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L5P a specific lipopeptide of *Mycobacterium avium* subsp. *Paratuberculosis*: feature and innovative diagnosis applications

Franck Biet, Christelle Ganneau, Thierry Cochard, Pierre Lafaye, Sylvie Bay

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The 3rd Workshop on Microbiology in Health
and Environmental Protection

MIKROBIOT 2013

LODZ, POLAND, 17–20 IX 2013

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The 3rd Workshop on Microbiology in Health and Environmental Protection

MIKROBIOT 2013

SEPTEMBER 17–20, 2013, LODZ, POLAND

EDITED BY WIESŁAWA RUDNICKA

PATRONAGE

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GENERAL MEETING INFORMATION

DATE AND CONFERENCE VENUE

From Tuesday, 17 September 2013 until Friday, 20 September 2013

MAIN AUDITORIUM

Faculty of Biology and Environmental Protection
141/143 Pomorska Street, 90-237 Łódź

Building D

MASS SPECTROMETRY AND PROTEOMICS IN MICROBIAL STUDY WORKSHOP-PRACTICALS

The Faculty of Biology and Environmental Protection
12/16 Banacha Street, 90-237 Łódź

Building A

FLOW CYTOMETRY WORKSHOP

Faculty of Biology and Environmental Protection
141/143 Pomorska Street, 90-237 Łódź

Building D

CAFETERIA

University of Łódź Campus Canteen
14 Lumumby Street, Łódź

GET-TOGETHER PARTY

21 Pomorska Street, 90-202 Łódź

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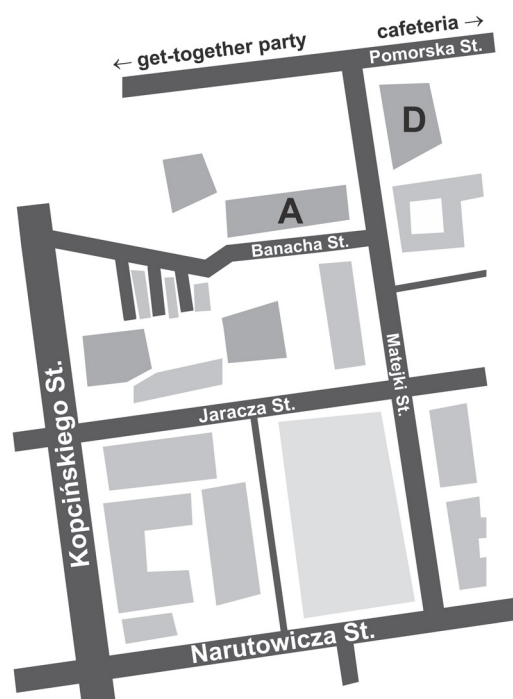
Registration Desk will be open within the entire Workshop.

Regular Participant: 350 PLN (90 €)

Student including doctoral students: 250 PLN (60 €)

Registration fee includes:

- access to all scientific sessions including plenary, oral and poster sessions,
- access to Opening and Closing Ceremony,
- access to the Exhibition Hall,
- delegate bag including Abstract Book and all conference materials,
- participation in The Flow Cytometry Workshop and The Mass Spectrometry and Proteomics in Microbial Study (subsequent registration required),
- certificate of attendance,
- lunches and coffee breaks,
- Get-together party



BADGES

Badges must be worn at all times during the Workshop and allow the admittance to the Cafeteria and Get-together party.

CERTIFICATE OF ATTENDANCE

The Certificate of Attendance confirming the participation in MIKROBIOT 2013 (33 education points) will be available at the Registration Desk.

PLENARY LECTURES AND ORAL PRESENTATIONS

The authors of plenary lectures and oral communicates are asked to prepare their presentations in PowerPoint format on CD/DVD/CD-RW device (signed with the name and date of presentation) and to provide it at the Registration Desk at least one hour before the presentation. Lecture and short oral presentation slots are respectively 30 and 15 minutes long. Please limit your presentation to 25 and 12 minutes respectively while leaving the remaining time for questions and comments.

POSTER SESSIONS

The authors (excluding oral presentations) are asked to prepare posters corresponding to the results presented in the accepted abstract. The size of the poster should be kept within 80 × 110 cm. Poster Sessions will be displayed in the Poster Session Area in the Building D, next to the Registration Desk. The abstracts with assigned numbers are published in the Conference Book. Presenters are requested to set up posters (in the morning of the designated day) and to remove them at the end of the each poster session.

Posters that are not removed by the Presenters will be stored in The Registration Desk. The presenting authors are asked to stand beside their posters during the chaired poster session.

EXHIBITOR BOOTHS

All participants are welcome to visit the stands of our Sponsors, Exhibitors and Partners located in the Exhibition Areas.

SCIENTIFIC PRIZES

Scientific Awards and Scientific Distinctions funded by the Sponsors and Organizers will be awarded to the authors of the most prominent research works presented on MIKROBIOT 2013 during The Closing Ceremony on the last day of the Workshop (20.09).

OFFICIAL LANGUAGE

The official language of the Workshop is English.

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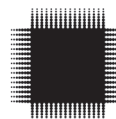
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Dear Friends and Colleagues,

On behalf of the Organising Committee, the Institute of Microbiology, Biotechnology and Immunology at the University of Lodz, the Committee on Microbiology of the Polish Academy of Sciences, the Institute of Medical Biology of the Polish Academy of Sciences, and Polish Society of Microbiologists, I have a great pleasure and honour to welcome you to 3rd International Workshop “Microbiology in Health and Environmental Protection – MIKROBIOT 2013” taking place in Lodz, Poland.

MIKROBIOT 2013 is aimed to provide an excellent platform for exchanging the most up-to-date advances in medical and environmental microbiology, microbial biotechnology, genetics and immunology. We are proud that outstanding experts in these fields have accepted our invitation to present plenary lectures. We hope to provide a meeting atmosphere conducive to constructive interactions of leading senior scientists with young researchers, students and postdoctoral fellows. The editions of MIKROBIOT 2008 and MIKROBIOT 2010 were a success. The MIKROBIOT 2013 promises to be even more successful. Four days of plenary lectures, the technical trainings in Flow Cytometry, Mass Spectrometry and Proteomics in Microbial Study, several oral presentations and over two hundred poster exhibitions will give an opportunity to exchange ideas, discuss and process findings for the benefit of the participants and development of new scientific collaborations.

The Organizers would like to express gratitude to The Mayor of Łódź, The Marshal of the Lodzkie Region, Rector of the University of Łódź, Dean of the Faculty of Biology and Environmental Protection, the Editorial Staff of Advances in Microbiology and all Sponsors. We would also like to cordially thank our colleagues and friends who helped organise the workshop.

We wish all MIKROBIOT 2013 participants a successful meeting and a very pleasant stay in Łódź.



Wiesława Rudnicka
Chairman of the Scientific Committee

MIKROBIOT 2013
– The 3rd Workshop on Microbiology in Health
and Environmental Protection

Day I (Tuesday, September 17)

08.00–08.45 **Registration**

(open throughout the entire Workshop)

08.45–09.15 **Welcome address and official opening of the Workshop**

chair: Antoni Różalski, Wiesława Rudnicka

SESSION I.

MOLECULAR HOST-PATHOGEN-ENVIRONMENTAL INTERACTIONS

session dedicated to prof. Adam Jaworski

chair: Jarosław Dziadek, John Blaho

09.15–10.00 **DNA STRUCTURE AND INSTABILITIES RELATED TO HUMAN GENOMIC DISORDERS:
WITH APPRECIATION AND ADMIRATION OF PROF. ADAM JAWORSKI**

ROBERT D. WELLS – Institute of Biosciences and Technology, Center for Genome Research,
Houston, Texas, USA

10.00–10.30 **ENVIRONMENTAL EFFECTS ON GENOME INTEGRITY IN PROKARYOTES**

RICHARD BOWATER – School of Biological Sciences, University of East Anglia, Norwich,
United Kingdom

10.30–11.00 **ONCOAPOPTOSIS: HARNESSING CELLULAR ANTIMICROBIAL RESPONSES AS A NOVEL
MOLECULAR THERAPEUTIC FOR CANCER TREATMENT**

JOHN BLAHO – The City College of New York, Center for Advanced Technology, New York, USA

11.00–11.15 Coffee break

11.15–11.45 **ANTI-ALLERGIC EFFECTS OF *LACTOBACILLUS PLANTARUM* L-137 ISOLATED FROM
A TRADITIONAL FERMENTED FOOD AND DEVELOPMENT OF ORAL VACCINE WITH
THE *LACTOBACILLUS***

YOSHIKATSU MUROOKA – Osaka University, Osaka, Japan

11.45–12.15 **CRYSTALLOGRAPHIC STUDIES OF THE TYPE III SECRETION SYSTEM: WATCHING AT
ATOMIC RESOLUTION HOW PATHOGENIC GRAM-NEGATIVE BACTERIA ATTACK AND
SECRETE THEIR LETHAL LOAD INTO EUKARYOTIC HOST CELLS**

ZYGMUNT DEREWENDA – Department of Molecular Physiology and Biological Physics,
University of Virginia School of Medicine, Charlottesville, USA

12.15–12.30 **MICROBIAL SEQUENCING WITH THE ION PGM™ SYSTEM**

MARCO PIROTTA – Manager Business Development Next-Generation Sequencing,
Life Technologies

12.30–14.00 **Lunch**

14.00–16.30 **Oral presentations of Ph.D. Students of Institute of Medical Biology, Polish Academy of Sciences**
(supported by Project Operational Programme – Innovative Economy (OP IE) 01.01.02–10–107/09
“Studies of the molecular mechanisms at the interface the human organism-the pathogen-
environmental factors (InterMolMed)”)

14.00–14.15 **PATHWAYS TO SYNTHESIS OF THE MALONYL-CoA IN *MYCOBACTERIUM***

JAKUB PAWEŁCZYK, supervisor – Jarosław Dziadek

14.15–14.30 **SELECTED INNATE IMMUNITY FACTORS IN PULMONARY TUBERCULOSIS PATIENTS**

ANNA SOKOŁOWSKA, supervisor – Maciej Cedzyński

14.30–14.45 **THE INTERACTION OF COMPLEMENT-ACTIVATING LECTINS WITH *MYCOBACTERIUM
TUBERCULOSIS* AND ITS CELL FRACTIONS**

MARCIN A. BARTŁOMIEJCZYK, supervisor – Anna Świerzko

14.45–15.00 **MOLECULAR GENOTYPING OF HUMAN INTESTINAL *E. COLI* STRAINS**

ANNA B. KUBIAK, supervisor – Paweł Parniewski

- 15.00–15.15 **CONSEQUENCES OF TLR2 ACTIVATION IN MONOCYTE-MACROPHAGE DIFFERENTIATION MODELS**
IWONA SACHRAJDA, supervisor – Łukasz Pułaski
- 15.15–15.30 **INFLUENCE OF ENZYMES DEGRADING CHOLESTEROL ON THE RESPONSE OF HUMAN MACROPHAGES TO *MYCOBACTERIUM TUBERCULOSIS* INFECTION *IN VITRO***
MARTA BRZEZIŃSKA, supervisor – Magdalena Klink
- 15.30–15.45 **COMPUTER-AIDED DISCOVERY OF NEW *M. TUBERCULOSIS* DNA LIGASE A INHIBITORS**
MARCIN NOWOSIELSKI, supervisor – Marcin Hoffmann
- 15.45–16.00 **EVALUATION OF PRIMASE DnaG OF MYCOBACTERIA AS A POTENTIAL TARGET FOR ANTIBIOTICS**
ANETA KURONŃ, supervisor – Jarosław Dziadek
- 16.00–16.15 **PARTICIPATION OF *MYCOBACTERIUM TUBERCULOSIS* PROTEINS OF DNA DOUBLE-STRAND BREAKS REPAIR SYSTEMS IN THE INFECTION OF HUMAN MACROPHAGES WITH MTB, *IN VITRO***
IZABELA SZULC, supervisor – Magdalena Klink
- 16.15–16.30 **TRS-BASED PCR AS A POTENTIAL TOOL FOR INTER-SEROVAR GENOTYPING *SALMONELLA* ENTERITIDIS, TYPHIMURIUM, INFANTIS, VIRCHOW, HADAR, NEWPORT AND ANATUM**
ANNA KRZYŻANOWSKA, supervisor – Paweł Parniewski
- 16.30–16.45 **Coffee break**
- 16.45–17.45 **Oral presentations of selected abstracts**
chair: Paweł Stączek, Robert D. Wells
- 16.45–17.00 **SENSITIZATION OF BREAST CANCER CELLS TO DNA DAMAGE AGENTS WITH DNA-PK INHIBITOR**
WOJCIECH M. CISZEWSKI – Northern Institute of Cancer Research, Newcastle University, Framlington Place, United Kingdom
- 17.00–17.15 **THE FUNCTION OF RNase H CLASS IS ESSENTIAL FOR SURVIVAL OF *MYCOBACTERIUM SMEGMATIS***
ALINA MINIAS – Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland
- 17.15–17.30 **TOPOLOGY-DEPENDENT DnaA INTERACTIONS WITH *HELICOBACTER PYLORI* ORIGIN OF CHROMOSOMAL REPLICATION**
RAFAŁ DONCZEW – Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Department of Microbiology, Wrocław, Poland
- 17.30–17.45 **A POTENTIAL REGULATORY SYSTEM OF *STAPHYLOCOCCUS AUREUS* IMPLICATED IN RESPONSE TO DIFFERENT STRESS STIMULI**
MICHAŁ BUKOWSKI – Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Cracow, Poland
- 17.45–18.45 **Poster session and coffee break**

Day II (Wednesday, September 18)

SESSION II.**MICROBIAL BIOTECHNOLOGY FOR ENVIRONMENTAL PROTECTION AND INDUSTRY***chair: Jerzy Długoński, Leo de Graaff*

- 08.30–09.00 **MICROBIOLOGICAL MUTAGENICITY TESTS WITH THE USE OF GENETICALLY MODIFIED STRAINS OF *VIBRIO HARVEYI* IN STUDIES ON MUTAGENIC POLLUTION OF ENVIRONMENT**
GRZEGORZ WĘGRZYN – Molecular Biology Department, University of Gdańsk, Gdańsk, Poland
- 09.00–09.30 **COMPATIBLE SOLUTES; THEN AND NOW**
MILTON S. DA COSTA – Departamento de Ciências da Vida and Centro de Neurociências e Biologia Celular, Universidade de Coimbra, Coimbra, Portugal
- 09.30–10.00 **SUB-CELLULAR PROTEOMICS TO DISCOVER MECHANISMS IN MICROBIAL PHYSIOLOGY**
LEO DE GRAAFF – Microbial Systems Biology, Laboratory of Systems and Synthetic Biology, Wageningen, University of Wageningen, Netherlands
- 10.00–10.15 **Coffee break**
- 10.15–10.45 **SPECIES BIOTYPIZATION, WHY *DE NOVO* SEQUENCING ALWAYS WINS?**
MARIO CINDRIĆ – Laboratory of System Biomedicine and Centre for Proteomics and Mass Spectrometry, “Rudjer Boskovic” Institute, Croatia
- 10.45–11.15 **NON-RIBOSOMAL PEPTIDES PRODUCED BY THE BALTIC CYANOBACTERIA – STRUCTURE AND ACTIVITY**
HANNA MAZUR-MARZEC – Institute of Oceanography, University of Gdańsk, Gdynia, Poland
- 11.15–11.45 **MASS SPECTROMETRY FOR MICROBIAL GLYCOMICS**
WŁODZIMIERZ JACHYMEK – Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, PAS, Wrocław, Poland
- 11.45–12.15 **DIASORIN ARROW – SMALL IN SIZE, BIG IN PERFORMANCE**
PAULINE BOYLE – Global Distribution Manager Molecular, DiaSorin, Symbios
- 12.15–13.45 **Lunch**
- 13.45–15.15 **Oral presentations of selected abstracts**
chair: Hanna Mazur-Marzec, Yoshikatsu Murooka
- 13.45–14.00 **MALDI-TOF/TOF ANALYSIS OF *METARHIZIUM VELUTIUM* PROTEOME DURING 4-*n*-NONYLPHENOL BIODEGRADATION**
RAFAŁ SZEWCZYK – Department of Biotechnology and Industrial Microbiology, Institute of Microbiology, Biotechnology and Immunology, Faculty of Biology and Environmental Protection, University of Lodz, Poland
- 14.00–14.15 **BALTIC BACTERIA – EFFECTIVE DEGRADERS OF CYANOBACTERIAL NONRIBOSOMAL PEPTIDES**
ANNA TORUŃSKA – Laboratory of Biochemical Ecology and Microorganisms, Institute of Oceanography, University of Gdańsk, Poland
- 14.15–14.30 **HALOTOLERANT MICROORGANISMS ASSOCIATED WITH THE PLANTS IN SALINE SOIL**
SONIA SZYMAŃSKA – Department of Microbiology, Nicolaus Copernicus University in Torun, Poland
- 14.30–14.45 **GENETIC MODIFICATION OF GLYCEROL FERMENTATIVE CATABOLISM IN WILD TYPE STRAIN *C. FREUNDII* AD970**
KATARZYNA CZACZYK – Department of Biotechnology and Food Microbiology, Poznan University of Life Sciences, Poznań, Poland
- 14.45–15.00 **THE TORTUOUS TRIMETHYLAMINE DEGRADATION PATHWAYS IN TWO STRAINS OF GENUS *PARACOCCLUS* (ALPHAPROTEOBACTERIA)**
KATARZYNA KUŹMICZ – Department of Bacterial Genetics, Institute of Microbiology, Faculty of Biology, University of Warsaw, Poland
- 15.00–15.15 **MOLECULAR METHODS FOR IDENTIFICATION OF BACTERIAL STRAINS**
- 15.15–17.15 **COLONIZATION OF HISTORIC BUILDINGS**
ANNA OTLEWSKA – Institute of Fermentation Technology and Microbiology, Lodz University of Technology, Poland
- 15.15–17.15 **Poster session and coffee break**
- 16.15–19.30 **Mass Spectrometry and Proteomics in Microbial Study – sample analysis**
- 20.00 **Get-together party**

Day III (Thursday, September 19)

SESSION III.**INNATE AND ADAPTIVE RESPONSES TO MICROBES.****THE BENEFICIAL AND HARMFUL ASPECTS***chair: Marek Niemiałtowski, Leif Percival Andersen*

- 09.00–09.30 **ALTERATION OF THE INNATE IMMUNE RESPONSE DURING COPD EXACERBATION**
PHILLIPE GOSSET – Institut Pasteur de Lille, France
- 09.30–10.00 **ROLE OF THE BACTERIAL CELL WALL GLYCOCONJUGATES IN ELICITATION OR SUPPRESSION OF EUKARYOTIC INNATE IMMUNITY**
ANTONIO MOLINARO – Department of Chemical Sciences, Università di Napoli Federico II, Napoli, Italy
- 10.00–10.15 **HIGH CONTENT SCREENING, NOVEL IMAGING TECHNOLOGY FROM MOLECULAR DEVICES**
MIKOŁAJ GMYR – Syngen Biotech, Wrocław, Poland
- 10.15–10.30 **Coffee break**
- 10.30–11.00 **MICROBIAL CELL ACTIVATION AND INFLAMMATION TRIGGERING**
JOËL PESTEL – Unité de Glycobiologie Structurale et Fonctionnelle, CNRS UMR 8576, IRF 147, Université Lille-Nord de France
- 11.00–11.30 **ZEBRAFISH EMBRYOS AS A NEW BIOLOGICAL MODEL TO STUDY THE PATHOPHYSIOLOGICAL EVENTS OF MYCOBACTERIUM ABSCESSUS INFECTION**
LAURENT KREMER – Laboratoire de Dynamique des Interactions Membranaires Normales et Pathologiques, Université de Montpellier 2, CNRS UMR 5235, Montpellier, France
- 11.30–12.00 **SEEING THROUGH HOST-PATHOGEN INTERACTIONS USING A ZEBRAFISH EMBRYO INFECTION MODEL FOR BURKHOLDERIA CEPACIA COMPLEX**
ANNETTE VERGUNST – INSERM U1047, Université de Montpellier 1, UFR Médecine, Nîmes, France
- 12.00–12.30 **L5P A SPECIFIC LIPOPEPTIDE OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS: FEATURE AND INNOVATIVE DIAGNOSIS APPLICATIONS**
FRANCK BIET – INRA, UMR ISP 1282, Infectiologie et Santé Publique (ISP-311), Centre Val de Loire F-37380 Nouzilly, France
- 12.30–14.00 **Flow Cytometry Workshop – Becton Dickinson**
- 14.00–15.30 **Lunch**
- 15.30–16.30 **Oral presentations of selected abstracts**
chair: Magdalena Chmiela, Phillipe Gosset
- 15.30–15.45 **GALLERIA MELLONELLA HEMOLYMPH PEPTIDES – A NEW WEAPON AGAINST LEGIONELLA BACTERIA**
ELŻBIETA CHMIEL – Department of Genetics and Microbiology, Maria Curie-Skłodowska University, Lublin, Poland
- 15.45–16.00 **PREPARATION OF GLYCOCONJUGATES HAVING OLIGOSACCHARIDE MOIETY FROM BACTERIAL SOURCE**
ALBERTO MARIA MARZAIOLI – Department of Chemical Sciences, Università di Napoli Federico II, Napoli, Italy
- 16.00–16.15 **IMMUNOPROTECTION AGAINST CHRONIC TOXOPLASMA GONDII INFECTION INDUCED BY RECOMBINANT ANTIGENS OF THE PARASITE**
JUSTYNA GATKOWSKA – Department of Immunoparasitology, Faculty of Biology and Environmental Protection, University of Lodz, Poland
- 16.15–16.30 **USE OF IL-2 PRESENTING BACILLUS SUBTILIS SPORES LEADS TO DEVELOPMENT OF CELLULAR IMMUNE RESPONSE TO ORALLY ADMINISTERED SPORE BASE VACCINE**
IWONA PIĄTEK – Department of Medical Biotechnology, Intercollegiate Faculty of Biotechnology UG-MUG, Poland
- 16.30–16.45 **THE EFFECT OF CpG ODN AND OXYDIZED CHOLESTEROL ON THE INTEGRITY AND IMMUNOMODULATORY PROPERTIES OF VASCULAR ENDOTHELIUM**
MACIEJ CHAŁUBIŃSKI – Department of Internal Diseases and Clinical Pharmacology, Medical University of Lodz, Poland
- 16.45–17.45 **Poster session and coffee break**

Day IV (Friday, September 20)**SESSION IV.****MICROBIAL VIRULENCE FACTORS IN HUMAN, ANIMAL AND PLANT INFECTIONS***chair: Barbara Różalska, Antonio Molinaro*

- 09.00–09.30 **GRAM-NEGATIVE BACTERIA AND LPS – SOME ASPECTS OF 65 YEARS OF RESEARCH**
OTTO HOLST – Division of Structural Biochemistry, Research Center Borstel, Germany
- 09.30–10.00 **HOW TO TURN A BACTERIAL PATHOGEN INTO A PROBIOTIC-LIKE ORGANISM**
CAMILLE LOCHT – Institut Pasteur de Lille, France
- 10.00–10.30 **EMERGING GLOBAL LANDSCAPE OF THE HOST RANGE AND GENETIC DIVERSITY OF HANTAVIRUSES**
RICHARD YANAGIHARA – Pacific Center for Emerging Infectious Diseases Research, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii, USA
- 10.30–11.00 **PREVENTING NOSOCOMIAL WATER-BORNE INFECTION BY CONTROLLING WATER AND WATER SYSTEMS**
LEIF PERCIVAL ANDERSEN – Copenhagen University Hospital (Rigshospitalet), Denmark
- 11.00–11.30 **STRUCTURE, SEROLOGY, AND GENETICS OF PROVIDENCIA O-ANTIGENS**
OLGA OVCHINNIKOWA – N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Russia
- 11.30–11.45 **Coffee break**
- 11.45–12.45 **Oral presentations of selected abstracts**
chair: Henryka Długońska, Otto Holst
- 11.45–12.00 **CHEMISTRY AND IMMUNITY OF LPSs ISOLATED FROM CYSTIC FIBROSIS PATHOGENS**
FLAVIANA DI LORENZO – Department of Chemical Sciences, Università di Napoli Federico II, Napoli, Italy
- 12.00–12.15 **ACTIVITY AND PRELIMINARY IDENTIFICATION OF THE ANTIMYCOBACTERIAL COMPOUND ISOLATED FROM METABOLITES OF THE EARTHWORM GUT BACTERIUM *RAOULTELLA ORNITHINOLYTICA***
MARTA FIOŁKA – Department of Immunology, Institute of Biology and Biochemistry, Maria Curie-Skłodowska University, Lublin, Poland
- 12.15–12.30 **DOES THE CONCENTRATION OF GLUCOSE AFFECT THE QUANTITY AND CHEMICAL STRUCTURE OF THE *P. AERUGINOSA* BIOFILM FORMED IN PATIENTS WITH CHRONIC DIABETIC FOOT WOUNDS?**
AGNIESZKA MACHUL – Department of Microbiology, Jagiellonian University Medical College, Cracow, Poland
- 12.30–12.45 **EFFECT OF STRESS FACTORS ON EXTRACELLULAR POLYSACCHARIDES SYNTHESIS IN *PROTEUS MIRABILIS* BIOFILM**
MAGDALENA MORYL – Department of Immunobiology of Bacteria, University of Lodz, Lodz, Poland
- 12.45–13.00 **DERIVATIVES OF 5-ARYLIDENEIMIDAZOLONE – NEW TOOLS TO COMBAT MDR BACTERIA**
EWA OTREBSKA – Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College, Cracow, Poland
- 13.00–13.15 **GOOD LABORATORY PRACTICE – QUALITY MAKES THE DIFFERENCE**
MONIKA ŻMUDA – Mettler Toledo
- 13.15–14.30 **Lunch**
- 14.30–15.30 **Poster session and coffee break**
- 15.30–16.00 **Closing remarks**

The award ceremony for the best oral and poster presentation of MIKROBIOT 2013.
INVESTIN award for the project with commercial potential.

ABSTRACTS OF THE PRESENTED WORKS

SESSION I

MOLECULAR HOST-PATHOGEN-ENVIRONMENTAL INTERACTIONS

PLENARY LECTURES

I-PL 1

DNA STRUCTURE AND INSTABILITIES RELATED TO HUMAN GENOMIC DISORDERS: WITH APPRECIATION AND ADMIRATION OF PROF. ADAM JAWORSKI

Robert D. Wells

Institute of Biosciences and Technology, Center for Genome Research, Houston Texas, USA

Professor Adam Jaworski has made profound contributions to the education, research, and administrative programs at the University of Lodz and at least two American Universities. Adam and I along with other associates coauthored at least 14 original scientific papers in premier journals over a 20 year time period. Further, he orchestrated the transition of more than 45 Polish scientists and their families from Poland to my laboratories in Alabama and Texas, USA over a 29 year time period. Our work was focused on simple repeating DNA sequences, which we showed to adopt non-B DNA conformations (such as left-handed Z-DNA, triplexes, cruciforms, slipped structures, and tetraplexes), as muta-

genic agents. The mutagenesis is due to the non-B DNA conformation rather than to the DNA sequence *per se* in the orthodox Watson-Crick B-form. The human genetic consequences of these non-B structures are ~20 neurological diseases, ~50 genomic disorders (caused by gross deletions, inversions, duplications and translocations) and several psychiatric diseases involving polymorphisms in simple repeating sequences. Thus, the convergence of biochemical, genetic, bioinformatic, and genomic studies has demonstrated a new paradigm implicating the non-B DNA conformations as the mutagenesis specificity determinants, not the sequences as such.

I-PL 2

ENVIRONMENTAL EFFECTS ON GENOME INTEGRITY IN PROKARYOTES

Richard Bowater

School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, United Kingdom

All cells contain a variety of pathways that recognise and then repair damage within their genomic DNA. These repair pathways are important to facilitate cellular reactions involving DNA and they help maintain genomic integrity. We have studied DNA repair pathways from various prokaryotic cells, and have become interested in the mechanisms by which the environment of the cell influences DNA damage and repair. We have characterised the physiological functions of a range of DNA repair processes, with a particular emphasis on the reaction steps that repair breaks within DNA molecules. Thus, our studies have focused on the role of DNA

ligases that act at the final step to join the breaks. We have analysed the biochemical activities of different DNA ligases and the way that interactions with other proteins influence these activities. As will be reported in this presentation, we have elucidated the effects at a physiological level of mutations in relevant genes, and have characterised recombinant versions of proteins involved in these DNA repair processes. Our data highlight that prokaryotic cells contain a wide variety of DNA repair mechanisms that assist their survival in diverse environments.

I-PL 3

ONCOAPOPTOSIS: HARNESSING CELLULAR ANTIMICROBIAL RESPONSES AS A NOVEL MOLECULAR THERAPEUTIC FOR CANCER TREATMENT

John Blaho

The City College of New York, Center for Advanced Technology, USA

Many cancer cells refractory to radiation treatment and chemotherapy proliferate due to loss of intrinsic programmed cell death (apoptosis) regulation. Consequently, the resolution of these cancers are many times outside the management capabilities of conventional therapeutics. We have developed a replication defective herpes simplex virus system which triggers apoptosis specifically in transformed human cells, termed oncoapoptosis. Susceptibility to virus induced cell death is dependent on the p53 protein status

in the tumor cells, indicating specific targeting of the treatment. Primary cells which produce functional p53 are resistant to oncoapoptotic killing but not to apoptosis induced by nonviral environmental factors. Thus, induction of apoptosis by nonreplicating virus is a feasible molecular therapeutic approach for killing human cancer cells. Our findings have important implications in designing novel virus-based anticancer strategies.

I-PL 4

ANTI-ALLERGIC EFFECTS OF *LACTOBACILLUS PLANTARUM* L-137 ISOLATED
FROM A TRADITIONAL FERMENTED FOOD
AND DEVELOPMENT OF ORAL VACCINE WITH THE *LACTOBACILLUS*

Y. Murooka^{1*}, Y. Yamamoto², K. Ono³, and M. Yamashita⁴

¹Emeritus Professor of Osaka University; ²House Wellness Foods Co., Hyogo

³Hiroshima University, Higashi-Hiroshima, and ⁴Shibaura Institute of Technology, Tokyo, Japan

METHODS AND RESULTS. *Lactobacillus plantarum* L-137 was isolated from a traditional fermented food in the Philippines. Orally fed live and heat-killed (HK) L-137 showed anti-allergic effects by suppression of IgE via stimulation of IL-12. HK L-137 also had anti-tumor effect in tumor-bearing mice and anti-viral effect against an influenza virus in mouse lungs. Clinical tests showed that HK L-137 increased levels of cellular immunity and IFN- β in serum that resulted in increasing health related QOL. We identified that a specific structure of lipoteichoic acid in the L-137 cell wall, but not peptidoglycan, was one of the important factors that stimulated

cytokine production. We developed an expression vector for *L. plantarum* that was based on one of its plasmids, pLTK2. Using this expression vector, we produced a cholesterol- degrading enzyme in *Lactobacilli*. To desensitize children against mite allergens, a main allergen from house dust mites was expressed in cells of NCL21, a derivative of L-137. Furthermore, we succeeded in generating the recombinant NCL21 expressed a major Japanese cedar pollen allergen. **CONCLUSION.** We identified the immunological effective factor in *L. plantarum* L-137, and showed that the strain has potential as the source of an oral vaccine for desensitization to several allergies.

I-PL 5

CRYSTALLOGRAPHIC STUDIES OF THE TYPE III SECRETION SYSTEM:
WATCHING AT ATOMIC RESOLUTION HOW PATHOGENIC GRAM-NEGATIVE BACTERIA ATTACK
AND SECRETE THEIR LETHAL LOAD INTO EUKARYOTIC HOST CELLS

Zygmunt S. Derewenda

University of Virginia School of Medicine, Department of Molecular Physiology and Biological Physics,
Charlottesville VA, 22908 USA

Gram-negative bacteria have several different secretion systems, of which the Type III Secretion System (T3SS) is the most complex. Among the most researched T3SSs are those from such pathogens as *Shigella* (dysentery), *Salmonella* (typhoid), *Vibrio* (diarrhea) and *Yersinia* (plague). The key structural feature of the T3SS is the ~3.5 MDa needle complex (NC), made up of at least 14 principal proteins, with other proteins in auxiliary roles. The needle complex, as the name suggests, is used to inject proteins from the bacterial cell directly into the host cell, after the membrane of that cell is pierced. It is made up of the inner and outer membrane rings joined

by the connector, and the actual hollow needle, 60–80 nm in length and 8 nm wide, with a tip. While this structure is admittedly very large, recent crystallographic and cryoEM studies of individual elements and even of the intact needle, provide us with unprecedented insight into the structure-function relationships in this important bacterial organelle. Our group contributed to this field through the determination of the atomic structure of the *Yersinia* LcrV protein, and we also developed methodologies that enabled other labs to crystallize some challenging components such as the EscJ protein. The talk will offer an overview of the field.

ORAL PRESENTATIONS**I-O 1**

PATHWAYS TO SYNTHESIS OF THE MALONYL-CoA IN *MYCOBACTERIUM*

Jakub Pawełczyk^{1*}, Anna Brzostek¹, Laurent Kremer^{2, 3}, Bożena Dziadek⁴,
Anna Rumijowska-Galewicz¹, Marta Fiołka⁵, Jarosław Dziadek¹

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Malonyl-CoA is the essential, two-carbon substrate for the synthesis of mycolic and other fatty acids in *Mycobacterium*. It is generated by the carboxylation of acetyl-CoA in a two-step reaction catalyzed by acetyl-CoA carboxylase (ACC) and incorporated into the growing acyl chain during the cycle of the fatty acid synthase I and II reactions. Each half reaction is catalyzed by a spe-

cific ACC subunit: the first step by biotin carboxylase (BC) and the second step by carboxyltransferase (CT). Since the CT subunit confers the substrate specificity of ACC, the large number of CT encoding genes in mycobacterial genomes reflects their ability to carboxylate not only acetyl-CoA but also several other distinct substrates. The main goal of this research was to identify the genes

encoding the CT component of ACC enzyme in pathogenic and non-pathogenic mycobacteria. By construction and lipid analysis of *M. tuberculosis* mutants we demonstrated that *accD6* (Rv2247) encodes the essential carboxyltransferase, responsible for synthesis of the malonyl-CoA in tubercle bacillus *in vivo*. Strikingly, our study revealed that although *accD6* homologue of *M. smegmatis* (*MSMEG_4329*) possesses the same essential function, it is dispensable. To identify alternative CT subunit of ACC in this species, we tested the essentiality and possible involvement in mycolic acid biosynthesis of all CT genes in *M. smegmatis*. Analysis allowed

the identification of *MSMEG_6391* and *MSMEG_1813* (*M. tuberculosis* *accD4* and *accD5* homologues) as the only essential CT genes in *M. smegmatis*. The study on the function of both genes led us to the conclusion that protein encoded by *MSMEG_1813* is bifunctional, carrying propionyl but also acetyl carboxyltransferase activity that allow replacing the *MSMEG_4329* (*accD6*) function in this species. The importance of *accD6* in *M. tuberculosis* as well as the difference in its essentiality, expression level and regulation between pathogenic and non-pathogenic mycobacteria will be further examined.

I-O 2

SELECTED INNATE IMMUNITY FACTORS IN PULMONARY TUBERCULOSIS PATIENTS

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⁴Masovian Center of Lung Diseases and Tuberculosis Treatment, Otwock, Poland; ⁵University of Łódź, Łódź, Poland

INTRODUCTION. Tuberculosis still belongs to the major health problems with incessantly high morbidity and mortality. Effective mechanisms of the innate immune response are crucial to control *Mycobacterium tuberculosis* expansion. **AIM.** The aim of our study was to investigate the role of selected pattern recognition molecules: mannan-binding lectin (MBL), pulmonary surfactant proteins A and D (SP-A, SP-D), H-ficolin, MBL-associated serine proteases-1 and -2 (MASP-1, -2) and Toll-like receptors (TLRs: -1, -2, -4, -6), in *M. tuberculosis* infection. **PATIENTS AND METHODS.** DNA and serum samples were obtained from patients with confirmed pulmonary tuberculosis (TB; n = 399). The control group consisted of adult volunteers with no history of tuberculosis (C; n = 260). Single nucleotide polymorphisms (SNPs) of *MBL2* (MBL), *SFTPA1* (SPA-1), *SFTPA2* (SP-A2), *SFTPD* (SP-D), *FCN3* (H-ficolin), *MASP2*, *TLR1*, *TLR2*, *TLR4* and *TLR6* were investigated with the use of PCR or PCR-RFLP methods. Serum levels of MBL, H-ficolin, SP-D and activities of MBL-MASP-2 complex were determined by

ELISA while activities of MBL-MASP-1 complex – by fluorescence assay. **RESULTS.** No significant differences in frequencies of SNPs of *MBL2*, *FCN3*, *SFTPA1*, *SFTPD*, *MASP2* and *TLR1*, *TLR2*, *TLR4*, *TLR6* genes were found. The frequency of *SFTPA2* gene A/A variant (codon 9; A > C polymorphism) was significantly higher in TB than in C group (p = 0.007). Median serum concentrations of MBL, SP-D and H-ficolin as well as activities of MBL complexes with MASP-1 and MASP-2 were markedly elevated in patients suffering from pulmonary tuberculosis compared with healthy controls. Furthermore, a significant increase in MBL and H-ficolin but not SP-D serum levels was observed in patients, during antituberculosis treatment. **CONCLUSION.** Our data suggest that the elevated MBL, H-ficolin and SP-D serum concentrations/activities and *SFTPA2* gene polymorphism might be associated with pulmonary tuberculosis. **ACKNOWLEDGEMENT.** This work was supported by the European Union within European Regional Development Fund, through grant Innovative Economy (POIG. 01.01.02-10-107/09).

I-O 3

THE INTERACTION OF COMPLEMENT-ACTIVATING LECTINS WITH MYCOBACTERIUM TUBERCULOSIS AND ITS CELLULAR FRACTIONS

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INTRODUCTION. Mannan-binding lectin (MBL) and ficolins (M-, L-, H-) play an important role in innate immune response. They bind to the carbohydrate-rich surface structures of microorganisms and may contribute to their clearance thanks to opsonic properties and ability to activate complement *via* the lectin pathway. Their deficiencies have various clinical associations, however, their role in pathogenesis of tuberculosis remains unclear. **AIM.** Investigation of the interactions of MBL and H-ficolin with *Mycobacterium tuberculosis* and identification of cell fractions responsible for these interactions. **MATERIALS AND METHODS.** *Mycobacterium tuberculosis* H37RV (MTB H37RV) came from the collection of the Laboratory of *Mycobacterium* Genetics and Physiology, IMB PAS. Lipoarabinomannan (LAM) was obtained from Nacalai Tesque, INC (USA), p85A/B antigens from Abcam (UK), whereas recombinant lectins came from R&D Systems (USA). Monoclonal anti-MBL antibodies were from BioPorto (Denmark), anti-H-ficolin – from HyCult (USA). Anti-LAM MAbs

were obtained from Gentaur (Belgium), and against *M. tuberculosis* p85A/B antigens – from Abcam. HRP-labeled secondary antibodies came from DAKO (Denmark). ELISA, TRIFMA, flow cytometry and Western blot techniques were employed. **RESULTS.** Recombinant MBL and H-ficolin were shown to interact with MTB H37RV inactivated cells. Western blot analysis demonstrated that they bound to fractions corresponding to 28–36 kDa molecular weight. MBL recognized LAM while both lectins – p85A and p85B antigens. These interactions were inhibited by mannan (MBL) or *H. alvei* 1200 lipopolysaccharide (H-ficolin). Immunoprecipitation confirmed the binding of both lectins either with surface-bound or secreted forms of p85 antigens. **CONCLUSION.** Both *M. tuberculosis* lipoarabinomannan and p85 complex are target structures recognized by MBL and ficolins. However, the significance of observed interactions requires further investigation. This work was supported by the grant Innovative Economy (POIG. 01.01.02-10-107/09).

I-O 4

MOLECULAR GENOTYPING OF HUMAN INTESTINAL *E. COLI* STRAINS

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INTRODUCTION. There are several techniques for fingerprinting of microorganisms, based on repetitive-elements polymerase chain reaction (rep-PCR). Trinucleotide repeat sequences (TRS) were detected in many bacterial genomes and were effectively used in our laboratory for discrimination uropathogenic *Escherichia coli*, *Mycobacterium gordonae*, *Mycobacterium kansasii*, *Mycobacterium avium* strains and *Salmonella enterica* ssp. *enterica* serovars. **AIM.** The goal of this work was to develop a novel genetic tests (CGG-PCR, GTG-PCR) for the differentiation and epidemiological investigation of human intestinal pathogenic *Escherichia coli* strains. **MATERIALS AND METHODS.** The collection of 101 human intestinal pathogenic *Escherichia coli* strains was tested using TRS-PCR assay designed in our laboratory. TRS-PCR tests using N₆(CGG)₄ and N₆(GTG)₄ primers were performed separately. Also, two multiplex-PCR protocols testing the presence of some virulence

factors specific for different IPEC pathotypes and for UPEC pathotype were conducted. Database of the band pattern profiles was created. **RESULTS.** The similarity comparisons of the CGG- and GTG-band pattern profiles revealed that CGG-PCR and GTG-PCR tests were able to distinguish strains with very good efficacy. It was confirmed by high values of discriminatory indices for these assays at levels 0.987 and 0.985 respectively, with reproducibility at very similar level. Composite data analyses of CGG and GTG assays had the highest discriminative power (DI = 0.990) for this collection of *E. coli* strains. **CONCLUSION.** We think that the CGG-PCR and GTG-PCR tests are powerful tools for discriminative genetic analyses of human intestinal pathogenic *Escherichia coli* strains. Therefore, they may be used not only for the discrimination of the isolates but also for tracing transmission routes of this pathogen.

I-O 5

CONSEQUENCES OF TLR2 ACTIVATION IN MONOCYTE-MACROPHAGE DIFFERENTIATION MODELS

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INTRODUCTION. Monocytes and macrophages are essential elements of innate immune system. These cells provide first line of defense against microorganisms, thanks to their ability to phagocytose pathogens, produce and release reactive oxygen species and cytokines. Receptors recognizing microorganisms play a key role in host defense. **AIM.** We investigated significance of TLR2 stimulation in undifferentiated (monocyte-like) and differentiated (macrophage-like) cells with regard to function and survival of cells. **MATERIALS AND METHODS.** We used THP-1 and Mono Mac 6 cell lines as a model of human monocytes. Cells were differentiated using PMA or mixture of vitamin D3 and TGFβ, respectively. We used synthetic lipopeptide pam3CSK4 for TLR2 activation. We investigated expression level of genes using real-time PCR. Activity of antioxidant enzymes was measured using enzyme-specific biochemical methods. Cell viability, after treatment with H₂O₂ or iron/ascorbate radical generating system, was assayed using resazurine-based method, and carbonyl group content was meas-

ured in reaction with fluorescein-5-thiosemicarbazide. **RESULTS.** In THP-1 cells, differentiation and TLR2 activation resulted in elevated level of superoxide anion generation. We observed lower cells susceptibility to oxidative stress generating compounds in differentiated cells and after pam3CSK4 stimulation of undifferentiated cells. Analysis of gene expression showed that differentiation and TLR2 activation result in higher expression of genes coding some antioxidant enzymes. Carbonyl group content was lower in differentiated and activated cells. Mono Mac 6 cells were more resistant to oxidative stress and this resistance was difficult to future modulate. **CONCLUSION.** Cells of innate immune system during differentiation and recognizing of pathogens are subjected to elevated level of reactive oxygen species. THP-1 cells during differentiation and TLR2 stimulation expressed elevated level of antioxidant compounds. Superoxide dismutase and the glutathione system had most conspicuous protective effects on cell viability under oxidative stress conditions.

