. One of the protective mechanisms in this case can be the release from the cells of the substances of various molecular mass at the cooling stage. At certain concentrations these substances act as cryoprotective agents.

ROLE OF BACTERIA OF THE REDUCING STAGE OF SULFUR CYCLE, ISOLATED FROM DIFFERENT BIOTOPES, IN THE PURIFICATION OF ENVIRONMENT FROM THE POLLUTANTS

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Oxidation of organic compounds and reduction of sulfur- and nitrogen-containing inorganic compounds by microorganisms in the processes of anaerobic respiration plays the significant role in the mineralization of these compounds. Pollution of aqueous and edaphic environment by sulfur and nitrogen oxoanions, organic compounds compels to search the methods of environment purification from these pollutants. Methods of biological purification are preferably used to purify wastewaters, which can be explained not only by the particularities of wastewaters composition, but also by the economical suitability of the usage of biotechnology. Physical and chemical methods are used mainly for the preliminary purification and in the complicated climate conditions.
Bacteria *Desulfovibrio desulfuricans* Ya-11 and *Desulfuromonas* sp. were isolated from Yavoriv Lake, *Desulfomicrobium* sp. and *Desulfotomaculum* sp. – from different areas of Lviv wastewater purification system. In the process of sulfur and its oxoanions reduction they produce hydrogen sulfide. Sulfidogenic activity of bacteria depends on the concentration of sulfur-containing compounds and donors of electrons, presence of alternative electron donors in the medium, pH, temperature.
Nitrite ions as the intermediate and ammonium ions as the final products of the dissimilatory sulfate reduction were accumulated during the growth of *D. desulfuricans* Ya-11 and *Desulfomicrobium* sp. in the medium with nitrate ions. Oxoanions of nitrogen inhibited sulfate reduction, which can be used for the regulation of hydrogen sulfide content in Lake.
Sulfate-reducing bacteria *Desulfuromonas* sp., *Desulfomicrobium* sp. and *Desulfotomaculum* sp. are able to utilize fumarate as the donor and acceptor of electrons. During the utilization of fumarate as the acceptor of electrons succinate is accumulated in the medium. If fumarate is utilized as a donor of electrons, small amounts of citrate, isocitrate and acetate are determined in the medium, besides succinate.
The studied bacteria utilize lactate, pyruvate, acetate, malate, propionate, fumarate, citrate, ethanol, glycerol, glucose, fructose, alanine, higher fatty acids as the donors of electrons.
The ability of sulfur- and sulfate-reducing bacteria to reduce sulfur, oxoanions of sulfur and nitrogen, to oxidize organic compounds to acetate, carbon dioxide and water under the anaerobic conditions makes them perspective objects in the bioremediation of polluted soils and waters.

STATE OF MEMBRANE OF *CHLOROBIUM LIMICOLA* IMV K-8 AND *DESULFUROMONAS ACETOXIDANS* IMV B-7384 UNDER THE INFLUENCE OF Cu^{2+} AND Fe^{3+}

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Bacteria, which carry out sulfur compounds transformation, play key role in functioning of biocenoses, influencing the geochemical cycles of carbon, sulfur and metals, which is highly important for technologically transformed territories. Cu^{2+} and Fe^{3+} ions are among the most common pollutants of water and soil environments. Toxicity of those metals is based on their participation in reactions of formation of reactive oxygen species. Maintaining the proper level of cytoplasmic membrane fluidity is an important mechanism of cell adaptation of cell to environment factors. Index of unsaturation of cell lipids and index of viscosity of membrane are used for estimation of membrane fluidity.

Two strains of microorganisms, in particular *Desulfuromonas acetoxidans* IMV B-7384 and *Chlorobium limicola* IMVK-8 were investigated in this research. *D. acetoxidans* IMV B-7384 is exoelectrogen that possesses resistance to heavy metals influence and is capable to organic compounds oxidation with simultaneous sulfur reduction and *C. limicola* IMVK-8 that carries out oxidation of organic compounds and hydrogen sulfide, formed in the process of sulfur reduction. These properties of investigated strains provide their application to microbial fuel cell that demonstrates the new approach for alternative energy generation. *D. acetoxidans* IMV B-7384 were cultivated in Postgate C medium with addition of ferric (III) citrate in concentration 10–20 mM. Suspension of *C. limicola* IMVK-8 cells was incubated during 1 hour in 0.05–0.5 mM cooper (II) sulfate solution. Metal compounds were not added into control samples. Methyl esters of fatty acids were analyzed using gas chromatograph Agilent 7890A (Agilent Technologies, USA). Standard kit of fatty acids methyl esters (Supelco, USA) was used for processing of obtained results.

In response to influence of Fe^{3+} ions in *D. acetoxidans* IMV B-7384 cells the decrease of content of unsaturated fatty acids occurred, that was displayed in diminishing of unsaturated index of lipids. Possibly, content of unsaturated fatty acids in *D. acetoxidans* IMV B-7384 cells under the influence of Fe^{3+} ions decreases because of their conversion to cyclopropane fatty acids. Decreasing of level of unsaturation of lipids could lead to decreasing of membrane fluidity that adversely affects the functioning of cell. Important part in increasing of membrane fluidity is taken by branched fatty acids. At the influence of Fe^{3+} ions on investigated bacteria cells content of 15-methyl-hexadecanoic acid increased. Enhance of content of those fatty acids, probably, caused decrease of membrane viscosity index that is observed by increasing of membrane fluidity. Thus, changes in the fatty acid composition of *D. acetoxidans* IMV B-7384 under the influence of Fe^{3+} ions provide a decreasing of content of unsaturated fatty acids, which are one of the main targets of the action of reactive oxygen species, and are aimed at maintaining a proper level of membrane fluidity.

Under the influence of 0.05–0.25 mM Cu^{2+} ions on *C. limicola* IMV K-8 cells index of unsaturation of lipids increased while membrane viscosity index decreased. It indicates the increase of membrane fluidity, compared with control. Under the influence of 0.5 mM Cu^{2+} ions investigated indices did not differ significantly from the control, however, the accumulation of biomass was reduced up to 70% at these conditions. We assume that under the influence of 0.05–0.25 mM of Cu^{2+} ions increasing of membrane fluidity is aimed at effluxing of Cu^{2+} ions from the cell. Probably, under the increasing of metal salt concentration to 0.5 mM in *C. limicola* IMV K-8 cells mechanisms that regulate membrane fluidity have been damaged.

PRODUCTION OF MOLECULAR HYDROGEN BY PURPLE NON-SULFUR BACTERIA *RHODOPSEUDOMONAS YAVOROVII* YA-2016

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The production of biohydrogen with the participation of microorganisms and its usage in different branches of the economy is promising because of the need of transition to environmentally friendly types of energy. Obtaining of molecular hydrogen using microorganisms is less energy-intensive process in comparison with chemical methods (Benemann, 1997). Both autotrophic and heterotrophic microorganisms are able to produce molecular hydrogen. According to the sourses of energy used by organisms, microbiological processes are divided into: dark anaerobic production of hydrogen; light-dependent hydrogen production; bioelectric hydrogen production using modified fuel cells (Shchurska and Kuzminsky, 2011).

The total yield of hydrogen produced by photofermentation of purple non-sulfur bacteria can be greatly improved by optimizing the culture conditions. The aim of our work was to investigate the ability of purple non-sulfur bacteria to produce hydrogen under different culture conditions.

Phototropic purple non-sulfur bacteria, isolated from lake Yavorivske, obtained in pure culture and identified as *Rhodopseudomonas yavorovii* Ya-2016 were investigated. Bacteria were cultured in modified medium of ATCC № 1449 without $NaHCO_3$ and $Na_2S \times 9H_2O$ with acetate and malate at concentrations of 12 and 36 mM and vitamin B_{12} (5 mcg/l) during 14 days under micro-aerophilic conditions, with light intensity of 200 lux and temperature of + 27…+ 30 °C. Biomasswas determinedby turbidimetricmethod. Plastic sterile syringes with volume of 2.5 ml ("Bayer") were used for obtaining samples of gas phase. The volume and composition of the gas were determined on the 7th and the 14th days of cultivation. Gas composition was determined using standard methods by means of gas chromatograph LCHM-8-MD (Drugov and Berezkin, 1981).