I-O 6

INFLUENCE OF ENZYMES DEGRADING CHOLESTEROL ON THE RESPONSE OF HUMAN MACROPHAGES TO *MYCOBACTERIUM TUBERCULOSIS* INFECTION *IN VITRO*

Marta Brzezińska*, Izabela Szulc, Anna Brzostek, Magdalena Klink, Michał Kiełbik,
Zofia Sułowska, Dziadek Jarosław

Institute of Medical Biology Polish Academy of Sciences, Lodz

INTRODUCTION. Cholesterol oxidase (ChoD) and ketosteroid dehydrogenase (KstD) are *Mycobacterium tuberculosis* (Mtb) enzymes involved in the cholesterol degradation but their role in

the human macrophages infection remains unclear. **AIM.** This study assessed the participation of enzymes: ChoD and KstD on the functional properties of human macrophages infected with

Mtb. We also determined Toll-like receptor 2 (TLR2), complement receptor 3 (CR3) and mannose receptor (MR) involvement in the bactericidal activity of macrophages during infection with Mtb. **MATERIALS AND METHODS.** Using the technique of gene replacement based on the process of homologous recombination, we obtained Mtb H37Rv mutants lacking the functional copy of *ChoD* ($\Delta choD$) or *KstD* ($\Delta kstD$). Complemented strains were obtained by insert intact *choD* or *kstD* genes in Mtb. The intracellular growth of bacteria, production of nitric oxide (NO), reactive oxygen species (ROS) and cytokines: TNF- α and IL-10 by macrophages infected with Mtb wild-type, its mutants and complementation strains were tested. **RESULTS.** $\Delta choD$ and $\Delta kstD$ mutants grew weaker in macrophages in comparison to Mtb wild-type and complemented strains. Inhibition of TLR2, CR3 or MR-mediated signaling pathways significantly increased the survival

of both mutants but not wild-type strain. Only mutants stimulated macrophages to NO production that was blocked in the presence of TLR2-signaling pathway inhibitor. Neither of the mutants inhibited ROS production by macrophages in contrast to wild-type and complemented strains. Blocking of TLR2-mediated signaling pathway abolished the ability of Mtb wild-type to the inhibition of ROS production by phagocytes. Only macrophages infected with $\Delta choD$ produced lower amounts of IL-10 than cells infected with other strains. **CONCLUSION.** Mtb using *ChoD* and *KstD* affect the antimicrobial mechanisms in macrophages via TLR2-signaling pathway. Both enzymes participate in the virulence of Mtb and promote pathogen survival in human macrophages. Research co-financed by the European Regional Development Fund under the Operational Programme Innovative Economy, grant POIG.01.01.02-10-107/09.

I-07

COMPUTER-AIDED DISCOVERY OF NEW *M. TUBERCULOSIS* DNA LIGASE A INHIBITORS

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²Quantum Chemistry Group, Adam Mickiewicz University, Poznan, Poland

INTRODUCTION. Despite great progress in wet experiments automation, a chemical phase space remains far too big to be systematically explored. Consequently, there is all the time increasing need for a use of analytical methods that can indicate a right research direction. **AIM.** Identification of new antituberculosis agents. We focus on an interplay between different approaches for the best efficiency. **MATERIALS AND METHODS.** Used methods include molecular mechanics (MM), quantum mechanics (QM), and machine learning. **RESULTS.** Several sets containing in total more than 11 000 compounds have been prepared for the virtual

high throughput screening (vHTS) experiments. Dockings have been performed and their results analysed. Twenty compounds were selected for *in vitro* screening. Two of them gave particularly encouraging results. Additionally, a mixed method involving docking, molecular dynamics and QM single point energy evaluation has been investigated, and a new piece of software for the workflow optimisation has been developed. **CONCLUSIONS.** Because of the complexity of the problem one method is usually not sufficient for a successful drug candidate prediction. When several approaches are combined the accuracy might be very high.

I-08

EVALUATION OF PRIMASE DnaG OF MYCOBACTERIA AS A POTENTIAL TARGET FOR ANTIBIOTICS

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INTRODUCTION. *Mycobacterium tuberculosis*, the bacterial causative agent of tuberculosis, currently affects millions of people. The emergence of resistant strains leads to the search for new efficient antibiotics and alternative drug targets. The potential targets should be essential for bacteria and absent in the human host. Primase DnaG has been considered as one of such candidates. It is key enzyme in DNA replication responsible for the synthesis of short RNA fragments. **AIM.** Evaluation of mycobacterial DnaG primase as a potential target for new drugs against tuberculosis. **MATERIALS AND METHODS.** In this work, we used a homologous recombination to analyze the indispensability of *dnaG* in mycobacteria also in AEP overproduction background. The affinity chromatography was used to purify the recombined DnaG. The activity of this protein and its sensitivity for inhibitors was examined *in vitro* using a radioactive assay. **RESULTS.** The essentiality

of *dnaG* was evaluated in wild type *M. smegmatis* strain and in engineered mutants in AEP primases overproduction background. The wild type gene was replaced with unfunctional copy when additional *M. tuberculosis* and *M. smegmatis* intact *dnaG* was introduced under control of chemically inducible promoter. The *E. coli dnaG* or *M. smegmatis* AEP primases (Prim 1, Prim 2, Prim 3) did not allow inactivating of native *dnaG* in *M. smegmatis*. We showed that the activity of DnaG is affected by doxorubicin and suramin *in vitro* but only doxorubicin appeared to be active against *M. smegmatis*. **CONCLUSIONS.** DnaG primase is essential for viability of mycobacteria and it can be considered as a potential target for new anti-tuberculosis drugs.

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I-O 9

PARTICIPATION OF *MYCOBACTERIUM TUBERCULOSIS* PROTEINS OF DNA DOUBLE STRAND BREAKS REPAIR SYSTEMS IN THE INFECTION OF HUMAN MACROPHAGES WITH MTB, *IN VITRO*

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INTRODUCTION. In *Mycobacterium tuberculosis* (Mtb) the non-homologous end-joining (NHEJ) and homologous recombination (HR) pathways are important DNA double-strand breaks repair systems, crucial for bacterial survival. **AIM.** In this study we determined the involvement of genes encoding proteins Ku, D ligase (NHEJ system) and RecA (HR system) in Mtb intracellular survival and their influence on macrophage response to tubercle bacilli infection. The involvement of ERK1/2-signaling pathway in the infection of macrophages with Mtb strains was also assessed. **METHODS.** MtbH37Rv strains with inactivated *ku*, *ligD* genes ($\Delta ku/ligD$), *recA* gene ($\Delta recA$) or all three genes ($\Delta ku/ligD/recA$) were obtained using the technique of gene replacement based on the process of homologous recombination. The macrophages differentiated from human THP-1 cell line were infected with Mtb strains. Intracellular growth of bacteria, production of nitric oxide (NO), reactive oxygen species (ROS) and tumor necrosis factor (TNF- α) were tested. **RESULTS.** The intracellular growth of triple mutant ($\Delta ku/ligD/recA$) was significantly weaker, compared to the

rest of Mtb strains. Inhibition of ERK1/2 pathway in macrophages, caused the increased intracellular growth of $\Delta ku/ligD/recA$ to the level of Mtb wild-type. Both, $\Delta ku/ligD/recA$ and $\Delta recA$ mutants, stimulated macrophages to produce NO and did not inhibit ROS production by phagocytes, compared to the wild-type and $\Delta ku/ligD$ strains. Nevertheless, inhibition of ERK1/2 pathway resulted in the lack of NO production and strong inhibition of ROS production by macrophages infected with $\Delta ku/ligD/recA$ or $\Delta recA$ mutants. Only the triple mutant did not inhibit PMA-stimulated ERK1/2 phosphorylation. We found that macrophages infected with $\Delta ku/ligD/recA$ or $\Delta recA$ mutant produced significantly less TNF- α than Mtb wild-type and $\Delta ku/ligD$ strains. **CONCLUSIONS.** We suggest that HR repairing pathway is essential for Mtb intracellular survival and plays an important role in the modification of macrophages antimicrobial activity via ERK1/2-signaling pathway. Research co-financed by the European Regional Development Fund under the Operational Programme Innovative Economy, grant POIG.01.01.02-10-107/09.

I-O 10

TRS-BASED PCR AS A POTENTIAL TOOL FOR INTER-SEROVAR GENOTYPING *SALMONELLA* ENTERITIDIS, TYPHIMURIUM, INFANTIS, VIRCHOW, HADAR, NEWPORT AND ANATUM

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INTRODUCTION. *Salmonella enterica* subsp. *enterica* comprise a number of serovars many of which pose epidemiological threat for humans and are a worldwide cause of morbidity and mortality. The rapid identification to determine the primary sources of bacterial contamination is important to improve public health. Among typing methods to differentiate *Salmonella*, techniques based on phenotypes or genotypes have been developed. **AIM.** Despite the undeniable advantage of employing the highly advanced molecular methods, the cost of equipment and need for skilled staff may exclude some methods from use in many of the countries which need them the most. That is why, there is still a need for new methods that are simple, inexpensive and able to discriminate *Salmonella* serotypes. In this work we show the efficacy of the manual rep-PCR approach based on the presence of trinucleotide repeat sequences (TRS) in *Salmonella* genome. **MATERIALS AND METHODS.** One hundred seventy *Salmonella* strains were examined in this work. These consisted of commonly isolated in humans serovars

including Enteritidis, Typhimurium, Infantis, Virchow, Hadar, Newport and Anatum. All of the isolates were grown in liquid LB broth at 37°C overnight and the genomic DNA was isolated using a commercial DNA extraction kit. Afterwards the TRS-PCR, electrophoresis, reproducibility assessments and bioinformatic analyses were performed. The primers were designed to conform to the 5'-N₆(TRS)₄-3' scheme (N = G, A, T, or C). **RESULTS.** Five of the TRS-primers, N₆(GTG)₄, N₆(CAC)₄, N₆(CGG)₄, N₆(CCG)₄ and N₆(CTG)₄ perfectly distinguished the Enteritidis and Typhimurium serovars, and the N₆(GTG)₄ primer grouped additionally the other five frequently isolated serovars. **CONCLUSIONS.** In our opinion, the TRS-PCR methodology has the potential for rapid and easy single-tube DNA-based assays for the discrimination of seven *Salmonella enterica* serovars. The determination of TRS-fingerprints for unknown *Salmonella* strains could serve as a useful predictor for their serovar affinity.

I-O 11

SENSITIZATION OF BREAST CANCER CELLS TO DNA DAMAGE AGENTS WITH DNA-PK INHIBITOR

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BACKGROUND. DNA-dependent protein kinase (DNA-PK) plays a key role in the repair of DNA double-strand breaks (DSBs) that are probably the most deleterious form of DNA damage. Inhibition of DNA-PK has been considered as an attractive approach to decrease resistance to therapeutically induced DNA DSBs. Ionizing radiation (IR) and the topoisomerase II inhibitor, doxorubicin, which induce DSBs are used in the treatment of breast cancer. **AIM.** To determine the therapeutic potential of DNA-PK inhibition with NU7441. **METHODS.** We determined the basal expression of DNA-PK, ATM and ATR, and the level of IR mediated DNA-PK auto-phosphorylation as well as the effect of DNA-PK inhibition by NU7441 on DNA-PK activity, DNA repair, cell cycle and survival after IR or doxorubicin treatment in three human breast cancer cell lines (MCF-7, MDA-MB-231 and T47D) representing different breast cancer subtypes. **RESULTS.** T47D cells

had the highest expression of all tested PIK kinases and the most rapid rate of DNA DSB repair. IR caused a 10-16-fold increase in DNA-PK activity and 2-3-fold induction of ATM in all 3 cell lines. NU7441 inhibited IR-induced DNA-PK activity in all cell lines with IC50s in the range 0.17 to 0.25 μ M. NU7441 retarded the repair of DSB and increased accumulation of cells in G2/M phase after DNA damage. NU7441 significantly increased the sensitivity of all cell lines to IR (4 to 12-fold) and doxorubicin (3 to 13-fold). The greatest sensitizing by NU7441 was observed in MDA-MB-231 cells that represent the triple-negative breast cancer phenotype. **CONCLUSIONS.** Our data indicate that DNA-PK is an effective target for chemo- and radio-potential in breast cancer and suggest that further development of DNA-PK inhibitors for clinical use is warranted particularly against those with the triple-negative phenotype which are currently difficult to treat.

I-O 12

THE FUNCTION OF RNase H CLASS I IS ESSENTIAL FOR SURVIVAL OF *MYCOBACTERIUM SMEGMATIS*Alina Minias^{1,2*}, Jarosław Dziadek¹¹Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland²Institute of Microbiology, Biotechnology and Immunology, University of Lodz, Lodz, Poland

INTRODUCTION. RNase H is a ubiquitous enzyme responsible for the removal of RNA from RNA/DNA hybrid. In other species it has been shown responsible for the removal of RNA primers during DNA replication and for the removal of ribonucleotides misincorporated within the DNA double helix. In *E. coli* the role of RNase H I can be at least partially substituted by polymerase I. **AIM.** We wanted to test the essentiality of RNase H class I encoding genes (MSMEG5562, MSMEG4305) in model organism *Mycobacterium smegmatis*. **MATERIALS AND METHODS.** We used the technique of gene replacement by homologous recombination in order to obtain the mutants deficient in either one or both genes encoding RNase H class I in *M. smegmatis*. In order to do so, we constructed plasmids containing large deletions within the investigated genes and introduced them into host cells. We also constructed plasmids

allowing the complementation of the deleted genes. The obtainment of the mutants was confirmed by Southern blot. **RESULTS.** Following the multistep selection process we were able to select the mutants defective in single RNase H class I genes. However, we were not able to obtain double mutant deficient in both of the genes. In order to confirm that not obtaining of the clone was due to the essentiality of the genes and not to a technical problem, we introduced a functional copy of one of the genes in *attB* site of mycobacterial genome. The obtained mutant containing deletions in both native genes and a functional copy at *attB* site was then used in an experiment where we tried to substitute the complemented gene for an empty vector. We were not able to obtain mutant deficient in RNase H class I function. **CONCLUSIONS.** The two genes encoding RNase H class I have an overlapping function essential for survival of *M. smegmatis*.

I-O 13

TOPOLOGY-DEPENDENT DnaA INTERACTIONS WITH *HELICOBACTER PYLORI* ORIGIN OF CHROMOSOMAL REPLICATIONRafał Donczew^{1*}, Thorsten Mielke², Rudi Lurz², Christoph Weigel³, Jolanta Zakrzewska-Czerwińska^{1,4}, Anna Zawilak-Pawlik¹¹Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Department of Microbiology, Wrocław, Poland²Max-Planck-Institute for Molecular Genetics, Berlin, Germany; ³HTW-Berlin, Department of Life Science Engineering, Berlin, Germany⁴University of Wrocław, Department of Microbiology, Wrocław, Poland

INTRODUCTION. Replication of the bacterial chromosome is initiated by the interaction of the DnaA protein with the *oriC* region. Binding of the DnaA protein to specific DnaA boxes in *oriC* leads

to the DNA melting within DNA unwinding element (DUE). Many factors regulate replication initiation and such systems differ significantly among bacteria. **AIM.** We have recently found that *H. pylori*

oriC exhibits a bipartite structure, being a first such origin discovered in a Gram-negative bacterium. DnaA specifically binds both regions (*oriC1* and *oriC2* flanking the *dnaA* gene), but the DnaA-dependent DNA unwinding occurs within *oriC2*. Surprisingly, *oriC2* is bound exclusively as a supercoiled DNA. The question arose what are the crucial DNA motifs responsible for DnaA binding in *oriC2* including the topology dependent interactions. **MATERIALS AND METHODS.** DMS footprinting method was utilized to study DnaA binding to both *oriC* subregions. Directed mutagenesis enabled us to characterize the role of particular *oriC* elements in orisome formation. The *oriC* mutants were analysed using electron microscopy and unwinding assay developed for studying *oriC* activity. **RESULTS.** Footprinting experiments allowed us to confirm previously identi-

fied DnaA boxes in *oriC1*. Subsequently, we determined two novel DnaA boxes in *oriC2*, one of which is bound only when supercoiled. The importance of newly discovered *oriC2* DnaA binding sites was confirmed by the analysis of DnaA-*oriC2* interactions (*wt* and mutated boxes) in EM. Additionally, preliminary results of the ongoing unwinding assays on *oriC* mutants lead to the same conclusions. **CONCLUSIONS.** Our experiments enabled us to identify the DnaA-box engaged in the topology-dependent interaction alongside with other *oriC2* elements and to evaluate their role in the DnaA-*oriC* complex formation. We showed for the first time that the DNA topology regulates DnaA-*oriC* interaction, what may be important for the coordination of the basic cellular processes (such as replication) with the environmental signals.

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THE ROLE OF TOPOISOMERASE I IN CHROMOSOME DYNAMICS OF *MYCOBACTERIUM SMEGMATIS*

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INTRODUCTION. Tuberculosis and other disorders caused by pathogens of the genus *Mycobacterium* have an enormous impact on global health. Due to the evolution of multiple drug resistant strains the need for understanding the biology of *Mycobacteria* and molecular basis of virulence is still a priority. Topoisomerases belong to a large and conservative family of enzymes, control topological tensions in DNA created during transcription, replication and segregation of daughter chromosomes. Based on the mechanism of catalyzed reaction, topoisomerases are divided into two types. Interestingly, it has been demonstrated that TopA *Mycobacterium* (topoisomerase of type I) differ from the other topoisomerases I due to their unique features, such as binding with high specificity to both, single and double stranded DNA and lack of typical zinc finger motif in an elongated C-terminal domain. **AIM.** Our study

focuses on TopA – topoisomerase I from *Mycobacterium* and its influence on dynamics of bacterial chromosomes. **MATERIALS AND METHODS.** Allelic gene replacement method was used to generate mutants of *Mycobacterium*. The growth and chromosome dynamics of mutant strains was analyzed. **RESULTS AND CONCLUSIONS.** It was shown that *TopA* gene is essential for the survival of *M. smegmatis* (a model organism for mycobacterial studies) while decreased TopA levels reduced the growth rate of the mutant strain. Additionally, chromosome dynamics was analyzed in TopA depleted strain by studies of subcellular localization DnaN-EGFP and ParB-mcherry using fluorescent microscopy. To elucidate the function of TopA C-terminal domain an attempt was made to create a *M. smegmatis* strain producing truncated protein.

I-O 14

A POTENTIAL REGULATORY SYSTEM OF *STAPHYLOCOCCUS AUREUS* IMPLICATED IN RESPONSE TO DIFFERENT STRESS STIMULI

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INTRODUCTION. Bacterial systems which are responsible for transcription regulation in response to stress conditions may play an important role in pathogenesis. Up till now, few such systems have been characterised for *Staphylococcus aureus* and were also demonstrated to control expression of virulence factors genes. **AIM.** In this study, the expression profile of three genes were analysed. These genes likely constitute an operon functionally linked with transcription regulation and controlled by two promoters. **MATERIALS AND METHODS.** Liquid cultures of *Staphylococcus aureus* CH91 strain were subjected to different stress stimuli such as osmotic stress, salt stress, oxidative stress, antibiotic exposure or acidification. The total RNA from samples, collected at different time points, was isolated and used as a template for reverse transcription. The obtained cDNA was utilised for Real Time PCR analysis of expression of the three genes. **RESULTS.** The results remain in agreement with the hypothesis stated that the operon

is regulated by two promoters. In unfavourable conditions, the amount of the transcript of one of the three genes was highly elevated when compared with the control setting. Nonetheless, during normal growth the gradual increase in the amount of the same transcript could be observed and remarkably was anti-correlated with the level of transcription of the remaining two genes. One of them was demonstrated to be a DNA-binding protein. **CONCLUSIONS.** The expression of the analysed genes is tightly linked with the exposure to some stress stimuli as well as with the phase of the bacterial culture growth. The presence of the gene coding for the DNA-binding protein strongly suggests that the operon constitutes an uncharacterised regulatory system, which is activated in response to stress stimuli.

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POSTERS

I-P 1

ANALYSIS OF APOPTOTIC PROPERTIES OF LIPID A FROM *PROTEUS MIRABILIS* AND *BULKHOLDERIA CEPACIA* BY FLOW CYTOMETRY

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INTRODUCTION. The cytotoxic properties of LPS are linked to its chemical structure. The toxic center of LPS is lipid A, its glycolipid fragment is the most conservative region in LPS structure. **AIM.** We analyzed the proapoptotic properties of lipid A from *P. mirabilis* and *B. cepacia* LPSs, which are characterized by one or two residues of Ara4N linked to phosphate residues, respectively. **MATERIALS AND METHODS.** After treatment of A549 cells by lipid A at doses of 100 or 500 ng/ml for 48 h, the frequencies of early/late apoptotic and necrotic cells were evaluated with the Annexin V-FITC apoptosis detection Kit I using flow cytometry or stained by trypan blue. **RESULTS.** The flow cytometric analyses of the A549 cells (passaged 15 times; high passage number increased

the % of apoptotic cells) showed that the distributions of early apoptotic cells in the presence of *P. mirabilis* and *B. cepacia* lipid A are lower (12.8% and 13.0%, respectively) than in control cells stained by Annexin V, in comparison to trypan blue analysis. **CONCLUSIONS.** Annexin V has a specific affinity for phosphatidylserine (PS). PS is translocated from the inner leaflet of the plasma membrane to the outer leaflet during early apoptosis. This interaction is mediated by calcium ions. We suggest that lipid A chelated Ca²⁺ and disturbed PS-Annexin V complex formation.

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I-P 2

MODULATION OF HUMAN MACROPHAGES ACTIVITY BY BACTERIAL CHOLESTEROL OXIDASE

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INTRODUCTION. Cholesterol in cellular membrane implicates in the regulation of uptake of mycobacteria by macrophages. Cholesterol oxidase (ChoD), a key enzyme of cholesterol metabolism, is considered as important virulence factor of mycobacteria, but its influence on macrophage activity is unknown. **AIM.** The study objective was to evaluate the influence of bacterial ChoD on macrophage activities *in vitro*. **MATERIALS AND METHODS.** ChoD from *Nocardia erythropolis* was used as a model with a similar structure to the mycobacterial enzyme. Macrophages derived from THP-1 monocyte line (24 h incubation, PMA-20 ng/ml) were exposed to ChoD (0.01–1.0 U/ml) for 1–24 h. Macrophage activity was investigated *in vitro* by ELISA, cytometric assays and fluorescence microscopy. **RESULTS.** ChoD was not toxic to macrophages in concentrations up to 0.5 U/ml, and was not pro-apoptotic, even in a large dose (1.0 U/ml). At higher concentrations, ChoD exerted a necrotic effect. Preincubation of macrophages with low concentrations of ChoD (0.1–0.5 U/ml) caused a considerable increase in the production of reactive oxygen species

in PMA-activated macrophages (four times, EC₅₀ = 0.04). Concomitantly, the production of interleukin-10 in non-activated cells was induced by ChoD. The MAPKs phosphorylation were down-regulated in activated macrophages. ChoD significantly down-regulated expression of inflammatory receptors of macrophages, TLR-2 and CR3. FITC-labeled ChoD was located in the macrophage cellular membrane and, partly, inside cells. It was important, that the enzyme from *Rhodococcus* sp. was ineffective. Thus, bacterial origin of ChoD is significant in modulation of macrophage function. **CONCLUSIONS.** Our results suggest that the response of macrophages to the enzyme is ambiguous. On the one hand ChoD induces reactive oxygen production in macrophages without induction of apoptosis, on the other hand, it down-regulates key inflammatory receptors and induces production of anti-inflammatory IL-10.

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I-P 3

DIFFERENTIATION BY RAPD-PCR OF *CANDIDA ALBICANS* ISOLATED FROM UPPER RESPIRATORY TRACT OF PATIENTS WITH NON-SMALL CELL LUNG CANCER

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INTRODUCTION. The patients with non-small cell lung cancer are predisposed to fungal infections caused especially by yeast, belonging to *Candida* spp., mainly *Candida albicans*. **AIM.** The aim

of this study was to estimate the genetic diversity by RAPD-PCR of *C. albicans* isolated from the upper respiratory tract of patients with non-small cell lung cancer. **MATERIAL AND METHODS.** A total

of 52 isolates of *C. albicans* colonizing upper respiratory tract from patients with non-small cell lung cancer were included in these studies. DNA from the isolates was prepared using GeneMATRIX Yeast DNA Purification Kit (EUR_x) according to the manufacturer's procedure. RAPD-PCR method was performed with RSD12 primer (5'-GGTCCGTGTTTCAAGACG-3'). The PCR products were electrophoresed in agarose gels at room temperature in TBE buffer. Reaction products were detected by ethidium bromide and visualized with UV light. Different banding positions of RAPD fingerprinting patterns of isolates of *C. albicans* were analysed using the BioGene program (Polygen). For the analysis of relationships among the strains, BioGene constructs dendrograms by the

unweighted pair group method. **RESULTS.** A value of a similarity coefficient $\geq 80\%$ was arbitrarily used as the threshold for clustering of similar genotypes, since it is roughly halfway between the mean value for dissimilarity and identity. On the basis of RAPD-PCR profiles, among 52 strains of *C. albicans*, 34 genotypes were defined within the overall yeast population, including 10 clusters containing from 2 to 6 isolates, which comprised 28 (53.85%) isolates and unique genotypes in 24 (46.15%) strains. **CONCLUSIONS.** The analysis of *C. albicans* genotypes by RAPD-PCR method revealed a large genetic variability among the strains isolated from the upper respiratory tract of patients with non-small cell lung cancer.

I-P 4

MODULATION OF GENES EXPRESSION BY SEQUENCE-SPECIFIC ENDORIBONUCLEASE IN *STAPHYLOCOCCUS AUREUS*

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INTRODUCTION. The impact of endoribonucleases of bacterial toxin-antitoxin systems on gene expression may strongly depend on their sequence specificity. Whilst the problem is still a matter of debate, the outcomes of many studies suggest that those of higher specificity can exert directional effects on the bacterial transcriptome. **AIM.** The aim of the research was the detailed characterisation of the PemIK_{sa} toxin-antitoxin system. The sequence specificity of the endoribonuclease PemK_{sa} was determined and its implications for bacterial growth and gene expression were demonstrated. **MATERIALS AND METHODS.** The genes of the PemIK_{sa} toxin-antitoxin system were cloned into plasmid vectors and expressed in *Escherichia coli* and *S. aureus* strains. The purified proteins were obtained, their interactions and effects of the PemK_{sa} activity on bacterial metabolism were investigated. The sequence specificity of the PemK_{sa} toxin was determined with Primer Extension. Bioinformatic analysis assessed the potential impact of PemK_{sa} activity on bacterial transcriptome. **RESULTS.** PemK_{sa} is an endoribonu-

lease recognising U↓AUU sequence and PemI_{sa} is its inhibitor. Expression of PemK_{sa} strongly inhibits growth of *S. aureus* cultures. The sequence specificity allows PemK_{sa} for selective degradation of transcripts and in effect silencing genes expression. Analysis of the genome of *S. aureus* ED98 revealed that the statistically significant number of virulence factors genes is underrepresented in the recognised sequence, whereas the overrepresented group consists mostly of those coding for transmembrane transporters. **CONCLUSIONS.** The operon *pemIK_{sa}* was demonstrated to be class II toxin-antitoxin system and to possess characteristics suggesting its regulatory role in *S. aureus* metabolism. The distribution of occurrence of the recognised sequence among genes implies a possibility of global directional changes in the bacterial transcriptome upon the system activation. This seems to be of even greater importance as the directional impact is strongly related to virulence factors genes. This study was supported by the grant NN303 813340 from the MNiSW to BW.

I-P 5

L- AND D-LACTATE MODULATE PRODUCTION OF CYTOKINES IN HUMAN AND MOUSE LYMPHOMA CELLS

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INTRODUCTION. Mucosal immune cells of the female reproductive tract (FRT) and the gastrointestinal tract (GT) are constantly exposed to L/D-lactate of bacterial origin. These compounds not only protect FRT/GT from pathogens colonization but may also modulate activity of mucosal cells through HCA1 receptor for L-lactate. Here we report a novel mechanism of interaction between human microbiota and lymphocytes and indicate lactate as a modulator of host immune system response. **AIM.** Evaluation of stimulatory/inhibitory effects of sodium lactate on cytokines

expression in human and murine lymphocytes. **METHODS.** Two lymphoma cell lines were used in experiments: HuT-78 (human) and EL-4 (murine) derived reporter cell lines stably transfected with transgene consisting of promoter regions from mouse β -actin, IL-2, IL-4, IFN- γ , and IL-10 and ORF for EGFP. EGFP mediated fluorescence of EL-4 cells was analyzed by flow cytometry. mRNA and protein levels of cytokines were determined using RT-PCR and ELISA assay, respectively. Receptor expression was measured by Western blotting. **RESULTS.** Both EL-4 and HuT-78 cells expressed receptor

for L-lactate (HCA1R). Treatment of EL-4 cells with 20 mM lactate for 24 h resulted in a slight increase in IL-4 and IL-10 promoter activity by 25% and 24%, respectively. Lactate stimulated IL-4 and IL-13 production in activated by PMA/Ionomycin Hut-78 cells by measuring mRNA and protein levels. Elisa assay demonstrated significant increase of IL-4 concentration in culture medium by 58% (D-lactate) and IL-13 by 95% (L-lactate), 310% (D-lactate),

264% (L/L-lactate). Similar effect was observed for cells activated with anti-CD3/CD28 antibodies. Interestingly, the observed stimulatory effect of lactate on cytokine production was higher for D- form than for L- form. **CONCLUSIONS.** This study indicated a role of D-lactate on modulation of cytokines production in lymphoma cells. It is tempting to speculate that D-lactate may serve as a messenger of microbial infections for the immune system.

I-P 6

MOLECULAR EVOLUTION OF CRUCIAL EUKARYOTIC-LIKE EFFECTORS IN *LEGIONELLA PNEUMOPHILA*

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Legionella pneumophila (LP) is an aquatic organism with protozoa as natural hosts. During evolution this interaction generated a pool of virulence traits allowed the infection of human macrophages. Dot/Icm is considered a key virulence system that modulates different host cell processes through the translocation of effectors. Among these, AnkB, AnkH and AnkJ contain eukaryotic domains mimicking eukaryotic proteins interfering with host functions. Legionellosis has an environmental origin so the study of the allelic diversity of virulence-related genes in strains isolated from distinct environments and disease-related is important to determine whether different hosts and environments influenced the ability of LP to grow in amoebae and/or macrophages and correlate these data with the allelic diversity of virulence genes absolutely required for intracellular multiplication, in this case *ankB*, *ankJ*, and *ankH*. Several software packages, namely PhyML, RDP3, GARD, DnaSP and Selecton were used to generate phylogenetic

trees, to estimate intergenic recombination, genetic variability and the selection at each amino acid site, respectively, for *ankB*, *ankJ*, *ankH* and *rpoB* genes from 28 LP strains. Signs of incongruent relationships between strains were identified for *ankB* and *ankJ* some corresponding to recombination events. Since AnkB is an essential effector for LP, the existence of horizontal gene transfer, the presence of positively selected codons and the amino acid substitutions could result in a profound change in effector function, reflecting an increase in the fitness of these LP strains in different environments and/or tropism towards different hosts. The similar results found for *ankJ* could explain the differential importance of this effector depending on the LP strain or host. Contrarily, *ankH* was highly conserved envisioning the involvement in cellular mechanisms preserved among various LP hosts. In fact, AnkH counterparts were identified in several members of the family *Legionellaceae* suggesting that its acquisition would have occurred in a common ancestor.

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EFFECTS OF TWO PHYTOECDYSTEROIDS ON *TOXOPLASMA GONDII* GROWTH

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INTRODUCTION. Toxoplasmosis is one of the most common parasitoses affecting a wide range of warm-blooded animals including humans. The parasite infection is usually asymptomatic in immunocompetent individuals, however, it may pose a serious threat to immunocompromised and congenitally infected hosts. Current therapy of toxoplasmosis with pyrimethamine and sulfadiazine is not satisfactory and associated with many side effects, thus there is a need for new, effective drugs and in this context ecdysteroids seem a promising alternative. Ecdysteroids are plant and insect-derived compounds with proven antiviral, antimicrobial, antifungal and antiprotozoan activity. These substances have been reported to have immunomodulating, antiarrhythmic and hepatoprotective effects in mammals. Furthermore, compounds are non-toxic and they do not activate steroid receptors on mammalian cells. **AIM.** Taking into account literature data on antiparasitic activity of ecdysteroids, the study was aimed at evaluating the influence of α -ecdysone and

ecdysterone (20-hydroksyecdysone) on *Toxoplasma gondii* invasion *in vitro*. **MATERIALS AND METHODS.** The experiments were performed on cell lines of mouse and human origin and on leukocytes obtained from inbred mice differing in their natural susceptibility to toxoplasmosis. All tested mammalian cells serving as host cells were infected with *T. gondii* tachyzoites and cultivated in the short-term (72 h) and long-term (1 month) presence of phytoecdysteroids. **RESULTS.** The obtained results revealed that a short exposure to α -ecdysone and ecdysterone results in a statistically significant inhibition of parasite proliferation, however long-term contact with the tested compounds is accompanied by the rise in *T. gondii* replication. **CONCLUSIONS.** Since the exact mechanism behind the observed phenomenon remains unknown, a further study is undoubtedly required.

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I-P 8

MUTAGENIC ACTIVITY OF THREE SELECTED THIOSEMICARBAZIDE DERIVATIVES

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INTRODUCTION. Thiosemicarbazides are compounds showing antibacterial properties that can potentially be used to treat infections. Many studies have shown that thiosemicarbazide derivatives often act with the same or higher efficacy than other antibiotics (e.g. penicillin, ampicillin, gentamicin, novobiocin), what makes them good candidates to become an alternative to drugs commonly used in the antimicrobial therapy. **AIM.** The main purpose of our investigations was to determine mutagenic activity of selected thiosemicarbazide derivatives and to define what types of mutations these agents induce. **MATERIALS AND METHODS.** Three selected compounds (P, TT, 3T) showing specific antibacterial activity and able to inhibit bacterial type IIA topoisomerases were tested for their mutagenic activity by the forward mutagenesis assay (Rif^R) and ability to reverse different types of mutations (substitutions, insertions, deletions) in the lactose operon using a specially constructed set of *E. coli* strains

carrying defined mutations. **RESULTS.** The forward mutagenesis assay (Rif^R) revealed the lowest mutagenic activity of P compound, and the highest for 3T. The most frequently observed mutation in the reverse mutation assay is the AT→TA transversion (for TT and 3T). None of the tested compounds caused the AT→GC transition. P compound did not induce analyzed mutations (substitutions, insertions, deletions). The most frequently observed spontaneous mutation was GC→TA transversion. **CONCLUSIONS.** The presented results show that three examined thiosemicarbazide derivatives have mutagenic activity. Point mutations can lead to inactivation of enzymes critical to bacterial survival. This effect is desirable and makes tested agents very attractive potential antibacterial drugs. However, there is a risk that the mutagenic effect may also appear in the genome of human cells or contribute to the rapid acquisition of resistance of bacterial strains to these compounds.

I-P 9

IS THERE A LINK BETWEEN *HELICOBACTER PYLORI* UREASE AND ATHEROSCLEROSIS OCCURRENCE?Iwona Konieczna¹, Weronika Gonciarz^{1*}, Inga Relich², Beata Kolesińska², Justyna Frączyk², Marek Kwinkowski¹, Zbigniew Kamiński², Wiesław Kaca¹¹Department of Microbiology, Institute of Biology, Jan Kochanowski University at Kielce²Institute of Organic Chemistry Technical University, Lodz

INTRODUCTION. Atherosclerosis is a disease with chronic inflammatory response affecting arterial blood vessels. The role of bacteria in the initiation of the inflammatory reaction was discussed. Infections caused by *Helicobacter pylori* concern approximately 50% of the world population. In the sera of atherosclerosis patients a significantly higher level of anti-*Helicobacter pylori* urease was observed. Sequence similarity between the “flap” epitope of bacterial urease and the human C-C chemokine receptor type 11 (CCRL1) was revealed. **AIM.** The aim of this study was to compare the level of antibodies against synthetic peptides mimicking the sequence of human CCRL1 protein (26–59 amino acids), in the sera of atherosclerosis patients and volunteer blood donors. **METHODS.** Sera of 19 atherosclerosis patients (AP) (median age: 62 ± 13), 13 old volunteer blood donors (oVBD) (median age 51 ± 6) and 16 young volunteer blood donors (yVBD) (median age 19 ± 0.5) were analyzed. Thirteen overlapping synthetic peptides mimicking the sequence of extracellular domain of CCRL1 were immobilized

on the cellulose membrane via a triazine derivative and used as an antigen for the detection of anti-CCRL1 antibodies. The level of specific IgG antibodies was determined by the quantitative dot blot method. **RESULTS.** In the sera of patients with atherosclerosis, contrary to VBD (yVBD as well as oVBD), a significantly higher level of antibodies recognizing peptides mimicking N- and C-terminal part of extracellular domain of CCRL1 protein was revealed. Amino acids sequence IKEDV (32–36 aa) in N-terminal part of CCRL1 share homology with flap epitope of *H. pylori* urease. Similarity with bacterial proteins and C-terminal part of this domain was also found. Reactions with peptides corresponding to the central fragment of CCRL1 extracellular domain showed a similar level in all sera. **CONCLUSIONS.** Presented results indicate the role of *H. pylori* urease in developing of atherosclerosis due to the molecular mimicry to human CCRL1.

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I-P 10

PCR-RFLP ASSAY AS AN USEFUL TOOL FOR DETECTION OF POLYMORPHISM OF COA GENE IN *STAPHYLOCOCCUS AUREUS*

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INTRODUCTION. *Staphylococcus aureus* is the most important bacterial pathogen involved in human diseases. About 20–40% of healthy humans are carriers of *S. aureus*, usually colonizing the upper respiratory tract. One of the important virulence factors is

coagulase. The typing procedure for methicillin-resistant *S. aureus* was developed based on PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism) method included amplification of the *coa* gene encoding coagulase and analysis of the

PCR product after digesting with a given restriction enzyme, e.g. *AluI*. **AIM.** The aim of this study was to analyze the possibility of using this method for typing methicillin-sensitive *S. aureus* isolated from the upper respiratory tract of children attending four day-care centers in Lublin. **MATERIALS AND METHODS.** Genomic DNA was isolated by using a commercial test (DNA-Gdańsk II). For restriction analysis, the PCR products of *coa* gene were digested with *AluI*. **RESULTS.** All 182 *S. aureus* strains showed a single band *coa* gene PCR product with molecular size ranging from 650–1080 bp. The products of size 650 and 750 bp were the most frequent and accounted for 31.87% and 47.8% of isolates, respec-

tively. *AluI* restriction enzyme digestion of the PCR products generated fifteen different, numbered as a1 to a15, restriction patterns. PCR-RFLP patterns a1, a4 and a5 were the most common and accounted for 57.14% of the isolates. The amplicons of 650, 750, 850, 910, and 1080 bp generated different quantities of fragments, varying from 2 to 4 bands, and sized between 81–648 bp. PCR products of 4 strains were not digested by *AluI* and therefore this method had 98% typability. The discriminatory index of this typing method was 0.86. **CONCLUSIONS.** The PCR-RFLP method based on *coa* gene polymorphism used in this study for MSSA typing possesses good discriminatory power and typability.

I-P 11

TOLL-LIKE RECEPTOR 2 AND 4 SINGLE-NUCLEOTIDE POLYMORPHISMS IN CYTOMEGALOVIRUS INFECTION

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INTRODUCTION. Toll-like receptors (TLRs), a family of evolutionary conserved transmembrane proteins, detect pathogen-associated molecular patterns from a variety of organisms leading to the activation of innate immune system. The response to human cytomegalovirus (HCMV) is initiated after recognition of its envelope glycoproteins (gB and gH) by TLR2. Recent study provides confirmation of an important role of TLR4 signalling in mediating the HCMV-induced cell cycle perturbation and effective HCMV infection. Several single nucleotide polymorphisms (SNPs) within TLR genes seem to be associated with host-pathogen defense mechanisms. Little is known about the role of TLR polymorphisms in the pathogenesis of HCMV infection. **AIM OF STUDY.** We investigated the possible association between the polymorphisms of TLR2 (Arg677Trp and Arg753Gln), and TLR4 (Asp299Gly) with HCMV infection. **MATERIALS AND METHODS.** Blood samples obtained from HCMV-infected children and

adults, and healthy control subjects were included in this study. The SNPs were genotyped by PCR-RFLP method. Digested PCR products were analysed by capillary electrophoresis. **RESULTS.** We did not observe differences in the frequencies of TLR2 (Arg753Gln) and TLR4 (Asp299Gly) genotypes among control and HCMV infected patient groups. However, we found heterozygosity for the TLR2 (Arg677Trp) SNP in 68% health adults. In contrast, none of the HCMV infected patients showed a heterozygous TLR2 (Arg753Gln) polymorphism. **CONCLUSIONS.** Our results demonstrated that TLR2 (Arg677Trp) polymorphism detected in adults may be a protective factor against HCMV infection. SNPs in the TLR2 (Arg753Gln) and TLR4 were not associated with HCMV infection.

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I-P 12

DIVERSITY OF BACTERIAL VIRUSES IN URBAN SEWAGE

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INTRODUCTION. Waste-water treatment plant Gdańsk-Wschód receives urban sewage from the city of Gdańsk (Poland) and the adjacent villages. Sewage is generated mainly by household wastes containing feces of human and animal origin which are the major source of microorganisms such as, for example, bacterial viruses (bacteriophages, phages). **AIM.** The analysis of urban waste-water phage diversity through their isolation from raw sewage and characteristics in terms of morphology, physiology and genetics. **MATERIALS AND METHODS.** For phage isolation we used the enrichment method consisting in the addition of overnight bacterial culture to the sewage sample, cultivation and chloroform extraction. Morphological studies were performed using electron microscopy. Plaque test was used to determine plaque morphology, host range and the susceptibility to various conditions, like exposure to low and high temperature, acid and alkaline pH, osmotic shock, organic solvents and detergent. For phage DNA sequencing we used 454-pyrosequencing. **RESULTS.** We created the collection

of 83 bacteriophages infecting laboratory, clinical and environmental strains of bacteria. All isolated phages were tailed and most of them belonged to the family *Siphoviridae*. All phages presented a very narrow host range, in some cases limited only to one bacterial strain. Ten phages infected biofilm forming *Pseudomonas aeruginosa* hospital strains. Two coliphages were able to lyse their hosts at 4–7°C. Most phages were stable in alkaline pH and susceptible to osmotic shock. The genomes of eight phages were sequenced and assembled. Two of them showed low similarity to the genomes of known phages deposited in GenBank database. **CONCLUSIONS.** Urban sewage is a source of diverse bacteriophages. Their detailed characteristics extends our knowledge about viruses in this environment. The abundance of phages in the collection infecting pathogenic bacteria confirms the presence of these pathogens in urban sewage. Such phages may be further analyzed as potential candidates for the phage therapy.