The results of the studies showed that bacteria grown in the medium where the content of sodium acetate was 12 mM, accumulated biomass 1.5 times less than those grown in the medium with malate. The highest biomass yield (2.07 g/l) *R. yavorovii* Ya-2016 accumulated on the 14th day of growth in the medium with 12 mM of malate.

Methane was not detected in the gas phase on the 7th and the 14th days. The strain *R. yavorovii* Ya-2016 did not produce hydrogen in the culture medium with sodium acetate. On the seventh day of cultivation concentration of oxygen was 2.32% and it decreased to 0.2% on the fourteenth day with the simultaneous increase of concentration of hydrogen. The increase of malate concentration in the culture medium from 12 to 36 mM resulted in the increase of hydrogen production from 1.49 to 7.64% on the 14th day of cultivation, respectively. Consequently, it has been found that the concentration of substrate significantly influences the production of hydrogen by purple non-sulfur bacteria *R. yavorovii* Ya-2016.Thus, the ability of hydrogen production by *R. yavorovii* Ya-2016 was investigated on the model substrate (malate). Obtained results are the basis for further research to optimize and increase the efficiency of hydrogen production from organic waste. We will be focused on genetic engineering improvement of the strain by means of the heterological overexpression of nitrogenase activators which are mediate positive regulators of hydrogen production.

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RECOVERY AT DIFFERENT TEMPERATURES OF PROBIOTIC *SACCHAROMYCES BOULARDII* IMMOBILIZED IN SODIUM ALGINATE BEADS

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Probiotic preparations immobilized in carriers based on biodegradable polysaccharide gels are becoming increasingly widespread. Technologies for long-term storage of such drugs need an experimental justification.

The aim of the research was to study the recovery of probiotic *S. boulardii* cells immobilized in granules of 1% sodium alginate at different temperatures. Gel granules were prepared by ionotropic gelation. The cross-linking agent was 0.2 M $CaCl_2$. The granules were dissolved in 2% EDTA solution. As the control were free cells suspended in a medium based on beer wort (8°B). The initial concentration of free cells was 10^9 CFU per 1 ml, concentration of immobilized cells was 10^9 CFU per 100 microgranules with the diameter of 200 μm. The samples were stored at the temperatures of 30, 40, - 20, - 40, -80, -196°C. The viability of cells was determined by the Koch's plate method on agar medium. Conditions simulating *in vitro* digestion process in human body were reproduced according to the patent (Chicherin IY et al., 2012). The respiratory activity of the cells were frozen either down to -80 or -196°C was examined by EPR method using TEMPON spin probe. The content of reactive oxygen spesies (ROS), the asymmetric distribution of phospholipids in CPM, DNA damages were investigated by flow cytometry using fluorescent probes $DCFH_2$-DA, Annexin V, 7-AAD.

It was found that at all storage temperatures the number of viable cells immobilized in gel beads exceeded the number of viable cells suspended in a liquid medium. At storage temperature of 30 °C free cells have died after 6 months, the number of immobilized cells has decreased after 1 year (observation period) by 6.2 lg CFU/ml. At the storage temperatures of 4, -20, -40, -80 °C the number of viable free cells decreased by 1.5; 5.47; 3.0; 0.7 lg CFU/ml, respectively. During storage of -196 °C free cells did not die. The number of viable immobilized cells after storage for 1 year at the temperatures of 30, 4, –20,–40°C has decreased by 6.2; 0.8; 3.1; 1.2 lg CFU/ml. During storage at -80, - 196°C the immobilized cells did not die.

Storage temperatures below 0°C did not affect the ability of gel granules to protect *S. boulardii* cells from damaging effects of simulated gastric juice and small intestine juice. Compared to free cells, the immobilized cells in the gel granules after freezing to -80, -196°C retained the initial characteristics of respiratory activity, decreased the content of ROS, the number of impairments of the asymmetric distribution of phospholipids in CPM. There were also fewer cells with fragmented DNA and late apoptotic changes.

The results of research indicated to the prospects of storage at low temperatures of probiotics immobilized in granules of sodium alginate gel.

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EVALUATION OF GENOMIC METHYLATION PATTERN OF *PHOTOBACTERIUM PHOSPHOREUM* EXPOSED TO ULTRA-HIGH FREQUENCY ELECTROMAGNETIC RADIATION

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Luminous bacteria are well-known microorganisms that widely used as indicators of various types of contamination. They change intensity of their luminescence as the response to stresses. Bacterial luminescence is a result of enzymes activity, in particular luciferase, and it was revealed several mechanisms that regulate activity of *lux*-operon. Results of previous researches showed influence of ultra-high frequency electromagnetic radiation (UHF EMR) on bacterial luminescence, cell morphological features as well as transcriptional activity of *luxb* gene encoding β-subunit of luciferase (Zelena et al., 2014), although any correlation between luminescence intensity and expression of *luxR* regulatory gene or genomic polymorphism were not detected under such treatment (Zelena et al., 2015). The aim of the present study was to characterize methylation patterns of *Photobacterium phosphoreum* genome exposed to UHF EMR. Bacterial cells were subjected to UHF EMR for 5 and 15 min; incubation of bacteria at 42°C instead of irradiation was used as the control of heating component (non-specific effect of UHF EMR). To assess methylation profile in *P. phosphoreum* genome bacterial DNA was digested by HpaII and MspI endonucleases and amplified with primer to dinucleotide repeat. Amplicon features were characterized and compared after amplification of DNA of different treatments. Results of comparison analysis between PCR products obtained after DNA cleavage by methylation sensitive/insensitive restriction enzymes revealed no differences in amplicon profiles, assuming invariability of methylation patterns in bacterial genome under UHF EMR exposure. Thus, it might suppose that impact of UHF EMR on bacterial features involves more specific regulatory mechanisms than altering whole-genome methylation pattern, however further detailed studies of genomic methylation are required.

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MOLECULAR MASS DETERMINATION OF BACTERIOCIN FROM *ENTEROCOCCUS ITALICUS* ONU547 BY TRICINE-SDS-PAGE

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Bacteriocins are cationic proteins or peptides that synthesized on ribosomes and have antagonistic activity against a number of gram-positive bacteria (H-Kittikun et al., 2015). The aim of this work was determination of molecular mass of bacteriocin, produced by *E. italicus* ONU547. Tricine-sodium dodecyl sulphate-polyacrylamide gel electrophoresis (Tricine-SDS-PAGE) was used for experiment. 20 µl of mixture of bacteriocin samples with buffer were charged in the wells and electrophoresis was performed at 40 mA, 10°C. After, the gel was fixed, stained and assayed for molecular mass and antagonistic activity against indicator *Lactobacillus sakei* INRA (H-Kittikun et al., 2015). As a result, the bands of partially purified bacteriocin (PPB), as well as of bacteriocin from cell-free supernatant (CFS), exhibited inhibitory activity against used indicator and in the case of CFS the molecular mass of the band was approximately 3 kDa. When PPB was analyzed, two bands with inhibitory activity were observed with molecular mass approximately 2 and 3 kDa. It could indicate the two-component nature of studied bacteriocin. It is known from literature data that *E. italicus* produced two types of bacteriocin – enterocin A and enterocin B, which are with molecular mass of 4.828 and 5.479 Da, respectively (Gaaloul et al., 2014). But in our study, the molecular mass of bacteriocin of *E. italicus* ONU547 was approximately 2-3 kDa. These results could indicate the possibility of production of a bacteriocin, other than enterocin A and B by this species of enterococci. It can be explained by transfer of genes that are responsible for bacteriocin production from others species of enterococci to *E. italicus* ONU547. The further study to prove these hypotheses will be performed.