I-P 13

ANALYSIS OF THE PRESENCE OF MUTATIONS A2143G AND A2142G IN 23S rRNA GENE ASSOCIATED WITH RESISTANCE OF *HELICOBACTER PYLORI* STRAINS TO CLARITHROMYCIN

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INTRODUCTION. Clarithromycin resistance is the major cause of *Helicobacter pylori* (*H. pylori*) treatment failure. Clarithromycin resistance is caused by point mutations in the region of domain V of the 23S rRNA gene. The most common mutations are: A2143 and A2142G. **AIM.** The aim of the study was to assess the presence of mutations A2143G and A2142G in 23S rRNA gene of *H. pylori* strains resistant to clarithromycin. **MATERIAL AND METHODS.** 21 *H. pylori* strains resistant to clarithromycin were isolated from patients with gastrointestinal tract diseases. Resistance to clarithromycin was quantitatively tested with the E-test which enables determining of the minimal inhibitory concentration (MIC value). Bacterial genomic DNA was extracted by using isolation kit. PCR was conducted to amplify the region of the 23S rRNA gene using appropriate primers and conditions. To detect point mutations related to clarithromycin resistance restriction, RFLP technique was carried out with two restriction enzymes. Enzyme

Eco3II(BsaI) was used to detect A2143G mutation and enzyme BbsI – to detect A2142G mutation. The occurrence of the mutations was carried out against the reference resistant to clarithromycin *H. pylori* ATCC 700684 strain. **RESULT.** The obtained MIC values ranged from 1.5 mg/L to 64 mg/L. Mutation A2143G was presented by 8 *H. pylori* strains, A2142G – 9 strains. There were 3 strains without any tested mutations. Average MIC values for resistant strains with A2143G mutation amounted to 6 mg/L, whereas for strains with A2142G mutation – 30 mg/L. **CONCLUSION.** A2143G and A2142G mutations were the main point mutations in the 23S rRNA gene. Strains with A2143G mutation had lower MIC values than strains with A2142G mutation. Moreover PCR-RFLP is a fast method of detecting the resistance to clarithromycin that may contribute to a faster introduction of a correct treatment. In the future the method to detect other mutations causing resistance to clarithromycin will be elaborated.

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ADHESION PROPERTIES OF BACTERIA MEASURED BY ATOMIC FORCE MICROSCOPY

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INTRODUCTION. Many bacteria exhibit the ability for adhesion to the surface of various materials. Since the primary adhesion between bacterial cell and surface is the first step in the colonization of materials, it is important to investigate the cell surface adhesion properties. **AIM.** We used atomic force microscopy (AFM) to study bacterial cell adhesion properties by registering force curves and adhesion maps. **MATERIALS AND METHODS.** We investigated the adhesive properties of two different bacterial strains *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 700603. Cell suspensions were prepared by inoculating TSB medium and incubating them overnight at temperature 30°C with shaking. Bacterial cells were immobilized on gelatin coated glass surfaces. AFM measurements in distilled water were performed using a Bioscope II AFM with silicon nitride tips. To localize individual cells, images

were obtained in intermittent contact mode. The adhesion forces between the AFM tip and the bacterial cell surfaces were determined from a series of tip retraction cycles. A matrix of 16-by-16 force-distance curves was defined and the adhesion force for each force curve was calculated and displayed as a grey pixel on an adhesion map. **RESULTS.** The adhesion map was a very good method to understand the adhesion properties of the cell surface. Each strain of the bacteria has a different and characteristic pattern of adhesion to the AFM tip. Our adhesion maps reveal a homogeneous distribution of adhesion properties for a single strain. Adhesive forces depend on the morphology of bacterial strain in terms of the force component, the distance components and the number of adhesion events. **CONCLUSIONS.** AFM provides a modern method to study differential adhesion patterns of bacterial cells.

I-P 15

MIRU-VNTR TANDEM REPEATS AS A MARKER OF GENETIC DIVERSITY OF *MYCOBACTERIUM TUBERCULOSIS*Anna Macieja^{1*}, Anna Brzostek², Arkadiusz Wojtasik², Jarosław Dziadek², Anna Sajduda¹¹Department of Genetics of Microorganisms, University of Łódź, Łódź, Poland²Institute of Medical Biology, Polish Academy of Sciences, Łódź, Poland

INTRODUCTION. Tuberculosis is an infectious disease caused by acid-fast bacilli *Mycobacterium tuberculosis* which belong to *Mycobacterium tuberculosis* complex. Presence of polymorphic sequences in genomic DNA allows to differentiate *M. tuberculosis* at the strain level. Besides the reference IS6110-RFLP and

spoligotyping, one of the most widely used genotyping methods of *M. tuberculosis* is MIRU-VNTR. It relies on the detection and analysis of variable number of tandem repeats (VNTRs) of mycobacterial interspersed repetitive units (MIRUs). **AIM.** To determine the usefulness of MIRU-VNTR tandem repeats in the

analysis of genetic diversity of *M. tuberculosis* strains. **MATERIALS AND METHODS.** The study sample included 108 clinical isolates of *M. tuberculosis* obtained in 2005–2007 from 107 tuberculosis patients living in Łódź. Twenty four MIRU-VNTR loci were PCR-amplified using specific primers. The PCR products were electrophoretically separated in 2% agarose gels. The size of the amplicons was determined and the number of repeats in each locus was estimated resulting in a 24-digit MIRU-VNTR profile characteristic for each strain. The analysis of genetic diversity of the strains and their comparison were performed. **RESULTS.** The MIRU-VNTR typing revealed 80 different patterns. Sixty-six of them were unique,

while the remaining 14 were shared by 2–8 strains with identical profiles. Allelic diversity of the 24 loci was also calculated. Five loci (MIRU 10, MIRU 40, VNTR 1955, VNTR 2163b and VNTR 4052) showed high allelic diversity. Ten loci were moderately and nine loci were poorly diverse. The discriminatory power was calculated and compared for sets of 12, 15, 19 and 24 MIRU-VNTR loci. The highest discriminatory potential (HGDI = 0.989) was obtained for sets of 19 and 24 loci. **CONCLUSION.** Nineteen-loci based MIRU-VNTR typing seems sufficient to study genetic diversity of *M. tuberculosis* strains. It could make the analysis less laborious, faster and cheaper compared to the use of 24 loci.

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IDENTIFICATION OF THE SpaCBA PILI POLYMORPHISM IN SELECTED *LACTOBACILLUS* STRAINS AND ANALYSIS OF ITS IMPACT ON ADHESION TO ENTEROCYTES

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INTRODUCTION. The ability to adhere to enterocytes is one of the key features of probiotics. This process involves a number of factors, among which the important role of pili was demonstrated. Some *Lactobacillus* species are confirmed to have heterotrimeric spaCBA type pili. **AIM.** The aim of this study was to identify SpaCBA pili in strains of selected *Lactobacillus* spp. and assess the impact of their presence and sequence polymorphism on the adhesion of these strains to enterocytes. **MATERIALS AND METHODS.** Total 30 bacterial strains of *L. rhamnosus*, *L. casei* and *L. paracasei* were tested. Bacterial cultures were grown in MRS liquid medium at 37°C for 18 to 20 hours. The presence of pilus specific proteins coding genes spaA, spaB and spaC was verified by PCR. In order to identify the presence of sequence polymorphism in the genes possibly affecting the structure of the SpaCBA pilus, a SSCP-PCR approach

was used. To correlate the spaCBA polymorphism to adhesion capability the adhesion assay was carried out using the Caco-2 cell line. The effectiveness of the adhesion was measured using a scintillation counter. **RESULTS.** SSCP analysis revealed the presence of polymorphism in the coding region of spaCBA operon. The *Lactobacillus* strains analyzed showed the adhesion to Caco-2 enterocytes capability from 0.6% to 15%. A correlation of polymorphism to adhesion rate is being analyzed. **CONCLUSIONS.** The presence of SpaCBA pili is a factor increasing the adhesion efficiency of *Lactobacillus* spp. to Caco-2 enterocytes. Lack of these structures on the surface of bacterial cells results in a significant reduction in adhesion efficiency, indicating its important role in the adhesion process. Occurrence of polymorphism in the spaCBA operon is another factor influencing the adhesion capability of the bacteria.

I-P 17

INFECTIVENESS OF PORCINE ENDOGENOUS RETROVIRUSES IN ASPECT OF XENOTRANSPLANTATION

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INTRODUCTION. The lack of sufficient availability of human donor organs has led to a search for alternative sources of organs for transplantations. Pigs are now considered as potential donors due to simplicity of their genetic manipulations and similarities in anatomical and physiological parameters between them and human. However, xenotransplantations are associated with the risk of zoonoses, among which Porcine Endogenous Retroviruses transmission is considered as the major danger. **AIM.** Study on PERV safety in xenotransplantation: *in vitro* research in viral infection opportunity of human cells after exposure to PERV suspension and estimation of viraemia level depending on Swine Leukocyte Antigen class I gene profiles within a transgenic herd. **MATERIALS AND METHODS.** *In vitro* infection of human cell lines using medium from PK-15 culture as a source of viruses. Each cell passage controlling of presence and stability of PERV genes and viral particles released from infected cells. Estimation of

PERV viraemia and SLA class I gene profiles in peripheral blood samples from a herd of transgenic pigs (fucosyltransferase I and/or α -galactosidase transgenesis). **RESULTS.** PERVs were able to infect human cells *in vitro* and initially were transcriptionally active. But following the elimination of PERV gene from the human cells genome, beginning from *envA* gene ending to LTR sequences, have been observed, what took effect on loss of its permissivity. No correlations between PERV titer and SLA class I gene profiles or type of transgene were noticed. **CONCLUSIONS:** PERV infection of human cells *in vitro* is possible but unstable. Its success depends e.g. on type of cells and conditions of process. Both transgenic and nontransgenic specimens differ in the level of PERVs as well as specific porcine organs, which may be used as xenografts. This project was supported by research grant N R12 0036 06/7/2009; DOP-D/138/09 subsidized by the National Centre for Research and Development.

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THE ROLE OF *LEGIONELLA MICDADEI* PHOSPHATIDYLCHOLINE IN TNF- α INDUCTION

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INTRODUCTION. *Legionella* are Gram-negative bacteria that cause a severe form of pneumonia, especially in immunocompromised patients. By means of different strategies, bacteria have evolved the capacity to evade the microbicidal activity of macrophages and to establish as obligate intracellular parasites. **AIM.** We asked whether changes in the PC content in *L. micdadei* membranes may induce changes in the host immune response measured by the level of TNF- α produced by human macrophages. **MATERIALS AND METHODS.** Lipids from the inner and outer membranes prepared from bacteria grown on a choline-supplemented and non-supplemented medium were extracted using the Bligh and Dyer method. To identify PC species, the precursor ion mode (PI) and neutral loss scan (NL) mass spectrometry techniques were employed in the positive ion mode. The multiple reaction monitoring (MRM) technique performed in the negative ion mode was employed to establish the PC amount in the membranes. The

macrophages were treated with alive or dead *L. micdadei* for 4 h at a MOI of 10 or 100. In another experiment, macrophages were treated with different concentrations (10–1000 ng/ml) of outer and inner membranes isolated from *L. micdadei*. TNF- α was measured in culture supernatants by the ELISA method. **RESULTS.** The outer membrane of *L. micdadei* was a more efficient inducer of TNF- α than the inner membrane. In our study, the outer (temperature-treated and -untreated) membrane isolated from bacteria cultured on choline caused a decline in TNF- α induction at all the doses applied, in comparison to bacteria cultured without choline. **CONCLUSIONS.** Incorporation of PC into *L. micdadei* membranes may lead to changes in their physicochemical properties and reduced TNF- α induction, thereby allowing the pathogen to escape some of the effectors of the innate immune system.

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I-P 19

RELATIONSHIP BETWEEN TOLL-LIKE RECEPTOR 9 POLYMORPHISM AND CYTOMEGALOVIRUS DISEASE

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INTRODUCTION. Toll-like receptors (TLR) play an important role in the innate immune responses to pathogens and have been implicated in infectious and autoimmune processes. Cytomegalovirus is a DNA virus which activates host cells via TLR2 and TLR9. TLR9 recognizes CpG DNA motifs that are frequently present in viruses and play a central role in the host defense against viral infection. The TLR9 polymorphism is located within the promoter region and may influence transcriptional regulation of the *tlr9* gene. **AIM OF STUDY.** Two polymorphisms within the TLR9 (T-1237C and T-1486C) were analyzed in the present study on patient groups with HCMV infection. **MATERIALS AND METHODS.** DNA was extracted from whole blood samples from HCMV-infected and uninfected (controls) children and adults. To analyse single nucleotide polymorphisms (SNPs) T-1237C and T-1486C, PCR-RFLP and capillary electrophoresis were used. **RESULTS.** We

found no association between genotype, haplotype or specific SNP alleles for the *tlr9* SNPs at -1237 and 1486 and HCMV infection. However, the homozygous CC genotype (T-1486C) and mutated C allele were more frequent in the HCMV-seropositive children in comparison with the control group ($p < 0.01$). Moreover, the frequency of C allele and homozygous CC genotype was higher in HCMV-infected adults (kidney recipients), compared with uninfected patients ($p < 0.05$). No association of the examined SNPs with the viraemia level in patients was detected. **CONCLUSION.** Our study demonstrated that TLR9 (T-1237C, T-1486C) polymorphism was not associated with HCMV infection. There was evidence suggesting increased frequency of C allele in seropositive patients. **ACKNOWLEDGEMENTS.** Project financed by the European Regional Development Fund under the Operational Programme Innovative Economy, Grant No. POIG.01.01.02-10-107/09.

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INTERACTION OF *PSEUDOMONAS AERUGINOSA* CYSTIC FIBROSIS ISOLATES WITH A549 CELLS

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INTRODUCTION. *Pseudomonas aeruginosa* cystic fibrosis (CF) isolates are an appropriate model for studies on the adaptation of bacteria to chronic infections. An important characteristic of

the isolates' phenotype is the mode of interaction with host cells. **MATERIALS AND METHODS.** We examined the morphological effects of co-cultivation of lung epithelial cells A549 with

two CF strains of *P. aeruginosa* isolated at one-year interval from the same patient, pre- (Pa48) and post-tobramycin (Pa64) inhalatory treatment. Strain PAO1 was used as a reference strain. Bacteria were applied for 2 hours to A549 confluent monolayers at a dose of 10^5 CFU/ml and the samples were colored with trypan blue or Giemsa for light microscopy, or TRITC-phalloidin for confocal microscopy. **RESULTS.** The trypan blue-exclusion cytotoxicity test showed penetration of the dye and coloring of the nuclei of all A549 cells co-cultivated with strain Pa48, or treated with 0.2% Tween 20 (permeabilised control). Strains Pa64 and PAO1 evoked similar effects – predominance of unlabeled A549 cells and the presence of focally organised cellular groups with more or less intensively colored nuclei. Giemsa staining showed that strain

Pa48 caused necrotic changes of the lung cells while both Pa64 and PAO1 caused disruption of the monolayer with detachment of some A549 cells from the substratum and formation of empty areas in the samples. To check for eventual alterations of the actin cytoskeleton, the cells were colored with the F-actin label TRITC-phalloidin and observed by confocal microscopy. Strains Pa64 and PAO1 induced loss of actin filaments. Cells co-cultured with Pa48 were characterised by extensive occurrence of stress fibres outlining the periphery of the otherwise apparently empty cells. **CONCLUSION.** The examined clinical strains are characterized by two distinct modes of interaction with A549 cells: Pa48 causes necrotic degeneration while Pa64 affects the actin cytoskeleton.

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CULTURE CONDITIONS RESULT IN O157- AND ENTEROBACTERIAL COMMON ANTIGEN-RELATED CELL-SURFACE VARIATIONS IN *ESCHERICHIA COLI* O157

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INTRODUCTION. The *E. coli* O157 serotype is the predominant cause of food-borne enterohaemorrhagic infections. For its rapid identification, PCR-based protocols have presently been elaborated. However, in both food materials and samples from infected patients, one major problem is the presence of the pathogen on the background of a rich microbiota. Therefore, selective extraction techniques like immunomagnetic separation are applied which are based on recognition of the O157 lipopolysaccharide. The effectiveness of these may expectedly be influenced by the presence of other surface molecules such as the enterobacterial common antigen (ECA) that may interfere with the O157 binding. **MATERIALS AND METHODS.** Here we examined the possible effects of growth conditions (exter-

nal vs. body temperature, solid vs. liquid medium) on the surface expression of O157 and ECA. We compared the results from immunofluorescence, whole-cell ELISA and qRT-PCR. **RESULTS.** ICC showed variability of surface phenotypes within all of the samples. Whole-cell ELISA, however, indicated distinct culture mode-related shifts in the bacterial populations, and these were in accordance with the results from qRT-PCR. **CONCLUSIONS.** The results confirm that growth in broth at 37°C selectively promoted the expression of ECA while growth on agar at 37°C stimulated O157 synthesis. Such differences were less pronounced in samples cultivated at 20°C. The observed shifts in the cell-surface glycome co-related with a variable recognition by glycan-binding proteins like MBL.

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IDENTIFICATION OF A NOVEL FACTOR AFFECTING INITIATION OF CHROMOSOME REPLICATION IN *HELICOBACTER PYLORI*

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INTRODUCTION. The replication of a bacterial chromosome is initiated by the DnaA protein, which binds to the specific chromosomal region *oriC* and unwinds duplex DNA within the DUE site. The unwound DNA provides the entry site for other proteins of the replication machinery, which synthesize new DNA. The initiation is tightly regulated by many factors, which ensure that the chromosome is duplicated only when the conditions favor the growth and survival of daughter cells. **AIMS.** *Helicobacter pylori* is a bacterium involved in the development of severe gastroduodenal diseases. The key *H. pylori* initiation factors, i.e. DnaA and *oriC*, as well as the oriC binding protein HobA have been characterized, but there have been no other regulatory factors identified so far in *H. pylori*. The aim of this work was to identify a new factor, which regulates the initiation of *H. pylori* chromosome replication. **METHODS.** DNA-affinity chromatography was used to fish proteins from *H. pylori* cell-free lysates, which might interact

with the *oriC* region. The fished proteins were identified by mass spectrometry and produced in *E. coli* as recombinant proteins to verify the affinity chromatography results by gel shifts, footprinting and immunoprecipitation. **Results.** From a few proteins fished by *oriC*-affinity chromatography, only one has been confirmed to specifically interact with *H. pylori oriC*. The identified protein belongs to the family of response regulators, which are known to be involved also in regulation of chromosome replication in other bacteria. The protein specifically interacts with one of the *H. pylori oriC* subregions and, as indicated by DMS-footprinting, stimulates DnaA binding to *oriC*. **CONCLUSIONS.** The novel regulator of the *H. pylori* chromosome replication has been identified. It belongs to the *H. pylori* two component system, which might suggest its involvement in coordination of the *H. pylori* chromosome replication with other cell cycle processes and/or environmental conditions.

I-P 23

THE USE OF THE FILM PRINTERS AS SUBSTRATES FOR IMMOBILIZATION OF FUNGAL CELLS IN THE ANALYSIS OF THE AFM IN LIQUIDS

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INTRODUCTION. The atomic force microscope (AFM) is a powerful instrument for studying the nanoscale surface structures of living cells and measuring their interactions with abiotic surfaces, other cells or specific biomolecules in liquid environment. The main problem in the application of the AFM in microbiological research is the immobilization of microorganisms to flat surfaces without changing their surface properties or viability. One commonly used immobilization method is using the gelatin-coated glass slide or mica sheet. **AIM.** The aim of the study was to verify whether the film for inkjet printers, which is pre-coated with gelatin, is a good surface for the immobilization of fungal cells to analyze the AFM. **MATERIALS AND METHODS.** Appropriate size pieces of film were prepared in a laminar flow, and then autoclaved. The culture of strain *Candida albicans* ATCC 10231 was carried out in liquid Sabouraud media. In the first variant of the analysis on the film

a few drops of fungi suspension were applied, after 1 h the samples were washed in PBS. In the second variant the film was placed in a Petri dish, where was added Sabouraud liquid medium and inoculum of *Candida albicans*, after 24 h of culture samples were washed in PBS. Sample blank was a clean section of the film. Images of samples were obtained in PBS with commercial atomic force microscopy in semi-contact mode. **RESULTS.** Zero sample surface was quite smooth, the amplitude of the amount was 300 nm. In the images of both variants of the tested samples were visible fungal cells, indicating effective immobilization on the surface of the film. **CONCLUSIONS.** The film for inkjet printers can be successfully applied in the analysis of the AFM in liquid microbiological samples. Compared to the method of coating slides with gelatin, the use of the film is an easier method.

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EFFECT OF HYDROGEN PEROXIDE ON THE PHAGE INDUCTION IN SHIGA TOXIN-PRODUCING *E. COLI* STRAINS (STEC)

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INTRODUCTION. Since 1982, all over the world, a series of epidemic and/or sporadic infections caused by strains of various serotypes of Shiga toxin-producing *E. coli* strains (STEC) have been reported. The fundamental role in the pathogenesis of severe human diseases caused by STEC is played by Stx (Shiga toxins). The genes coding for Stx are located in lambdoid prophages and effective toxin production occurs only after prophage induction (caused by factors like antibiotics, UV or H₂O₂). **AIM.** The aim of the study was to determine the effect of H₂O₂ on prophages induction and on *stx* genes expression in the STEC strains. **MATERIALS AND METHODS.** In the present study, three reference *E. coli* strains and five own isolates were used: one collected from a calf and four from sheep. Hydrogen peroxide (to a final concentration of 3 mM) was added to bacterial cultures and incubated at 37°C for 3.5 hours. After the exposure, the *stx* genes expression was

checked by qRT-PCR and the phage titer was calculated by the double agar overlay plaque assay and morphology of plaques was analyzed as well. The same strains of bacteria, incubated under the same conditions but without the addition of H₂O₂, were used as controls. **RESULTS.** The plaques were observed only in the strains exposed to H₂O₂. The significant differentiation of plaques morphology was also noticed. The increased genes expression (*stx1* and *stx2*) corroborates the initial results of the experiment. **CONCLUSION.** The results demonstrate the effective impact of H₂O₂ on the induction of Shiga toxin-converting lambdoid prophage and, as a consequence, the increase in the *stx1* and *stx2* genes expression. Phages carrying *stx1* or *stx2* genes induced cell lysis of three STEC strains, while the other strains of STEC maintained their lysogenic state, despite the exposure to the induced prophage *stx1* or *stx2*.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL 1,2,4-TRIAZOLE – CIPROFLOXACIN CONJUGATES

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INTRODUCTION. Literature data shows that it is difficult to obtain the fluoroquinolone derivatives for which the increase in the antibacterial activity against Gram-positive bacteria would not be related to the decrease in the activity towards Gram-negative bacteria. However, it was shown that 1,2,4-triazole deriva-

tives offer enormous possibilities of modification of antibacterial activity of ciprofloxacin. **AIM.** The main purpose of our investigations was to obtain derivatives of ciprofloxacin showing considerable activity both against Gram-negative and Gram-positive bacteria, and to find the possible structure-activity relationships

connected with inhibitory property towards gyrase. **MATERIALS AND METHODS.** Three of new hybrids containing ciprofloxacin moiety and 1,2,4-triazole derivatives were prepared using the so-called Mannich reaction. Structures of the compounds were determined using ^1H -, ^{13}C -NMR, IR and elemental analysis. The antimicrobial activity of the compounds was determined, by broth microdilution technique, on the Gram-negative (*E. coli* ATCC 25922, *P. mirabilis* ATCC 12453, *P. aeruginosa* ATCC 9027) and the Gram-positive bacterial strains (*S. aureus* ATCC 25923, *S. aureus* Microbank 14001, *S. epidermidis* ATCC 12228, *B. subtilis* ATCC 6633, *B. cereus* ATCC 10876, *M. luteus* ATCC 10240). The inhibitory activity of gyrase was performed using *E. coli* Gyrase Supercoiling Assay Kit. **RESULTS.** All of the synthesized hybrids

of ciprofloxacin and 1,2,4-triazole-based compounds possessed significant antibacterial activity, that was several times higher as compared to the reference drugs (ciprofloxacin, vancomycin). MIC values ranged between 0.011 and 0.41 μM . They also displayed a strong inhibitory mechanism of gyrase with IC_{50} value from 1,2 to 3,5 μM . **CONCLUSIONS.** The presented results revealed that our novel 1,2,4-triazole – ciprofloxacin conjugates show improved antibacterial activity against both Gram-negative and Gram-positive species of bacteria. The mechanism of action is similar to ciprofloxacin – inhibition of gyrase. These features make them good candidates to become an alternative to commonly used antibiotics.

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HEAT SHOCK PHENOMENON AND *GALLERIA MELLONELLA* – *BACILLUS THURINGIENSIS* INTERACTION

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INTRODUCTION. All living organisms interact with their environment and have to deal with many kinds of stress and infections. During infection, host immune response is activated to fight off disease. On the other hand, pathogen expresses virulence factors to overcome host's defense mechanisms. The development of infection depends on factors such as: host predisposition, environmental conditions and pathogenicity of the intruder. We show the effect of temperature on the interaction between insect host – the greater wax moth *Galleria mellonella*, and entomopathogenic bacteria *Bacillus thuringiensis*. **AIM.** We followed the kinetics of the host's immune response after infection and checked whether temperature stress influenced *Galleria mellonella* resistance to virulence factors, such as bacterial proteases. **MATERIALS AND METHODS.** In our research *G. mellonella* caterpillars were injected with *B. thuringiensis* vegetative cells (6×10^3 per larvae). The activity against *E. coli* and *B. circulans* in cell-free hemolymph was tested using zone inhibition and bioautography assays. The presence of transcripts for

defense peptides and their protein products were detected using Real-Time qPCR and Tris-Tricine electrophoresis, respectively. **RESULTS.** In larvae injected with *B. thuringiensis* anti-*E. coli* activity was gradually increasing from 3 to 15 hours and was reduced at 24 hours after infection. This was probably due to the degradation of immune-relevant proteins/peptides by bacterial proteases. In heat-shocked, infected larvae, the immune response was stronger in comparison to non-heat shocked ones. We have also found that heat shock increases the resistance of defense peptides to the degradation by bacterial protease-thermolysin. We observed 12% and 24% survivors in non-shocked and heat-shocked infected larvae, respectively. **CONCLUSIONS.** Experience of temperature stress improves resistance of *G. mellonella* larvae to infection with *B. thuringiensis*. This can partially explain the phenomenon of so-called behavioral fever.

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EVALUATION OF ANTIOXIDANT CAPACITY OF SOME SELECTED LACTOBACILLUS STRAINS

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INTRODUCTION. Free radicals are highly reactive and dangerous species that play a key role in the pathogenesis of IBD (Inflammatory Bowel Disease). One of the possibilities of treatment in patients with IBD is the use of antioxidant compounds that allow the elimination of free radicals and the inhibition of damages associated with their activity. **AIM.** The purpose of this study was an *in vitro* evaluation of hydrogen peroxide decomposition kinetics by some selected strains of *Lactobacillus* and identification of genes encoding their antioxidant enzymes (heme catalase and manganese catalase). **MATERIALS AND METHODS.** A total of 15 strains of *Lactobacillus* species (13 strains of *L. plantarum* and 2 strains of *L. fermentum*) were examined for their ability to decompose chemically pure H_2O_2 (the final concentration of hydrogen peroxide in the culture was 60 mg/L). Measurement of the kinetics of H_2O_2 decomposition was performed using two methods: Peroxide Test Strip (Merck, Germany) and OxiSelect Hydrogen Peroxide Assay

(Cell Biolabs, USA). Furthermore, Columnar DNA Genomic Mini set (A&A Biotechnology, Poland) was used for the isolation of the genomic DNA from all tested strains and PCR was performed to detect the presence of genes encoding antioxidant enzymes: heme catalase (primers: 5'-CCAAATATGCAATGGGATTT-3' and 5'-ACCTTGTAGTAATTTGTCGGG-3') and manganese catalase (primers: 5'-AAGATGCCCCATTTGGTCCAGAA-3' and 5'-TTAAGCATG TCACGCACACCTTC-3'). **RESULTS.** Seven of the tested strains of *Lactobacillus* completely decomposed H_2O_2 within 24 hours from its addition to the culture. The other strains were able to decompose H_2O_2 only partially at the same time. All strains contained gene encoding heme catalase while the gene for manganese catalase was present in 10 strains. **CONCLUSIONS.** Some strains of *Lactobacillus* species can effectively eliminate H_2O_2 from the environment, therefore it is possible that they will reduce symptoms of acute and chronic inflammatory bowel disease.

I-P 28

SUSCEPTIBILITY AND RESISTANCE OF INSECT SPECIES
TO FUNGAL INFECTION MAY RESULT FROM DIFFERENTIAL HYDROLYSIS RATES
OF THEIR CUTICLES BY FUNGAL PROTEASES AFFECTED BY CUTICULAR LIPIDS

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Entomopathogenic fungi are important natural regulatory factors of insect populations and have a potential as biological control agents of insect pests. They are able to invade insects through the cuticle, composed of proteins and chitin covered by lipid layer, by a combination of mechanic pressure of growing hyphae and enzymatic degradation. Insecticidal fungi produce a number of cuticle degrading enzymes: proteases, chitinases and lipases. Although mechanisms of enzymatic degradation of cuticle are intensively studied, the reasons for insects' differential susceptibility to fungal infection remain obscure. The soil fungus *Conidiobolus coronatus* selectively attacks insect species. Prompt death of invaded insects is attributed to the action of toxic metabolites released by the invader incapacitating innate defense response of blood cells. Susceptibility or resistance of insects to fungal invasion may result from several factors, including composition of the cuticular lipids as some of them decrease enzymatic activity of *C. coronatus* when added to the culture medium. The aim of the present work was to check correla-

tions between the proteolysis rates of cuticles dissected from species susceptible and resistant to *C. coronatus* by enzymes produced by this fungus and the composition of insects' cuticular lipids. *C. coronatus* was cultivated in minimal medium. The proteolytic activity of filtrates was determined using N-suc-Ala-Ala-Pro-Leu-p-Na as a substrate. Hydrolysis of cuticle from insects with known both, reaction to *C. coronatus* and cuticular lipids composition was determined. Statistical analysis of time-dependence of the cuticle hydrolysis data concerning insects susceptible to fungal infection showed significantly higher rates compared with data obtained from resistant insects. Correlations between the composition of cuticular lipids and cuticle hydrolysis rates were found. Cuticular lipids may exert antifungal effect decreasing proteolytic activity of *C. coronatus* enzymes. Further experiments are needed to resolve mechanisms of this action.

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ANALYSIS OF THE ROLE OF THE RadA PROTEIN IN DNA REPAIR IN MYCOBACTERIA

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INTRODUCTION. *Mycobacterium tuberculosis* is constantly exposed to several DNA – damaging agents during infection; therefore, mycobacteria developed many mechanisms to repair DNA and survive inside of macrophages and within granuloma. Post-genomics era allows to reveal the presence of genes encoding well known proteins involved in DNA repair systems and genes, including *radA* gene, encoding proteins potentially involved in metabolism of DNA in these bacteria. **AIM.** We analyzed the role of the RadA protein in the DNA repair pathways in mycobacteria, due to its potential contribution to homologous recombination in different species of microorganisms. **MATERIALS AND METHODS.** There were constructed series of mutants of *Mycobacterium smegmatis* carrying the inactivated genes, whose protein products are potentially involved in the genetic material repair, including the gene encoding the RadA protein. Next, genetic constructs were exposed to selected DNA-damaging agents (ultraviolet radiation – UV, mitomycin C – MMC,

methyl methanesulfonate – MMS, and ofloxacin – OFX). Then the analysis of gene expression levels was performed by quantitative *radA_{MS}* real-time PCR and Western Blot. **RESULTS.** Despite the fact that RadA protein is not essential for mycobacterial cells, based on phenotypic analysis of *M. smegmatis* $\Delta radA$ it was showed that the strain devoid of functional RadA protein is more sensitive to DNA damage caused by the action of MMS, compared with wild – type strain. On the other hand, inactivation of the *radA* gene in the mutant lacking the ability to synthesize RecA – the key protein of homologous recombination process – reduces its sensitivity to mutagenic agents. Moreover, it was evidenced that the level of RadA protein in the mycobacterial cells grows significantly under DNA damaging conditions. **CONCLUSIONS.** Taking under consideration all the above results, we can suggest that the RadA protein can be considered an agent, which preferentially directs the repair processes for homologous recombination.

MICROBIAL BIOTECHNOLOGY FOR ENVIRONMENTAL PROTECTION AND INDUSTRY

PLENARY LECTURES

II-PL 1

MICROBIOLOGICAL MUTAGENICITY TESTS WITH THE USE OF GENETICALLY MODIFIED STRAINS OF *VIBRIO HARVEYI* IN STUDIES ON MUTAGENIC POLLUTION OF ENVIRONMENTGrzegorz Węgrzyn^{1*}, Beata Podgórska²¹Department of Molecular Biology, and ²Department of Molecular Evolution, Faculty of Biology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland

Mutagenic pollution of environment is a global and important problem. This includes marine environment. Although many mutagenicity assays have been developed, there are specific problems with testing marine water and sediments for mutagenic contamination. Chemical methods, although precise, are relatively expensive, and considering very low concentrations at which mutagens can act, they require very high volumes of water for tests. Therefore, screening of waters for the presence of mutagenic pollution must involve biological assays. The use of bacteria in such assays was

proposed a long time ago. However, most of genetically modified bacterial strains used in commonly available microbiological mutagenicity assays, like *Escherichia coli* or *Salmonella*, survive relatively poorly in marine waters. Thus, alternative assays have been developed, in which bacteria occurring naturally in marine habitats, like *Vibrio harveyi*, are employed. These assays appear to be useful in testing not only marine samples but also they can be used in other approaches, which involve detection and estimation of the amount of mutagenic compounds.

II-PL 2

COMPATIBLE SOLUTES; THEN AND NOW

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Until the mid 1990s compatible solute accumulation had been examined primarily in food spoiling yeasts, fungi and bacteria. These were circumscribed to low molecular solutes like polyols and sugars, a few amino acids and amino acid derivatives. Then microbiologists began to look at the accumulation of compatible solutes in bacteria and archaea that live at high temperatures, isolated from shallow marine and abyssal thermal environments. These organisms, like all other microorganisms must adjust, within intrinsic limits, to alterations in the water activity of the environment, but found that the majority of these solutes were unique to hyper/thermophiles. These compatible solutes include mannosylglycerate, di-myo-inositol-phosphate and the very rare compatible solutes diglycerol-phosphate, di-mannosyl-di-myo-inositol-phosphate and mannosylglyceramide. In recent years we have studied the synthesis

of mannosylglycerate (MG) and trehalose in the thermophilic bacteria *Thermus thermophilus*, *Rubrobacter xylanophilus* and *R. radiotolerans* and *Persephonella marina* as well as several archaea of the genera *Pyrococcus* and *Thermococcus*. But these studies led to the finding of other rare compatible solutes in mesophilic bacteria of different lineages, such as members of the phylum Planctomyces which accumulate a plethora of solutes and *Mycobacterium* spp. that synthesize a mannosylglycerate analogue named glucosylglycerate, not as a compatible solute, but as a component of a polysaccharide. Other compatible solutes have also been encountered and their biosynthetic pathways described. These compatible solutes have a considerable biotechnological value for the preservation of proteins and cells; they also have an important niche in the cosmetic industry as moisturizers and sun screens.

II-PL 3

SUB-CELLULAR PROTEOMICS TO DISCOVER MECHANISMS IN MICROBIAL PHYSIOLOGY

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Recent advances in mass spectrometry-based proteomics and the bioinformatics of data analysis allow shot-gun proteomics analysis of proteins fractions. These protein fractions may result from (partial) protein purifications, but can also be cell organelles fractions. Using differential conditions a proteomics analysis may give a strong contribution to study of physiological changes as a part of environmental changes in microorganisms. In our research on

filamentous fungi like *Aspergillus niger* and *Rhizopus oryzae* we apply sub-cellular proteomics to study the cellular changes that result from different growth or induction conditions. We examined in particular study the change in proteome in relation of up-take of sugars and organic acids. We also used isolated mitochondrial fractions for the identification of mitochondrial transporters. For these studies we have developed fermentor based controlled culture

conditions that form the basis for the differential conditions studied, e.g. low and high oxygen or organic acid production versus no production. Mycelial biomass generated under these controlled conditions was used for organelle isolation that is based on organelle specific enzyme markers. The fractions are used for MS/MS analysis,

the resulting MS spectra are via a bioinformatics pipeline correlated to proteins. In this presentation examples of research strategies to apply a proteomics approach will be given to study microbial processes and how this can be correlated with e.g. transcriptome analysis for the interpretation of the physiological effect of the change.

II-PL 4

SPECIES BIOTYPIZATION, WHY *DE NOVO* SEQUENCING ALWAYS WINS?

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A novel method for peptide amino acid sequence determination upgraded with genome fingerprint scanning (GFS) was designed to investigate gene expression of the clinical organisms at the first instance but it can be used in further development as ultimate *de novo* sequencing method in “bottom up” proteomics. The method links proteomics data, consisting of determined tryptic peptides amino acid sequences obtained by negative and positive ion modes. MALDI MS/MS sequencing data read and compared to sequenced genome of the observed organism is confirmed by overlapping of the sequence data from both modes (e.g. GAAGAK read in neg. and pos. ion modes; GAAGAK b ions neg. ion mode and KAGAAG y ions pos. ion mode). The idea that lies behind this new technology is enhanced *de novo* sequencing of unknown peptide amino acid sequences in negative MS/MS (enabled by derivatization that contains two negatively charged groups without observed side reactions or peptide degradation) and positive MS/MS of the same peptide used as quality control (MS/MS of derivatized ions in both cases). The enhancement of negatively charged ions’ ionizability (similar to fluorescence effect in spectroscopy) and dissociation in negative ion mode ensures data confidence without ion adduction or in-source decay interferences characteristic for positive ion mode peptide mixture analysis. This technology enables unambiguous determination of at least five to six amino acids and often more in a row from

a MS/MS experiment only in one ion mode, but it can be combined as b-ion series from N-terminus (negative ion mode) and *vice versa* y-ion series from C-terminus (positive ion mode), even though derivatized ions are “invisible” as precursor ions in positive ion mode. Short peptide of five to six amino acids in a series (it is important to create a continual series without gaps or statistical uncertainties which could influence the scoring data) can be used to detect gene that codes for the analyzed protein. Chemical derivatization is non-destructive for tryptic peptides and without any known side reactions. Aforementioned mass spectrometry features are followed by reading software called The Protein Reader (TPR). The protein reader comprises uniquely designed algorithms specifically adjusted to the simplified MS/MS readings (neg. MS/MS b-ions and pos. MS/MS y-ions). The difference between TPR and other software products for protein identification (GPS, Protein Pilot, MASCOT, Peaks etc.) is the reading concept. It does not match masses of the fragments to the theoretical masses, it reads them and after the reading searches strings in the database. There is a TPR feature for *de novo* reading without using the database. Point mutations, wrong database annotations and unknown species protein function should not represent database problem in proteomics in future. Clinical application of the developed technology, methodology and TPR program package are underscored.

II-PL 5

NON-RIBOSOMAL PEPTIDES PRODUCED BY THE BALTIC CYANOBACTERIA – STRUCTURE AND ACTIVITY

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INTRODUCTION. Non-ribosomal peptides (NRPs) belong to the most widely studied group of cyanobacterial metabolites. Some of the compounds have deleterious effect on living organisms. Other peptides have gained attention due to their potential biotechnological application. Members of the Nostocales and Oscillatoriales orders have proven to be exceptionally rich sources of NRPs. **AIM.** In this work, the metabolic diversity of the Baltic cyanobacteria was studied. In addition, the potential application of cyanobacterial metabolites as a source of pharmaceuticals was explored. **MATERIALS AND METHODS.** The activity of crude extracts, collected fractions and compounds isolated from the Baltic cyanobacteria of Nostocales order was tested in an array of enzymatic assays. The extracts and active samples were screened for peptides using nano-LC-Q-TOF MS/MS (AB Sciex). Then, structural analyses of peptides were performed with LC-MS/MS system equipped with a hybrid triple quadrupole/linear ion trap mass spectrometer (QTRAP5500,

AB Sciex). The principal component analysis (PCA) ordination was performed to study the differences in peptide pattern among the analyzed strains. **RESULTS.** In the work, over 60 cyclic and linear non-ribosomal peptides, including novel structures, were elucidated. Significant differences in the number and structure of peptides produced by cyanobacteria were observed. Among the compounds, the cyclic anabaenopeptins were most numerous and diverse. They inhibited the activity of elastase, carboxypeptidase and/or protein phosphatase. The linear tetrapeptides, sumigins and aeruginosins, showed strong (nM) activity towards trypsin and/or thrombin. **CONCLUSIONS.** The Baltic strains of *Nodularia spumigena* were shown to be an exceptionally rich source of NRPs. However, among the strains, significant diversity in peptide profiles was recorded. The strong and selective activity of some NRPs revealed in this work, corroborates the potential of cyanobacteria as a source of lead compounds for drug development.

II-PL 6

MASS SPECTROMETRY FOR MICROBIAL GLYCOMICS

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Carbohydrate structures play extremely important role in building bacterial envelope and the environment contact edge zone properties modification. One of the main glycolipids of Gram-negative bacteria is the lipopolysaccharide – the endotoxin. Another example of bacterial building blocks concerns glycosylated proteins such as the S-layer proteins. Bacterial glycosylation genes encoding for glycosyltransferases were found to be directly linked with virulence genes. Numerous bacterial pathogens virulence factors were also found to be covalently modified with carbohydrate structures. Thus the importance of these structures as either bacterial antigens or the moieties involved in bacteria – host as well as bacteria – bacteriophage interactions gives us the reason to undertake studies of these molecules, by the means providing the highest sensitivity. Thus we can use the mass spectrometry hybrid systems that can

complement the NMR spectroscopy data. The carbohydrates can be studied directly in and on the cell (with no extraction or isolation necessary). The isolation step is provided by mass spectrometry. This the whole cell bacteria mass spectrometry. MALDI (matrix assisted laser desorption) enables ionization and the TOF (time of flight) and hybrid TOF-TOF and Fourier transform systems are the methods of choice in these studies. This approach can allow for bacteria typing (O-antigen). Bacterial protein glycosylation is another example of MALDI MS-MS application. The research concerning endotoxin structures and in particular LPS core structures modified with e.g. aminoacid substitution is further application of multiple stage mass spectrometry. These structures were studied with either ESI MSn or the MALDI TOF-TOF systems.