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CORRECTION OF IMMUNE DISORDERS WITH CRYOPRESERVED CORD BLOOD IN MODEL OF ACUTE PURULENT PERITONITIS

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Acute purulent peritonitis (APP) remains one of the most severe inflammatory diseases and it is the cause of the development of sepsis and multiple organ dysfunction syndrome in patients. Despite a constant improvement of the methods of diagnosis and treatment of APP, the rate of post-surgery complications in the most severe forms of the disease remains high. Existing methods of surgical treatment of APP and the procedure itself (either open or laparoscopic) do not have a significant effect on the number of post-surgery complications. The studies aimed at substantiating the therapy of pathogenetic impairments at the cellular-molecular level are very limited and are mainly devoted to the study of individual immunity units, whereas a small number of studies have been devoted to the problem of the complex study of immune homeostasis in general and the development of methods for its correction.
The aim of the study was to evaluate the immunocorrecting efficiency of cryopreserved cord blood in the treatment of experimental acute purulent peritonitis.
Materials and methods. The experiments were performed in Wistar male rats of 6 months' age, weighing 180-200g. APP was modeled in rats after appendectomy. The appendix was left loosely in the abdominal cavity (AC). After 24 hours, all the experimental animals under ether anesthesia underwent relaparotomy and AC sanation with a 0.02% aqueous solution of furacilin. Cryopreserved human cord blood (cHCB) was injected into the tail vein in 0.5 ml ($5 * 10^6$ cells per 1 ml). Ampicillin was administered intravenously at a dose of 40 mg / kg. All the animals were divided into 5 groups: 1 - intact (control); 2 - with inducted APP; 3 - APP + injection of ampicillin; 4 - APP + introduction of cHCB + injection of ampicillin; 5 - APP + introduction of cHCB. The rats were examined to the 1st, 3rd, 5th, 7th days before and after treatment. The quantitative composition of $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, $CD25^+$, $CD72^+$ spleen cells by the method of cytofluorimetry; blood counts; the level of immunoglobulins (IgM, IgG IgA), the concentration of CIC, cytokines (IFNγ, IL-10), and C-RB blood; adhesive and phagocytic activity of cells of the peritoneal cavity were analyzed.
Results. The experimental study revealed that intra-abdominal infection occurring in APP was closely associated with destabilization of the immune system characterized by a deficiency of cellular immunity, a decrease in phagocytic activity of neutrophils, activation of humoral immunity (increased concentration of CIC, IgM and IgG) and the number of neutrophils in combination with proinflammatory cytokines increase. cHCB inclusion in to the treatment protocol of APP ensured the adequacy of the immune system response to a surgery trauma, as evidenced by the normalization of a number of immune status indices, the normalization of leukocounts for almost 3 days and was confirmed by the results of histological studies and clinical observations.
Conclusions. The obtained results testify to the need for the use of cHCB which has a pronounced immunocorrecting and clinical effects under the conditions of inflammatory process in combined therapy of patients with APP.

CRYOPRESERVED CORD BLOOD TO TREATE EXPERIMENTAL ISCHEMIC STROKE

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Currently, the treatment of ischemic stroke (IS) is associated with the use of neurotropic drugs for repairing the nervous tissue, as well as for reducing the inflammatory response. A search for new approaches to the treatment of IS is under way. Human cord blood (HCB) can be used as an agent for cell therapy. Due to the multicomponent cell composition and as well as the complex of biologically active compounds in it, it was favorable to apply the cryopreserved human cord blood (cHCB) to treat IS in the experiment.
Objective. The research aim was to assess the possibility of using the cryopreserved cord blood in the combined treatment of experimental IS.
Materials and methods. The experiments were performed in 6-month-old male Wistar rats. IS was modeled by occlusion of the middle cerebral artery (CMAo). The HCB were cryopreserved (cHCB) with the programmable freezer UOP-6 (SDCB with EU at the IPC&C NASU). The cHCB were intraperitoneally introduced in 0.5 ml in a dose of 5 x 10^6 cells 6 hrs later the induction of IS. Cerebrolysin was injected for 3 days in 0.1 ml per 100 g of animal weight. The parameters of immunological and neurological status of rats in dynamics of the IS development after the treatment were analyzed. The subpopulation composition of spleen cells ($CD3^+$, $CD4^+$, $CD8^+$, $CD25^+$), the concentration of cytokines (IFN-γ, IL-10), content of C-reactive protein (C-RP) and circulating immune complexes (CIC) were determined; as well as phagocytic and adhesive activity of MPS cells were done. The structure of the brain and lymphoid organs was studied. There was performed behavioral and neurological testing of animals. The experimental data were statistically processed by the Student-Fisher method using the Statistica 7.0 program, (Stat Soft Inc), adapted to the tasks posed.
Results. Treatment of IS by the introduction of cHCB with cerebrolysin contributed to the restoration of clinical blood counts; normalization of C-RP and CIC concentrations; phagocytic and adhesive activity of MPS cells. The percentage of $CD3^+$, $CD4^+$, $CD8^+$ T-lymphocytes increase with a decrease of $CD4^+$ $CD25^+$ cells. There were a correction of the indices of cytokine concentration, i.e. a decrease in the concentration of pro-inflammatory and an increase in anti-inflammatory levels. The structure of the brain and lymphoid organs was restored; the neurological status of animals was normalized.
Conclusions. Based on the experimental study findings on the combined treatment of IS with the administration of cHCB and cerebrolysin, it could be recommended to use it in clinical practice.

IMMUNOCORRECTION BY CRYOPRESERVED FETAL LIVER CELLS IN EXPERIMENTAL ATOPIC DERMATITIS

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Recently there has been a steady increase in the incidence and prevalence of allergic skin diseases: atopic dermatitis (AD), contact dermatitis, eczema. Everywhere there is an increase in the severity of the course and torpidity of allergic dermatoses to the therapy. The developing immune-dependent inflammation is aggravated by a genetically determined defect in the cutaneous barrier due to impaired function of the filagrin gene. The multifactority of pathogenetically significant factors for the development of AD is recognized, including immunological disorders in the cellular and humoral units of immunity. There is a hyperproduction of immunoglobulins of class E (IgE), which not only can directly bind the antigen that causes the development of the atopic process, but also actively participates in its presentation by dendritic cells to T-lymphocytes (Th2). Overlay the secondary infection, which is chronic and recurrent, against the background of imbalance of the state of local and systemic immunity in AD is an indication for the treatment of this disease by immunomodulators. In this work, a suspension of cryopreserved fetal liver cells (cFLC), which have a pronounced immunomodulating activity, was used as those mentioned. Samples of cFLC were stored at a low-temperature bank IPC&C NASU at -196° C.

The aim of this investigation is to evaluate the effectiveness of cryopreserved fetal liver cells for correction of the immune system in the rats with atopic dermatitis.

Materials and methods. The experiments were performed in Wistar rats at the age of 6 months, weighing 180-200g. The AD was modeled by the method of Zalkan PM. and Ivleva EA (1965). Dinitrochlorobenzene in 0.5 ml of 5% alcohol-acetone solution was rubbed into the skin of the back (3x4 cm2) of animals daily through 21 days. The FLC were cryopreserved with the program freezer UOP-6 (Special Designing and Technical Bureau with Experimental Unit of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukrain). Animals were divided into groups: 1 - intact (control); 2 - AD without treatment; 3 - AD + standard treatment (prednisolone ointment); 4 - AD + nFLC; 5 - AD + cFLC. Immunocorrecting effect of cFLC was assessed by the degree of recovery of cellular (CIU) and humoral (GIU) immunity units and monocyte-phagocytic system (MPS). A clinical blood test was performed; the content of the C-RP was determined in it. The structure of the skin and organs of the lymphohematopoietic complex was studied on the 3rd, 7th, 14th, 21st days after the induction of AD and treatment.

Results of the study. The obtained data indicate that the therapeutic effect of standard therapy with prednisolone was observed in rats with AD induction on the 7th day. While after treatment with FLC that was observed even on the 3-5th days of the beginning of therapy and regardless of which bioobject was used, i.e. native (nFLC) or cryopreserved (cFLC).