ORAL PRESENTATIONS

II-O 1

MALDI-TOF/TOF ANALYSIS OF *METARHIZIUM VELUTINUM* PROTEOME DURING 4-*n*-NONYLPHENOL BIODEGRADATION

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INTRODUCTION. 4-nonylphenol is a xenobiotic and endocrine disrupting compound causing serious problems in the environment. Microbial biodegradation is one of the most advantageous ways of the compound removal, however, the metabolic background of the process has not been explained. **AIM.** Identification of proteins involved in 4-*n*-NP biodegradation by *Metarhizium velutinum*. **MATERIALS AND METHODS.** Trend and quantitative analysis of 4-*n*-NP and its derivatives during culturing was done with GC-MS and LC-MS/MS. Proteins extraction involved mechanical homogenization of the mycelium followed by 20% TCA protein precipitation. The obtained protein residue was re-suspended in 0.2 M NaOH with SSB buffer. Proteins were separated on 2-D gels and selected spots were digested with trypsin for MALDI-TOF/TOF sequencing. The MASCOT Search Engine was used for protein database searches followed by Principal Component Analysis (PCA) of the peptide maps. **RESULTS.** Trend and quantitative analysis of 4-*n*-NP biodegradation established the best conditions for proteome analysis after 24 h of culture incubation when majority of the by-products

reached their maximum relative concentration. 2-D gels revealed that 13 protein spots were present only in control samples and 19 protein spots were present only in xenobiotic containing cultures. Other protein spots were observed in both sets of the samples with the similar intensity. 20 proteins were identified on the basis of MALDI-TOF/TOF sequencing and Mascot database search. Collected data allowed us to formulate the possible explanation of microorganism strategy towards the tested xenobiotic. The major groups of proteins involved in the biodegradation process were: oxidation-reduction systems related to nitroreductase-like proteins, ROS defense systems, TCA cycle and energy-related systems. PCA analysis applied for unidentified proteins let us formulate initial classification of these proteins. **CONCLUSIONS.** Proteome analysis of 4-*n*-NP biodegradation by *M. velutinum* resulted in more than 60% proteins identified or initially classified by PCA. Collected data let us propose the proteomic background of 4-*n*-NP biodegradation. The work was supported by the National Center for Science, Poland (Project No. UMO-2011/01/B/NZ9/02898).

II-O 2

BALTIC BACTERIA – EFFECTIVE DEGRADERS OF CYANOBACTERIAL NONRIBOSOMAL PEPTIDES

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INTRODUCTION. In the Baltic, the blooms of cyanobacteria are formed mainly by the toxic *Nodularia spumigena*. The concentration of nodularin (NOD) in surface waters can temporarily reach

over 40 000 µg/L. Apart from NOD, the cyanobacterium produces a variety of other biologically active nonribosomal peptides. Elimination of NOD from contaminated waters undergo mainly via

microbial degradation. **AIM.** The objectives of the work were (1) to determine the factors regulating microbial degradation of NOD by the Baltic bacteria (2) to test the ability of these microorganisms to degrade other cyanobacterial nonribosomal peptides (3) to isolate single bacterial strain with NOD-degrading ability. **MATERIALS AND METHODS.** Biodegradation was studied in the batch laboratory experiments. Sediment and water samples with a natural community of microorganisms were collected from the Gulf of Gdańsk. The influence of light, temperature and NOD concentration on the degradation rate of NOD was tested. During the experiments, peptides and their degradation products were analyzed by HPLC and LC-MS/MS. The identification of *mlrA-D* genes in NOD-degrading bacteria was performed by PCR. **RESULTS.** NOD-degrading activity of bacteria living in sediments was observed during the

whole year. NOD degradation in the presence of natural bacterial community from sediments took 3–24 days. The time, during which NOD concentration fell below the detection limit depended on the previous contact of microorganisms with the toxin. Baltic microorganisms were also capable of degrading microcystins, aeruginosins, anabenoheptins and spumigins. 10 bacterial strains isolated from sediments and 3 strains isolated from *N. spumigena* CCNP1401 culture showed NOD-degrading activity. *mlrA-D* genes were not found in the analyzed samples. **CONCLUSIONS.** Natural microbial communities from the Baltic sediments are very effective in the degradation of cyanobacterial non-ribosomal peptides. As some of the peptides belong to acute toxins, the observed activity may have potential application in their removal from waters used by humans.

II-O 3

HALOTOLERANT MICROORGANISMS ASSOCIATED WITH THE PLANTS IN SALINE SOIL

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INTRODUCTION. Soil salinization increased significantly during last decades and causes major problems for plant productivity. A promising strategy to improve crop salt tolerance could be the application of salt-tolerant (halotolerant) plant growth promoting microbes. However, high salinity constitutes an environmental stress also for microorganisms. **AIM.** Evaluation of density and metabolic biodiversity of microorganisms associated with halophytic plants. **MATERIALS AND METHODS.** The research has been done in the industry and natural saline areas in Central Poland: salty meadow in the vicinity of soda factory (S1) and area affected with natural brine in the health resort (S2). Investigated plants belong to Chenopodiaceae – *Salicornia herbacea* L. Bacterial and fungal strains were isolated and quantified from three zones with different influence of analysed plant: interior of plant roots – endophytes (E), rhizosphere – part of the soil closely adjacent to the

plant roots (R) and soil not influenced by root exudates (S). Bacteria and fungi were obtained by plating the dilutions on appropriate agar maums with different concentrations of NaCl (0, 100, 200, 400, 600 mM). The metabolic biodiversity was investigated with the use of Biolog EcoPlate™ microplates. For isolated strains a preliminary genetic analysis were carried out. **RESULTS.** Significant differences between density and metabolic activity of halotolerant microorganisms associated with analysed zones of isolation: (E) – endophytes, (R) – rhizosphere and (S) – soil were observed. We have revealed changes in analysed parameters between two investigated test sites (with industrial – S1 and natural – S2 salinity). **CONCLUSIONS.** The site of higher salinity were characterized by a higher density of bacteria and fungi. Higher sensitivity to NaCl concentrations were determined for fungi. The highest abundance and total metabolic activity was observed for rhizosphere microorganisms.

II-O 4

GENETIC MODIFICATION OF GLYCEROL FERMENTATIVE CATABOLISM IN WILD TYPE STRAIN *C. FREUNDII* AD970

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INTRODUCTION. It has been suggested that over-expression of additional copy of 1,3-propanediol oxidoreductase (PDOR) may improve yield of 1,3-propanediol (1,3-PD) production from glycerol. **AIM.** The aim of this study was to investigate if over-expression of additional copy of PDOR will influence 1,3-PD production by the wild type strain *Citrobacter freundii* AD970. **MATERIALS AND METHODS.** DhaT gene was amplified on genomic DNA of *S. blattae* and cloned under the control of rhamnose-induced-promoter. An empty vector (V) and a construct with insert (V+I) were used for transformation of *C. freundii* AD970. The strains were analyzed regarding expression of DhaT through SDS-PAGE and DhaT activity – through in-gel-assay, where after separation, the gels were incubated in PDOR-activity buffer. The strains performance during cultivation in minimal media containing glycerol as the sole carbon source was also assessed. The cultures were analyzed regarding OD600, production of 1,3-PD and accumu-

lation of 3-hydroxypropanal. **RESULTS.** SDS-PAGE analysis of proteins from V and V+I allowed identification of a single more distinctive band in the rhamnose-induced V+I strain lane. Native-PAGE electrophoresis did not provide clear response on increased PDOR activity due to strong activity of native proteins. The three strains were cultured on minimal media with glycerol as the sole carbon source. Expression of DhaT triggered increased biomass production ($p < 0.05$) and a tendency to reduce accumulation of 3-HPA, the toxic intermediate of 1,3-PD production. No statistically important improvement in 1,3-PD production was observed. **CONCLUSIONS.** We continue our studies on modification of the genetic construct by exchange of the rhamnose-induced-promoter, since the inducer was probably not efficiently utilized in this system. **ACKNOWLEDGEMENTS.** The study was financially supported by the project POIG01.01.02-00-074/09.

II-O 5

THE TORTUOUS TRIMETHYLAMINE DEGRADATION PATHWAYS IN TWO STRAINS OF GENUS *PARACOCCLUS* (*ALPHAPROTEOBACTERIA*)

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INTRODUCTION. *Paracoccus aminophilus* JCM 7686 and *Paracoccus aminovorans* JCM 7685 (*Alphaproteobacteria*) are methylotrophic strains isolated from a sample of polluted soil. They are able to degrade many toxic compounds, including trimethylamine (TMA), dimethylamine (DMA) and methylamine (MA). **AIM.** We studied molecular basis of TMA degradation pathways of JCM 7686 and JCM 7685 to reveal their biotechnological potential. **MATERIALS AND METHODS.** Complete nucleotide sequences of the JCM 7686 and JCM 7685 genomes were obtained (454 Life Sciences) and annotated (GeneDB). The genes involved in TMA degradation have been identified by amino acid sequence similarity to known proteins and their predicted role was analyzed by mutational analysis (gene replacement). The growth of mutants (JCM 7686) was analyzed in minimal media containing TMA, DMA or MA as a sole carbon and energy source. The protein profiles of JCM 7686 cultured on minimal media with arabinose, methanol or trimethylamine were analyzed and allowed detecting

many proteins (mass spectroscopy) upregulated during methylotrophic growth. **RESULTS.** The multireplicon genomes of the analyzed strains consist of: (i) main chromosomes, (ii) chromids and (iii) plasmids. Bioinformatic and functional analyzes revealed that chromid pAMI6 of JCM 7685 carries two functional copies of *tmm* gene encoding TMA monooxygenase, as well as many other genes, which are upregulated in the presence of TMA. This replicon may, therefore, significantly shape methylotrophic pathway of *P. aminophilus*. A closely related chromid of *P. aminovorans* (pAMV1) also carries the *tmm* gene. Another chromid of this strain (pAMV3) carries an additional set of genes encoding TMA and DMA dehydrogenases as well as denitrification pathway, which enables degradation of TMA under anaerobic conditions. **CONCLUSIONS.** Both studied *Paracoccus* spp. contain genes involved in TMA degradation. Our results indicate crucial role of chromids in providing adaptive and niche-specific genetic information, which influence the metabolic networks of their hosts.

II-O 6

MOLECULAR METHODS FOR IDENTIFICATION OF BACTERIAL STRAINS COLONIZATION OF HISTORIC BUILDINGS

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INTRODUCTION. It is established knowledge, that microorganisms can be responsible for the deterioration of different materials, and cultural heritage. Therefore, for the correct conservation and restoration of the historical objects, it is important to identify microorganisms colonization the structural elements and surfaces. **AIM.** The objective of this study was isolation and identification of bacterial strains colonization of museum objects. **MATERIALS AND METHODS.** The taxonomic position of 40 isolated bacterial strains was confirmed by molecular methods based on 16S rRNA gene sequencing. The genomic DNA was isolated using Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland) according to manufacturer's instruction. The 16S rRNA gene was amplified by polymerase chain reaction (PCR). The PCR products were detected by 1% agarose in 0.5 × TBE buffer with ethidium bromide and purified

using Clean Up Mini Kit (A&A Biotechnology, Gdynia, Poland). The obtained nucleotide sequences of the 16S rRNA gene were compared, using the BLAST 2.2.27+ software, with the sequences available from the National Center of Biotechnology Information. **RESULTS.** The predominant microflora consisted of bacteria of the species *Bacillus atropheus*, *B. cereus*, *B. mycooides*, *B. subtilis*, *B. gibsonii*, *B. muralis*, *Psychrobacillus psychrodurans*, *Sporosarcina aquimarina*, *Staphylococcus equorum*, *Micrococcus luteus*, and *Pseudomonas fluorescens*. **CONCLUSIONS.** Analysis of nucleotide sequences of 16S rRNA gene is a fast, simple, and suitable alternative method to conventional identification procedures for reliable characterization of bacterial strains colonization of structural elements of historic buildings.

POSTERS

II-P 1

CHARACTERIZATION OF NEW THERMOPHILIC SPECIES OF GENUS *RUBROBACTER* ISOLATED FROM A HOT SPRING IN AZORES

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INTRODUCTION. The genus *Rubrobacter* belongs to family *Rubrobacteraceae*, a family of the order *Rubrobacteriales*, of the class *Actinobacteria* and currently this genus embraces three species:

Rubrobacter xylanophilus, *R. taiwanensis* and *R. radiotolerans*. **AIM.** We recently isolated four thermophilic strains from a hot spring at Ribeira Grande on the Island of São Miguel in the Azores, Portugal.

MATERIALS AND METHODS. For isolation we used the membrane filtration method with filters pore size 0.45 µm. The medium utilized for the isolation of the organisms was Thermus medium (4) and the temperature of incubation was 60°C. The strains were characterized on the basis of phenotypic, quimiotaonomic, genotypic and phylogenetic parameters. **RESULTS.** Comparisons of 16S rRNA gene sequences show these strains to be most closely related (98% similarity) to species of the genus *Rubrobacter*. The strains RG-1^T, RG-6, RG-3^T and RG-8 had an optimum growth temperature of about 60°C, an optimum pH for growth between 6.0 and 7.0, form cocci that stain Gram-positive, are aerobic, oxidase and catalase positive and hydrolyze casein. Strains RG-1^T and RG-6

are pink-pigmented, had a growth range in medium containing between 0 and 11% NaCl and hydrolyze starch; strains RG-3^T and RG-8 are non-pigmented, had a growth range in medium containing between 0 and 2% NaCl and do not hydrolyze starch. The major fatty acids are iso-C_{17:0}, iso-C_{18:0} and an unknown fatty acid (ECL 19.893). Menaquinone 8 is the major respiratory quinone. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, one glycolipid, one phosphoglycolipid and one phospholipid. **CONCLUSIONS.** Based on 16S rRNA gene sequence analysis, physiological and biochemical characteristics we intent to describe two new species of the genus *Rubrobacter*.

II-P 2

COMPARISON OF FILAMENTOUS BACTERIA FROM WASTEWATER TREATMENT PLANT IN LODZ AND WASTEWATER TREATMENT PLANT IN ZGIERZ USING IMAGE ANALYSIS TECHNIQUES

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INTRODUCTION. Computer analysis techniques can be useful in the analysis of activated sludge in wastewater treatment plant. They can be used to analyze the filamentous bacteria, which play an important role in the operation of activated sludge systems. **AIM.** The aim of this work was to compare the contribution of filamentous bacteria in activated sludge coming from the first and last part of the activated sludge chambers in wastewater treatment plants in Lodz and Zgierz. It was made with the use of computer image analysis techniques. **MATERIALS AND METHODS.** Samples were collected once a month from the first and last part of the activated sludge chamber. For each test at least two independent slides were made. Slides were stained using the Neisser staining. Then, images were made and they were analyzed using an automated procedure using the computer program NIS ELEMENTS AR. Total length of

filamentous bacteria in the image, total surface area of filamentous bacteria in the image and the total aggregate surface area of the image was determined. **RESULTS.** The average length of strands of filamentous bacteria in an aeration chamber in samples from Lodz was 229.55–8127.55 µm, while samples from Zgierz was 686.83–3500.93 µm. The results for anaerobic chamber was 2892.12–7008.31 µm for samples from Lodz and 2489.29–3428.32 µm for the samples with Zgierz. **CONCLUSIONS.** There was a greater length of the filaments of filamentous bacteria and the surface area of filamentous bacteria for both anaerobic chamber for samples of sludge from wastewater treatment plant in Lodz and in Zgierz. The ratio of the surface of filamentous bacteria to the surface area of aggregates was significantly higher for samples from Lodz.

The work was done as part of a development project NR14-0004-10.

II-P 3

SEARCH FOR NEW NATURALLY OCCURRING STRAINS OF *STREPTOMYCES* SPP. TO IMPROVE BIODEGRADATION OF PETROLEUM SUBSTANCES

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INTRODUCTION. Oil contamination is recently one of the main problems in soil remediation. For bioremediation processes different species of bacteria from the genus of *Bacillus*, *Pseudomonas* and *Streptomyces* have been used. However, there is still a need for searching for new naturally occurring strains with specific properties to improve the biological degradation of petroleum substances. **AIM.** Isolation of *Streptomyces* spp. from soil sample contaminated with petroleum substances and screening for their ability to degrade different petroleum substances. **MATERIALS AND METHODS.** Prior to this study, four strains of *Streptomyces* isolated from soil contaminated with petroleum products for over 20 years were used for determination of their ability to grow in media contained different petroleum substances. In this study, petroleum-based fuels, including petrol, petroleum and diesel and petroleum-derived naphthalene and anthracene were used. Bacteria were inoculated on Bushnell Haas Agar medium containing different concentrations of petro-

leum substances and incubated at 25°C for 28 days. As controls, the same *Streptomyces* strains, cultured on the same medium but containing glycerol instead of petroleum substances were used. During the incubation, growth and morphological changes of bacterial colonies were observed. **RESULTS.** The colonies of bacteria cultured on media containing petrol, petroleum, diesel and anthracene appeared after 7 days of incubation. This time was the same as compared to the control cultures. On media containing naphthalene bacterial growth was slower and colonies were observed after 14 days of incubation. **CONCLUSIONS.** Screening tests conducted in this study allowed for the isolation of four strains of *Streptomyces* with the ability to grow in the petroleum contaminated media. These strains were able to utilize petroleum substances, including toxic naphthalene and anthracene as sole source of carbon. Further studies are in progress in order to investigate the potential of the isolated strains to decompose crude petroleum and different petroleum products.

II-P 4

HYDROPHOBICITY OF YEAST STRAINS ISOLATED FROM BIOFILMS
IN WATER DISTRIBUTION SYSTEMS

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INTRODUCTION. Yeast cell adhesion can cause a variety of problems in the food industry and medicine. Recent studies have revealed that hydrophobic cells are more virulent than hydrophilic cells. Therefore, hydrophobicity may be a critical parameter in the cell adhesion to solid surfaces. **AIM.** Aim of this study was to evaluate cell hydrophobic properties of yeasts during incubation in different environmental conditions in contact with PVC surface – the material commonly used in water distribution systems. **MATERIALS AND METHODS.** The five species of yeasts isolated from natural biofilms in water distribution systems were used in this study. Incubation of the strains was conducted with PVC carrier in enriched (peptone water) and poor (Ringer solution) media at 25°C for 12 days. Hydrophobicity of yeast cells was evaluated using

MATH method (Microbial Adhesion to Hydrocarbons). **RESULTS.** Tested yeast strains showed different hydrophobicity properties. The highest values of hydrophobicity were recorded for *Debaryomyces hansenii* K and *Asterotremella humicola* S strains. Ringer solution as the culture medium decreased the hydrophobicity of all yeast strains. Enrichment culture medium with peptone increased the hydrophobicity of *Debaryomyces hansenii* Z strain. Starved yeast cells in 12-day of incubation showed significantly decreasing this parameter. **CONCLUSIONS.** The obtained results showed that the hydrophobicity of yeast cells forming biofilms was strain-specific and variable at the incubation time. These values depended on the kind of strain, the kind of medium, the availability of nutrients, as well as the age of cultures.

II-P 5

ADHESION OF YEAST STRAINS ISOLATED FROM BIOFILMS
IN WATER DISTRIBUTION SYSTEMS TO PVC SURFACE

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INTRODUCTION. Biofilms on pipe walls in water distribution systems are composed mainly of bacteria in a polymeric matrix. Presence of extracellular polymeric substances may cause the yeast secondary colonization. **AIM.** The aim of our study was to characterize the adhesive properties of environmental yeast isolates to PVC surface. **MATERIALS AND METHODS.** The five species of yeasts (*Asterotremella humicola* AA, *Asterotremella humicola* S, *Debaryomyces hansenii* Z, *Debaryomyces hansenii* K and *Rhodotorula mucilaginosa* 2L.) isolated from natural biofilms in water distribution systems were used in this study. Incubation of the strains was conducted with PVC carrier in enriched (peptone water) and poor (Ringer solution) media at 25°C for 12 days. The analysis of yeast adhesion to the carriers involved the luminometry measurement and microscopic obser-

vations after ether-ethanol denaturation and methylene blue staining. **RESULTS.** Tested strains showed various levels of cell adhesion. For tested PVC surfaces, the maximum RLU values were noted for *Debaryomyces hansenii* Z and *Asterotremella humicola* S strains. Ringer solution as the culture medium increased the ATP concentrations per cm² on fourth and eighth day of incubation. Starved yeast cells after 12-day incubation showed significantly decreasing of this parameter in both media. The heterogeneous nature of adhesion resulted in the surface coverage ranging approximately 20–60% of the total PVC area. **CONCLUSIONS.** The obtained results showed that the adhesive abilities of tested yeast cells were strain-specific and the RLU values were variable in the incubation period according to starvation process and nutrient limitation.

II-P 6

CHANGES IN PHOSPHOLIPIDS COMPOSITION OF FILAMENTOUS FUNGUS
CUNNINGHAMELLA ELEGANS IN RESPONSE TO TRIBUTYLTIN CHLORIDE (TBT)

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INTRODUCTION. Phospholipids (PLs) have been important target research chemicals due to their role as barriers between subcellular compartments and PLs functions in signal transduction. One of the compounds which disturb lipids homeostasis is tributyltin (TBT). This biocide is reported to be immuno-, neuro-, hepato-, nephro-, gastro- toxic and also causes testicular damage. However, the mechanism(s) by which TBT induces toxicity have not been fully established. **AIM.** In order to reveal the correlations between PLs composition and microbial tolerance towards TBT, a comparative lipidomics study was carried out using a fungal strain *Cunninghamella elegans*. **MATERIALS AND METHODS.** *C. elegans* was incubated in Sabouraud medium with TBT

(10 mg/l) or without the biocide. Fungal biomass from the stationary phase of growth was applied to PLs extract using Folch procedure. Profiling of fungal phospholipids was performed using high performance liquid chromatography (HPLC)-MS/MS, leading to identification of 52 species. Individual phospholipids were then quantified by a multiple reaction monitoring method (HPLC-MRM). **Results.** Analysis of the distribution of the hydrocarbon chains showed that palmitic acid (C16:0) and stearic acid (C18:0) were the most predominant saturated fatty acids, whereas oleic acid (C18:1), linoleic acid (C18:2) and γ -linolenic acid (C18:3) were the major unsaturated species. TBT induced a decline in the amounts of many molecular species of phosphatidylcholine (PC)

or phosphatidylserine (PS), and an increase in the levels of phosphatidic acid (PA), phosphatidylinositol (PI) and phosphatidylethanolamine (PE). **CONCLUSION.** Obtained data indicate that the resistance of *C. elegans* to TBT is associated with the ability

of the fungus to modulate phospholipid composition in response to the biocide.

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II-P 7

REGULATORY PROTEIN KORSA HOMOLOGUE FROM pSAM2-LIKE INSERTION SEQUENCE INFLUENCES THE SYNTHESIS OF SECONDARY METABOLITE, POLYKETIDE COELIMYCIN IN *STREPTOMYCES COELICOLOR* A3(2)

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Streptomyces are bacteria with complex life cycles involving mycelium differentiation and production of a broad range of secondary metabolites with a number of biological activities including the majority of antibiotics of natural origin like erythromycin or tetracycline. One of such substances is coelimycin – the product of a type I polyketide synthase from *Streptomyces coelicolor* A3(2) called Cpk. Polyketide synthases and understanding their regulation are crucial for the production of new drugs by synthetic biology and biotechnology methods. The *cpk* genes are influenced by several regulatory mechanisms like gamma-butyrolactone signaling or nitrogen and phosphate metabolism regulation. Here we present the results of searching for direct regulators of coelimycin producing gene cluster. We discovered a regulatory link between *cpk* cluster and an pleiotropic GntR regulator – protein SCO3932. Method used in the study: DNA-protein affinity chromatography, Electrophoretic Mobility Shift Assay, Footprint, homologous pro-

tein overexpression, mutant construction, immunoblotting. Using the DNA region between genes *cpkA* and *cpkD* in affinity chromatography we caught the protein SCO3932. The protein is a KorSA homolog and it is also homological to GntR protein family containing wHTH DNA binding domain and UTR effector domain. Surprisingly we found that protein SCO3932 coded within p-SAM2 – like element binds to *cpkA* promoter and influences the synthesis of coelimycin. We identified the sequences binding motif upstream of SCO3932 gene. That motif is also present within genes *CpkA* and *CpkB*. Overexpression of protein SCO3932 delays onset of coelimycin production by about four hours. This may be caused by blocking transcription of main synthase subunits by binding in the coding regions of *cpkA* and *cpkB* genes. However, in the wild strain the protein SCO3932 is expressed on a constant level during growth for 40 hours. We suspect that another factor overcomes the repression effect of protein SCO3932.

II-P 8

EFFECT OF POTATO JUICE ON THE GROWTH OF BACTERIA WITH THE POTENTIAL INDUSTRY APPLICATION

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INTRODUCTION. According to the EU directive, usage of antibiotics as growth stimulants in animals feeding is banned. Therefore, there is a need for innovative technology solutions, which would provide a satisfactory efficiency at relatively low expenses. Application of probiotic strains of lactic acid bacteria may be an absorbing issue. Simultaneously, in spite of potentially attractive strains selection, hitting a high biomass growth is an essential point. At the same time, it is significant, that culture medium makes up almost 70% of all production costs. Thus, both effectiveness of bacteria growth and as low as possible outlays constitute a fundamental point. **AIM.** The purpose of the study, was to obtain an increase in growth yield of chosen microorganisms by enrichment of starch

culture medium in potato juice. **MATERIALS AND METHODS.** In the presented study strains of lactic acid bacteria that belong to The Department of Biotechnology and Food Microbiology in Poznań were used. Culture mediums from rye, barley and wheat flour were tested. In a control sample a tap water was used as a solvent, while in the proper samples fresh potato juice was exerted. Analysis of variations in microorganisms growth was performed by Koch Method. **RESULTS.** One has proved, that replacement of water by potato juice ensures an increase in the yield of bacterial biomass in a shorter time. **CONCLUSIONS.** Potato juice, as a post-production waste product, may be a valuable source of bioactive compounds stimulating growth of lactic acid bacteria.

II-P 9

APPLICATION OF THE IMPEDANCE MICROSENSORS FOR BIOFILM GROWTH MONITORING

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INTRODUCTION. Among various monitoring methods of cell culture growth, the impedance methods deserve special attention, displaying a number of favorable features, such as: non-invasive-

ness, label-free detection (no markers needed), real-time measurement and provision of data concerning electrical properties of environment. **AIM.** To investigate the possibility of application

of the impedance sensors and impedance spectroscopy method for monitoring of bacterial biofilm growth in real time. **MATERIALS AND METHODS.** Study was performed on two species of biofilm-forming bacteria: *P. aeruginosa* ATCC15442 and *K. pneumoniae* ATCC4352. Bacteria, suspended in culture medium, were placed in microtitre 24-plate wells containing impedance sensors. Impedance of each sensor was measured using impedance spectroscopy method in four minutes intervals for 24 hours. Obtained data were analyzed using electrical equivalent circuit method. Ability of bacteria to adhere and to form biofilm on sensors surface was confirmed by means of Electron Microscopy and quantitative culturing. **RESULTS.** The changes of impedance of the sensor-medium

structure were observed during the course of experiment. This phenomenon was caused by the following factors: adhesion of bacterial cells on the surface of the sensor and subsequent biofilm formation; increase of the concentration of bacterial metabolites in the medium and aging of culture medium. The most distinctive changes were visible in reactance at low frequency (20 Hz). The approximation of impedance spectra with equivalent electrical circuit show that the best indicator of the bacteria growth on the surface of the sensor is constant-phase element in series with a resistor depicting medium resistance. **CONCLUSIONS.** The results obtained confirm the possibility of application of electrical measurement methods for monitoring of bacterial biofilm growth in real time.

II-P 10

S. CEREVISIAE YEAST CELLS AS A MATERIAL FOR ENCAPSULATION OF LIPID-SOLUBLE FOOD INGREDIENTS

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INTRODUCTION. *Saccharomyces cerevisiae* yeast cells can be considered as natural micro-vehicle for producing capsules of micrometer size range. Strong cell wall of *S. cerevisiae* cells provides high stability and their phospholipids membranes behaves like liposomes whereby they could be used to protect sensitive compounds from adverse effects caused by light, moisture and oxygen. **AIM.** The aim of this study was to evaluate the feasibility of bakers' yeast cells as an encapsulation carrier for functional food ingredients: essential oils, cod liver oil and vitamin E (α -tocopherol). **MATERIALS AND METHODS.** Before encapsulation, yeast biomass was pre-treated with various physical (high temperature, osmotic stress, sonication) and chemical (ethyl alcohol and alkali) factors. Cells were then added to the o/w emulsion of a particular oil or to ethanol solution of vitamin E and stirred for 24 h at 25–70°C. After incubation, biomass was separated by centrifugation and washed three times with water to remove unbounded compounds. Resulting

wet biomass was then freeze-dried. Encapsulated substances were extracted from yeast cells with organic solvent (ethanol, methanol/chloroform) and their amounts were measured either by GC or HPLC. **RESULTS.** Final content of oil ingredients was dependent from the type of oil and ranged from 13% for oregano to 86% for basil oil and 25% for cod liver oil. Encapsulation efficiency for vitamin E approached 35%. Pre-treatment of the yeast biomass with 10% ethanol solution resulted in higher efficiency of the process. Other treatments did not affect the final substance content. **CONCLUSIONS.** This study shown that *S. cerevisiae* yeast cells could be used with good result as a simple carrier for functional food ingredients. Microcapsules with up to 86% of active compound could be prepared by simple mixing such compound with yeast cells in water or ethanol solution. **ACKNOWLEDGEMENTS.** This work was supported by the Polish Ministry of Science and Higher Education Project No. 2136/B/P01/2010/38.

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DEFINING THE STAGES OF CHROMOSOME SEGREGATION IN *STREPTOMYCES VENEZUELAE* AND THE ROLE OF TOPA IN THIS PROCESS

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INTRODUCTION. *Streptomyces* are famous as producers of diverse secondary metabolites including many antibiotics. These organisms are characterized by morphologically complex life cycle, during which they initially form a vegetative mycelium of branched hyphae. Upon nutrients depletion *Streptomyces* form sporogenic hyphae which differentiate into chain of exospores in the process called sporulation. ParA and ParB are the major proteins responsible for proper segregation of multiple chromosomes into spores. ParB forms nucleoprotein complexes around *oriC* while ParA polymers align ParB complexes along the hyphae. **AIM.** We focus on the dynamics of the ParA and ParB assembly during *S. venezuelae* sporulation. We also examine the localizations of ParA and ParB proteins in the TopA-depleted strain. **MATERIALS AND METHODS.** In order to localize proteins of interest within *S. venezuelae* hyphae we used PCR targeting to construct different strains, which produce chromosomally encoded EGFP or Cherry-tagged ParB and

ParA instead of the wild type proteins. In the TopA-depleted strain *topA* gene was under the control of an inducible promoter and the native *topA* gene was deleted using PCR targeting. Using time-lapse fluorescence microscopy we analyse the timing of protein complexes formation and their disassembly. **RESULTS.** Time lapse microscopy enables to define the subsequent stages of the sporogenic hyphae development. It indicates close correlation of ParAB dependent segregation with stopping of hyphae extension. In the wild type strain ParA is visible as dispersed fluorescence spreaded along the hyphae, whereas ParB forms regularly spaced complexes. In TopA-depleted strain ParA and ParB form randomly distributed fluorescent foci. **CONCLUSIONS.** We propose the model of sporulation, where active chromosome segregation occurs at the time when the sporogenic hyphae stops growing. Lowering the *topA* expression level changes the pattern and timing of ParAB complexes formation and blocks sporulation.

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OPTIMIZATION OF CARBON AND NITROGEN SOURCES FOR 1,3-PROPANEDIOL PRODUCTION BY *CITROBACTER FREUNDII*

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INTRODUCTION. 1,3-propanediol (1,3-PD) is a valuable molecule that finds application in synthesis. It is used as a monomer for the synthesis of polytrimethylene-terephthalate (PTT) – a novel polymer. 1,3-PD can be produced from glycerol by microorganisms such as *Clostridium* spp., *Klebsiella* spp., *Lactobacillus* spp. and *Citrobacter freundii*. **AIM.** The aim of this study was to investigate the influence of various carbon and nitrogen sources on the production of 1,3-PD by *Citrobacter freundii*. **MATERIALS AND METHODS.** *Citrobacter freundii* AD119 was used. The strain was cultivated for 7 days at 30°C in media containing various carbon and nitrogen sources. The mixture of glycerol and other carbon sources such as: sucrose, glucose, xylose, citric acid, mannose, lactose, starch, galactose, arabinose and fructose were tested. Nitrogen sources such as a yeast extract, meat extract, bactopectone, ammonium sulphate, urea and corn steep liquor were also investigated. After incubation fermentation broths were analyzed by HPLC (Agi-

lent Technologies 1200 series). **RESULTS.** The experiment revealed that among tested nitrogen sources, 1,3-production was stimulated by organic nitrogen sources to the greatest extent, except for corn steep. The highest final 1,3-PD concentration (18.3 ± 0.4 g/L) was obtained on a medium containing meat extract. 16.5 ± 0.2 and 14.8 ± 0.6 g/L of 1,3-PD were produced when, respectively, yeast extract and bactopectone were used. Results of experiments with inorganic sources on 1,3-PD production were not satisfactory. Concentration of the diol determined in the media containing inorganic nitrogen sources did not exceed 5 g/L. Partial replacement of glycerol with carbohydrates or citric acid had negative effect on 1,3-PD production as the final 1,3-PD concentration was 4.3–44.4% lower than the control. **CONCLUSIONS.** Organic nitrogen sources act also as energy sources and thus are suitable for 1,3-PD production. Partial replacement of glycerol with other carbon sources did not increase 1,3-PD production.

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LACTIC ACID BACTERIA STARTER CULTURES – INHIBITORY AGENTS FOR PATHOGENIC BACTERIA IN SILAGES

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INTRODUCTION. To help increase the quality and storage stability, the process of forage ensiling is being stimulated with the addition of starter cultures of lactic acid bacteria. Currently there is a significant range of bacterial preparations for forage ensiling on the market, but there is still a need for new formulations to improve not only the quality of the silages, but also their safety. **AIM.** The aim of the study was to determine the effect of three starter cultures of lactic acid bacteria to reduce the level of some pathogenic bacteria (*Listeria* sp., *Clostridium* sp. and coliform bacteria) during ensiling of meadow grass, corn grain and maize whole crop. **MATERIALS AND METHODS.** Half of the silages were prepared with the addition of the bacterial or bacterial-enzymatic preparation containing starter cultures of specially selected strains of lactic acid bacteria of the genus *Lactobacillus* in a dose of 5 g/t of raw

material. The process of ensiling lasted for 12–14 weeks. **RESULTS.** All silages, which were made with the addition of preparations, were characterized by very high quality. Silages made without the addition of starter cultures were characterized by satisfactory or good quality. The number of coliforms and *Listeria* sp. was 10-fold lower in average in the silages with the preparation in comparison to untreated ensiled forages. There was observed 10 to 100-fold decrease in number of molds between the experimental and control silages. No *Clostridium* sp. were identified in silages made with the addition of lactic acid bacteria preparation. **CONCLUSIONS.** The results of the study confirmed the effectiveness of researched starter cultures of lactic acid bacteria in inhibiting growth of molds and potentially pathogenic for animals bacteria such as *Listeria* sp., *Clostridium* sp. and coliform bacteria during the process of ensiling.

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MODULATION OF FUNCTIONAL PARAMETERS OF SELECTED MICROORGANISMS THROUGH ROTATING MAGNETIC FIELD EXPOSURE

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INTRODUCTION. Recently, different types of magnetic fields are used as potential agents to modulate the properties of microorganisms involved in biotechnological processes. Possibility of controlling the microbial viability and activity using magnetic fields can be

widely applicable in agriculture, food science and medicine. **AIM.** The aim of the present study was to determine the effect of the rotating magnetic field (RMF) on the viability, growth rate, cell metabolic activity and biofilm formation by selected bacteria and

yeasts. **MATERIALS AND METHODS.** Nine species of bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Streptococcus mutans*, *Cronobacter sakazakii*, *Klebsiella oxytoca*, *Staphylococcus xylosum* and two species of yeast: *Candida albicans*, and *Saccharomyces cerevisiae* were chosen for this study. Microorganisms were exposed to the RMF of different frequency and magnetic induction at various time intervals. The persistence of the effect of exposure on microorganisms after further incubation was also studied. **RESULTS.** The present study showed, that the exposition to the RMF, compared to the unexposed controls caused strong increase in the growth dynamics, cell metabolic activity and percentage of biofilm-forming bacteria

including *S. aureus*, *E. coli*, *S. marcescens*, *S. mutans*, *C. sakazakii*, *K. oxytoca* and *S. xylosum*, however inhibited cell metabolic activity and biofilm formation by *A. baumannii* and *P. aeruginosa*. It was also found, that exposition to the RMF caused a decrease in growth rate and cell metabolic activity of *C. albicans* and *S. cerevisiae*. The observed effect of the RMF exposition depended on the RMF frequency and magnetic induction. **CONCLUSIONS.** The results obtained in this study proved, that the RMF can be used as a novel tool for modulation of growth dynamics, cell metabolic activity and biofilm formation by different species of microorganisms. The desired effect can be achieved by regulation of frequency and magnetic induction of the generated RMF.

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MICROBIAL DIVERSITY AND DYNAMICS OF A GROUNDWATER AND A BOTTLED NATURAL MINERAL WATER

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INTRODUCTION. The current perception of the autochthonous microbial diversity present in bottled natural mineral waters (NMW) sold in Europe is based mainly on traditional culture-dependent methods. **AIM.** In order to retrieve more reliable information about the microbial diversity and dynamics at source, after bottling and through six months of storage of a commercial NMW, we applied a tandem approach, based on culture-dependent and -independent methods. **METHODS.** The application of Fluorescence *in situ* Hybridization (FISH) using domain-specific probes in conjunction with DAPI cell counts allowed the monitoring of the dominant microbial communities. Shifts of the prevailing communities were assessed by Denaturing Gradient Gel Electrophoresis (DGGE) and by 16S rRNA gene 454 pyrosequencing. The culturable heterotrophic community was characterized by the recovery of 3626 isolates. **RESULTS.** DGGE profiles revealed a shift of the communities present at source during the bottling process and storage, and a reproducible population dynamics on two consecutive

years. Borehole waters displayed low cell densities that increased two orders of magnitude upon storage, reaching a maximum value within the first 15 days after bottling. Borehole and bottled waters microbial communities were dominated by representatives of *Bacteria* as demonstrated by FISH. Pyrosequencing revealed a decrease in diversity throughout storage. The culturable community was primarily affiliated with the *Alpha*-, *Beta*- and *Gammaproteobacteria* that were also the most representative groups in the pyrosequencing dataset. *Archaea* was present in very low numbers, and belonged mainly to the *Thaumarchaeota* and the *Euryarchaeota*. **CONCLUSIONS.** The bottling procedure induces dramatic changes in the community structure present at source, as revealed by shifts of DGGE profiles and sample clustering based on structure and composition of the culturable community and pyrosequencing. The autochthonous population changed with the bottling and storage processes, but certain phylotypes and isolates were present in the boreholes and the bottles.

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ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM PIGS AGAINST PATHOGENIC BACTERIA *E. COLI*

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INTRODUCTION. Most probiotic strains occur among lactic acid bacteria, particularly *Lactobacillus*, *Bifidobacterium* and *Enterococcus*. These bacteria are part of the normal intestinal microflora of animals and can be used as probiotics. Their biological activity is closely linked with the ability to produce bioactive metabolites. **AIM.** The aim of this study was to isolate lactic acid bacteria strains from pigs showing antagonist activity of pathogenic *E. coli* enterotoxigenic. **MATERIALS AND METHODS.** Screened bacterial isolates were obtained from the upper and lower gastrointestinal tract of healthy pigs. Determined their activity against pathogenic strains of *E. coli* indicator. Screening was performed by point by measuring

the diameter of the clearing zones around the isolates deposited on the agar medium inoculated with bacteria indicator. **RESULTS.** We examined 178 isolates collected from the upper and lower gastrointestinal tract of pigs. Shown forming ability LAB normal gastrointestinal microflora of pigs to the antagonist effects on *E. coli*. Antagonistic activity against more than one strain of the indicator showed 53 isolates from pig manure and 17 isolates from swabs of the mouth. **CONCLUSIONS.** In the gastrointestinal tract of pigs lactic acid bacteria have been identified that can be used as an effective probiotic for pigs.

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1,3-PD PRODUCTION WITH NEW STRAINS
OF *CLOSTRIDIUM BUTYRICUM* OBTAINED BY GENOME SHUFFLING

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INTRODUCTION. In the face of shortage of fossil fuel supplies alternative resources for chemical industry have gained considerable attention. Biotechnological process of 1,3-propanediol (1,3-PD) production is based on usage of waste product which is glycerol from biodiesel production plants. The anaerobic synthesis of 1,3-PD from glycerol by *Clostridium butyricum* is performed under conditions generating various kinds of stress factors that may negatively influence the growth and metabolism of bacteria cells. Thus, strong effort is made to develop resistant and industrially attractive strains. **AIM.** The aim of this study was to verify fermentation profiles of new *Clostridium* strains obtained via genome shuffling grown on high concentration of glycerol. **MATERIALS AND METHODS.** The wild type of *Clostridium butyricum* was isolated from environmental samples. Other species were obtained from mutants with high tolerance to glycerol via genome shuffling method. The metabolic profiles

of the new *Clostridium butyricum* strains were verified during batch fermentations in a medium containing high initial concentration of glycerol (above 100 g/L). The fermentation was carried out with pH regulation. For the batch culture a 2 L glass fermentor was used. The metabolites levels were determined by HPLC assay. **RESULTS.** Complete utilization of glycerol and improvement of 1,3-PD production were observed. Prolonged fermentation time were also displayed. **CONCLUSIONS.** It has been demonstrated that in case of new *Clostridium butyricum* strains high initial concentration of glycerol (100 g/L) neither prevents bacteria growth nor decreases the efficiency of the 1,3-PD biosynthesis. **ACKNOWLEDGMENTS.** This work was prepared within the framework of project PO IG 01.01.02-00-074/09 co-funded by The European Union from The European Regional Development Fund within the framework of the Innovative Economy Operational Programme 2007–2013.

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TECHNICAL NONYLPHENOL ELIMINATION AND DETOXIFICATION
BY THE FILAMENTOUS FUNGUS *UMBELOPSIS ISABELLINA* IN THE HEAVY METALS PRESENCE

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INTRODUCTION. Chemical compounds belonging to the group of EDCs have a negative impact on living organisms by modulating their immune system. They get into the environment with waste water and as a result of industrial processes. The ongoing development of the industry enhances this negative process. Technical nonylphenol (tNP) is a compound exhibiting structural similarity to 17 β -estradiol, which is used widely as a surfactant or a plasticizer. Due to its structure, biodegradation of this compound is slow. An additional difficulty in the removal of tNP from the environment by microorganisms may be caused by the presence of toxic heavy metals (zinc, manganese, nickel and lead). **AIM.** The aim of this study was to determine the ability of the elimination and detoxification of tNP by *Umbelopsis isabellina* IM 833 strain both in the absence and presence of heavy metals. **MATERIALS AND METHODS.** The culture of IM 833 strain was conducted in Sabouraud medium.

The elimination analysis was performed by gas chromatography with a mass detector. Detoxification was tested using the toxicity test *ARTOXKIT M*. The initial concentration of tNP in the growth medium was 25 mg/l, the concentration of the tested metals was 2.5 mM. **RESULTS AND CONCLUSIONS.** IM 833 fungus has the ability to grow in the presence of tNP at the concentration of 25 mg/l with no apparent inhibition of growth, and also effectively removes the xenobiotic from the culture medium during incubation. Additionally, studies have shown high tolerance of the tested strain to heavy metal ions: zinc, manganese, nickel and lead at the concentration of 2.5 mM. In the presence of these metals the tested xenobiotic is also eliminated.

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GENERATING MUTANT LIBRARY
OF 1,3-PROPANEDIOL OXIDOREDUCTASE USING ERROR-PRONE PCR

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INTRODUCTION. The use of biocatalysts in industrial chemical production gives an expectation for green and economical process chemistry. However enzymes are products of natural evolution adjusted to fulfill their biological and physiological function. The most of naturally occurring enzymes do not have appropriate features necessary for production in chemical industry. The development in protein engineering allows for optimization of particular

enzyme traits which made them more suitable to chemical process. Error-prone PCR is technique which enables introducing point mutations in protein-encoding gene during amplification of DNA and thereby generate different variants of protein to clarify the relationship between protein sequence, structure and function. 1,3-propanediol dehydrogenase (PDOR, EC 1.1.1.202) is involved in the conversion of glycerol to 1,3-propanediol, which is a key chemical

bulk applied for synthesis of Polytrimethylene terephthalate with desired properties for large volume markets. PDOR is encoded by *dhaT* gene. **AIM.** Generating different variants with random mutations of 1,3-propanediol oxidoreductase. **MATERIALS AND METHODS.** The *dhaT* gene was obtained from *Clostridium butyricum* DSMZ 10702 and used as template for the construction mutant library. Error-prone PCR was performed using Genemorph® II Random Mutagenesis kit (Agilent Technologies) according to the manufacturer's instructions. The PCR products were digested with restriction enzymes *Bam*HI and *Xho*I, and inserted into plasmid pET28b+ that was digested with the same enzymes. The recombinant plasmids were then transformed into *E. coli* DH5α. Individual colonies were

picked and the recombinant plasmids were extracted. The obtained plasmid library were randomly sequenced. **RESULTS.** Error-prone PCR was carried out to introduce 2–4 nucleotide substitutions per *dhaT* by varying the amount of DNA template. Recombinant plasmids with mutated *dhaT* were transformed into competent *E. coli* DH5α. The total error-prone library size was 180 clones. **CONCLUSIONS.** The constructed library of random protein variants gives starting point for next step, which is high-throughput screening. This work was supported within the framework of project PO IG 01.01.02-00-074/09 co-funded by The European Union from The European Regional Development Fund within the framework of the Innovative Economy Operational Programme 2007-2013.

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PLASMID ENCODING CADMIUM RESISTANCE IN *VARIOVORAX* SP. FROM THE INDUSTRIAL AREA OF UPPER SILESIA

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INTRODUCTION. Upper Silesia is the territory heavily contaminated with heavy metals. Highly toxic element – cadmium – exceeds its concentration in this area, mainly due to the anthropogenic activity. Microorganisms are known to easily adapt to these harsh conditions and metal resistant bacteria may reach value of 90% of total population. Most of the genes encoding resistance to the heavy metals are located on extrachromosomal DNA – plasmids. **AIM.** The aim of this study was the recognition of the presence of plasmids in strain *Variovorax* sp., which has been isolated from the industrial area of Upper Silesia and determination that genes located on them participate in cadmium resistance. **MATERIAL AND METHODS.** Strain *Variovorax* sp. has been isolated from the industrial soil from Upper Silesia and identified through 16S rDNA sequence. The resistance to cadmium was verified on LB agar medium supplemented with CdCl₂. Plasmids have been isolated using modified Kado & Liu method and further purified

with Plasmid- Safe ATP-Dependent DNase (Epicentre). PCR reaction was used for the detection of genes encoding *cadA* and *czcD* genes from operons *cadAC* and *czcCBA* known for taking part in the cadmium resistance. Different chemical and physical agents as temperature, novobiocine, ethidium bromide, SDS have been used to eliminate plasmids from the cells. **RESULTS.** *Variovorax* sp. demonstrated the resistance to 1 mM CdCl₂ on LB agar medium. In this strain plasmids have been present, however the agents used for their curing, where enabled to remove plasmids from the cells at used concentrations. *cadA* and *czcD* genes where not detected. **CONCLUSION.** Strain demonstrated the presence of plasmids, however their participation in the cadmium resistance was not proved. Lack of *cadA* and *czcD* genes indicates other operons taking part in the cadmium resistance.

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THE ROLE OF MICROORGANISMS IN THE REDUCTION OF PETROLEUM PRODUCED WATER SALINITY

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Exploitation of hydrocarbons deposits beyond the acquisition of oil and natural gas causes extraction of the produced water. These wastes are extremely dangerous to the environment because of their composition, especially high levels of hydrocarbons and sodium chloride. Storage and disposal of petroleum wastes has become a major challenge in recent times. However, various organic pollutants have been shown to be transformed and mineralized by microorganisms able to grow in the presence of salt (Margesin and Schinner, 2001). Therefore, it is important to test the natural removal potential of microorganisms in petroleum wastes discharge. The main aim of the study was to obtain active, indigenous groups of aerobic microorganisms isolated from the petroleum produced water, capable of utilizing sodium chloride in metabolic processes and to estimate the biodegradability of the selected organic compounds in high concentrations of sodium chloride. Water samples were collected

from south-eastern Carpathians hydrocarbons deposits. Salinity of these waters was ranged from 3,5% to 7%. Aerobic microorganisms isolated from produced water were mainly represented by *Pseudomonas* sp. and *Bacillus* sp. Aerobic stationary microbial cultures were incubated on liquid, minimal M9 medium with sodium acetate, phenol and BTEX as sole carbon. All cultures have been treated with sodium chloride at concentrations range from 2% to 10%. The experiment has shown lack of potential for permanent reducing salinity by microorganisms in aerobic stationary microbial cultures. However there has been noticed decrease of sodium chloride level at exponential microbial growth phase, indicating its use in metabolic processes. As could be expected, sodium chloride was returned to the environment after the death of microorganisms. The utilization of the carbon sources was similar in all cultures, especially for phenol and sodium acetate and range from 60% to 83%.

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MALACHITE GREEN SORPTION ONTO *MYROTHECIUM RORIDUM* IM 6482 DEAD BIOMASS

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INTRODUCTION. Malachite green (MG) is a commonly used triphenylmethane dye which may cause mutagenic, carcinogenic and teratogenic effects in humans. **AIM.** The sorption of MG from aqueous solutions by dead biomass of fungal strain *Myrothecium roridum* IM 6482 was studied in the batch mode. The influence of parameters such as contact time, pH of dye solution, dye concentration, biosorbent dosage, the presence of other synthetic dyes and metal ions on the sorption efficiency was investigated. **MATERIALS AND METHODS.** Autoclaved biomass of *M. roridum* IM 6482 obtained from a 96-hour-old liquid culture was centrifuged and washed with deionized water. Biosorption experiments were conducted in shaking conditions in Erlenmeyer flasks containing 25 ml of the MG solution and appropriate amount of fungal biomass. At the pre-determined time intervals the adsorbent was separated from the solution by centrifugation. To determine the

MG concentration the absorbance of the supernatant was measured with the spectrophotometer SPECORD 200 (Analytik Jena) at the wavelength of 615 nm. Experiments were conducted in triplicates. **RESULTS.** MG adsorption equilibrium was attained after 30 minutes of incubation. The pH value of MG solution in the range from 5 to 7 did not significantly affect MG decolorization. The highest adsorption efficiency (10 mg/g) was achieved when 25 mg of the fungal biomass and MG in the initial concentration of 10 mg/l was used. The colorant was effectively eliminated from solution containing another synthetic dye (Acid Orange II or Methylene Blue), whereas the presence of metal ions, mainly Cd²⁺, limited decolorization by approximately 30%. **CONCLUSIONS.** The obtained results indicate that the dead biomass of *M. roridum* IM 6482, due to the suitable adsorption capacity, could be utilized for synthetic dyes removal from wastewater.

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FACTORS AFFECTING MALACHITE GREEN DECOLORIZATION BY MICROSCOPIC FUNGUS *MYROTHECIUM RORIDUM* IM 6482

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INTRODUCTION. The microbial decolorization of dyes is strongly influenced by numerous operational parameters such as composition of growth medium, pH and temperature values, as well as oxygen availability. **AIM.** The aim of the study was to determine the influence of selected factors on MG removal from a submerged culture of microscopic fungus *Myrothecium roridum* IM 6482. The effect of the initial dye concentration (1–50 mg/l), carbon source (glucose or sucrose at 0,75; 1,5 or 3%), medium pH value (4–7), temperature (28; 38 or 48°C) and shaking speed of the cultures (0; 75 or 150 rpm) on MG decolorization was studied. **MATERIALS AND METHODS.** Liquid Czapek-Dox medium supplemented with MG was inoculated with a 10% homogenous second-step preculture. At appropriate time intervals samples of

the fungal culture were centrifuged. Decolorization of the obtained supernatants was monitored spectrophotometrically and expressed as a percentage of the removed dye. Biotic and abiotic controls were also performed (without dye or mycelium supplementation, respectively). **RESULTS.** MG decolorization by *M. roridum* IM 6482 was promoted in the presence of 0.75% sucrose or 1.5% glucose. The process was more efficient when the fungus was cultured at 28°C in a medium with pH 4. MG decolorization occurred to be independent on the used conditions of culture shaking. **CONCLUSIONS.** Using the culture conditions favoring MG decolorization by *M. roridum* IM 6482 it eliminated 94% of the dye within 8 hours. The results obtained suggest that used in the study strain could be valuable for MG bioremediation.

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IDENTIFICATION AND SELECTION OF SULPHITE RESISTANCE GENES IN WINE YEASTS *SACCHAROMYCES CEREVISIAE*

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INTRODUCTION. Sulphite is widely used as a preservative for its antimicrobial and antioxidant activities. These features caused that SO₂ is frequently added in winemaking, hence sulphite resistance mechanisms have been studied extensively in *Saccharomyces cerevisiae*. Research turned out that yeasts possess plasma membrane protein encoded by the SSU1 gene (lies on chromosome XVI), which excretes sulphite from yeast cells. What more, some highly sulphite-resistance wine strains have an SSU1 allele (SSU1-R) on chromosome VIII. **AIM.** We tried to elucidate, how long adaptation to high sulphite concentrations affects to the evolution of yeast strains. **MATERIALS AND METHODS.** After 10 years of storage with

SO₂ (concentration ~ 400 mg/ml) the pool of 12 strains *S. cerevisiae*, which include 11 sulphite strains and 1 negative control strain, were tested for the presence of the SSU1 gene and its SSU1-R allele. We analysed them by using PCR assays, gel electrophoresis and DNA sequencing. Obtained sequences were aligned, in CLUSTAL W tool, with the known sequences from databases (BLAST and SGD). Gene expression was tested during alcoholic fermentation for few chosen strains. The four sample time points were collected for each strain and then analysed by real-time PCR. **RESULTS.** The presence of the SSU1 gene was confirmed in 6 strains and SSU1-R gene in 3 strains. None of those genes was detected in

2 strains. Surprisingly, the control strain also has SSU1 gene. **CONCLUSION.** The results indicate that possession of the SSU1 or its allele is independent of the long-termed storage in must with

SO₂. It is well-known that evolution in yeast cell occurs easily and dynamic. In our research it was confirmed by the presence of SSU1 in negative control strain.

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THE MICROFLORA OF SELECTED WATER RESERVOIRS IN ŚWIĘTOKRZYSKIE VOIVODSHIP

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BACKGROUND. Microbiological analysis of water is an essential part of environmental monitoring. Such studies allow to control the condition of homeostasis and biodiversity in nature. Periodical sanitary analysis is used to determine typical indicators of microbial contamination. Despite the increased use of metagenomic methods in identifying environmental bacteria species, traditional microbiological methods are still commonly used. **AIM.** of this study was to analyze water and bottom sediments of selected water reservoirs in Świętokrzyskie Province in winter 2013. **MATERIALS AND METHODS.** 16 water and 14 bottom sediment samples of five water reservoirs were diluted and plated on growth and selective medias (Yeast Extract, TSC, SS, Endo, Bile Esculin Azide). Analysis were carried out according to Polish Committee of Standardization Norms. Grown colonies were classified into various types and Gram stained to observe them under light microscope. **RESULTS AND**

CONCLUSIONS. Studies checked the presence of total coliforms, *Salmonella* spp., *Shigella* sp., *Enterococcus faecalis*, *Clostridium perfringens*. Total number of mesophilic and psychrophilic bacteria was the sanitary indicator. Bacterial strains isolated from cold (~4°C) water grew *in vitro*, suggesting potentially high adaptive properties. There were different numbers of colonies and colony types identified basing on cell morphology. Nevertheless, sanitary norms were exceeded in each reservoir. Further chemo-physical, geographical and microbiological correlations will allow wider determination of mutual dependencies of mentioned components in given environment. **ACKNOWLEDGEMENTS.** The study was supported by National Science Centre 2990/B/P01/2011/40, project leader: Lukasz Bak from Kielce University of Technology and project for young staff: 046/R/11 from Jan Kochanowski University, Kielce, Poland.

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GROWTH INHIBITION OF *EUROTIUM* SPP. BY THE USE OF ESSENTIAL OILS FROM SAVORY AND THYME

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Spoilage and poisoning of food by fungi are a major problem for the food industry and consumers. The aim of this work was to investigate the influence of essential oils from savory and thyme on mycelium growth *Eurotium* spp. Essential oils from the herbs of *Satureja hortensis* L. 'Saturn' and *Thymus vulgaris* L. 'Słoneczko' collected from Stargard Szczeciński (north-western Poland) were isolated by hydrodistillation and analysed by means of GC-MS. The main constituents of the savory oil were carvacrol (70.5%), γ -terpinene (15.1%) and p-cymene (5.5%). The most important components of thyme oil were thymol (61.5%), p-cymene (7.7%) and γ -terpinene (6.3%). The ability of the two oils to inhibit the growth of myce-

lium of *Eurotium* sp. (*E. amstelodami*, *E. chevalieri*, *E. herbariorum*, *E. repens*, *E. rubrum*) was tested *in vitro* (at 25°C) on agar medium with discs containing different concentration of the essential oils (1.25–10 μ l/disc). The size of inhibition zones of the tested fungi depended on kind and dose of oil, species of fungus and duration of action. Both oils at the lowest concentration (1.25 μ l/disc) showed inhibitory activity against all the tested strains of fungi. At dose 10 μ l/disc the inhibitory effect of tested essential oils maintained for 30 days. The results of this study suggest that essential oils of savory and thyme can control the growth of fungi species of *Eurotium* sp. and they can be considered as natural fungicides.

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SERS AS A MOLECULAR METHOD FOR THE IDENTIFICATION OF BACTERIAL METABOLITES AND FOR ANALYZE THE DIFFERENCES IN CHEMICAL COMPOSITION OF THE MYCOBACTERIAL CELL WALL AFTER EXPOSURE TO ANTIBACTERIAL SUBSTANCES

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INTRODUCTION. SERS (Surface Enhanced Raman Spectroscopy) is a technique for measuring the radiation intensity of Raman scattering of molecules adsorbed on the surface of a metal or coated by

metal (gold or silver) nanoparticles. SERS technique is characterized by a significant strengthening of the measurement signal in relation to the classical Raman Spectroscopy. SERS can be an useful tool to

conduct microbiological studies. **AIM.** The aim of the study was to show SERS as a molecular method for the identification of compounds from bacterial metabolites and for analyze the differences in chemical composition of the *Mycobacterium smegmatis* cell wall after exposure to antibiotic substances. **MATERIALS AND METHODS.** Measurements for identification of the substances were carried out by placing the sample on a Renishaw Diagnostics Klarite plate. The instrumentation used in this study was a Renishaw InVia Raman Microscope. Excitation was provided using a 785 nm semiconductor laser. For determination of chemical changes in composition of mycobacterial cell wall after incubation with antibiotic substance, *M. smegmatis* cells were silver-coated to enhance Raman signal. FTIR (Fourier Transform Infrared Spectroscopy) and colorimetric methods

was used to confirmed SERS results. **RESULTS.** The SERS analysis identified the compounds obtained from metabolites of bacteria *Raoultella ornithinolytica* as protein and carbohydrate. The Raman spectra before and after incubation of *M. smegmatis* with antibiotic substance show significant differences in the intensities of the individual characteristic peaks. These differences reflect the increase in the intensity of bands corresponding to the bands assigned to carbohydrates and a decrease in the intensity of the bands assigned to fats. The antibacterial substance has been identified as the complex containing peptide and carbohydrate component. **CONCLUSIONS.** SERS technique can be a great tool to complement standard microbiological methods because it provides direct information about the chemical nature of the obtained antibacterial substances.

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BIOCONVERSION OF CRUDE GLYCEROL TO FUMARIC ACID BY *RHIZOPUS ORYZAE* THROUGH SUPPLEMENTATION WITH SOYBEAN COMPONENTS

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INTRODUCTION. Glycerol is a good carbon source utilized by many microorganisms for value added chemical production. Fungi of the genus *Rhizopus* are important producers of key metabolites such as fumaric acid, lactic acid and ethanol as co-products. Crude glycerol is the waste byproduct of biodiesel production process and its conversion mainly to organic acids brings significant economic benefits. **AIM.** 1. Enhancement of crude glycerol consumption in fungal bioreactor cultures. 2. Increase the amount of fumaric acid biosynthesis by addition of soytone and soybean oil into fermentation medium based on crude glycerol. **MATERIALS AND METHODS.** Each of three selected isolates of *Rhizopus oryzae* were grown in shaking flask culture to choose optimal dose of soytone and soybean oil respectively, as the potential factors affecting fumaric acid production when cultivated under different fermentation medium conditions. Crude glycerol was added in the amount of

30 g/L. Second stage of the research was a culture of *R. oryzae* ATCC 20344 in a 2-L stirred bioreactor. Fermentation medium contained 30 g/L of crude glycerol and 2 g/L of soytone. 20 g/L of soybean oil was added after 48 h of cultivation. To obtain biomass in the form of pellets pH ratio was 3.0 for 48 h, then 4.5 for the next 96 h. **RESULTS.** The crude glycerol consumption rate was observed to reach 0.083 g/L/h in the stationary phase of fungus growth. Final amount of fumaric acid was 20 g/L. **CONCLUSIONS.** *R. oryzae* has a metabolic potential, which can be used to dispose of waste glycerol from biodiesel production while its bioconversion to fumaric acid.

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APPLICATION OF ACID-SENSITIVE BACTERIAL UREASE IN ALCOHOL PRODUCTION

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INTRODUCTION. Ethyl carbamate (urethane) is a carcinogenic compound with a short latency period. It is founded in many food products, mainly in fermented food and beverages. It arise in reaction of ethanol and certain carbamyl precursors like urea or citrulline. Distilled alcohols like sake, wine and beer contained the highest residual ethyl carbamate level. Urease hydrolyze urea to ammonia and carbon dioxide. However, application of urease in alcohol production process is difficult, because require an acid-resistance enzyme active in alcohol presence. Most of bacterial ureases is active in neutral conditions. **AIM.** The aim of this study was to find bacterial urease active in presence of alcohol and in different pH. **METHODS.** Over 100 bacterial strains isolated from different soil samples were analyzed. Ureolytic activity was determinate in spectrophotometer in Christensen liquid medium supplemented with 5, 10, 15 and

20% of alcohol in neutral or acidic conditions. Assay was also done for bacterial cells immobilized by alginate. Bacterial strains were identified by sequencing of 16S rDNA. **RESULTS.** From among over 100 bacterial strains isolated from different soil samples only two (belonging to *Staphylococcus* sp.) were able to hydrolyzing of urea in medium supplemented with even in 20% of alcohol in neutral as well as acidic conditions. However, after sonication of bacterial cells ureolytic activity was completely lose. Immobilization of whole microbial cells into alginate beads allowed for hydrolysis of urea in alcohol presence in acidic conditions. **CONCLUSIONS.** Presented results indicate that by alginate immobilization, acid-sensitive urease may be successfully used to urea removal during alcohol production. **ACKNOWLEDGEMENTS.** This work was supported by grant MNiSzW no. N N304 044639

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THE INFLUENCE OF MEDIA ON THE PROCESS OF 1,3-PROPANEDIOL PRODUCTION BY *CLOSTRIDIUM BUTYRICUM*

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INTRODUCTION. *Clostridium butyricum* is a species that has been known for decades to have the ability to produce 1,3-propanediol from glycerol. 1,3-PD has many applications in chemical synthesis. The fact that it can be produced biologically from crude glycerol can also improve the economical viability of biodiesel production. **AIM.** The aim of this work was to investigate the ability of different *Clostridium butyricum* strains to synthesize 1,3-propanediol from glycerol in a medium designed for minimal nutrient content. The capacity of the medium to sustain bacteria metabolism depending on the medium used for inoculation culture was verified. Furthermore, cultures showing significant glycerol utilisation were switched from batch to fed-batch operation to further test the medium capability for 1,3-PD production support. **MATERIALS AND METHODS.** Six isolates used were obtained previously from various environmental samples or were mutants of these. The car-

bon source in the culture medium was crude glycerol from biodiesel production containing 86% (w/w) of pure glycerol. Batch and fed-batch cultivations were carried out in 5-litres stirred-tank reactors Biostat B-Plus (Sartorius, Germany) with a working volume of 2 L. Two different inoculum media were used – Reinforced Clostridial Medium (RCM) and Rich Medium (RM) according to Himmi et al. 1999. Modified RM was used for the production tests. 1,3-propanediol concentration and glycerol consumption in culture samples was quantified by HPLC. **RESULTS AND CONCLUSIONS.** The designed minimal medium allowed the synthesis of 1,3-propanediol. However, in all cases better results were obtained in processes where RCM was used for inoculum preparation. The tested medium did not provide conditions for satisfactory 1,3-propanediol formation in fed-batch fermentation, thus it is not suitable for obtaining high concentrations of this metabolite.

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AN ATTEMPT TO USE WASTE GLYCEROL AND POTATO JUICE TO COMPOSE THE PRODUCTION MEDIUM FOR THE PRODUCTION OF 1,3-PROPANEDIOL BY *CLOSTRIDIUM BUTYRICUM*

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INTRODUCTION. One of the most important aspects of industrial biotechnology is optimization of composition of the production medium used in the production of metabolites, which are commercially attractive. The cost of the whole process, and thus the metabolite formed, depends on the composition of the medium and its price. Biotechnological synthesis from glycerol to 1,3-propanediol allows use of waste glycerol from the production of biodiesel and obtaining attractive substrate for chemical synthesis. **AIM.** The aim of this study was to attempt to fully or partially replace yeast extract with dried or frozen potato juice, waste product from the starch production process. **MATERIALS AND METHODS.** In this study a strain of bacteria of the genus *Clostridium butyricum* DSP1 was used, deposited in the Polish Collection of Microorganisms in Wrocław. Control medium contained: waste glycerol

(Vratislavia Bio), nitrogen source, mineral salts and yeast extract. Tested medium contained instead of yeast extract, freeze-dried or fresh juice of the potato. Analysis of the products of fermentation was carried out by high performance liquid chromatography (HPLC). Microbiological analysis was performed by Koch method. **RESULTS.** Experiment has shown that it is possible to replace an expensive ingredient of culture medium (yeast extract) from the potato juice. However, better results were obtained with potato juice lyophilisate, which certainly is associated with a higher content of nutrients ingredients. **CONCLUSIONS.** Potato juice, which is waste product of starch production, can be an attractive source of active ingredients that stimulate the growth and production capacity of bacteria of the genus *Clostridium*.

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EFFECT OF CATIONIC GEMINI SURFACTANTS (GS) ON DIFFERENT MORPHOLOGICAL FORMS OF *ASPERGILLUS* AND *PENICILLIUM* MOULDSAnna Koziróg^{1*}, Bogumił Brycki²¹Institute of Fermentation Technology and Microbiology, Lodz University of Technology, Poland²Laboratory of Microbiocides Chemistry, Adam Mickiewicz University in Poznań, Poland

INTRODUCTION. Comparing in earlier study the sensitivity of conidia and mycelium to hexamethylene-1,6-bis-(*N,N*-dimethyl-*N*-dodecylammonium bromide) (C6) and pentamethylene-1,5-bis(*N,N*-dimethyl-*N*-dodecylammonium bromide) (C5) were determine that this gemini surfactants GS inhibit the devel-

opment of mycelia from 3 to 12 times higher concentrations (0.31 µM/mL for *A. niger*, 0.31 µM/mL (C6) and 0.76 µM/mL (C5) – *P. chrysogenum*) than conidia (0.12 µM/mL for *A. niger*, 0.06 µM/mL – *P. chrysogenum*). **AIM.** Identify changes in the morphology of conidia and mycelia two moulds species after exposure

to GS in concentrations 1/2 MIC, MIC and 2 MIC. **MATERIALS AND METHODS.** Cultures of conidia and two- days mycelium with gemini surfactants were carried out in liquid medium MEB (Merck) for 48 h, 27°C. At this time, conidia were observed by light microscopy and mycelia in addition to scanning electron microscope. **RESULTS.** Development of conidia and mycelia treated of GS at concentrations 1/2 MIC was delayed for the control sample, but there were no visible changes in morphology. In culture, *A. niger* with the addition of test compounds at concentrations MIC and 2 MIC in microscopic image conidia were seen covered with a substance, having a different optical translucency. Conidia of *P. chrysogenum* after 24 h remained unchanged, but

without the possibility of development. For mycelium increased concentrations of to MIC and 2 MIC caused complete inhibition of their further development. Hyphae of both species of mold were covered with a substance also observed unsticking the cytosol of the cell wall. Images obtained in a scanning electron microscope confirm the observations made by light microscopy. In culture, with the addition of compound C6 after 48 h of its operation in a concentration MIC and 2 MIC difficult to discern the individual hyphae, they were completely destroyed. **CONCLUSIONS.** The conidia and mycelia are coated by tested GS at concentrations MIC, what probably causes lack of access inter alia to nutrients and progressive cell death.

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THE GENE CLONING, OVEREXPRESSION, PURIFICATION AND BIOCHEMICAL CHARACTERISATION OF A NEW COLD-ADAPTED β -GALACTOSIDASE FROM *ARTHROBACTER* SP. VII-4

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INTRODUCTION. β -Galactosidase [EC 3.2.1.23] is an enzyme that catalyzes the hydrolysis of O-glycosidic linkages in galactosides. It is commercially used in dairy industry for the production of milk with reduced lactose content. Potentially, the best method for lactose removal under cooling conditions should be carried out with a cold-adapted enzyme. **AIM.** The aim of this study was to determine the taxonomic affiliation of the isolate labeled as Spitzbergen VII-4, and obtain the sequence of the gene encoding cold-active β -galactosidase and the construction of expression system, biosynthesis and biochemical characterisation of the enzyme. **MATERIALS AND METHODS.** Research methods included the use of the PCR technique, cloning, and expression of the gene. The protein was purified to homogeneity by using ion exchange chromatography and gel filtration. Then an analysis of the enzyme was done. **RESULTS.** The isolate Spitzbergen VII-4 was classified as *Arthrobacter* sp. based on 16S rRNA gene sequence. Sequence of the gene encoding β -galactosidase was obtained by construction

of genomic DNA library, and its analysis showed that it consists of 2064 bp, and encodes an enzyme belonging to the glycoside hydrolase family 42. The biosynthesis of protein was carried out in *Escherichia coli*. After purification the enzyme with specific activity of 9.2 U/mg was obtained. The enzyme is a trimer with molecular mass 227 kDa. Optimal conditions for enzyme activity are 32°C and pH 7.5. Cations Mg^{2+} are activators of β -galactosidase whereas Ca^{2+} , Co^{2+} , Mn^{2+} and Ni^{2+} are inhibitors of the enzyme. Moreover, the thiol group-containing compounds such as dithiothreitol, tris(2-carboxyethyl)phosphine, reduced glutathione and cysteine decreased the activity of the glycoside hydrolase. The enzyme exhibits hydrolytic activity with β -D-galactosides, β -D-glucuronide, and β -D-fucoside. **CONCLUSIONS.** Biochemical properties of β -galactosidase *Arthrobacter* sp. VII-4 show that enzyme can be potentially used for lactose removal from dairy products, however, comprehensive research must be done.

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LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) TECHNIQUE FOR CHARACTERIZATION OF *PANTOEA ANANATIS*

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INTRODUCTION. *Pantoea ananatis* is a well known polyphagic plant pathogen recently described in Poland on maize plants. In favourable environmental condition it may cause up to 50% losses in maize (*Zea mays*) crops on particular fields. It is closely related to *P. agglomerans* and its identification is often difficult. **AIM.** We used LAMP technique to design fast and efficient method for characterization and identification of *P. ananatis* a causal agent of maize diseases. **MATERIALS AND METHODS.** A bioinformatic analysis of genomic sequences of *P. ananatis*, *P. agglomerans*, *P. vagans* and *Escherichia coli*, were performed to find a region of genome specific and characteristic for *P. ananatis*. Total genomic sequences obtained from GenBank database Genomic sequences were analyzed using freeware Mauve v2.3.1 program. A potentially

specific for *P. ananatis* region of genome was selected and five sets of LAMP primers were design on its basis and tested on genomic DNA of bacteria obtained using CTAB protocol. **RESULTS.** One of five tested primer sets, designed on the fragment of genome of *P. ananatis* of our choosing, enabled us creating an LAMP assay for fast and specific characterization and identification of *P. ananatis* from other bacterial plant pathogens including closely related *P. agglomerans*. **CONCLUSIONS.** This experiment is an approach that combines the LAMP technology with a bioinformatic data mining strategy and comparative genomics for characterization and identification of plant pathogenic bacterium. The LAMP assay is a rapid, specific and easy to perform protocol for microbial characterization and detection with a large potential for its application in agricultural industry.

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IMMOBILIZED SPRAY-DRIED *LACTOBACILLUS RHAMNOSUS* GG
AND *LACTOBACILLUS PLANTARUM* 299v
WITH OMEGA-3 FATTY ACIDS AS A POTENTIAL PROBIOTIC FOOD ADDITIVE

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INTRODUCTION. Probiotic microorganisms, when ingested in certain numbers, exert health benefits beyond inherent basic nutrition. Combined with polyunsaturated omega-3 fatty acids (EPA and DHA), essential for normal metabolism and not synthesized by the human body, in one easy-to-handle product, can form an innovative food additive/supplement. **AIM.** The aim of the study was to develop a potential health promoting food additive/supplement containing immobilized probiotic bacteria and unsaturated fatty acids with natural antioxidants obtained by spray-drying and examine its microbiological and chemical quality. **MATERIALS AND METHODS.** The developed formulation containing probiotic bacteria: *L. rhamnosus* GG and/or *L. plantarum* 299v together with omega-3 fatty acids and natural antioxidants (patent pending) was spray-dried using Anhydro MS-150 spray dryer and packaged at modified atmosphere. Microbiological analyses included enumeration of lactic acid bacteria, mesophilic and psychrotrophic aerobes, yeasts, moulds, sulphate-reducing anaerobes, Enterobacteriaceae, coliform bacteria, *E. coli*, *Salmonella* spp., *L. monocytogenes*, coagulase-positive staphylococci, *B. cereus*, *Enterococcus* spp., and amylolytic and lipolytic bacteria. Chemical analyses of PUFA stability included the determination of total fat and free fat contents,

peroxide, acid, anisidine and Totox values, and PUFA, EPA and DHA determination by gas chromatography. **RESULTS.** Both probiotic strains used: *L. rhamnosus* GG and *L. plantarum* 299v, showed high survival rates during spray drying process. The total number of live probiotic bacteria in obtained preparations after spray-drying reached 3.14×10^9 CFU/g. Microbiological quality of the product was very high with no pathogenic bacteria detectable. The contents of PUFA, EPA and DHA were 183.9–240.1, 64.7–81.6 and 69.2–91.4 mg/1 g of lipid, respectively, with fat content being 28.1–33.1 g/100 g. High stability of contained PUFA was proved by peroxide value of 3.9–5.1 meq O/fat kg and anisidine value of 7.2–8.9. Performed analyses showed the possibility to obtain highly stable immobilized probiotic bacteria with omega-3 fatty acids by spray-drying.

The research was performed within the project: “ProBioKap – Health promoting food additives containing immobilized unsaturated fatty acids and probiotic bacteria obtained by spray drying” co-funded by the European Union under Action 1.3. Sub-measure 1.3.1, co-funded by the European Regional Development Fund in the framework of Innovative Economy Operational Programme 2007-2013 (POIG.01.03.0-32-193/09-00).

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ASAIA BOGORENSIS, AN UNUSUAL SPOILAGE BACTERIA
OF FRUIT-FLAVORED BOTTLED WATER

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INTRODUCTION. The recent study in Poland has demonstrated a higher consumption of fruit-flavor bottled waters, particularly by children. However, the microbiological quality of these products seems to be not safe. **AIM.** Our aim was to identify the spoilage microflora in final products. **MATERIALS AND METHODS.** Bacteriological analysis of spoiled fruit-flavoured bottled mineral water was done. Quantitative examination of samples was conducted using pour plate method by inoculation of GC agar medium with D-glucose and CaCO₃. The genomic DNA was isolated using Genomic Mini Kit according to manufacturer's instruction. The 16S rRNA gene were amplified by polymerase chain reaction. The nucleotide sequence of the amplified 16S rRNA gene were obtained using the BigDye Terminator Ready Reaction Cycle Sequencing kit and the reaction products were analyzed by using an Applied Biosystems model 3730 Genetic Analyzer. The obtained nucleotide sequences were compared with 16S rRNA gene sequences of

Asaia sp. available in NCBI by using the program BLASTN 2.2.27. **RESULTS.** The incubations demonstrated the presence of mixed cultures consisting of a two main morphotypes. One of these morphotypes was dominant – the bacteria formed orange-pink, small colonies with clear zones. The obtained nucleotide sequence 1395 bp was compared with 16S rRNA gene sequences of genus *Asaia* available in NCBI. Direct sequencing of 16S rRNA gene identified these cells as *A. bogorensis* (99.6% homology with *Asaia bogorensis* NBRC 103528). **CONCLUSIONS.** Tropical-originated *Asaia* spp. become a frequent bacterial infection of soft drinks. This genera is very difficult to identify with the classical methods. These bacteria should be recognized using molecular techniques, such as 16S rRNA gene sequencing. Therefore, genetic methods for routinely identification of these unusual bacteria in commercial soft drinks should be considered.

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COLILERT-18® FOR DETECTION OF COLIFORMS AND *ESCHERICHIA COLI* IN DIFFERENT FRESHWATERS

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INTRODUCTION. In Poland, coliform bacteria are the recommended hygienic indicator organisms for all kinds of water. *Escherichia coli* could serve as a more specific indicator for fecal contamination. However, many bacteria in the genera of *Klebsiella*, *Enterobacter*, *Citrobacter*, and *Serratia* meet the definitional criteria for coliforms even though they are not of fecal origin. Polish standards have approved the use of two methods for detection and enumeration of *E. coli* in water samples: a membrane filtration (MF) and a most-probable-number (MPN) method. The Colilert® system (IDEXX) is a new method for detection of *E. coli* and total coliforms in waters. Substrates ONPG and MUG included in the media, are metabolized by enzymes of coliforms (β -galactosidase) and/or *E. coli* (β -glucuronidase) present in the sample, resulting in the release of a yellow and/or fluorescent products. The high specificity of the enzymes used in this system eliminated the need for confirmatory testing. **AIM.** Our aim was to use the Colilert-18/

Quanti-Tray 2000 system, being commercial application of defined substrate technology, which ensures simultaneous detection of total coliforms and *E. coli* directly in different water samples. **MATERIALS AND METHODS.** The water samples were collected from different sites between March and June 2012. Detection of coliforms and *E. coli* by using the Colilert-18® method was done according to the manufacturer's instructions. The results were compared with those obtained using standard MF method and agar medium with lactose, TTC and Tergitol7 (Merck Millipore). **RESULTS.** The "background" of total microflora significantly affected the interpretation of results on the reference agar medium. High specificity of enzymes in Colilert system caused that the results did not interfere with the accompanying microflora. **CONCLUSIONS.** Test Colilert® system is simple and useful tool in the sanitary analysis of water, providing to obtain, in a short time, with high sensitivity and precision, easy-to-interpret results.

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SELECTION AND CHARACTERIZATION OF ENDOPHYTIC BACTERIA FOR IMPROVING PHYTOREMEDIATION OF PETROLEUM HYDROCARBONS

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INTRODUCTION. Endophytic bacteria live inside plant tissues without causing any apparent symptoms of disease. Recent attention has been paid on the partnership between plants and plant growth-promoting endophytes (PGPEs), which can enhance phytoremediation of soils contaminated by petroleum hydrocarbons. The latest researches suggest that beneficial effect of endophytes relies on degradation of xenobiotic compounds and plant growth-promoting activities. **AIM.** The purpose of this research was the selection and characterization of endophytic bacteria from the different plants which grew on soil contaminated by petroleum hydrocarbons. Endophytic bacteria were evaluated for their ability to degrade crude oil and plant growth-promoting activities. **MATERIALS AND METHODS.** Endophytic bacteria were isolated from surface-sterilized plant tissues growing on hydrocarbon-contaminated soil around the refinery in Czechowice-Dziedzice and near the smelter in Zabrze. The production of indole acetic acid, hydrogen cyanide,

cellulase and ability to phosphate solubilization were screened in specific media. The genes encoding enzymes for utilize of hydrocarbons such as cytochrome P450-like enzyme, alkane monooxygenase and dioxygenase, were detected by PCR reaction. The emulsifying index were used to quantify biosurfactants production by isolates. **RESULTS.** Isolated strains possess a number of properties that are thought to be responsible for plant growth-promoting activities such as production of IAA, HCN, siderophores, solubilize of phosphate. The genes encoding enzymes involved in hydrocarbon degradation were detected in strains of the genus *Rhodococcus* and *Pseudomonas*. In addition, the ability to motility, cellulase and biosurfactants production were showed. **CONCLUSIONS.** The isolated endophytic bacteria showed a potential for use in phytoremediation. The real role of the endophytic community is not yet understood and its diversity is poorly characterized. Thus, understanding the plant-endophyte interactions is a key to enhance phytoremediation.

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BIOSYNTHESIS OF 2,3-BUTANEDIOL BY *RAOULTELLA PLANTICOLA* CECT 843

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INTRODUCTION. 2,3-Butanediol (2,3-BD) is a chiral bivalent alcohol which exhibits a wide range of potential utilizations as a solvent, liquid fuel or precursor for 1,3-butadiene – an intermediate for synthetic rubber production. Several microorganisms are able to synthesis 2,3-BD. So far the highest yields were reached using risk

class 2 strains e.g. *Klebsiella* species, however an industrial process with risk class 1 microorganisms are cheaper and less recalcitrant. **AIM.** The main study objective was to statistically optimise the biosynthesis process of 2,3-Butanediol by *Raoultella planticola* CECT 843 strain. **MATERIALS AND METHODS.** Response surface

methodology (RSM) was used in this work to optimise the biosynthesis of 2,3-butanediol by *Raoultella planticola* CECT 843 strain. The optimisation experiment was planned according to D-optimal design and consisted of 25 runs. The effect of yeast extract (YE) and concentration of CH_3COO^- , Fe^{2+} and Mg^{2+} ions was evaluated on the final level of 2,3-butanediol. Sugars and other carbon compounds concentration was determined preferably by HPLC or GC. **RESULTS.** Obtained results showed statistically significant influence ($p < 0.05$) of the yeast extract, CH_3COO^- and Mg^{2+} ions concentration on the studied process. On the basis of statistic analysis, optimal values of analysed variables were determined as follows

($\text{g} \cdot \text{dm}^{-3}$): yeast extract – 4; $\text{CH}_3\text{COONH}_4$ – 4; $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ – 0.1; $\text{MgSO}_4 \cdot \text{H}_2\text{O}$ – 0.3; that corresponded to $13.6 \text{ g} \cdot \text{dm}^{-3}$ final 2,3-Butanediol concentration. **CONCLUSIONS.** Under optimised conditions *Raoultella planticola* CECT 843 can effectively convert glucose to 2,3-BD. Production of diol achieved nearly $14 \text{ g} \cdot \text{dm}^{-3}$ and efficiency of production reached $0.44 \text{ g} \cdot \text{g}^{-1}$ glucose in 24 h of cultivation. It shows that risk class 1 bacteria strains are promising direction in research of microbial production of 2,3-Butanediol. This work was supported by ERA-IB EU-project called "Production and Upgrading of 2,3-Butanediol from Biomass" (ERA-NET-IB/03/2009)

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INFLUENCE OF ADHESION SURFACE OF POROUS CARRIERS ON THE BIOCONVERSION OF GLYCEROL TO 1,3-PROPANEDIOL BY *CLOSTRIDIUM BUTYRICUM*

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INTRODUCTION. Using crude glycerol from a biodiesel production process for producing 1,3-PD is a good solution from the economical as well as ecological point of view. Biotechnological production of 1,3-PD from waste biomass is a promising and attractive alternative to the traditional chemical synthesis. The productivity of 1,3-PD can be improved through the application of fermentation with cell immobilization on porous carriers. **AIM.** The aim of this study was to investigate the influence of physical properties of five porous carriers on the bioconversion yield. **MATERIALS AND METHODS.** Adsorption parameters for porous carriers were determined on the basis of experimental low-temperature nitrogen adsorption isotherms. The nitrogen isotherms were measured at -196°C on ASAP 2020 volumetric analyzer. The BET (Brunauer-Emmett-Teller) specific surface area S_{BET} was calculated from adsorption isotherms in the range of relative pressures from 0.05 to 0.3. Obtained results for surface areas were calculated per volume

(m^2/dm^3) units for each sample. *Clostridium butyricum* PK was used in this study, derived from the collection of Department of Biotechnology and Microbiology, Poznań University of Life Sciences. Fermentations were carried out at 37°C under anaerobic conditions in the vessels with a medium volume of 20 ml with an initial level of glycerol 50 g/l. **RESULTS.** S_{BET} ranged from 168,03 to 2931,55 [m^2/dm^3]. For carrier with the highest S_{BET} the bioconversion yield was the highest and reached 0,441 [mol 1,3-PD/mol glycerol]. **CONCLUSIONS.** The higher total area resulted in the higher yield of bioconversion. The area of porous carriers available for the adhesion of microorganisms, affect the amount of immobilized biomass, which in turn affects the efficiency of the bioconversion process. This work was funded within the framework of project no. 01.01.02-00-074/09 co-funded by The EU from the European Regional Development Fund within the framework of the Innovative Economy Operational Programme 2007–2013.

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EFFECT OF SORBIC ACID ON THE LUMINAL LARGE INTESTINE MICROFLORA METABOLITES OF RATS

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INTRODUCTION. Sorbic acid is a preservative used in the food products and pharmaceutical preparations for prevention from microbial spoilage. The preliminary studies have proven the possibility and advantages of application of chromatography-mass spectrometry for microbial markers determination for the purpose of animal microflora monitoring. **OBJECTIVE.** To study the effect of sorbic acid on the rats' large intestine fatty acid composition. **MATERIALS AND METHODS.** The animal subjects were orally administered hydroglyceric solution of the studied preservative with the feeding tube within 14 days in the amount of 25 mg per 1 kg of body mass. For the experiment the large intestine contents samples were taken, subjected to the acid methanolysis and N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) treatment with the result that lipidic elements escaped in the form of methyl esters and trimethylsilyl derivatives that were twice extracted with hex-

ane. There were concentrated and dilute solutions analyzed – 1 ml was brought in the Agilent Technologies chromatograph injector with 5973N mass selective detector (USA). **RESULTS.** It has been found that the large intestine includes over 80 lipidic components, among which the fatty acids of microbial origin are the main part. On the 7th and 14th day the fatty acids – marker for the separate microorganism groups (among which there are *Selenomonas*, *Bacteroides*, *Prevotella*, *Eubacterium*, *Clostridium*, *Staphylococcus*, *Candida* etc.) content is changed. On the 21st day (the 7th day after the preservative injection is finished) the content of most fatty acids is normalized. **CONCLUSIONS.** Administration of antimicrobial preservative to the animal subjects causes irregularity in the fatty acid composition of the large intestine content that, in its turn, proves the change in the proportion of the separate microorganism groups.