ECOLOGICAL TROPHIC GROUPS OF MICROORGANISMS OF YAVORIVSKE LAKE WATER

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The ratio of ecological trophic groups of microorganisms depends on the loadings on the environment and determines the direction of substances transformation in it. Changes in the species composition and the quantitative ratio of different groups of microorganisms are a complex signal of ecological changes (Patyka et al., 2014). Microbiological control of the main ecological trophic groups of microorganisms of technogenic formed Yavorivske lake, with an area of more than 700 ha and a depth of more than 90 m, and their interconnections are carried out by us since 2001 (Baran et al., 2003; Moroz et al., 2008). The purpose of this work was to investigate the chemical composition of lake water and to study the main ecological trophic groups of microorganisms, involved in transformation of carbon, nitrogen and sulfur compounds, to estimate the ecological state of reservoir. The chemical analysis of water samples taken from different depths (0–70 m) of Yavorivske lake in the autumn of 2016 showed, that acidity, mineralization and Na^+, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^- and NO_3^- content at all depths, K^+, $P_2 0_5$ and NH_4^+ at depths up to 20–50 m were insignificant and did not exceed the permissible norms. At all depths the content of SO_4^{2-} (913–1530 mg/l) and H_2S (30–34 mg/l) at depths over 30 m significantly exceeded the norms. It was established that the change in the quantity of ecological trophic groups of microorganisms and their ratio in the lake water, compared with the source type reservoir of Roztochya reserve, is an adaptive response of the microbial community to stress environmental factors. The total quantity of all ecological trophic groups of microorganisms in water of Yavorivske lake with depth decreases from 5.24 to 2.45 CFU×10^6/ml of water and is 2.4–5.2 times lower than in the control: 1.28×10^7 CFU/ml of water. In the lake is formed new microbocenosis, in which on the surface and at depth of 30 m the most numerous ecological trophic groups are neutrophilic colorless sulfur oxidizing bacteria (39.3 and 13.3%), microorganisms that use organic forms of nitrogen (21.6 and 42.9%), nitrifying (7.3 and 9.4% of phase I, 13.5 and 4.9% of phase II of nitrification), denitrifying (7.1 and 11.3%), oligonitrophilic bacteria (4.4 and 5.1%), microorganisms that use mineral forms of nitrogen (5.8 and 1.4% respectively) and phototrophic nonsulfur bacteria (4.9% at depth of 30 m). Sulfate and sulfur reducing (42.9 and 44.9%), phototropic sulfur (2.7 and 4.5%) and nonsulfur bacteria (4.4 and 3.7% respectively) are the most numerous groups of microorganisms at depths of 50 and 70 m of the lake. The quantity of acidophilic colorless sulfur oxidizing bacteria, cyanobacteria, cellulose degrading microorganisms and microscopic fungi at all depths was insignificant. In control the most numerous ecological trophic groups are microorganisms that use organic forms of nitrogen (28.3%), oligonitrophilic (25.6%), nitrifying (23.5% of phase II and 15.7% of phase I of nitrification), neutrophilic colorless sulfur oxidizing bacteria (3.0%), microscopic fungi (1.9%). Their number, besides neutrophilic colorless sulfur oxidizing bacteria, is much higher than in the lake water. The sulfur oxidizing and sulfidogenic microbial community of Yavorivske lake may be a site for isolation of new strains valuable for biotechnological application.