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THE EFFECT OF ESSENTIAL OILS ON MORPHOLOGY AND METABOLISM OF *CANDIDA* YEAST

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INTRODUCTION. Members of *Candida* species cause significant problems for many industrial branches. In order to prevent from *Candida* sp. development, essential oils are more and more frequently applied in food and cosmetic industry as well as in denture materials. Essential oils are natural, non-toxic, non-pollutive and biodegradable compounds with a broad spectrum of antimicrobial activity. **AIM.** The aim of the research was to determine changes in morphology and metabolic properties of *Candida* sp. in the presence of essential oils. **MATERIALS AND METHODS.** The research was carried out for *Candida albicans* ATCC 10231 and the influence of thyme, peppermint, clove and tea tree oils was examined. Yeasts were cultured on YPG agar medium (BTL) supplemented with essential oils in a concentration of 0.0075 – 2% v/v. From the morphologically different colonies isolates were obtained and their biochemical and enzymatic profiles were analyzed by the mean of API 20C AUX and API-ZYM tests (bioMerieux). In microscopic preparations morphological indexes of isolates cells were also deter-

mined. **RESULTS.** Changes of enzymatic activity of isolates were observed in the presence of all the tested essential oils and they were associated with loss of activity of esterase, lipase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -glucosidase. Furthermore, for some isolates additional activity of N-acetyl- β -glucosaminidase, alkaline phosphatase, cystine arylamidase and α -fucosidase was detected. Some isolates, after treatment with tea tree and clove oils, lost their ability to assimilate xylitol, sorbitol and trehalose. However, there were no significant differences in morphology of isolates compared to the control strain, and the morphological indexes ranged from 1.0 ± 0.2 to 1.2 ± 0.1 for spherical cells, 1.5 ± 0.4 – 1.8 ± 0.2 for ovoid cells and even 3.5 ± 0.7 for pseudohyphae. **CONCLUSIONS.** All the examined essential oils caused changes in metabolic properties of *Candida albicans*. However, the extent of differences depends on the type and concentration of essential oil.

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METHODS COMPARISON FOR ASSESSMENT OF FIVE ESSENTIAL OILS ACTIVITY AGAINST *CANDIDA ALBICANS*

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INTRODUCTION. *Candida albicans* is a yeast species recognized as the most frequent etiological agent of systemic and invasive candidiasis in humans. Invasions can affect all tissues, organs and systems of human in various stages of development. In addition, the biofilm formed by the yeast is a serious problem for many industries, and its activity may be the cause of losses in food, feed, cosmetic, paper, leather and textile processing. Study of the activity of essential oils that may be effective in preventing the spread of *Candida albicans* and search for the most accurate and sensitive methods to assess yeast sensitivity are necessary. **AIM.** The aim of this study was to estimate a reliability of two methods of the essential oils activity assessment against *Candida albicans* ATCC 10231. **MATERIALS AND METHODS.** The following commercial essential oils were used: cinnamon, tea tree, clove, peppermint and thyme (Pollena Aroma SA, Warsaw). The minimum inhibitory concentration (MIC)

of the essential oils was determined by the serial dilution and microdilution method with resazurin. However, the minimum fungicidal concentration (MFC) of the essential oils was determined by the first one. **RESULTS.** The biological activity of essential oils differed depending on the method used. The MIC values estimated by the serial dilution method ranged from 0.25% to 2% v/v, while using resazurin microplates method MIC varied from 0.5% to 4% v/v. MFC values were from 0.5% to 4% v/v. **CONCLUSIONS.** All tested essential oils showed activity against *Candida albicans* regardless of the method used. The microdilution method with resazurin has a number of advantages as a MIC end-point indicator. A comparison showed that all MICs were higher by resazurin microplates method. The majority of microorganisms reduce resazurin and give clearly defined end-points. However, the assay was not suitable for *Candida albicans* perhaps because of slow reduction kinetics.

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SEASONAL MICROBIAL RIBOTYPE SHIFTS IN THE ACTIVATED SLUDGE BIOCENOSES FROM SILESIA WASTEWATER TREATMENT PLANTS

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INTRUDUCTION. Efficiency of sewage treatment in wastewater treatment plants mainly depends on composition and activity of activated sludge. Significant microbial richness enables multidirectional chemicals conversions but, when its disorders are observed, unfavourable processes may also occur, e.g. sludge bulking. Amplified rDNA Restriction Analysis (ARDRA) is not used for identification

of precise species in sludge, but allows to observe genetic changes of the community over time or subjected to different environmental conditions as well as compare sludges derived from several WWTPs. **AIM.** Observation of genetic changes in activated sludge communities during seasons or consequent on changeable environmental conditions. Detection of genetic markers enabling early detection

of sludge bulking. **MATERIALS AND METHODS.** Genomic DNA from 72 samples of activated sludge assembled from 5 municipal WWTPs located in Silesia served as templates in amplification of 16S rRNA gene fragment. Amplimers underwent hydrolysis by seven endonucleases, separately (*AluI*, *BsuRI*, *DdeI*, *HhaI*, *HinfI*, *MboI*, *MspI*), polyacrylamide gel electrophoresis of digestion products and following electrophoregram analysis by the use of GeneTools image analysis software. For each collected sample, initial comprehensive physicochemical analysis has been conducted as well. **RESULTS.** From among seven endonucleases, *AluI* and *MspI* are the most suitable for observation of genetic changes of activated sludge biocenosis.

Each WWTP characterize its appropriate products profile, but there are many bands common with all WWTPs. DNA fragments characteristic only for bulking sludge were also noticed, what gives possibility of bulking symptoms molecular detection. **CONCLUSIONS.** Variations in the number and length of restriction products point at the permanent transformation of sludge community. The rearrangement is most intensive at the turn of winter and spring, what is expressed by different fingerprints after ARDRA. However, not all genetic changes of biocenoses relate to bulking process. This project was financially supported by research grant no KNW-1-110/P/2/0, subsidized by Medical University of Silesia.

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TRACE AMOUNT OF BACTERIAL DNA AS A SOURCE OF FALSE POSITIVE RESULTS OF RECOMBINANT DNA POLYMERASE APPLICATION

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INTRODUCTION. PCR technique, mainly due to its high sensitivity, is widely used in most microbiological laboratories. Therefore it is also prone to produce false-positive signals. Obeying strict protocols and safeguards may be insufficient solutions in case of using recombinant DNA polymerase. In spite of purification methods, during bioproduction recombinant DNA polymerases proteins could contain bacterial DNA which affects reaction results, especially when universal bacterial DNA targeting primers are used. **AIM.** Comparison the accuracy, efficiency and presence of false-positives results in PCR reaction while using native and recombinant DNA polymerases. Attempt to make use of *AluI* restriction endonuclease in order to PCR contamination elimination. **MATERIALS AND METHODS.** Amplification of highly conservative bacterial 16S rRNA genes region by using universal primers (SDBact0509S17, SDBact0784A22), several DNA polymerases: *Tfl* or native polymerase with appropriate buffers was carried out. As a template DNA isolated from activated sludge microbial community was used. Moreover additional reaction – incubation

PCR mixture with *AluI* (before primers and template had been added) was performed. **RESULTS.** To exclude false-positive results as a consequence of recombinant DNA enzyme applying native polymerase was used. In spite of applied pre- and post-amplification preventing methods contamination was detected using recombinant and native polymerase. Amplification efficiency of native polymerase was much lower than other polymerases. Application of *AluI* on PCR mixture bring on false-positives results elimination without negative effect on reaction efficiency. **CONCLUSIONS.** Contaminant DNA endonuclease digestion may be use as a preventing, pre-amplification method releasing recombinant DNA polymerases from trace amounts of bacterial DNA. Native DNA polymerase application may not be enough to eliminate false-positive results. To precise evaluation of the amplification efficiency and level of contamination reduced Real Time PCR should be carried out.

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SYNTHESIS OF BIOPOLYMERS BY *PSEUDOMONAS* SP. G101 USING SAPONIFIED WASTE PALM OIL

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INTRODUCTION. Polyhydroxyalkanoates (PHAs) are a class of biological polyesters produced intracellularly by many bacteria as discrete granules and used as a storage material for carbon and reducing equivalents. They have interesting characteristics similar to those of synthetic plastics, and therefore can be used instead of the conventional petroleum-based plastics. Moreover, these polymers are biodegradable and biocompatible, hence can be used for a variety of applications including packing, chemical production and in medical areas such as tissue engineering and drug delivery. **AIM.** The aim of the present study was to investigate the ability of *Pseudomonas* G101 strain to produce mcl-polyhydroxyalkanoates during growth on saponified waste palm oil (SWPO). **MATERIALS AND METHODS.** Two bioreactor experiments were conducted to determine the effect of a different feeding strategy on cell growth and PHAs content. Mcl-PHAs were isolated and purified to determine the composition by gas chromatography of the 3-hydroxyalkanoates methyl esters and evaluate their physical properties.

Reverse transcription real-time PCR approach was applied to analyze biosynthesis process at molecular level. **RESULTS.** *Pseudomonas* sp. G101 was capable of utilizing saponified waste palm oil towards biopolymer accumulation. The obtained results reflect the influence of the feeding strategy on the mcl-PHAs content. By employing pulse-feeding of SWPO, mcl-PHAs were synthesized at a much higher productivity (57.8 mg/l·h) and a higher final mcl-PHAs amount was extracted (42.69% of CDW) than was reported in continuous feeding fermentation. The analyzed strain showed a tendency to accumulate large amounts of 3-hydroxyoctanoic and 3-hydroxydecanoic acid. The transcriptional analysis of key genes revealed that the *pha* depolymerase gene (*phaZ*) was transcribed simultaneously to the *pha* synthesis genes (*phaC1* and *phaC2*). **CONCLUSIONS.** Our results clearly demonstrate that *Pseudomonas* sp. G101 is a suitable organism for additional mcl-PHAs research because of robust growth on waste plant oil and the accumulation of high levels of biopolymer.

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THE GENOME PROFILE OF UNIQUE ENTOMOCIDAL STRAIN *BACILLUS THURINGIENSIS* IS5056 HARBORING PLASMID WITH A NEW *cry1Ab21* GENE

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INTRODUCTION. The alternative to chemical pest reducing tools providing to environment degradation can be using the bioinsecticides based on *Bacillus thuringiensis* toxins. The δ -endotoxin, also called Cry protein, acts through the larvae alimentary tract only one to a few species of insects. The specificity of the Cry toxin in relation to the larvae causes that they are harmless for humans and animals and do not affect natural ecosystems. The *B. thuringiensis* IS5056 strain, producing newly described Cry1Ab21, shows high activity against Lepidoptera larvae. **AIMS.** In this research plasmid and genome profile of *B. thuringiensis* IS5056 in comparison to reference strains were performed. **MATERIALS AND METHODS.** The genome profiles were obtained by pulsed field gel

electrophoresis (PFGE) of DNA digested with restriction enzymes NotI and SmaI. To receive plasmidial profile, plasmids were extracted with HiSpeed isolation kit Qiagen and separated electrophoretically. Southern blot analysis for detection of *cry1* and *cry2* genes of plasmid profiles were performed. **RESULTS.** The plasmid profiling of IS5056 allowed to determine the number and size of plasmids of this strain, three of which carry the *cry* genes. Plasmid and genome profile of the tested strain are unique regarding more than 30 reference strains of *B. thuringiensis*. **CONCLUSION.** *B. thuringiensis* IS5056 is a newly described isolate, which can be the basis for the bioinsecticide production.

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THE MODULAR STRUCTURE OF PLASMID pIS56-63 CARRYING A NEW *cry1Ab21* GENE IN *BACILLUS* SUBSP. *THURINGIENSIS* IS5056 HIGHLY TOXIC TO *TRICHOPLUSIA NI* LARVAEEmilia Murawska^{1*}, Krzysztof Fiedoruk², Izabela Świącicka¹¹Department of Microbiology, University in Białystok, Poland²Department of Microbiology, Medical University in Białystok, Poland

INTRODUCTION. *Bacillus thuringiensis* indicate high entomocidal potential due to the δ -endotoxins production. These proteins, known also as crystal proteins (Cry), usually are coded on megaplasmids and their expression is always simultaneous to sporulation process. Polish strain *Bacillus thuringiensis* subsp. *thuringiensis* IS5056, revealing strong toxicity to *Trichoplusia ni* larvae, was recognized to harbor three megaplasmids enclosing *cry* genes and, what is more interesting, the medium size plasmid pIS56-63 with new *cry1Ab21*. **AIMS.** The sequence analyses of this plasmid gave the chance for better understanding of functional and evolutionary role of this mobile molecule. **MATERIALS AND METHODS.** The genome of *Bacillus thuringiensis* IS5056 was obtained using high-throughput DNA sequencing methods (454 and Illumina). The potential coding elements were annotated and verified manually

by comparison with the public nucleotide, protein and conserved domain databases. Moreover, the sequence comparisons pIS56-63 to plasmids with the highest coverage and similar structure were conducted. **RESULTS.** The pIS56-63 revealed presence of four modules: replication, regulation, conjugation as so as mobile cassette including *cry1Ab21*. Almost all mobile sequences (transposon, insertion sequences, phage element) were present within the cassette in toxin gene neighborhood. The homologue genes in the same (or other) order were found in the database sequences. **CONCLUSION.** The theta type replication plasmid is potentially able to conjugation, but the horizontal transfer probability of *cry1Ab21* increases because of mobile sequences surrounding. The presence of δ -endotoxin gene beyond pathogenicity island and on conjugative plasmid can be the key element in differentiation and dispersion of this protein family.

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INVESTIGATION OF THE ROLE OF CpkN PROTEIN IN THE REGULATION OF EXPRESSION OF CPK POLYKETIDE SYNTHASE GENE CLUSTER OF *STREPTOMYCES COELICOLOR* A3(2)Małgorzata Nowaczyk¹, Krzysztof Pawlik^{1,2}, Magdalena Kotowska¹¹Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, R. Weigla 12, 53-114 Wrocław, Poland²Department of Toxicology, Wrocław Medical University, Borowska 211, 50-556 Wrocław, Poland

One of the widely used bacteria are *Streptomyces*. What makes them so special and useful? These bacteria synthesize extracellular enzymes of industrial importance and a lot of biologically active substances – antibiotics, fungicides, cytostatics. To reap the benefits from these organisms it is needed to investigate the regulatory network of their metabolism. One of the newest examined 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, which expression is tightly controlled. CpkN is a regulatory

protein homologous to SARPs (*Streptomyces Antibiotic Regulator Proteins*). 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 study involved construction of *S. coelicolor* mutant strains with over-expression and deletion of *cpkN* gene. Expression profiles of selected structural and regulatory genes from *cpk* cluster were created using qRT-PCR. The phenotype of strains with changed level of CpkN protein was strongly medium dependent. No differences in coelimycin production were observed on rich media, as opposed to minimal medium.

The compound synthesis was inhibited in *cpkN* deletion mutant and slightly increased by constitutive overproduction of the SARP protein. Results of the qRT-PCR experiment suggest indirect effects and involvement of additional factors in the regulation. Deletion of *cpkN* decreased *cpkC* (one of the main subunits of polyketide synthase) expression. Surprisingly, overproduction of CpkN did

not stimulate transcription of the structural gene. CpkN protein plays an important role in control of coelimycin production on minimal medium, but it is not necessary on rich media. Therefore we conclude that it is a non-essential activator of the polyketide synthase Cpk. We also found that its transcription is subject to feedback control.

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PROBIOTIC LACTOBACILLI REDUCE ACTIVITY OF B-GLUCURONIDASE AND B-GLUCOSIDASE IN HUMAN FAECAL WATER IN THE PRESENCE OF 2-AMINO-3-METHYL-3H-IMIDAZO[4,5-*f*]QUINOLINE (IQ)

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INTRODUCTION. High activity of bacterial β -glucuronidase and β -glucosidase in human colon are recognised as biomarkers of harmful action of the intestinal microbiota. **AIM.** The aim was to assess the activity of β -glucuronidase and β -glucosidase after incubation of intestinal microbiota with carcinogenic heterocyclic aromatic amine IQ and probiotic *Lactobacillus* strains: *Lb. casei* ŁOCK 0900, *Lb. casei* ŁOCK 0900 and *Lb. paracasei* ŁOCK 0919). **METHODS.** The stool samples derived from 15 healthy individuals (up to 18 months of life; 30–40 and 75–85 years old), on basic diet. The faeces were incubated for 72 h at 37°C in anaerobic conditions with IQ (50 μ g/ml) and appropriate probiotic strain (10^{10} CFU/ml). The enzyme activity was measured by spectrophotometric method. **RESULTS.** Incubation of faeces with IQ increased activity of both

enzymes in all individuals and it was the highest in the group of elderly. Also in the group the activity was decreased (to the control level) the most effectively after addition of probiotic strains, and the reduction was: by 76.0% (*Lb. paracasei* 0908) in FW of children, 23.4% (*Lb. casei* 0908) in adults and 82.0% (*Lb. paracasei* 0919) in elderly (β -glucuronidase); and by 55.0% in children (*Lb. casei* 0908), 90.0% (*Lb. paracasei* 0919) in elderly, with little reduction in adults (β -glucosidase). **CONCLUSIONS.** Carcinogenic potency of faecal microbiota (activity of faecal enzymes) is observed mostly in old persons. Probiotic lactobacilli effectively decrease activity of faecal enzymes after incubation with IQ. The decrease depends on the probiotic strain, the person's age and its individual faecal microbiota.

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PHOSPHOLIPIDS MODIFICATION IN HEAVY METAL TOLERANT FUNGUS *PAECILOMYCES MARQUANDII* UNDER COPPER AND NICKEL EXPOSURE

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INTRODUCTION. Heavy metals toxicity to living cells can be manifested by an increase in membrane permeability leading to a disruption of membrane function. Cellular phospholipids play a crucial role in the membrane fluidity and permeability regulation. **AIM.** In this work we studied the effect of copper and nickel on phospholipids of *P. marquandii* strain, isolated from high metal polluted environment (Silesia, Poland). **MATERIALS AND METHODS.** Liquid cultures of *P. marquandii* with the addition of nickel and copper were incubated on a rotary shaker at 28°C for 5 days. The washed fresh mycelia were homogenized and extracted with an organic solvent mixture of chloroform and methanol (2:1, v/v). Next, all samples were prepared for analysis of phospholipids by HPLC MS/MS. **RESULTS.** A total of 28 lipid species were identified in *P. marquandii* mycelium. It has been shown that tested heavy

metals cause modification in the profile of phospholipids, involving changes in the percentage of main classes (phosphatidylcholine and phosphatidylethanolamine). Furthermore, nickel contributes to a significant increase in the fatty acid saturation. **CONCLUSIONS.** The changes in the profile of phospholipids observed under the metals stress, may be associated with the tested strain adaptation to grow in the environment contaminated with heavy metals and also affect the uptake of metal ions into the mycelium. On the other hand, a rise in the fatty acids saturation of nickel exposed mycelium is probably caused by nickel oxidative damage of double bonds in fatty acids.

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APPLICATION OF EPIFLUORESCENCE MICROSCOPY FOR VIABILITY TESTING OF *LACTOBACILLUS* SPP. IN CRANBERRY JUICE SAMPLES

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INTRODUCTION. The viability of *Lactobacillus* spp. is crucial for their applications in food industry. Great interest in functional products containing *Lactobacillus* spp. is expressed worldwide and is con-

nected with a demand for developing new lactobacilli-based products, such as vegetable and fruit juices. However, these approaches establish that the *Lactobacillus* spp. are subjected to various stress

conditions that may affect the physiological state of the microbes. For this reason, the selection of suitable viability tool is important. **AIM.** The objective of this study was the qualification of the viability of chosen *Lactobacillus* strains in controlled cranberry juice models. The aim of this research was to investigate the usefulness of epifluorescence microscopy (EFM) for viability assessment of *Lactobacillus* spp. **MATERIALS AND METHODS.** The esterase substrate carboxyfluorescein diacetate (CFDA) and the DNA binding probe propidium iodide (PI) were tested for live/dead discrimination using fifteen *Lactobacillus* strains. EFM-CFDA results were compared with plate count estimations. The viability of each strain was studied during storage (30°C for 7 days) of cranberry juice samples (99.8% and 5.9% cranberry content). **RESULTS.** In accordance to viability assessment, *Lactobacillus* strains survived well during tested

period in cranberry juice samples, but better survival of lactobacilli was established in juice with 5.9% of cranberries. The EFM-CFDA and plate counts in investigated models were significantly different, as a comparison in both cranberry juices revealed. Significant differences in lactobacilli counts were also obtained during the 7-day storage period. Viability assessment showed coexistence of three subpopulations representing active/culturable (CFDA⁺/CFU⁺), permeabilized/dead (PI⁺/CFU⁻) and dormant cells (CFDA⁺/CFU⁻). **CONCLUSIONS.** CFDA/PI and plate counts gave reliable results for subpopulations discrimination, showing VBNC (viable but non-culturable) cells remarkable visible as a consequence of incubation in cranberry juice. EFM is a useful assay in targeting different cellular functions and provides accurate tool to assess the bacterial functionality in versatile products.

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CHARACTERIZATION OF FACTOR(S) RESPONSIBLE FOR ANTAGONISM OF PSEUDOMONAS SP. P482 AGAINST PATHOGENS OF POTATO

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INTRODUCTION. *Pseudomonas* genus is intensively studied in the field of biological control due to a wide range of produced secondary metabolites. Rhizosphere isolate *Pseudomonas* sp. P482 secretes unidentified antimicrobial compound(s) acting against selected bacterial (*Dickey* spp., *Pectobacterium* spp.) and fungal (*Rhizoctonia solani*, *Fusarium culmorum*) plant pathogens. **AIM.** Analysis of the compound(s) responsible for antibacterial and antifungal activity of *Pseudomonas* sp. P482 by isolating them and identifying genetic background for their production. **METHODS.** Production of antimicrobial compounds by P482 was tested in different liquid media. The active compounds were extracted from the filtered bacterial culture supernatant with organic solvent. Obtained extracts were used for pathogens growth inhibition tests. Further, the extracts were separated with thin layer chromatography (TLC) to establish method for better purification. The presence of genes involved in production of antimicrobial compounds previously

described for *Pseudomonas* spp. was analyzed by PCR. Random mini-Tn5 transposon mutagenesis was conducted on P482, and transposon mutants were screened for loss of antimicrobial activity. **RESULTS.** The highest relative antimicrobial activity was obtained for ethyl acetate extract from filtered supernatant of P482 cultured in 10% TSB medium. This extract was separated using reverse phase TLC and retention factor (Rf) of antimicrobial compound(s) was established by overlaying the TLC plate with agar-solidified culture of the pathogen. PCR analysis showed no presence of genes involved in the synthesis of common *Pseudomonas* spp. antimicrobials. Transposon mutant lacking antimicrobial activity is under investigation. **CONCLUSION.** The method for extraction of active compound from culture supernatant of P482 was established. Currently efforts are undertaken to purify compound(s) using HPLC technique and to generate transposon mutant defected in antimicrobial activity.

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β-GALACTOSIDASE ARTHROBACTER SP. 32 cB – OBTAINING THE GENE SEQUENCE, CONSTRUCTION OF THE EXPRESSION SYSTEM, BIOSYNTHESIS AND BIOCHEMICAL CHARACTERIZATION OF THE ENZYME

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INTRODUCTION. β-Galactosidase is an enzyme which catalyzes the hydrolysis of O-glycosidic bond in β-galactosides. Another activity of β-galactosidase is a transglycosylation activity. The main industrial use of this protein is the hydrolysis of lactose in milk in a cooling conditions. Synthesis of galactooligosaccharides, which are mostly used as a prebiotics added to some foods or available as dietary supplements, is only one of many applications of transglycosylation activity of β-galactosidases. Glycosidase-catalysed transglycosylation is a promising alternative to classical chemical glycosylation methods. **AIM.** The aim of the work was to obtain the sequence of the gene encoding cold-active β-galactosidase *Arthrobacter* sp. 32 cB, construction of the expression system, biosynthesis and biochemical characterization of the protein. **MATERIALS AND METHODS.** In the first stage of research methods used included Polymerase Chain Reaction (PCR), cloning of DNA fragments and the gene expression. Second stage of research included purification

of the protein with a use of ion-exchange and size-exclusion chromatography and biochemical analysis of the protein obtained. **RESULTS.** Sequence of a β-galactosidase gene from a psychrotolerant bacteria *Arthrobacter* sp. 32 cB was obtained and expressed in an *Escherichia coli* expression system. The specific activity of a purified enzyme was 213 U/mg for ONPG at 30°C. The molecular mass of native enzyme estimated by gel filtration was 256.6 kDa. Hence, it is assumed that the *Arthrobacter* sp. 32 cB β-galactosidase is a dimeric protein composed of 109.7 kDa subunits. It belongs to glycoside hydrolase family 2. Moreover, the maximum activity of the enzyme was determined at pH 8.0 and 28°C. **CONCLUSIONS.** Biochemical tests of β-galactosidase *Arthrobacter* sp. 32 cB testify that enzyme can be used for the production of a low-lactose milk. However, the enzyme shows high transglycosylation activity therefore main use of it could be production of galactooligosaccharides and heterooligosaccharides.

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MICROBIAL PRODUCTION OF 1,3-PROPANEDIOL BY NEWLY ISOLATED *HAFNIA ALVEI* AD27

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INTRODUCTION. Nowadays, global interest is being directed towards research and commercialization of microbial synthesis as a way to obtain chemicals from renewable sources. The natural environment can be a source of many microorganisms (e.g. *Hafnia alvei*) which show potential for industrial application. The methods of isolation permit to obtain attractive new producers of valuable substances. 1,3-propanediol (1,3-PD) is one of such chemicals. **AIM.** The purpose of this work was to isolate new strains of bacteria which are able to synthesize 1,3-propanediol from glycerol. **MATERIALS AND METHODS.** Microorganisms were isolated from various sources from environmental samples and food products. The individual colonies were isolated by employing streak plate technique. Plates were incubated for 24–48 h at 30°C. Then single colonies were transferred to various media to be tested for 1,3-PD production. After 7-day incubation at 30°C without pH regulation, the broths were collected and analyzed by HPLC. 16S rRNA

gene sequencing was used to identify selected strains. Batch fermentations were carried out in a 5-L bioreactor (Sartorius B plus). **RESULTS.** A total of 3800 isolates were screened for the ability to grow on glycerol and synthesize 1,3-PD. 28% of the examined isolates were able to synthesize 1,3-PD. Selected 1,3-PD-producing bacteria were identified by 16S rRNA sequences as *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Citrobacter freundii* and *Hafnia alvei*. The latter was never before reported as capable of 1,3-PD production. For *Hafnia alvei* AD27 bioreactor experiments were conducted at temperatures of 30°C and 37°C and final 1,3-PD concentrations of 13.0 and 10.3 g/l were obtained, respectively. **CONCLUSIONS.** Natural environment probes can be a good source to isolate bacteria with potential for industrial application. *Hafnia alvei* AD27 was acquired, a strain from a species never before proven to possess the feat of 1,3-PD production.

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IDENTIFICATION OF MICROSCOPIC FILAMENTOUS FUNGI CAPABLE OF DEGRADING BISPHENOL A

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INTRODUCTION. Microscopic fungi can be classified to a species rank by macroscopic and microscopic characterization, but these observations sometimes lead to misidentification. Therefore a method of genetic identification based on ITS fragments sequencing has been developed. **AIM.** The aim of this study was to identify strains that effectively eliminate bisphenol A from the culture medium. **MATERIALS AND METHODS.** Axenic cultures of strains IM 6449, IM 4659, IM 6358 and QF 10 were used. Total DNA was isolated from strains using a Plant DNA Mini Kit. Then, the ITS fragment of the nuclear genome was amplified in the PCR. The reaction products were separated on an agarose gel, from which they were isolated using a PCR and Gel Kit. After purification of the amplification products a sequence reaction was conducted. The nucleotide sequences obtained were compared to all available sequences of fungi in the GenBank sequence database using the

BLASTN algorithm. Moreover, for every strain microscopic observations were done using a confocal microscope. **RESULTS.** The obtained results allowed us to assign the strains to particular species. IM 6449 strain isolated from soil from the area of a dye factory “Boruta-Zachem kolor s.a.” in Zgierz, was identified as *Fusarium oxysporum*. IM 4659 strain derived from Nigeria was identified as *Emericella corrugata* (also called *Aspergillus corrugatus*). IM 6358 strain isolated from municipal waste from Moscow was identified as *Penicillium chrysogenum* and QF 10 strain from Hungary was identified as *Trichoderma atroviride*. **CONCLUSIONS.** The results of molecular analysis are concordant with those of morphological observation and enable classification of the tested strains to a species or a genus.

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DEGRADATION OF BISPHENOL A BY FILAMENTOUS FUNGI IM 6449 AND QF10

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INTRODUCTION. The continuous increase in environmental pollution has caused changes in the environment, and has had a negative effect on living organisms. Compounds classified as EDCs (Endocrine Disrupting Chemicals) get into environment as municipal and industrial wastewater posing a major threat to humans and animals. One of such troublesome compounds is bisphenol A (BPA) used in the production of high-quality transparent baby bottles or pacifiers. Small amounts of BPA can be released from the resin coating getting into foods and drinks. This xenobiotic, with a complex structure, occurs in the surface water and

is resistant to degradation. A good solution to this situation has become the search for safe and natural methods for the elimination of the pollutant from the environment. **AIM.** The aim of this study was to identify the degradation products of BPA by strains: IM 6449 and QF10 and to confirm occurrence of monocyclic derivatives of this process. **MATERIALS AND METHODS.** The culture of filamentous fungi *T. atroviride* QF10, *F. oxysporum* IM 6449 and was conducted in Sabouraud medium. The analysis was done by liquid chromatography coupled with tandem mass spectrometry using deuterium-labeled bisphenol A. The initial concentration

of bisphenol A in the growth medium was 50 mg/l. **RESULTS.** A method based on the deuterium-labeled bisphenol A was developed and derivatives of this compound 4-hydroxybenzaldehyde and 4-hydroxybenzoic acid were determined. It has allowed typing of monocyclic products derived from the decomposition of BPA. Strains QF 10 and IM 6449 showed the ability to degrade BPA to the

above-mentioned compounds. **CONCLUSIONS.** Strains IM 6449 and QF10 showed an ability to degrade BPA. The conducted studies have confirmed formation of derivatives resulting from a split in the methyl bridge in the structure of BPA.

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THE MICROORGANISMS SENSITIVITY TO NANOSILVER MISTING DISINFECTION

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INTRODUCTION. Nanosilver is used as an antimicrobial agent because of its high stability, long-term and broad spectrum of activity and low probability of resistance acquisition by microorganisms. **AIM.** The effectiveness of nanosilver misting disinfection on bacteria and fungi on different technical materials and on the various morphological forms were determined. **MATERIALS AND METHODS.** The inoculums of ATCC microorganisms (*A. niger*, *B. subtilis*, *E. coli*, *S. aureus*) and isolates from historical objects (*B. subtilis*, *A. versicolor*) were applied on different samples of technical materials (leather, paper, textile, wood). The vegetative cells were obtained from 12 hours (bacteria) or submerged culture (fungi), while the spore cells by heat shock (bacteria) and by rinsed 7-day culture with water and Tween® (fungi). Inoculums were applied on cotton samples. All samples were subjected to nanosilver misting (90 ppm, 8 cycles) in disinfection chamber. The results were

compared with the control samples without silver and the reduction (R) was calculated. **RESULTS.** The highest reduction level was obtained on paper (70%) and on textile (69%). The worse results were noted for leather (67%) and wood (64%). The most vulnerable microorganism was *A. niger* (76%), moderately sensitive *E. coli* (74%) and *S. aureus* (67%), the most resistant to nanosilver preparation were endospore forming bacteria *B. subtilis* (54%). The studies on morphological forms revealed that for *B. subtilis* vegetative cells of environmental isolate (81%) were more susceptible for nanosilver than collection strain (37%). In case of spores, the ATCC strain had more resistant ones (58%) than isolate (10%). For fungi, the obtained reduction level was similar for vegetative cells and spores for collection *A. niger* and isolate *A. versicolor* strains (71–81%). **CONCLUSIONS.** Fungi are more sensitive to nanosilver misting. The best disinfection results were obtained on paper and textiles.

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THE FITNESS EFFECTS OF STRUCTURALLY UNSTABLE PROTEIN VARIANTS CODED BY THE ADE2 AND LYS2 GENES OF SACCHAROMYCES CEREVISIAE

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It is believed that the process of protein synthesis is under strong control of natural selection which works to maintain it possibly least burdensome for the cell. This is because translation consumes a major, and often the largest, share of cell's resources. Moreover, polypeptides are constantly endangered by misfolding and aggregation which can make them not only unproductive but also toxic to the cell. The fact that the most abundantly expressed proteins are also the most conserved is taken as an evidence that in case of these proteins natural selection operates especially effectively because any non-optimal changes of their amino acid sequence would be especially costly in terms of fitness. This explanation is plausible but lacks direct experimental evidence. We created sets of thermosensitive (structurally unstable) mutations in the *ADE2* and *LYS2* genes of the budding yeast. We overexpressed

the mutated proteins and showed that those of them that were the least efficient in adopting a correct three dimensional structure were also the most damaging to fitness. We were able to demonstrate that those protein molecules that did not adopt native shapes tended to form aggregates. The negative fitness effects were not seen when the mutated proteins were expressed at low levels. Together our data show that the observed malfunctioning of the cell was caused not by malfunctioning of the synthesis of adenine and lysine or by de-regulation of other pathways but by the presence of redundant and probably toxic protein material. Our results provide a rare insight into the fitness effects caused by single amino acid substitutions altering the protein (in)stability. They will help to understand protein evolution and may be useful in protein designing.

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THE APPLICATION OF PRO-BIOTECHNOLOGY IN LAKES RECLAMATION

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INTRODUCTION. The lack of programs of protection of natural environment, leads to degradations (eutrophication) of many lakes. The reclamation of these natural water areas is the very important

issue of increasing of attractiveness of our region. It may be done among other by artificial oxygenation of lakes, mechanical removes of sediments, chemical inactivation of phosphorus and some bio-

logical methods with use of microorganisms. **AIM.** Application of pro-biotechnology in limiting of organic pollutions and undesirable microflora (including cyanobacteria) in the Konin Lake. **MATERIALS AND METHODS.** 10 probes of water and sediments from the Konin Lake were investigated. Into 9 of them the consortium of useful microorganisms were added. The 10th was a control probe. The pH of water and sediments, the level of oxygen, the quantity of phosphorus, nitrates, and nitrites, and the chemical request of oxygen were tested. Additionally, microbial analyzes were done (the test for presence of mezophilic and psychrophilic bacteria, lactic acid bacteria, moulds and yeast, bacteria from coli group, *Clostri-*

dium spp. and other anaerobic bacteria were done). The presence of cyanobacteria was investigated by flow cytometry. **RESULTS.** It was found out that the addition of useful microorganisms into water increased its quality and its microbial state. Also the increasing of physic-chemical properties of water was observed. **CONCLUSIONS.** The used of pro-biotechnology in reclamation of natural water reservoirs is a promising methods to increasing its quality. However, it is necessary to precisely investigate the mechanisms of biological reclamation and optimization of conditions of microorganisms' cultivation and inoculation.

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CYANOBACTERIA STIMULATES ENERGETIC PLANTS GROWTH

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INTRODUCTION. Cyanobacteria (Blue-green algae) are cosmopolitan, photosynthetic, prokaryotic microorganisms. Some species are capable of fixing nitrogen, due to the presence of an enzyme nitrogenase. Cyanobacteria secrete plant growth hormones and wide spectrum of secondary metabolites beneficial for plant development, increases bioavailability of macro and microelements and improve soil physical and chemical properties. Efficient, environmental friendly and cost effective production of plant biomass for energetic purposes is an important element of Polish Energy Policy. **AIM.** Evaluation of the *Anabaena* sp. AGG0203 strain as a energetic plants growth stimulating agent. **MATERIALS AND METHODS.** Preselected strain of diazotrophic cyanobacteria *Anabaena* sp. AGG0203 was used in this experiment in form of (1) living cells in log phase, centrifugated and resuspended in water and (2) cell homogenates. *Sida hermaphrodita* – energetic plant used in this research was cultivated in pots for 3 months using standard soil fertilized with recommended doses of N:P:K. *Anabaena* sp.

AGG0203 was applied in the second and fourth week of cultivation. Growth of plants, chlorophyll content and photosynthesis intensity were measured during the vegetation. Fresh and dry biomass was measured at the end of the season. **RESULTS.** *Anabaena* sp. AGG0203 applied in both forms significantly increased the plants growth, chlorophyll content, photosynthesis intensity and fresh/dry weight of plants. Cells homogenate increased the height of plants by 75.4%, fresh weight by 217% and dry weight 186.79% The effectiveness of homogenates was greater than cell suspension for all measured parameters especially for fresh and dry weight of biomass. **CONCLUSIONS.** *Anabaena* sp. AGG0203 produce and accumulate chemicals that significantly simulate the growth of *Sida hermaphrodita*, but only a small portion of these compounds is secreted by living cells. Cell disintegration releases the intracellular content and creates, a mixture of bioactive compounds, plant hormones and nutrients that is an effective stimulating factor for energetic plant.

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PREVALENCE OF OF THE GENE ENCODING TRANSGLUTAMINASE IN STRAINS OF *STREPTOMYCES*

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INTRODUCTION. *Streptomyces* are one of the most metabolically active bacteria. They produce ¾ known antibiotics and many biologically active substances including transglutaminase. Transglutaminase is an enzyme which catalyzes formation of bonds between proteins. Polymerization of the protein chains is often used to modify functional properties of food products. **AIM.** The aim of this study was to determine the prevalence of the gene encoding for transglutaminase in new strains of *Streptomyces* isolated from soils derived from different climatic zones. **MATERIALS AND METHODS.** We studied one hundred twelve soil samples from twenty six countries belonging to sixteen different climate zones. Isolated strains were classified morphologically to the genus *Streptomyces*. Confirmation to the genus *Streptomyces* and the presence of the

transglutaminase encoding gene in isolated strains was performed by real-time PCR assay. **RESULTS.** We studied one hundred twelve samples of soil from twenty six countries. We isolated nine hundred thirty eight strains, of which eighteen (1.9%) had the gene encoding for transglutaminase. These strains were isolated from five soil samples (4.4%) from four different climate zones. Two strains were from the temperate climate zone (Poland and Spain), one of the circumpolar zone (Norway) and fourteen from the hot zone (Egypt). **CONCLUSIONS.** Studies have confirmed that *Streptomyces* spp. which carry transglutaminase encoding gene are extremely rare in the environment. They accounted for less than 2% of the total number of analyzed strains.

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MICROBIAL-ENHANCED PHYTOEXTRACTION

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INTRODUCTION. Phytoextraction is an environmental friendly, cheap and large scale method which uses plants and their associated microorganisms to remove pollutants from the environment. Phytoextraction using energetic plants is a relatively new concept that shows good potential for the removal of heavy metals from large areas with relatively low concentrations of pollutants. Phytoextraction does not solely rely on plant functions, and must always be considered in combination with the effect of soil microorganisms. **AIM.** Evaluation of influence of commercially available effective microorganisms (EM) on growth, development and uptake of heavy metals by energetic plant *Helianthus tuberosus*. **MATERIALS AND METHODS.** Mixture of effective soil microorganisms (*Rhizobium* sp., *Azotobakter* sp., *Clostridium* sp., *Bacillus extorquens*, *Bacillus amylobakter*, *Begiatoa mirabilis*, *Leptotrix* sp., *Galionela* sp., *Crenothrix* sp.) enhancing uptake and bioavailability of crucial macro and microelements. Plant model: *Helianthus tuberosus* – an energetic plant that is used for biomass production for combustion,

biogas and bioethanol production. Plants were cultivated in soil contaminated with mixture of cadmium, copper and lead. Composition of EM was applied twice during the 6 months of growing season. Growth of plants, chlorophyll content and photosynthesis intensity was measured during the whole season. Cd, Cu, Pb content, fresh and dry biomass was measured and analyzed at the end of experiment. All data was statistically analyzed. **RESULTS.** Phytoextraction efficiency of cadmium and lead characterized by bioaccumulation factor (BAF) was increased significantly. Applied microorganisms increased the uptake of cadmium by 28.1%, by copper by 7% and lead by 34.8%. The translocation factor (TF) and transfer factor (TFR) values were calculated to complete information about heavy metal ion transport from soil to plant. Applied EM reduced the stress in plants and increased the biomass accumulation. **CONCLUSIONS.** Application of EM combination to enhance phytoextraction is a viable method of increasing the efficiency, and the cost-effectiveness of the process.

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CONTRIBUTION OF MICROORGANISMS IN THE FORMATION OF CARBONATE MINERAL PHASES DURING THE BIODEGRADATION OF MONOAROMATIC HYDROCARBONS

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A major problem of modern civilization is the contamination of soil and groundwater of petroleum and its products, which are very toxic. Particularly negative influence on organism growth have monoaromatic hydrocarbons from the BTEX group: benzene, toluene, ethylbenzene and xylene, which have mutagenic properties. Many groups of microorganisms have a natural ability to transform monoaromatic hydrocarbons into non-toxic compounds or mineralize them (Wolicka, 2010). Biodegradation of petroleum compounds is a multistep process, which take place with the participation of different groups of microorganisms, including sulfate reducing bacteria (SRB). The aim of this study was to identify mineral phases, resulting in cultures of sulfidogenic microbial communities during mineralization of BTEX. These microorganisms were isolated from formation waters and oils from certain mines in the Flysch Carpathians (SE Poland). Microorganisms were grown using the *microcosms* method with Postgate C medium with BTEX as the

sole carbon source. The molecular analysis of isolated microorganisms confirmed the presence of typical representatives of SRB e.g. *Desulfomicrobium baculatum* and other microorganisms, which can biodegrade toluene (*Geobacter metallireducens*) and xylene (*Cellvibrio Ueda*). All microbial cultures indicated reduction of sulfates – max. 75% and biodegradation of organic compounds about 72%. The X-ray diffraction analysis of post-culture sediments confirmed the presence of carbonate minerals such as calcite and rare vaterite which may formed as a result of microbial oxidation of organic compounds (including hydrocarbons), while dissimilative sulfate reduction. The obtained results, which confirm the involvement of microorganisms in the formation of carbonate minerals, are an important application in determining the ownership of porous reservoir rocks. High activity of microorganisms which degrade petroleum hydrocarbons can cause a marked reduction in the pore space of reservoir rocks and adversely affect the exploitation of oil.