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ADDITIONAL POSTER

Poster # 53

CLINICAL AND IMMUNOLOGICAL EFFICIENCY OF CRYOPRESERVED CORD BLOOD APPLICATION IN THE MODEL OF GENITAL HERPES

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One of the most serious actual medical and social problems is genital herpes (GH) caused by herpes simplex virus -1 (HSV-1). Certain results have been achieved in the study of the etiopathogenesis of GH, which is defined as immuno-mediated dermatosis. The main goal of the optimized approaches to its treatment is the suppression of HSV-1 reproduction during exacerbation and formation of an adequate immune response. For this, it is necessary to use the drugs with systemic immunomodulatory activity. The cryopreserved (cHCB) and lyophilized (lHCB) human cord blood, which proved to be a functional broad-spectrum immunomodulator, were used here for the treatment of GH in rats induced by HSV-1. The cHCB when combined with the drug "Acyclovir" (ACV), whose action is aimed to inhibition of the viral DNA polymerase by influencing the patient's IS can provide an effective treatment of GH induced by HSV-1.
This research aim was to assess the clinical and immunological efficacy of cryopreserved cord blood application in a combined treatment of genital herpes in rats.
Materials and methods. The experiments were performed in Wistar female rats of 6 months' age, weighing 180-200g. GH was simulated by intraperitoneal injection of 1 ml of HSV-1 (titer 1: 20000). The HCB (cHCB) was cryopreserved with the programmed freezer UOP-6 (Special Designing and Technical Bureau with Experimental Unit of the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukrain). The cells (0,5 ml) were intraperitoneal injected at a concentration of $5 * 10^6$ cells the day of GH induction. ACV was administered at a dose of 50 mg / kg of animal weight daily for 5 days. The rats were divided into groups: 1 - intact (control); 2 - GH; 3 - GH + ACV; 4 - GH + cHCB; 5 - HG + cHCB + ACV. Indices of cellular (CLI) and humoral (HLI) link of immunity; structures of uterine endometrium in rats were estimated to the 3rd, 7th and 14th days after GH induction and treatment.
Results. Our findings testify to a high clinical efficacy of cHCB application in most animals with GH. A pronounced corrective effect of cHCB on the state of immune homeostasis of experimental animals was established. This therapy contributed to the recovery of cytolytic function of NK, the normalization of cytokine and interferon status. The recovery of intercellular association eliminated disproportions in T-cell immunity, hyperactivation of the humoral link of immunity and neutrophilic granulocytes. A distinct correlation was found between the change in the number of ICC of the spleen, which determined the profile of inflammatory and anti-inflammatory cytokines of a body and the endometrial structure. The maximum pronounced normalization of the CIC in such treatment provided a reduction of inflammatory responses and a proper approximation of the structure of uterine endometrium in rats to a normal structure than in animals treated with ACV only.
Conclusions. A high clinical efficacy of a combined therapeutic method of GH animals by means of cHCB with ACV was proved. Favorable tolerability of this therapy and no side effects allow us to recommend this therapeutic method for a widespread use in patients with GH.

Poster # 54

CONSTRUCTION OF *CANDIDA FAMATA* YEAST STRAIN WITH HETEROLOGOUS EXPRESSION OF THE KEY GENE OF ROSEOFLAVIN BIOSYNTESIS *rosB*

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The non-pathogenic Gram-positive bacteria *Streptomyces davawensis* and *Streptomyces cinnabarinus* synthesize the riboflavin (vitamin B_2) analogs roseoflavin (RoF) and its biosynthetic precursor aminoriboflavin (8-demethyl-8-amino riboflavin, AF). Both compounds are antibiotics and show a strong suppressive effect on Gram-positive bacteria as *Staphyloccus aureus.* AF has a lower toxic potential and may be better suited as a lead structure to develop antimicrobial compounds. Fortunately, AF is not toxic for mammal cells, in contrast to RoF. AF is synthesized in *Streptomyces* from flavin mononucleotide (FMN). Available FMN overproducing strain of yeast *Candida famata* could serve as a promising platform for construction of the heterologous yeast AF overproducing strains.
Gene *rosB* of *S. davawensis*, encoding N,N-8-demethyl-8-aminoriboflavin-5'-phosphate synthetase, is the key gene of AF biosynthesis. The synthetic *rosB* gene with codons adapted for *C. famata*, was introduced into genome of the recipient yeast strain under the control of constitutive promoter of gene *TEF1 Debaryomyces hansenii*, encoding elongation translation factor. Selected transformants were stabilized, and the presence of *rosB* gene expression cassette in the genome of the recipient was confirmed by PCR. Constructed recombinant strains of *C. famata* acquired the ability to produce fluorescent product, the putative AF, in concentration of around 5 mg/L.

AUTHORS INDEX

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