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BIODEGRADATION OF NONYLPHENOL BY *METARHIZIUM ANISOPLIAE* COMPLEXSylwia Różalska^{1*}, Sandra Frączak², Anna Różycka², Milena Piątek¹, Jerzy Długoński¹¹Department of Industrial Microbiology and Biotechnology, University of Łódź, Poland²Biotechnology and Microbiology Students Society "Bio-Mik" Department of Industrial Microbiology and Biotechnology, University of Łódź, Poland

INTRODUCTION. EDCs (Endocrine Disrupting Compounds) are man-made compounds interfering with the endocrine system, by mimics natural hormones. One of the most common, dangerous

EDCs is nonylphenol. Species belonging to *M. anisopliae* complex are persistent in the environment and have permanent contact with various toxic substances. There is a probability that these filamen-

tous fungi have defense mechanisms against toxic substances and have the ability to utilize or biotransform these substances. **AIM.** The aim of this work was the evaluation of the 4-*n*-NP utilization ability in the *M. anisopliae* complex. **MATERIALS AND METHODS.** Fungal cultures on Lobos medium were supplemented with 50 mg/l of 4-*n*-NP. After appropriate time points, the cultures were extracted with ethyl acetate and subjected to analysis on GC-MS. **RESULTS.** All species belonging to *Metarhizium anisopliae* complex have ability to utilize 4-*n*-NP. The most efficient species were *M. velutinum*, *M. anisopliae*, *M. robertsii*, *M. globosum* and *M. guizhouense*, which remove about 95% of the 4-*n*-NP during 48 h

of incubation. This species also differs in the dynamic of 4-*n*-NP utilization and the fastest removal was observed for *M. robertsii* and *M. globosum*. The species *M. majus* and *M. lepidiotae* had the weakest ability to remove this xenobiotic and at the end of the incubation the amount of the 4-*n*-NP in the culture medium was 74 and 43%, respectively. **CONCLUSIONS.** All species belonging to *M. anisopliae* complex have the ability to remove 4-*n*-NP from the culture medium and this unique feature seems to be typical for the genus of *Metarhizium*.

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BIODEGRADATION OF 4-*n*-NONYLPHENOL AND PATHOGENICITY TO INSECT LARVAE AS A MULTI-FACETED APPROACH TO THE DIFFERENTIATION OF THE *METARHIZIUM ANISOPLIAE* COMPLEX

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INTRODUCTION. The fungal genus of *Metarhizium* involves number of valuable entomopathogenic strains and for hazardous xenobiotics e.g. 4-*n*-NP (4-*n*-nonylphenol) elimination. **AIM.** The purpose of this study was the revision of the *Metarhizium* genus with molecular biology methods. We also checked whether 4-*n*-NP degradation or pathogenicity to *Galleria mellonella* have a taxonomic values. **MATERIALS AND METHODS.** For phylogenetic analysis ITS sequences were obtained. Virulence experiments were conducted on *G. mellonella* larvae. For biodegradation experiments, fungal isolates were cultivated in Lobos medium supplemented with 50 mg/L of 4-*n*-NP. After appropriate incubation times samples were extracted and gas chromatographic analyses of the extracts were done. **RESULTS.** Sequences obtained seems to be a suitable tool for proper classification. Most isolates belong to the *M. velutinum* clade, but single isolates of *Metarhizium brunneum* and *Metarhizium lepidiotae* were also found. All *M. veluti-*

num strains were able to utilize 4-*n*-NP efficiently and after 48 h of incubation the concentration of the toxic compound in the medium was below 5 mg/l. Utilization of 4-*n*-NP by *M. velutinum* strains differs in its dynamics and the most efficient degraders were strains 2.11, 3.11, 4.11, IM 484 and IM 6511. All tested *Metarhizium* isolates were pathogenic to *G. mellonella* larvae, but mortality caused by different isolates varied. **CONCLUSIONS.** All strains analyzed in this work belong to *Metarhizium anisopliae* group. Degradation ability of 4-*n*-NP is a specific biochemical feature of *Metarhizium* genus and could be taxonomically valuable feature for identification to the genus. All tested *Metarhizium* isolates were pathogenic to *G. mellonella* larvae and it can be assumed that the virulence to *G. mellonella* larvae is also typical for the investigated fungal genus.

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SODIUM CHLORINE EFFECT ON SYNTHETIC ESTROGENS UTILIZATION BY FILAMENTOUS FUNGI

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INTRODUCTION. Endocrine disrupting chemicals (EDCs) are known to interfere with the endocrine systems and have influence on many physiological functions in aquatic organisms. The most common EDCs are synthetic estrogens e.g. mestranol (MES) and ethinylestadiol (EE2), which can be detected even in sea. The removal of xenobiotic substances by microorganisms (including filamentous fungi) is a very efficient method, but a special interest should be noticed on utilization in the presence of substances which have impact on microbial activity (e.g. salinity). **AIM.** In this work we assessed the ability of several filamentous fungi for MES and EE2 utilization. Moreover, we checked the removal of this EDCs in the presence of salinity. **MATERIALS AND METHODS.** Filamentous fungi were cultivated on Lobos medium (with or without NaCl in different concentrations 0.8; 1.8 or 2.8%) and with EE2 or

MES (10 mg/l). After 72 h of incubation the samples were extracted using QUECHERS method analyzed by HPLC MS/MS. **RESULTS.** The obtained results show that among 45 fungal strains, 12 strains were able to utilize EE2 and 3 were able to utilize MES in more than 90%. In the presence of salinity (0.8 or 1.8 or 2.8%) the most efficient degradation of EE2 was observed for the strains IM 6324 and *Aspergillus versicolor* IM 2161. The EE2 utilization conducted by the strain IM 6324 did not differ in the presence or absence of NaCl. The EDCs utilization by the *A. versicolor* IM 2161 was slightly inhibited (in 15%) only in the presence of 2.8% of NaCl. **CONCLUSIONS.** Among all tested fungi, two selected strains were able to utilize one of the EDCs in presence of salinity without decrease of efficiency. This study was supported by the grant of the National Centre for Science in Cracow, Poland, no UMO-2011/01/B/ NZ9/02898.

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THE INFLUENCE OF NONYLPHENOL
ON *METARHIZIUM VELUTINUM* CELL WALL COMPOSITIONEwelina Janiec¹, Sylwia Różalska^{2*}, Jerzy Długoński²¹ Biotechnology and Microbiology Students Society "Bio-Mik" Department of Industrial Microbiology and Biotechnology, University of Łódź, Poland² Department of Industrial Microbiology and Biotechnology, University of Łódź, Poland

INTRODUCTION. Microorganisms, including filamentous fungi have permanent contact with various wide spread, hazardous contaminants. One of them is nonylphenol which causing a multiply, strong toxic effects on eukaryotic organisms, but its harmful influence on filamentous fungi is poorly estimated. Fungal cell wall provides the integrity to the hyphae and also protects the fungus against toxic substances. As a fungal model *M. velutinum* was chosen, because it is an ubiquitous, ecologically relevant, entomopathogenic species persistent in soils. **AIM.** The aim of this study was to evaluate of a technical nonylphenol (tNP) influence on *M. velutinum* cell wall, as a primary protecting barrier against toxic substances. **MATERIALS AND METHODS.** In order to extract the cell wall, fungal mycelium was sonicated and mechanically disrupted. Afterwards, it was purified from proteins and lyophilized. The amount of

reducing sugars was determined with Dubois colorimetric method after 20 min extraction with H₂SO₄. **RESULTS.** It was observed, that the presence of tNP in concentration of 100 mg/l caused the cell wall amount decrease. tNP also affect on polysaccharides cell wall content and the amount of reducing sugars was 116 and 180 mg/l (for tNP concentrations of 50 and 100 mg/l, respectively) as compared to the controls, where the total amount of reducing sugars was estimated to 230 mg/l. **CONCLUSIONS.** The obtained results revealed, that tNP had a strong negative effect on cell wall amount and content of *M. velutinum* which could have an influence on its persistence in polluted environment.

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MICROFLUIDIC DEVICES FOR STUDIES OF THE ACQUISITION
OF BACTERIAL RESISTANCE IN A CHEMICALLY HETEROGENEOUS ENVIRONMENT

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Microfluidics is a relatively new field of science which is focused on the understanding of the behavior of the fluids flow in microscale. We are able to generate from millions to billions small droplets with final volume of pico- or nanolitres, which can serve as separated microreactors. Most recently, microfluidics is considered as a powerful tool in numerous applications, particularly in biological and chemical sciences. The versatility of this technology lies in simplicity of controlling and measuring of conditions and reactions inside a chip. The goal of our studies is to construct microfluidic devices, which can be used to investigate the emergence of the antibiotics resistance. In this approach we want to design a microchip with a network of interconnected chambers which enable obtaining

a temporary spatial gradient. Every compartment serves as a separate niche with different chemical conditions. In devices with such a design we are able to track motion pathways of bacteria with the expression of Green Fluorescent Protein (GFP). Movements toward higher concentrations of antimicrobial agents prove the acquisition of bacterial resistance. Results of this work will give us an answer how quickly bacteria gain resistance to the set of antibiotics, whether antibiotics show antagonistic, synergistic or additive activity and what types of changes in genetic material of mutants occur. In further steps we want to use the same chip to observe eukaryotic cells (for instance macrophages stimulated by bacterial antigens, drugs, etc.).

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THE POSSIBILITY TO USE OF RAW ENZYMATIC EXTRACTS IN DECOLORIZATION
OF ANTHRAQUINONE DYES

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INTRODUCTIONS. Extracellular ligninolytic enzymes synthesized by fungi can be used in decolorization of synthetic dyes. The application of fungi in decolorization of industrial wastewater requires the development of methods, which next to the effective decolorization, would limit formation of secondary waste, requiring additional utilization. **AIM.** The aim of our work was evaluation of possibility to use raw enzymatic extracts from cultures of

selective microscopic fungi in anthraquinone dyes decolorization. **MATERIALS AND METHODS.** In the study we used 3 selective strains: *F. solani* BwIII43, K37 *T. harzianum* BsIII33 with high efficiency in decolorization of Alizarin Blue solutions (50–70%). The research was carried out on 8 days post-cultures fluids characterized by high peroxidases activity: horseradish like (HRP-like), ligninase (LiP) and manganese dependent (MnP). In the case of HRP-like

it was maximum of activity. Post-cultures fluids (raw enzymatic extracts) were mixed with Alizarin Blue solution in relation 1:1 for final concentration 0.03%. As a main evaluation rate was received degree of decolorization of anthraquinone dye. Control variants were established by cultures of these microfungi on liquid medium. **RESULTS.** The study has proven that effective decolorization of Alizarin Blue was noted in non-cellular variants and the rate of decolorization was related to decolorization rate in cultures of this

microfungi. **CONCLUSIONS.** The results of the study demonstrated possibility to use raw peroxidase extracts in decolorization of Alizarin Blue. A closely related effect of anthraquinone dye decolorization in cultures of fungi and in non-cellular variants form grounds to carry out research on the use of raw enzymatic extracts from cultures of microfungi in industrial dyes decolorization. This study was partially financed by project No N N304 292 440 from the National Science of Center

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STUDIES ON BIOTECHNOLOGICAL POTENTIAL OF BACTERIA FROM THE SPECIES *CLOSTRIDIUM BUTYRICUM*

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INTRODUCTION. In response to the demand of industry for attractive microorganisms with biotechnological potential we may observe a worldwide search for bacteria and fungi, exhibiting interesting metabolic properties. An example of microorganisms of economic importance for industry may be provided by non-pathogenic bacteria from the genus *Clostridium*, particularly *C. butyricum*. **AIM.** The main aim of this study was to investigate interesting potential applications of *C. butyricum*, which may find potential applications including production of 1,3-propanediol. **MATERIALS AND METHODS.** Microorganism: *C. butyricum* DO14 (our original environmental isolate) from the Department of Biotechnology and Food Microbiology (Poznań University of Life Sciences). Phylogenetic analysis: the 16S rRNA sequences of *C. butyricum* DO14 was compared with the *Clostridium* strains available at the GenBank. The phylogenetic tree was constructed in the MEGA 4.0 program. Biochemical properties: metabolic potential of the analyzed microorganism was assessed using an ANAEROTest 23® biochemical test by Pliva-Lachema Diagnostica. Antibacterial activity: activity of *C. butyricum* DO14 against

pathogenic microorganisms was researched by the agar-well diffusion method. **RESULTS.** *C. butyricum* DO14 is related to other representatives of the same genus capable of glycerol conversion to 1,3-propanediol. Investigated bacterial isolate from the species of *C. butyricum* exhibited biochemical properties characteristic of the species to which they belong. The biochemical profile of novel *C. butyricum* isolate indicate the capacity to ferment a wide range of sugars (simple and complex). The tested strain did not utilize hexahydroxyl alcohols, except for glycerol as a carbon source. *C. butyricum* DO14 exhibit antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli*. **CONCLUSIONS.** Results of these studies broaden our knowledge on characteristics of specific isolate of the species *C. butyricum* in terms of their phylogenetic affiliation, fermentation capacity, as well as antibacterial properties.

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ELIMINATION OF NATURAL ESTROGEN AND IDENTIFICATION OF THE METABOLITES PRODUCED BY MICROSCOPIC FUNGUS *PENICILLIUM CHRYSOGENUM* IM 879

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INTRODUCTION. Estrogens are important members of the group of endocrine disrupting compounds (EDCs). Natural estrogens, 17 β -estradiol (E2) and estrone (E1) are discharged into the environment as a result of rapidly increasing production and use of a wide variety of drugs, as the production wastes, discarded products, metabolites contained in excrements of humans etc. **AIM.** The objective of this study was to identify metabolic pathways that might be involved in the elimination of microbial estrogens. To achieve the objective, fungal strain *Penicillium chrysogenum* IM 879 was applied. **MATERIALS AND METHODS.** A sample preparation procedure based on the quick, easy, cheap, effective, rugged and safe (QuEChERS) method was developed. Then, a sensitive method for simultaneously determining free and conjugated estrogens was developed using liquid chromatography-(electrospray) tri-

ple quadrupole-mass spectrometry (LC-(ESI)MS-MS) in a negative mode. **RESULTS.** After 5-day incubation in Sabouraud dextrose broth medium, fungal mycelium was homogenized, then estrogens and their metabolites were determined. During an E2 degradation experiment, strain *P. chrysogenum* IM 879 metabolized E2 (at the initial concentration 10 mg/l) almost completely. The main metabolites obtained in the fungal culture were estrone sulphate and estradiol-17-sulphate. Additionally, other metabolites, estradiol-17-glucuronide, estrone-3-glucuronide, hydroxyestrone and estrone were found. **CONCLUSION.** Estrogen metabolites produced in the fungus were identified and used to construct a proposed estrogen transformation pathway.

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STUDY OF HEAVY METALS TOXICITY
TO *PAECILOMYCES MARQUANDII* AFFECTING FUNGAL MEMBRANE INTEGRITY

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INTRODUCTION. Among different mechanisms of heavy metals toxicity to microorganisms, there is one involving a disruption of membrane function. Membrane damage is manifested by an increase in membrane permeability, enhanced electrolyte leakage and loss of potassium. **AIM.** In this work we studied the effect of zinc, lead, cadmium and copper on membrane condition of *P. marquandii*, a heavy metals tolerant strain. **MATERIALS AND METHODS.** Liquid cultures with the addition of metal at the concentration, which inhibited fungal growth by 50% were incubated on a rotary shaker at 28°C for 168 h. The efflux of electrolytes from the metal exposed mycelia was estimated conductometrically. Plasma membrane integrity was assessed using an intact cells impermeable dye, propidium iodide. Additionally, to test membrane integrity, the 24 h washed mycelia were digested with concentrated nitric acid and potassium content was measured. **RESULTS.** All tested metals caused enhanced electrolyte leakage from mycelium in comparison

to control samples. Confocal microscopy inspection showed intensive dye influx for copper treated hyphae. In the case of lead the intensity of fluorescence was also significant. Although cadmium caused the most significant growth inhibition it did not enhance propidium iodide fluorescence. Only in Cu²⁺ stressed mycelia the content of potassium was lower than in control samples, whereas in all the other metal treated samples it was higher. **CONCLUSIONS.** The obtained results revealed that copper most strongly affects fungal membrane integrity. Additionally, a rise in the potassium content in zinc, lead and cadmium exposed mycelia can show membrane adaptive changes enabling their survival in heavy metal polluted environment.

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CUNNINGHAMELLA ELEGANS PROTEOME ANALYSIS DURING
CORTEXOLONE BIOTRANSFORMATION AND TBT BIODEGRADATION

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INTRODUCTION. Proteomics is a branch of science dealing with the study of structure and function of proteins, and the interactions between them, through the analysis of a proteome. The advantage of proteomics is the possibility to observe the proteins that are directly involved in changes in the cell. Filamentous fungi are a group of organisms of high industrial importance. Several studies have been conducted on adaptations of this group of organisms in the removal or disposal of harmful substances from the environment. TBT is one of the xenobiotics, which have a negative impact on living organisms. Cortisolone transformation plays an important role in the production of steroid hormones. **AIM.** The study of expression of the proteome during the biodegradation of tributyltin chloride (TBT) and biotransformation of cortisolone by *Cunninghamella elegans*. **MATERIAL AND METHODS.** Proteins were extracted using the optimized procedure. Mycelia were

homogenized using mechanical and supernatant was treated using 20% TCA. Precipitated proteins pellets were pretreated with 0.2 M NaOH and dissolved in SSB buffer. Proteins were separated using 1D SDS-PAGE. **RESULTS.** *C. elegans* transform more than 90% of cortisolone to hydrocortisone and ephydrocortisone during 9 hours of culture and TBT is converted to less toxic derivatives: monobutyltin and dibutyltin. 5 differences in the *C. elegans* protein profiles between the test and control sample for both the TBT and cortisolone study were found. **CONCLUSIONS.** The presence of cortisolone and TBT in the environment causes changes in the expression of proteins in filamentous fungi. The obtained results provide a good starting point for further research using 2D SDS-PAGE and mass spectrometry.

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INFLUENCE OF SELECTED ENVIRONMENTAL PARAMETERS
ON BACTERIAL CONSORTIUM ISOLATED FROM THE CORRODING GAS PIPELINES

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INTRODUCTION. In the gas exploration and production sectors biocorrosion could account for 15–30% of the total corrosion occurring in the natural gas industry. Biocorrosion refers to corrosion influenced by the presence and/or metabolic activity of micro-

organisms on a metallic surface. **AIM.** The aim of this study was to evidence the bacterial diversity along corroded gas pipelines and microbial growth in selected temperatures and pH. **MATERIALS AND METHODS.** In the study bacteria isolated from high pres-

sure pipelines DN200 (Piotrkow Trybunalski – Lodz) and DN300 (Piotrkow Trybunalski – Czestochowa) was used. Swab samples method was used in assessment of microbial contamination inner pipelines surface. Microorganisms consortium were grown in natural gas atmosphere, in the dark, under static conditions for 14 days. Influence of the tested environmental parameters (such as: temperature 5°C and 22°C, or pH 6.0 and pH 7.5) on growth of bacteria was observed as medium turbidity. **RESULTS.** In the experiments the effect of extreme temperatures recorded for operator gas pipelines was observed. At the temperature of 5°C weak

growth of tested microorganisms was obtained. At the temperature of 22°C a larger increase in the microbial biomass was observed. Other results refers to the effects of selected environmental pH (such as pH 6.0 and 7.5) on growth of bacterial consortium. In the case of lower pH (6.0) elongation of adaptive phase -up to 7 days was observed. From day 10, in both pH 7.5 and 6.0 the growth of tested consortium were very similar. **CONCLUSIONS.** Winter temperature limits the growth of used bacterial consortium, but tested microorganisms are tolerant to pH changes.

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DENSITY AND DIVERSITY OF ECTOMYCORRHIZAL COLONIZATION OF TREES IN SALINE SOILS

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INTRODUCTION. The problem of salinity concerns about 20% of cultivable and approximately half of irrigated lands around the world. Soil salinity reduces plant growth and development by osmotic action and direct ionic toxicity. Scientific research indicates a high mycorrhizal fungi adaptability to adverse environmental conditions. Mycorrhizal fungi can alleviate salt stress plants by: increased mineral nutrition of plants, improved plant water uptake and reduced salt uptake by the host plant. Despite the fact that NaCl has a harmful effect on spore germination and growth of fungi, arbuscular structures formed on the roots of halophytes are rather common. However, so far there are no reports on the occurrence and tolerance of ectomycorrhizal fungi to salt stress. **AIM.** The aim of this study was to determine density and biodiversity ectomycorrhizal fungi in relation to seasonal changes. **MATERIALS AND METHODS.** The roots of trees *Salix alba*, *Salix caprea* and

Betula pendula growing in saline soil were collected in two seasons: autumn and spring. The tested stand was located near the soda factory in Inowroclaw. Salinity values measured at the stand were about 53 dS/m (530 mM NaCl). Morphological and anatomical abundance and diversity were assessed based on Colour atlas of ectomycorrhizae (AGERER R., 1987–1998). From selected morphotypes DNA material was isolated. Then ITS region (primers ITS1/ITS4) was amplified, obtained PCR products were cleaned and subjected to a sequencing process. The sequences were analyzed using BLAST program and compared with generally available sequences deposited in the NCBI. **RESULTS.** Morphological, anatomical and molecular analysis of isolated ectomycorrhizal structures allowed to identify different morphotypes formed by ectomycorrhizal fungal species. **CONCLUSIONS.** Abundance and diversity of ectomycorrhizal fungi were correlated with investigated tree species and seasons.

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RESISTANT DEXTRINS FROM POTATO STARCH AS POTENTIAL PREBIOTIC

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INTRODUCTION. A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and activity of one or, a limited number, of bacteria in the colon that can improve host health. **OBJECTIVE.** The objective of the present study was to prepare, and characterize new enzyme resistant, chemically modified dextrins from potato starch of potential prebiotic effect. **METHODS.** The enzyme-resistant dextrin, prepared by heating of potato starch in the presence of hydrochloric (0.1% dsb) and citric (0.1% dsb) acid at 130°C for 3 h (CA-dextrin), was tested as the source of carbon for probiotic lactobacilli and bifidobacteria cultured with intestinal bacteria isolated from faeces of three healthy 70-year old volunteers. The dynamics of growth of bacterial monocultures in broth containing citric acid (CA)-modified dextrin was estimated. It was also investigated whether lactobacilli and bifidobacteria cultured with intestinal bacteria in the presence of resistant dextrin would be able to dominate the

intestinal isolates. Prebiotic fermentation of resistant dextrin was analyzed using prebiotic index (PI). **RESULTS.** It was shown that all of the tested bacteria were able to grow and utilize CA-dextrin as a source of carbon, albeit to varying degrees. In co-cultures of intestinal and probiotic bacteria, the environment was found to be dominated by the probiotic strains of *Bifidobacterium* and *Lactobacillus*, which is a beneficial effect. The PI values in media with CA-dextrin were positive; furthermore the prebiotic index increased with time of culture (from 0.033 at 24 h of incubation to 0.176 at 168 h), which proves that bacteria beneficial for humans (*Bifidobacterium* and *Lactobacillus*) can dominate their environment containing a mixture of intestinal bacteria cultured with the addition of resistant dextrin. **CONCLUSIONS.** The experiments showed that CA-dextrin may have prebiotic properties. The study was supported by the Ministry of Science and Higher Education, grants No. NN312335339.

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ACIDOTHERMOPHILIC BACTERIA *ALICYCLOBACILLUS* SP. ISOLATED FROM THE NATURAL ENVIRONMENT

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INTRODUCTION. Processed fruit industry is more frequently subjected to the contamination with *Alicyclobacillus* sp., acidophilic thermophilic bacteria (ATB). These bacteria are nonpathogenic aerobic microorganisms, characterized by high resistance towards low pH and high temperature. Despite not being harmful for human health, their development in final food products is undesirable because the production of off-flavors due to guaiacol formation without swelling of the product packaging is observed. The spoilage of fruit juices or concentrated fruit products is strictly connected with the condition of raw materials. **AIM.** The presented study was focused on the isolation and characterization of *Alicyclobacillus* sp. inhabiting natural environments in central area of Poland. **MATERIALS AND METHODS.** Conducted research involved several genera of apple trees and the soil. Samples were collected from different materials – fruits, bark, soil and transferred to physiological salt solution (0.85% NaCl). In the case of fruits, the swab method was preferable. All samples were extracted for 45 minutes

and inoculated into different liquid media: BAT and glucose broth. Spread plate technique was used for bacteria isolation. Plates with Plate Count Agar were used for total microorganisms enumeration while selective media (BAT agar, K agar) aimed for determination of acidophilic thermophilic bacteria. Additionally, each sample was subjected to the heat shock treatment (80°C for 10 minutes) and inoculated onto selective media as well. Phenotypic features of all obtained isolates were analyzed by macro- and microscopic observations. Staining procedures were applied as well. **RESULTS.** The work resulted in isolation of 64 different bacterial strains, where only 18 were nonsporulating neither acido- nor thermotolerant bacteria. 23 isolates were proved to be strains of sporeforming alicyclobacilli. **CONCLUSIONS.** The research proved the frequent occurrence of *Alicyclobacillus* sp. in natural environment of different location. The wide spread of these bacteria may indeed lead to the spoilage of processed fruits products.

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ACTIVITY OF SELECTED ESSENTIAL OILS AGAINST ENVIRONMENTAL BACTERIAL STRAINS OF *ALICYCLOBACILLUS* GENERA

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INTRODUCTION. Acidophilic thermophilic bacteria *Alicyclobacillus* sp. are still threat for fruits processing environment. Alicyclobacilli are aerobic, spore-forming, soil-borne microorganisms expressing high tolerance towards temperature and heavily acidic environment (growth observed even at pH level 2). Attempts for prevention of their growth in concentrated fruit products and fruit juices have been undertaken, however, the usage of essential oils seems to be promising as well. Essential oils are plant secondary metabolites which exert biological activities against various microorganisms, however, their contribution in foods as additives is limited. **AIM.** The objective of the research was to establish the effectiveness of several plant essential oils against *Alicyclobacillus* sp. isolated from natural environments. **MATERIALS AND METHODS.** Oils selected for the experiment are common food additives including: cinnamon (*Cinnamomum Zeylanicum*), clove

(*Eugenia Caryophyllus*), eucalyptus (*Eucalyptus Globulus*), geranium, grapefruit, peppermint (*Mentha Piperita*) and tea-tree (*Melaleuca Alternifolia*) plant extracts with thyme (*Thymus Vulgaris*) essential oil as the control sample. The commercial oils purchased by Pollena Aroma SA, Warsaw Poland were used. For each essential oil the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were estimated by the disc-diffusion and microdilution methods. **RESULTS.** All of tested essential oils expressed activity towards bacteria but their effectiveness differed substantially. The vegetative cells were much more susceptible than the spores. **CONCLUSIONS.** Biological effect against microorganisms was rather dependent on the chemical composition of essential oils than the bacterial strain. Some of the oils tested seem to be suitable for biological stabilization of fruit concentrates acting against alicyclobacilli.

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BIODIVERSITY OF YEASTS ISOLATED FROM CAVES OF THE POLISH TATRA MOUNTAINS, AND ITS BIOTECHNOLOGICAL POTENTIAL

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INTRODUCTION. Most of the Earth's biosphere is permanently or periodically exposed to temperatures below 5°C. Cold habitats harbor a wide diversity of psychrophiles. For many years Antarctica

has been the geographic area preferred for studying the diversity and properties of cold-adapted microorganisms. However, the non-Antarctic habitats like Himalayas or Alps are also extensively

explored. The Tatra Mountains are Europe's second-highest massif after the Alps. The Polish part of the Tatra has tens caves. The microclimatic conditions in the Tatra caves are very severe, with temperature ranging from below freezing to a maximum of 6°C. **AIM.** The aim of this study was isolation and characterization of yeasts that inhabit selected caves of the Polish Tatras. **MATERIALS AND METHODS.** Samples of water were collected in the Zimna Cave, Wielka Śnieżna Cave, Miętusia Cave and Kasprowa Niżnia Cave. Yeasts were isolated by growing on agar plates supplemented with ampicillin and chloramphenicol. The effect of temperature was examined at 5–37°C. Assimilation of carbon and nitrogen compounds was tested at 18°C. The proteolytic activity was examined on agar plates containing milk. Other enzymatic activities were tested using API ZYM. Isolates were identified by analysis

of D1/D2 sequences of 26S rDNA. **RESULTS.** Nine yeast strains were isolated from water samples collected in four Tatra caves. Six of them were considered as psychrotolerants. Only one cold-adapted isolate belongs to the ascomycetous yeasts and it was classified as *Candida sake*. The psychrotolerant basidiomycetous yeasts were identified as *Guehomyces pullulans*, *Rhodotorula glutinis* or *Cystofilobasidium capitatum*. Two yeast strains had biotechnological potential. *R. glutinis* exhibited high proteolytic activity, whereas *G. pullulans* was able to produce β -galactosidase and α -amylase. **CONCLUSIONS.** The Tatra Mountains are the geographic region useful for studying the diversity and properties of cold-adapted microorganisms. The isolated strains can have biotechnological potential, e.g. as sources of cold-active enzymes.

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BIODEGRADATION OF BIODIESEL IN SOIL BY *ACHROMOBACTER XYLOSOXIDANS G21*

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INTRODUCTION. The commercialization of biodiesel or the biodiesel/diesel blend may cause environmental damage due to spills. Studies demonstrate that biodiesel is more easily biodegraded and less toxic than diesel oil in water and sediments but practically there is no information about bioremediation process of biodiesel in soil. **AIM.** Verification of biodiesel biodegradability with antioxidants at different concentrations in the soil using bacterial strain *Achromobacter xylosoxidans G21*. **MATERIALS AND METHODS.** In our experiments were used commercial product in non-sterile soil. The control over bioremediation process conducted for 3 months included: control of water content in soil at 20%, chromatographic analysis of FAME (reduction of biodiesel), changes in pH level the potentiometric method measured and dehydrogenase activity was monitored in soil. **RESULTS.** Biodiesel was introduced into the soil at a concentration of 3%, 5% and 8% w/w. After 90 days of

bioremediation a decrease in soil pollution ranged between 63% and 97% depending on treatment conditions. The influence of biofuel on dehydrogenases activity was observed. Higher doses of biodiesel, within a longer period of time, had a negative influence on microorganisms. Decrease in pH to 4–5 in variants of higher biodiesel concentration (5%, 8% w/w) and presence antioxidants during bioremediation were the main reasons for decreased microbial activity too. The results show, that biodegradation of biodiesel using *A. xylosoxidans G21* is possible, but the efficiency process was comparable to performance bioremediation of traditional diesel fuel the same time (90 days). Microbial degradation of soil contaminated with biodiesel became a new ecological problem and elaboration on efficient method for the reduction of FAME in soil requires more research.

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THE ASSESSMENT OF POLYMORPHISM OF STORAGE PROTEIN IN SEEDS OF WINTER RYE INBRED LINES

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INTRODUCTION. Plant gene resources of commercial due to the development of industry and agriculture from year to year impoverished. As a result of this process, irretrievably lost valuable old varieties of plants, for example. in China in the 40-ies of the last century was grown 10,000 varieties, but now only 1000. Hence, special attention shall be given to the diversity within the existing plant genotypes. Significant variability of the characteristics observed in inbred generations of winter rye, the fore aim of the study was to compare the diversity of inbred lines of rye at the pattered of storage proteins and their genetic similarity. **MATERIALS AND METHODS.** In this experiment, research was carried out into chosen 13 inbred lines of winter rye S_{20} generation, which were selected from different varieties and dwarf forms. The SDS-PAGE method was used to analysis storage proteins in these inbred lines of rye. Genetic similarity was

calculated by the analysis of phylogenetic tree constructed on the basis of Jaccard's coefficient. **RESULTS.** The assessment of storage protein polymorphism, using the SDS-PAGE technique, was carried out on 13 inbred lines of rye, defining the mass and amount of protein bands as well as genetic distance among lines. The most significant polymorphism (around 70%) was observed between lines L13 and L148, whilst the greatest similarity – 65% between lines L10 and L159. However, these lines were selected from different varieties. Electrophoresis of storage protein using SDD-PAGE technique constitutes a helpful tool in the identification of inbred lines of rye. **CONCLUSIONS.** Investigated inbred lines characterized by different type of growth as well as dissimilar content of protein storage in kernels. Investigated inbred lines of rye have changeable patterns of electrophoretic storage proteins, independently on their origin.

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GENETIC MODIFICATIONS ALLOWING FOR OBTAINING NON-CLUMPY *SACCHAROMYCES CEREVISIAE* POPULATIONS OF VARIOUS ORIGIN

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Saccharomyces cerevisiae is important economically and serves as a model organism in modern biology. Its stationary phase populations, consist of clumps of cells. These include old cells not able to divide, bud-forming “mother” cells, and “virgin daughter” cells. It is believed that under nutrient limitation the “virgin daughters” tend to become quiescent cells (QC). These uniform, non-budded cells with dense cytosol provide “reservoir of viability” for population. Fractioning of yeast cultures into quiescent and non-quiescent cells is greatly hampered by cell clumping and therefore has been restricted to selected laboratory strains. The aim of the present study was to find method to get rid of the clumps in natural yeast strains isolated from different environments. The strains were first deleted for *HO* and *URA3* genes using replacement cassettes with antibiotic markers. Native alleles of the *AMNI* gene in these strains were replaced by an allele from laboratory strain. Stationary phase populations of such modified strains were frac-

tionated in the density gradient. Each of the 5 transformed strains changed its natural phenotype into “non-clumpy” irrespectively of the media (YPD, SC, SD), stability (agar, liquid), and incubation temperature (23°C, 30°C, 38°C). Fractions of cells isolated as more dense were homogenous and showed features of QC (including higher viability). Our method allows to obtain solitary populations of *Saccharomyces cerevisiae* irrespectively of their origin. Such “not clumpy” populations could be analyzed more accurately using flow cytometry, optical density and colony forming unit counts. We hope that our method will be interesting for researchers dealing with not yet known abilities of strains isolated from different environments. The method allows also for isolation of homogenous, more viable QC fraction from different yeast populations, which could be useful in industrial applications.

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THE ELIMINATION OF CARBAZOLE BY FILAMENTOUS FUNGUS *CUNNINGHAMELLA ECHINULATA* IM 2611

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INTRODUCTION. Carbazole is an N-heterocyclic aromatic compound present in crude oil and its derivatives, coal tar and oil shale. It is used in the production of pharmaceuticals, dyes, insecticides or polymers. Presence of carbazole in the environment is a serious problem and is particularly harmful because of its toxic and mutagenic character. Numerous studies have shown that bacteria are able to use carbazole as a sole source of carbon, nitrogen and energy. However, little is known about the degradation of carbazole by microscopic filamentous fungi. **AIM.** The aim of this study was to evaluate the ability of the filamentous fungus *Cunninghamella echinulata* IM2611 to eliminate of carbazole. **METHODS.** To evaluate the effect of carbazole on the growth of microscopic filamentous fungus *Cunninghamella echinulata* IM 2611 a culture of the microorganism

was carried out in a minimal Czapek-Dox medium supplemented with carbazole at concentrations of 100, 200, 300, 400 and 500 mg/L, and the amount of biomass in the samples was assessed. In order to assess elimination of carbazole by IM2611 strain, carbazole samples of each concentration were extracted with ethyl acetate and then examined using the gas chromatograph. **RESULTS AND CONCLUSIONS.** *Cunninghamella echinulata* IM2611 has a high level of tolerance to carbazole. It has been noted that with an increasing concentration of carbazole the amount of filamentous fungi biomass grows, which may indicate that the microorganism uses carbazole as an additional source of carbon and energy. Based on chromatographic analysis, it was found that the *Cunninghamella echinulata* IM2611 eliminates carbazole from its growth environment.

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ANTIFUNGAL PROPERTIES AND TAXONOMIC IDENTIFICATION OF SURFACTANT PRODUCING *BACILLUS* ISOLATES

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INTRODUCTION. *Bacillus* strains produce a broad spectrum of bioactive compounds including biosurfactants of a potent antifungal activity. Therefore these microorganisms may have numerous

biotechnological applications, for example the utility in biocontrol against fungal phytopathogens. **AIM.** The aims of this study were: 1) the estimation of antifungal activity of *Bacilli* (IM47, IMb22

and IM B61) against strains of *Fusarium graminearum*, *Fusarium culmorum*, *Trichoderma harzianum* and *Trichoderma atroviride* 2) the comparison of the surface activity of cell free broths obtained from *Bacillus* submerged cultures 3) the taxonomic identification of *Bacillus* isolates. **METHODS.** The antifungal activity of *Bacillus* strains was examined on PDA plates. Surface activity of *Bacillus* culture supernatants was evaluated by a modified quantitative microdilution method. The *Bacillus* species affiliation was evaluated using the Microgen Bacillus MID-68 test. **RESULTS AND CONCLUSIONS.** Based on morphological, physiological and biochemical features *Bacillus* isolates IM b2, IM b47 and IM b61 were

classified to *B. subtilis*, *B. cereus* and *B. laterosporus*, respectively. It was established that growth of *Botrytis cinerea* as well as *Fusarium* strains was inhibited by all used in the study *Bacillus* isolates, but no antifungal activity against *Trichoderma* strains was exhibited by *B. subtilis* IM b2 and *B. laterosporus* IM b61. Surface activity examination revealed that *B. subtilis* IM b2 and *B. laterosporus* IM b61 were more effective producers of biosurfactants as compared to reference strain of *Bacillus subtilis* DSM 3257 with proven ability of surfactin synthesis. Based on the obtained data mixed formulation of biosurfactant producing *Bacillus* isolates and *Trichoderma* strains as biocontrol of fungal diseases is proposed

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APPLICATION OF MOLECULAR METHODS TO IDENTIFICATION OF FILAMENTOUS FUNGI

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INTRODUCTION. Filamentous fungi from genera *Aspergillus*, *Penicillium* and *Fusarium* are cereal pathogens, which produce dangerous secondary metabolites called mycotoxins. They include aflatoxins, ochratoxins and large group of fumonisins derived from *Fusarium*. All these compounds are lethal for humans due to low quantum satis. **AIM.** There is a need for fast and reliable method of filamentous fungi identification. It can be achieved by application of molecular methods. **MATERIALS AND METHODS.** The subject of study is a collection of wheat grain samples taken from 30 different places in Poland. From every sample, 150 g of grain was weighed, in the next step from these portions 100 grains were selected according to their appearance, which indicated fungal contamination. Next, grains were rinsed with distilled water and 70% ethanol, then dried.

Filamentous fungi were isolated from grains by placing single grains on Petri dishes with potato dextrose agar (PDA) and incubated for 7 days at room temperature. Obtained fungi were transferred to potato dextrose broth (PDB) and incubated for 5 days also at room temperature. Such prepared material was used for DNA isolation and further amplification with application of ITS primers. Identification was proceed on basis of sequencing PCR products compared to available ITS databases as well as according to classical, morphology based methods. **RESULTS.** Initial results indicate about 30% share in total fungal contamination with *Fusarium* sp. On the basis of morphology there were identified 3 species: *Fusarium culmorum*, *Fusarium graminearum* i *Fusarium poae* During further research other fungi, isolated from grain, will be analyzed.

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LACTOBACILLUS STRAINS RESISTANT TO GASTROINTESTINAL CONDITIONS – IN VITRO STUDIES

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INTRODUCTION. According to FAO/WHO Report probiotics are “live microorganisms which when administered in adequate amounts confer a health benefit on the host.” While selecting a preferable probiotic strain several aspects of functionality have to be considered. Most important are acid tolerance and tolerance to human gastric juice and bile tolerance (a property for survival in the small bowel). **AIM.** The aim of this study was to evaluate the probiotic *in vitro* properties of *Lactobacillus* isolated from plant material (homemade pickled cucumbers and cabbage). **METHODS.** Bacteria were cultivated for 1 d in MRS medium (Merck), centrifuged and re-suspended to final concentration 10^9 CFU/mL in PBS. Bacteria were incubated in 0.5% NaCl at pH 1.5, 2.5 and 3.5 at temp. 37°C for 2 h to study the impact of pH. Effect of the bile was estimated in 0.2, 2 and 4% bile salt solutions (Ox gall, Sigma Aldrich) after a 2nd treatment. The bacteria were then

plated on MRS agar medium for 2 d at 37°C. The measurement was repeated at least three times. *Lactobacillus* survival (in %) was calculated as follows: $N_i/N_x \times 100$, where $N_i = \log$ CFU/mL after incubation, $N_x = \log$ CFU/mL before incubation. **RESULTS.** The survival of these strains after incubation at pH 2.5 did not change markedly, and remained at above 90% (10^9 CFU/mL). The strains also exhibited a high survival rate at pH 3.5 (> 90%), whereas pH 1.5 were lethal. Just four strains could survive 90 min. at pH 1.5 (< 39%). The incubation with 0.2% bile salt solution resulted in a survival rates of 81–94% after 24 hour, whereas after incubation in 2% and 4% bile salt solution it was 59–94%. **CONCLUSIONS.** Most resistant to gastrointestinal conditions were *Lactobacillus pentosus* O22 and *Lactobacillus plantarum* K1 strains. All *Lactobacillus* strains isolated from pickles have the potential for use as probiotics.

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PRODUCTION OF RECOMBINANT PROTEINS MLrA AND MLrC
FROM *SPHINGOMONAS* SP. ACM-3962 INVOLVED IN THE BIODEGRADATION
OF CYANOBACTERIAL TOXINS

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INTRODUCTION. Bacterial strain *Sphingomonas* sp. ACM-3962 has a unique system of cyanobacterial toxins degradation regulated by a cluster of four genes: *mlrA*, *mlrB*, *mlrC* and *mlrD*. The first three encode enzyme proteins that sequentially hydrolyze bonds in the molecule of microcystin-LR (MC-LR), whereas *mlrD* gene encodes a protein likely to fulfill transport functions. **AIM.** The study was intended to improve knowledge of MC-LR degradation pathway present in bacteria *Sphingomonas* sp. strain ACM-3962 by receiving recombinant proteases MlrA and MlrC and their characteristics. **MATERIALS AND METHODS.** Genetic constructs were obtained by cloning *mlrA* and *mlrC* genes into pET-21a vector. Furthermore by site-directed mutagenesis we received muteins of MlrA to investigate postulated function of HAIHNE motif in enzymatic activity. Histidine residue in the beginning of the motif and glutamic acid residue terminating it has been replaced with

alanine residues (H260A and E265A). Proteins were produced in *E. coli* expression strain BL21(DE3) and purified from cell lysate using a nickel-charged affinity and ion exchange chromatography. Ability of degradation microcystin-LR by purified proteins and cell lysates was examined. **RESULTS.** We achieved successful heterologous expression of two proteins involved in cyanobacterial toxins degradation. Recombinant MlrA was able linearise of MC-LR in contrary to its muteins that were inactive. MlrC degraded linear form MC-LR into oligopeptides and aminoacids. **CONCLUSIONS.** It was proved that postulated sequences of open reading frames *mlrA* and *mlrC* are complete and allow synthesizing functional recombinant proteins. It was shown that recombinant proteins demonstrate degradative effects against MC-LR. H₂₆₀ and E₂₆₅ residues have a crucial role in hydrolyzing toxin molecule and its replacement with alanine abolished the enzymatic activity of MlrA.

II-P 89

HYACINTH ENDOBACTERIA – HOPES FOR EFFICIENT CLEANING
OF FRACKING FLOWBACK WATER POLLUTED WITH BARIUM – PRELIMINARY STUDY

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INTRODUCTION. Desperation for new energy resources has directed people towards shale – geologic formations containing imprisoned gas and/or oil within its pores. The exploration and exploitation of shale gas resources depends on available technologies requiring large amounts of water for drilling and fracking. Such technologies produce flowback waters contaminated by different pollutants. One of the significant water pollutants is barium (Ba). The concentration of Ba was found to reach on average, 661.5 mg dm⁻³ and even up to maximum concentrations of 15 700 mg dm⁻³ (according to the dSGEIS report). One of the successful hyperaccumulators efficiently absorbing metals from wastewater is water hyacinth, that was used in our research. **AIM.** Isolation and identification of endobacteria allows selection of microbial isolates capable of cooperating optimally to enhance phytoextraction

– plant adaptation and barium accumulation processes in water hyacinth. **MATERIALS AND METHODS.** In the present study, 42 days experiment with water hyacinth (*Eichhornia crassipes*) was conducted in a greenhouse. Plants were grown in quarter-strength modified Hoagland's solution with 400 mg dm⁻³ Ba (as BaNO₃) in slightly acidic conditions (pH = 6.2). At the end of experiment after root disinfection, endobacteria isolation was conducted. Hyacinth endobacteria were cultivated in 30°C on solid 10% LB agar medium containing hyacinth extract and 400 mg Ba dm⁻³ in the form of the barium nitrate. **RESULTS AND CONCLUSIONS.** After one week incubation – 34 bacteria colonies resistant to barium were chosen for future detailed analysis. Four different types of colonies were observed. Physiological, taxonomic and experimental research is in progress and results will be presented on conferences.

INNATE AND ADAPTIVE RESPONSES TO MICROBES THE BENEFICIAL AND HARMFUL ASPECTS

PLENARY LECTURES

III-PL 1

ALTERATION OF THE INNATE IMMUNE RESPONSE DURING COPD EXACERBATION

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BACKGROUND. Acute episodes of exacerbations marked the progression of chronic obstructive pulmonary disorder (COPD), a disease mostly due to cigarette smoke exposure. These exacerbations often result in an increased inflammation of the lung causing death in many cases. Nontypeable *Haemophilus influenzae* (NTHI) and *Streptococcus pneumoniae* are the most commonly isolated bacteria during these episodes. Mechanisms responsible for the increased susceptibility to this pathogen are not well defined. **OBJECTIVES.** Our aim was to define the alteration of the immune response to bacterial infection during COPD by using both murine experimental model, cell culture and clinical approaches. **RESULTS.** An experimental model of COPD mice was developed by daily exposure to cigarette smoke (CS) during 12 weeks. Mice with COPD developed exacerbated lung inflammation in response to

S. pneumoniae and NTHI. A profound reduction of bacterial clearance in the lung from COPD mice was detected. Analysis of lung host defences revealed a default in Th17 cytokines, associated with an altered phenotype in DC. Treatment of COPD mice with recombinant cytokine allow to limit the consequences of lung infection. To address this mechanism, the effect of *in vitro* exposure to CS on response to *S. pneumoniae* was analyzed in human cells. Although bacterial endocytosis was not altered, exposure of human DC to CS inhibited the pneumococcus-induced maturation and IL-23 production (a pro-Th17 factor). Moreover, exposure of mononuclear cells to CS altered the production of Th17 cytokines by innate lymphocytes. **CONCLUSIONS.** Exposure to CS results in a reduced production of Th17 cytokines induced by bacteria, a phenomenon that might explain the increased susceptibility to infections.

III-PL 2

ROLE OF THE BACTERIAL CELL WALL GLYCOCONJUGATES IN ELICITATION OR SUPPRESSION OF EUKARYOTIC INNATE IMMUNITY

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Innate immunity is the first line of defence against invading microorganisms in vertebrates and the only line of defence in invertebrates and plants and therefore plays a crucial role in the early recognition and subsequent triggering of a pro-inflammatory response to invading pathogens. This mechanism relies on recognition of evolutionarily conserved structures on pathogens, termed microbe-associated molecular patterns (MAMPs), through a limited number of germ line-encoded pattern recognition receptors. MAMPs are characterized by being invariant among entire classes of pathogens, essential for the survival of the pathogen, and distinguishable from "self". Gram-negative lipopolysaccharide and peptidoglycan are two very important cell wall glyco-conjugates and act as MAMPs in

eukaryotic/bacteria interactions. Besides their general architectural principle, a number of subtle chemical variations are at the basis of the dynamic host-guest recognition that in case of pathogens is followed by the innate response and in case of symbiosis is followed by its suppression. Therefore, the structural study of such glycoconjugates involved as virulence factors in animal or plant infections is a pivotal pre-requisite for the comprehension at molecular level of the innate immunity mechanisms. In this communication I will show some examples of isolation, structure determination and elicitation and/or suppression of plant and animal innate immunity by peptidoglycan and lipopolysaccharides from pathogen and symbiotic Gram-negative bacteria.

III-PL 3

MICROGLIAL CELL ACTIVATION AND INFLAMMATION TRIGGERING

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Microglial cells are considered to be originated from bone marrow-derived monocytes. Following activation by various stimuli, quiescent microglial cells change in morphology from ramified to amoeboid and modify the cell surface expression of the panel of receptors required to interact with environmental chemotactic and activating factors. Several studies emphasized the implication of Toll

receptors in the activation of microglial cells. In this communication I will show some examples of isolation, structure determination and elicitation and/or suppression of plant and animal innate immunity by peptidoglycan and lipopolysaccharides from pathogen and symbiotic Gram-negative bacteria.

like receptors (TLRS) and complement receptors in the microglia activation. Moreover interesting reports showed that chemokines play important role in microglia activation. However until now the mechanism by which chemotactic factors interact with microglial cells are not well understood. Recent results suggest that microglial cells might use similar signalling pathways as described for other immune cells (as monocytes) involved in inflammatory reactions. In a first series of experiments, we demonstrated that a recombinant protein homologous to C1q might behave as a chemotactic factor. Moreover this factor was shown led to interact with C1q-binding protein identified as having functional similarities with *gC1qR* present at human monocyte cell surface. In a second series

of experimental approaches, the presence of CD147 at the microglial cell surface was investigated. Original studies have emphasized the chemotactic activities of some cyclophilins. Cyclophilins A and B have been reported to significantly induce the direct chemotaxis of hematopoietic cells such as T cells and monocytes via a CD147-dependent pathway. Recent cytofluorometry cell analysis indicated that microglial cells might express CD147 as other immune cells in inflammatory conditions. In conclusion the presented data suggest that microglial cells might express receptors or specific binding proteins similar as those described on monocyte surface, allowing their potential recruitment and cell activation in inflammatory reactions.

III-PL 4

ZEBRAFISH EMBRYOS AS A NEW BIOLOGICAL MODEL TO STUDY THE PATHOPHYSIOLOGICAL EVENTS OF *MYCOBACTERIUM ABSCESSUS* INFECTION

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The emerging pathogen *Mycobacterium abscessus* is a rapidly-growing mycobacterium causing severe lung infections, particularly in cystic fibrosis patients. The Smooth (S) morphotype expresses glycopeptidolipids (GPL) at the bacterial surface while the Rough (R) morphotype is characterized by the loss of surface GPL. Previous studies suggested that the R variant is involved in more severe clinical forms, associated with a hyper-proinflammatory response. However, the molecular mechanisms responsible for the virulence associated to the R variant are unknown. The zebrafish embryo offers many advantages that motivated and validated its use for a better understanding of infectious diseases related to mycobacteria. Therefore, we have developed a zebrafish model of infection to investigate and compare the pathogenesis of R and S variants. A microinjection protocol was first developed and the fate and progression of the infection was monitored at a spatiotemporal level by videomicroscopy. A transcriptomic approach by qRT-PCR, an antisense technology using morpholinos and transgenic zebrafish lines were used to evaluate the contribution of the innate immune system during infection. In addition, the potential of embryo as a model in pharmacology has been used to evaluate and visualize *in vivo* the activity of antibiotics on *M. abscessus* infections. In contrast to the S variant, the R morphotype induced a more robust and lethal infection in embryos, characterized by the rapid development of bacterial foci within the Central Nervous System. An

intense pro-inflammatory response with TNF- α production was detected and the role of TNF- α in the control of *M. abscessus* infection was confirmed using TNFR morpholinos; TNFR morphants were extremely susceptible to *M. abscessus* infections. Next, the use of a *mpeg1:mCherry* transgenic zebrafish line, harbouring red fluorescent macrophages, demonstrated the presence of isolated or small aggregated bacilli within macrophages during early infection. Later stages were characterized by the presence of extracellularly-replicating mycobacteria, inducing a strong inflammatory response, leading to rapid tissue damage (abscess) and to larval death. In addition, the high propensity of the R variant to form cords *in vivo* prevented the bacilli from being phagocytosed by macrophages or granulocytes, representing a mechanism of immune evasion. We also showed that the activity of imipenem on infected-embryos was associated with a reduced mortality, with a lower bacterial burden and a significant decrease in the physiopathological signs, which could be imaged in real-time and at high resolution. We propose the zebrafish embryo as a suitable model, particularly relevant to i) the study of *M. abscessus* virulence, and to ii) monitor, at spatiotemporal level, the effects of antibiotics in an infected vertebrate. Moreover, the embryo is particularly conducive to high-throughput screening, thus allowing this biological system to be exploited in the search for new therapeutic molecules against *M. abscessus*, the most antibiotic resistant mycobacterial species.

III-PL 5

SEEING THROUGH HOST-PATHOGEN INTERACTIONS USING A ZEBRAFISH EMBRYO INFECTION MODEL FOR *BURKHOLDERIA CEPACIA* COMPLEX

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Chronic respiratory infection in cystic fibrosis patients is characterized by high levels of pro-inflammatory cytokines, leukocyte infiltration, and inflammation due to colonization by pathogenic bacteria. Particularly, infections caused by *Burkholderia cepacia* complex (Bcc) are associated with poor prognosis, and can progress into fatal respiratory inflammation and septicaemia. It has been shown that *B. cenocepacia* induces a particularly high pro-

inflammatory response (including IL-8 and IL-1 β), and that bacterial virulence factors, including LPS and flagellin, play a role in this. Importantly, *B. cenocepacia* manipulates macrophage host cell biology, allowing bacterial survival and intracellular multiplication, and this intracellular survival strategy has been proposed to contribute significantly to pathogenesis. The zebrafish has now been firmly established as a potent vertebrate model for the study of

infectious human disease. Especially, the possibilities for real time imaging of zebrafish embryos expressing phagocyte-specific fluorescent reporters, in combination with a developing innate immune system with remarkable similarity to that of humans, makes it an exciting model to study the role of phagocytes and the early innate immune response to bacterial infection. We developed a zebrafish embryo infection model to study host-pathogen interactions of the Bcc. We confirmed the importance of an intracellular strategy for *B. cenocepacia* in vivo; intravenously injected *B. cenocepacia* K56-2 – an isolate belonging to one of the major epidemic lineages – survives and multiplies in macrophages of zebrafish embryos prior to

dissemination and systemic fatal infection. However, embryos can control infection with less pathogenic isolates such as *B. stabilis* LMG14294, which cause a persistent infection. Using a combination of transgenic reporter fish, antisense strategies, RNAseq, and bacterial mutants, we aim to better understand (1) how these bacteria manipulate the host macrophage by interfering with host cell biology, (2) how some strains can cause such an extreme inflammatory response, and (3) what induces the transition from persistent to fatal systemic infection? We will describe the role of host phagocytes during infection, and our current analysis of the global host transcriptome during virulent versus persistent infection.

III-PL 6

L5P A SPECIFIC LIPOPEPTIDE OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS*: FEATURE AND INNOVATIVE DIAGNOSIS APPLICATIONS

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Johne's disease or paratuberculosis, caused by *Mycobacterium avium* subsp. *paratuberculosis* (*Map*), is a major worldwide health problem of domestic and wild animals. All control programmes developed until now against this large epizootic have failed due to the lack of sensitive and specific diagnostic assays and to the lack of efficient vaccine. The research of new cell wall antigens represent an original alternative to protein antigens typically used in commercial sero-diagnostic tests. Biochemical analysis of a large set of characterized *Map* isolates showed that all *Map* strains tested produce a lipopentapeptide (L5P) instead of GPLs produced by many non-tuberculous mycobacteria such as *Mycobacterium avium* subsp. *avium* (*Mav*). To provide a genomic basis for the synthesis of this compound, the genome of *Map* was explored. We showed that the module composition of the non-ribosomal protein synthase (Nrp) of *Map*, the enzyme involved in the synthesis of the peptidyl

moiety, is dramatically different from that of other GPL producers such as *Mav* and is in agreement with the amino acid content of the L5P. To circumvent the problems of challenging native purification, L5P was chemically synthesized which allows the large-scale production of pure L5P. We further showed that L5P is the target for a highly specific humoral and that the major epitopes of the L5P are localized in the peptidyl moiety of the molecule. We also showed L5P is the target for a specific humoral response in a subset of human patients with Crohn disease (CD). The L5P, a molecular signature of *Map*, will open the way to an innovative ELISA diagnosis to unambiguously distinguish *Map* from *Mav*, *M. bovis* or other environmental mycobacteria in sera from livestock or from CD patients. T cell assay and direct detection using specific monoclonal antibodies available should complete diagnosis of *Map* from blood, milk, and lesions.

ORAL PRESENTATIONS

III-O 1

GALLERIA MELLONELLA HEMOLYMPH PEPTIDES – A NEW WEAPON AGAINST *LEGIONELLA* BACTERIA

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INTRODUCTION. Antimicrobial peptides (AMPs) are produced by *Galleria mellonella* as a part of their innate immune system against bacteria and fungi. They are released into the hemolymph. The positively charged peptides interact preferentially with the negatively charged lipids of microbial membranes, leading to the destabilization and permeabilization of the cell membrane. Due to the increasing resistance of pathogenic bacteria to antibiotics and other antimicrobial agents, AMPs are a promising therapeutic alternative, hopefully also for *Legionella gormanii*, an etiological agent of atypical pneumonia. **AIM.** The purpose of the experiments was to examine the sensitivity of *L. gormanii* to *G. mellonella* immune

hemolymph extract and to determine the changes in *L. gormanii* cell morphology under the influence of hemolymph peptides. **MATERIALS AND METHODS.** The antimicrobial activity of the hemolymph peptides was assessed by counting live bacteria using a colony-forming unit (CFU) assay. Five microliters of the *L. gormanii* suspension were incubated with various concentrations of hemolymph extract at 37°C for 1h. The mixtures were then plated in triplicate on buffered charcoal yeast extract (BCYE) agar and the CFU was determined after four days of incubation at 37°C in a 5% CO₂ atmosphere. The effect of the hemolymph extract on the morphology of bacterial cells was investigated using transmission

electron microscopy (TEM). **RESULTS.** Our results indicate that the *G. mellonella* hemolymph extract treatment apparently reduced the survival rate of the bacteria in a dose-dependent manner. TEM examination of hemolymph extract-treated *L. gormanii* cells showed morphological changes and extensive damage to the cell membranes. **CONCLUSIONS.** *G. mellonella* immune hemolymph

extract contains potent bactericidal agents against *L. gormanii*. They cause severe damage to the bacterial envelope and changes in the cytoplasm appearance, which contributes to the strong reduction in *L. gormanii* viability.

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III-O 2

PREPARATION OF GLICOCONJUGATES HAVING OLIGOSACCHARIDE MOIETY FROM BACTERIAL SOURCE

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The lipopolysaccharides (LPSs) are amphiphilic components present in the cell wall of almost all Gram-negative bacteria; in particular they are localized in the external leaflet of the lipid bilayer constituting the outer membrane in which they represent more than the 75% of the outer surface. Owing to their external location, LPSs are involved in all the interactions of the microbe with the external environment and in particular in the many aspects of host-bacterium interactions: recognition, adhesion, colonization, virulence in the case of pathogenic bacteria, symbiosis, tolerance for commensal bacteria. From biosynthetic and chemical point of view, three different parts are usually identified in the LPS: an external polysaccharide (known as O-side chain) which is covalently linked to an oligosaccharide part (core), in turn, linked to a glycolipid portion (Lipid A). Lipid A is recognized by mammal immune innate system receptors (PRR) as a PAMP (molecular pattern associated to a potential pathogen), while the O-chain polymers are the target of the mammals adaptive immune system and plant innate immune system and their reaction with specific antibodies is at the basis for serotype classification among the different bacterial species. Since O-chain polysaccharide is exposed on the very outer surface of the bacterial cell it is a target for recognition by host antibodies;

actually, it is largely used in vaccines synthesis in conjugation with a non carbohydrate part. A conjugate vaccine is created by covalently attaching the O-chain polysaccharide antigen to a carrier protein, thereby conferring the immunological attributes of the carrier on the attached antigen and vice versa. This technique for the creation of an effective immunogen is very often applied to bacterial O-chain polysaccharides for the prevention of invasive bacterial disease. In this work we describe two different methods to obtain new macromolecules having an oligosaccharidic moiety from bacterial source: 1) Glicodendrones as immune elicitant, and 2) Liposome functionalized with lipooligosaccharides (LOS) molecules. With regard to the first method the aim of this project is the synthesis of new macromolecules, by azide-alkyne cycloaddition, capable to reproduce polysaccharide blend which is present on the microbial surface. The lipooligosaccharide chosen for this work is an LOS from *Escherichia coli K-12*. With regard to the second method, LOS were used to functionalize synthetic liposome with the purpose to enhance their immunotolerance and therefore their time of permanence in the organism. LPS from *Rhizobium rubi T* was chosen because this bacterial strain produces an lipooligosaccharide with the external region of the Core similar to human Lewis B structures.

III-O 3

IMMUNOPROTECTION AGAINST CHRONIC *TOXOPLASMA GONDII* INFECTION INDUCED BY RECOMBINANT ANTIGENS OF THE PARASITE

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Toxoplasmosis caused by an obligatory protozoan parasite *Toxoplasma gondii*, although usually asymptomatic or accompanied by mild flu-like symptoms, may also prove severe or even fatal in immunocompromised hosts. Moreover, numerous studies point out the possible link between parasite invasion and incidence of certain disorders affecting the central nervous system. Furthermore, the protozoan seems to be capable of altering intermediate host's behaviour to favour its transmission in environment. In the light of these facts the necessity for efficient immunoprophylactic measures is fully understandable. Thus, the study was aimed at testing the immunogenic and protective activity of a few recombinant parasite antigens using a mouse experimental model. In the preliminary experiments carried out on male C3H/HeJ mice the antigen com-

bination promoting the highest protection against chronic toxoplasmosis was selected. Out of two tested vaccine compositions: ROP2 + ROP4 + SAG1 and ROP2 + ROP4 + SAG1 + MAG1 the latter enriched in the parasite cyst wall antigen showed greater efficacy and was classified for further study. In the following evaluations performed on C3H/HeJ and BALB/c females the vaccine's protective activity reached the levels of 58% and 65%, respectively. Consecutive experiments on *T. gondii* – specific cellular and humoral immune responses (splenocyte proliferation, key cytokines release and antibodies production) induced by vaccination are in progress and their results will be presented at the conference.

The study was supported by the Polish Ministry of Science and Higher Education/National Science Centre (grant N N302 636340).

III-O 4

USE OF IL-2 PRESENTING *BACILLUS SUBTILIS* SPORES LEADS TO DEVELOPMENT OF CELLULAR IMMUNE RESPONSE TO ORALLY ADMINISTERED SPORE BASE VACCINEIwona Piątek^{1*}, Krzysztof Hinc¹, Małgorzata Stasiłojć¹, Adam Iwanicki¹, Grażyna Peszyńska-Sularz², Ezio Ricca³, Michał Obuchowski¹¹Department of Medical Biotechnology, Intercollegiate Faculty of Biotechnology UG-MUG, Poland²Tri-City Animal Laboratory, Medical University of Gdansk, Poland³Department of Structural and Functional Biology, Federico II University of Naples, Italy

INTRODUCTION. Bacterial spores have been successfully used to expose various heterologous proteins. Carrying antigens, eg. urease – important virulence factor of human pathogen *Helicobacter pylori*, may serve as vaccine delivery vehicles. **AIM.** We were interested if recombinant spores of *Bacillus subtilis* expressing subunit B of urease (UreB) and IL-2 would be able to elicit an immune response in mice upon oral immunization. What is more, we wanted to answer the question, whether IL-2-presenting spores would help to induce and direct the immune response. **MATERIALS AND METHODS.** To obtain recombinant *B. subtilis* spores expressing UreB and IL-2 on their surface, we used two coat proteins, CotC and CotB as carriers, respectively. To confirm the presence and surface localization of heterologous proteins on the spores, western blot and immunofluorescence microscopy analysis were performed. For quantitative determination of the amount of fusion proteins (CotC-UreB and CotB-IL-2) dot-blot analysis was used. The immune response was

analyzed by detection of UreB-specific antibodies in sera as well as the UreB-specific production of IFN- γ and IL-4 by sensitized splenocytes isolated from immunized mice in ELISpot experiments. **RESULTS.** After immunization with all tested recombinant strains, we did not detect any UreB-specific antibodies, which suggests that no humoral response had been elicited. The results of IFN- γ ELISpot experiments revealed that co-administration of CotC-UreB and CotB-IL-2 spores led to the induction of cellular immune response. Moreover, an increase in the immune response along with subsequent immunizations was clearly visible. Interestingly, we were not able to detect IL-4 produced by UreB-sensitized splenocytes isolated from immunized animals. **CONCLUSION** These observations led us to conclude that the use of IL-2 presenting spores induces strong shift towards cellular immune response. What is more, the presented combination of recombinant spores expressing UreB and IL-2 seems to be a promising vaccine candidate against *H. pylori* infections.

III-O 5

THE EFFECT OF CpG ODN AND OXYDIZED CHOLESTEROL ON THE INTEGRITY AND IMMUNOMODULATORY PROPERTIES OF VASCULAR ENDOTHELIUM

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As a source of inflammatory mediators and cytokines, vascular endothelium regulates the immune homeostasis within the vascular wall. Endothelial integrity may play an important role in this process, too. **AIM.** of the study was to assess the effect of CpG ODN (TLR9 agonist) and oxidized cholesterol on the integrity of human aortic endothelium, its apoptosis and immunomodulatory properties. **METHODS.** The effect of CpG ODN 2006 (2, 10 and 20 $\mu\text{g/ml}$) and 7-ketocholesterol (7-kCH, 1 and 10 $\mu\text{g/ml}$) on the integrity of primary human aortic endothelial cells (HAEC) was assessed in the Real-time Cell Electric Impedance Sensing system (RTCA-DP) based on the normalized Cell Index (nCI), which reflects endothelial electrical impedance. HAEC were induced with CpG ODN 2006 (2, 10 $\mu\text{g/ml}$) and 7-kCH (1 $\mu\text{g/ml}$) for 24 h in order to analyze occludin, MCP-1 and IL-35 mRNA expression in Real-time-PCR, and apoptosis in flow cytometry. **RESULTS.** CpG ODN (2, 10, 20 $\mu\text{g/ml}$) decreased nCI of HAEC as compared to the unstimulated control (0.85 ± 0.07 , 0.69 ± 0.06 and 0.63 ± 0.04 versus 0.92 ± 0.05 ; $p < 0.01$) and induced a 2- and 3-fold increase

in the occludin mRNA expression ($p < 0.05$). nCI of HAEC stimulated with 7-kCH (10 $\mu\text{g/ml}$) was significantly lower in 48 h as compared to the control (0.24 ± 0.08 versus 1.06 ± 0.08 ; $p < 0.001$). 7-kCH (10 $\mu\text{g/ml}$) caused an increase in apoptotic cells percentage (62.2 ± 2.65 versus $46.6 \pm 5.8\%$; $p < 0.05$) and a decrease in living cells percentage (33.9 ± 2.5 versus $48.4 \pm 4.9\%$; $p < 0.05$). 7-kCH in a lower concentration (1 $\mu\text{g/ml}$) slightly decreased nCI of HAEC (1.11 ± 0.02 versus 1.01 ± 0.01 ; $p < 0.05$). However, CpG ODN 2006 when added to 7-kCH (1 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$) did not change nCI as compared to 7-kCH alone (1.01 ± 0.01 and 1.01 ± 0.01 versus 1.01 ± 0.01 ; $p > 0.05$). CpG ODN (2, 10 $\mu\text{g/ml}$) caused a 3-fold decrease in MCP-1 mRNA expression ($p < 0.05$) and didn't affect IL-35 p35 subunit mRNA expression. **CONCLUSION.** CpG ODN and oxidized cholesterol may destroy the endothelial barrier and enable the inflammatory factors circulating in the peripheral blood affecting deeper parts of the vascular wall and modulating local immune processes.

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POSTERS

III-P 1

MODULATION OF *MORAXELLA CATARRHALIS* UPTAKE BY THP-1 MACROPHAGES IN THE PRESENCE OF NEUROPEPTIDES NPY, CGRP AND SP

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INTRODUCTION. *Moraxella catarrhalis* as a mucosal pathogen of human respiratory tract is one of a key etiological factor in chronic obstructive pulmonary disease (COPD) in adults. Phagocytosis by pulmonary macrophages is pivotal mechanism of effective microbial eradication. Neuropeptides (NPs) may be released from nerve endings during airflow obstruction. **OBJECTIVE.** The aim of the study was to determine the phagocytic uptake of this bacterium by THP-1 macrophages in the presence of neuropeptides NPY, CGRP and SP. **METHODS.** The PMA-stimulated THP-1 macrophage differentiation was performed on 96-well transparent-flat-bottom black microplates. The phagocytic uptake of fluorescently-labelled bacteria in opsonic and non-opsonic conditions was measured with spectrofluorometer. For opsonization 10% heat inactivated normal human serum was used. Data of relative fluorescence are presented

as mean \pm SEM for n observations. **RESULTS.** We found the modulatory action of neuropeptides that was observed in distinct types of pathogen recognition scheme, namely opsonic versus non-opsonic. The phagocytic uptake of non-opsonized *M. catarrhalis* by differentiated THP-1 macrophages was markedly decreased (up to 60%) in the presence of all studied neuropeptides. The uptake of opsonized bacteria by NPs treated THP-1 macrophages was slightly decreased or remained unchanged. The results suggest that mainly FcR-independent phagocytosis of *M. catarrhalis* is affected by studied neuropeptide treatment. **CONCLUSION.** Decreased *in vitro* uptake of *Moraxella catarrhalis* by THP-1 cell line macrophages in neuropeptide milieu may have implication in diminished bacterial clearance in COPD patients *in vivo*.

III-P 2

THE HUMORAL IMMUNITY IN DIAGNOSIS OF THE CYTOMEGALOVIRUS INFECTIONS

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INTRODUCTION. Over recent years a rise of the awareness of the influence of the cytomegalovirus (CMV) on fetus has been observed. The consequences of the infection are hazardous cause they might trigger off numerous diseases as well as proved teratogenic viral activity. CMV is widespread in human population what favours the spread of latent infections and the lack of characteristic symptoms in case of contagions. That is why an early and appropriate diagnosis particularly in case of women planning to get pregnant is essential. Serologic tests are the best methods confirming CMV infections an account of low availability and time-consuming procedures. **AIM.** The aim of the research was to detect the antibodies IgM and IgG-anti CMV among women planning to get pregnant serum to find the cytomegalovirus infections. **MATERIALS AND METHODS.** Serum samples taken from woman (came from Podlasie region) in their reproductive age planning pregnant between

2010–2012 constituted the research material. The level of IgM and IgG has been marked immunoenzyme techniques, with the use of Liaison CMV-IgM and Eti-Cytok-G. **RESULTS.** Among 21 women (17%) the level of IgM and IgG was negative. The numerous group are 96 patients (76%) amid whom the IgM were negative and IgG were positive which prove the contact with cytomegalovirus. The group of 9 women (7%) among whom the IgM have been classified as doubtful, and IgG as positive is worth noticing. In this case, it is relevant to mark the level of antibodies again to confirm infection. **CONCLUSIONS.** In the research population of women in 83% IgG anti-CMV were found. The humoral immunity diagnosis is a significant procedure enabling the observance of the course of the cytomegalovirus infection. Assessment of the dynamics of anti-CMV should be introduced as a routine test to minimize the number of premature birth, miscarriage and congenital infections.

III-P 3

INTERFERON AND TUBERCULIN SKIN TESTING IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS (TB)

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INTRODUCTION. Early detection and treatment of TB remains the most important strategy for reducing the incidence of this disease. Unfortunately, traditional methods of TB diagnosis are not only time-consuming but also unreliable. **AIM.** The study compared diagnostic utility of tuberculin skin test (TST) and interferon

γ -based assay (IGRA) (QuantiFERON-TB Gold In Tube) in diagnosis of active TB cases which could or could not be confirmed by acid fast staining or *M. tuberculosis* (Mtb) culture. **MATERIALS AND METHODS.** The study population consisted of 80 patients with active pulmonary TB. In 43 patients TB was confirmed by

a positive sputum culture and in 37 patients TB was diagnosed based on symptoms, chest x-ray and the effects of antituberculous therapy because of negative results of the *Mtb* culture. Moreover, 40 *Mtb* culture-negative patients with nonmycobacterial lung diseases (NMLD) were included as controls. **RESULTS.** IGRA was positive in 65% TB confirmed, 56% TB unconfirmed and 13% NMLD patients. TST was positive in 65% TB unconfirmed, 56% TB confirmed and 28% NMLD patients. The IGRA sensitivity in confirmed and unconfirmed TB patients was similar, 65.1% and 55.6%, respectively. The sensitivity of TST did not differ from the parameters designated for IGRA, 55.8% in confirmed and 64.9% in unconfirmed TB. The sensitivity of TST and IGRA was age-dependent and decreased significantly with the age of patients. No differences in the frequency or intensity of *Mtb*-stimulated IFN- γ

production assessed by IGRA testing between confirmed and unconfirmed TB were noticed. Significantly lower concentration of IFN- γ in patients with advanced TB compared to those with mild TB form was observed. The frequency and intensity of IFN- γ production did not correlate with the presence of lung cavities or infiltration shadows. **CONCLUSIONS.** The clinical utility of IGRA and TST in the diagnosis of culture-negative TB was demonstrated. The *M. tuberculosis*-stimulated IFN- γ level might help in the assessment of the extent of pulmonary TB lesions.

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III-P 4

VIRULENCE OF CLINICAL *M. TUBERCULOSIS* STRAINS FROM LODZ, POLAND

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INTRODUCTION. Poland, especially the Lodz region, has relatively high rates of tuberculosis (TB) morbidity compared with the other countries of the European Union. **AIM.** The virulence diversity of *M. tuberculosis* (*Mtb*) clinical isolates (previously evaluated to determine their genetic profile) was examined regarding their sensitivity to human neutrophil peptide-1 (HNP-1) and intracellular growth within mononuclear phagocytes. Additionally the nitric oxide (NO) and IL-12 production by *Mtb*-stimulated macrophages was quantified. **MATERIALS AND METHODS.** The study involved 36 clinical *Mtb* isolates collected from Lodz region, virulent *Mtb* H₃₇Rv and attenuated *M. bovis* BCG bacilli. The sensitivity of mycobacteria to HNP-1 and their survival inside THP-1 cell line was determined by bacteria plating and CFU enumeration. The level of NO and IL-12 in supernatants of mycobacteria-stimulated murine peritoneal exudate macrophage (PEM \emptyset) cultures was quantified. **RESULTS.** The sensitivity of clinical isolates to HNP-1 was concentration dependent and highly diverse. 69% isolates displayed greater resistance to the highest doses of HNP-1 than H₃₇Rv

strain did. At day 8 post-infection, only two isolates represented the level of multiplicity comparable to that of H₃₇Rv. Interestingly, two isolates demonstrated extremely high multiplication rate which suggests their great ability to survive within macrophages ($p < 0.05$). Surprisingly, 3 isolates revealed the intracellular growth ability similar to that of BCG strain. The intracellular growth of clustered strains and those with unique genotypes were similar. 31 out of 36 *Mtb* or 7 out of 36 *Mtb* isolates induced NO or IL-12 production in PEM \emptyset at a lower level than the H₃₇Rv strain did. **CONCLUSIONS.** Although more than a half of studied clinical *Mtb* isolates were characterized by a higher intracellular multiplication rate and greater resistance to HNP-1 than H₃₇Rv, the correlation between their virulence and transmission in population is more complex and not yet fully explained.

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III-P 5

CHANGES IN THE MITOCHONDRIAL NETWORK DURING ECTROMELIA VIRUS INFECTION OF PERMISSIVE L929 CELLS

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INTRODUCTION. Mitochondria are highly dynamic organelles which play an important role in the life of a cell. They are involved in the production of type I interferons (IFN) and apoptosis which are crucial in limiting viral infection. In many cell types, mitochondria have a tubular morphology and undergo continuous cycles of fusion and fission, which are opposing processes. There is known evidence that mitochondrial dynamics modulate apoptosis, and play an important role in other antiviral immunity pathways.

However, many viruses, including orthopoxviruses can escape from the immune system and they use mitochondria in their replication cycle and morphogenesis process. **AIM.** The aim was to elucidate how the Moscow strain of ECTV (ECTV-MOS) infection affects the mitochondrial network organization of L929 fibroblasts. **MATERIALS AND METHODS.** ECTV-MOS was propagated and titrated in Vero cell culture (kidney epithelial cells extracted from an African green monkey), and was subsequently used to infect L929 (mouse

fibroblastic haplotype H-2^k) cells. After a predetermined time, L929 cells were incubated with Mitotracker Red CMXRos (staining of mitochondria) and anti-ECTV antibody conjugated with FITC. Mitochondrial network was analyzed by fluorescence microscopy (Olympus BX60 equipped with Color View III cooled CCD camera and Cell[^]F software). **RESULTS.** The uninfected control cells were characterized by a normal, branched mitochondrial network. In the perinuclear or periplasmic region extended wavy mitochondrial tubules were arranged in a concentric or radial manner, respectively. Moreover, a microtubule organizing centre (MTOC), from

which mitochondrial tubules appeared to radiate, was observed in uninfected cells. In contrast, the infected cells destroyed mitochondrial network, as well as MTOC organization. During later stages of infection mitochondria showed different morphology with single, separated and circular tubules. Moreover, the perinuclear region was almost completely depleted of mitochondria. **CONCLUSION.** During infection ECTV contributes to destruction of a mitochondrial network in a culture of L929 cells.

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III-P 6

IMMUNIZATION EFFECT OF *DENDROBAENA VENETA* WITH *ESCHERICHIA COLI* ON THE CYTOTOXIC ACTIVITY OF ITS COELOMIC FLUID AGAINST TUMOR CELLS *IN VITRO*

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INTRODUCTION. Today lung and ovarian cancers are a major public health problem all around the world. Lung cancer is one of the few whose incidence is still increasing. However, ovarian cancer is the leading cause of death from gynecologic cancer. Thus, there is a need for discovery and development of new therapeutic agents to treat these two types of cancer. **AIM.** The aim of our research was to determine the cytotoxic effects of coelomic fluid, isolated from earthworm *D. veneta* (Annelida) before and after immunization with strain bacterial *E. coli* towards tumor cell TOV-112D and A549 lines. **MATERIALS AND METHODS.** The cell lines used in this study included: mouse fibroblast L929 (control), human ovar-

ian cancer (TOV-112D) and human lung cancer (A549). Coelomic fluid isolated from earthworm *D. veneta* before and after immunization with the strain of bacteria *E. coli* was used at a dose of 100 µg/ml. The cytotoxicity was determined using the MTT assay after incubation of 24, 48 and 72 hours. **RESULTS.** In all cell lines we observed high cytotoxicity in coelomic fluid both before immunization (≥ 60%) and after immunization (≥ 70%) with a strain of *E. coli*. **CONCLUSIONS.** Analysis of the tumor cell TOV-112D and A549 lines showed higher cytotoxic activity of the coelomic fluid of the earthworms after immunization with the bacterial strain *E. coli* than before immunization.

III-P 7

ANTIBODIES AGAINST SYNTHETIC PEPTIDES LIBRARY BASED ON *HELICOBACTER PYLORI* UREASE, AS AN INDICATOR OF INSTABILITY OF IMMUNE SYSTEM FOR RHEUMATOID ARTHRITIS AND ATHEROSCLEROSIS PATIENTS

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INTRODUCTION. Autoimmune diseases are characterized by multifactorial background, including chronic bacterial or viral infections. *Helicobacter pylori* is one of the most often infecting agents in human population. These bacteria are connected with development of several autoimmune diseases like rheumatoid arthritis. **AIM.** The aim of this study was to establish, in human sera, the level of antibodies against peptides library, corresponding to the flap region of *H. pylori* urease. This library is composed of 361 synthetic peptides, where each peptide differs in substituted amino acid in defined position. **METHODS.** Rheumatoid arthritis patient (RAP), atherosclerosis patient (AP) and volunteer blood donor (VBD) sera were analyzed. Synthetic peptides, immobilized on the cellulose membrane via a triazine derivative, were used as antigens for the detection of anti-urease antibodies. The level of specific IgG antibodies was determined by the quantitative dot

blot method. **RESULTS.** The matrix of reaction with peptides in the library corresponding to the flap region of *H. pylori* urease revealed, that the level of reaction for each serum (RAP, AP and VBD) depended on the peptide sequence. Both patients' sera showed a higher level of antibodies against used antigens, in comparison to the VBD. However, in the case of RAP serum a stronger reaction was observed. Moreover, reactions of patients' (RAP and AT) sera revealed less variety. **CONCLUSIONS.** Relatively small diversity of the level of reactions of patients sera with a panel of synthetic peptides, that differed from each other by single amino acid substitution only, was observed. It may suggest the presence of not-specific antibodies as well as instability of the humoral response in rheumatoid arthritis and atherosclerosis. **ACKNOWLEDGEMENTS.** This work was supported by grant UMO-2011/03/D/NZ6/03316 from the National Research Center.

III-P 8

THE INFLUENCE OF COLLOIDAL SOLUTION OF NANOSILVER AND NANOCOPPER ON CHEMILUMINESCENCE AND VITALITY OF NEUTROPHILS

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INTRODUCTION. Nanotechnology is used in many areas of daily life and industry. One example is using the bactericidal activity of silver and copper nanoparticles. It is important to examine whether these particles have an influence on first line defense immune cells – neutrophils in human body. **AIM.** The goal of this study was to examine whether silver nanoparticles affect the human immune system by stimulating or inhibiting the respiratory burst in neutrophils and if they change neutrophil vitality. **MATERIALS AND METHODS.** Neutrophils were isolated from 14 healthy donors' peripheral blood. To examine the influence of nanoparticles two methods were used. Firstly, the MTT assay which measures cell vitality with a tetrazolium salt reduction to an insoluble formazan. The amount of the reduced MTT dye is proportional to the mitochondrial cell oxidative activity which is connected with the number of metabolically active (live) cells in a population. Secondly, the physiological condition of neutrophils rated by the ability to gener-

ate reactive oxygen forms was measured with chemiluminometry. **RESULTS.** Both incubations of neutrophils with low concentrations – 0.4 ppm and 2 ppm of nanosilver and nanocopper solution did not affect the mitochondrial activity of neutrophils. The use of 10 ppm solution of nanosilver decreases neutrophils activity, contrary to nanocopper solution which does not have a negative effect. The mitochondrial activity of neutrophils is significantly decreased by 50 ppm nanosilver and nanocopper solution. The studies of chemiluminescence showed that silver and copper nano particles do not affect the leverage or the inhibitory effect on human neutrophil. Presence of silver and copper nanoparticles did not change chemiluminescence level of neutrophils activated by standard stimulator. **CONCLUSIONS.** The commercial colloidal nanosilver and nanocopper solutions with high concentrations can decrease mitochondrial neutrophils activity but they do not affect the ability to generate the reactive oxygen forms by neutrophils.

III-P 9

MYCOBACTERIA STIMULATED DENDRITIC CELLS INDUCE PREFERENTIALLY THE INTERLEUKIN-10 PRODUCTION BY MEMORY T CELLS FROM ALLERGIC PATIENTS

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INTRODUCTION. Dendritic cells (DCs) as professional antigen presenting cells possess the unique ability to present antigens to naive T cells and thus play a key role in the polarization of immune response towards the Th1 or Th2 profile. Allergy is caused by an excessive development of Th2 profile in immune response to environmental allergens. Mycobacteria as strong Th1 inducers are potent candidates to redress the Th1/Th2 balance in allergic disorders. Still little is known about the activity of memory T cells in response to mycobacterial antigens presented by DCs in the context of house dust mite allergy. **AIM.** We asked a question whether in the presence of Der p 1, the major allergen of *Dermatophagoides pteronyssinus* house dust mite, *Mycobacterium bovis* BCG vaccine and a recombinant BCG producing human IL-18 (rBCGhIL-18) were able to polarize Th2 towards Th1 memory T cell response. **MATERIALS AND METHODS.** Monocyte-derived dendritic

cells (MoDC), prepared from asthmatic patients who responded to Der p 1 in skin prick test and healthy BCG vaccinated donors, were stimulated with *M. bovis* BCG or rBCGhIL-18 bacilli in the presence/absence of Der p 1. The response of memory T cells to pulsed MoDC was evaluated by determining the IL-10 production by ELISA test. **RESULTS.** Memory T cells from allergic patients produced significantly more intensively IL-10 in response to BCG and rBCGhIL-18-pulsed DCs, as compared to the identically stimulated memory T cells from healthy donors. This effect was also observed when BCG and rBCGhIL-18-pulsed DCs were in the presence of Der p 1 allergen. This process was independent of IL-18. **CONCLUSIONS.** Our results indicate that memory T cells from allergic patients are more receptive to downregulating signals from BCG. Supported by the Polish Ministry of Science and Higher Education grant N N401 015236.

III-P 10

GENETICAL MARKERS OF INFLAMMATION IN CORONARY HEART DISEASE PATIENTS WITH COEXISTING *HELICOBACTER PYLORI* INFECTIONAgnieszka Matusiak^{1*}, Tomasz Rechciński², Maciej Chałubiński³, Karolina Rudnicka¹, Eliza Miszczyk¹, Maria Walencka¹, Marlena Broncel³, Magdalena Chmiela¹¹ Department of Immunology and Infectious Biology, University in Lodz, Poland² 2nd Department of Cardiology, Medical University, Lodz, Poland³ Department of Internal Medicine and Clinical Pharmacology, Medical University of Lodz, Poland

INTRODUCTION. *Helicobacter pylori* (Hp), common human pathogen, inducing gastritis and gastric ulcers is considered one of the risk factors of coronary heart disease CHD. Many studies

have shown that polymorphisms of the genes associated with biosynthesis of the inflammatory proteins, not related to the CHD process, may modulate its development. This encouraged us to

examine whether variants of two IL-1 β gene single nucleotide polymorphisms, C>T, at positions -31 and -511 may correlate with the general inflammatory markers C-reactive protein (CRP) and lipopolysaccharide binding protein (LBP). **MATERIAL AND METHODS.** The study group consisted of 27 patients with myocardial infarction (MI) and 30 healthy volunteers. The mean age of the CHD patients, diagnosed at the Department of Cardiology of Medical University in Lodz, was 59.7 ± 7.4 and of healthy donors was 45 ± 5 . Males comprised 65.8% of patients and 18% of healthy volunteers. Statistical analysis was performed with STATISTICA 10 PL software. The levels of LBP and CRP were estimated by ELISA and the latex test, respectively. The polymorphism of IL-1 β gene was detected by PCR. **RESULTS.** The elevated levels of CRP

were more prevalent in the MI group as compared to the healthy controls, $p = 0.00001$, without differences as regards Hp status. The level of LBP was significantly higher in the Hp-infected MI patients than in Hp infected healthy individuals, $p = 0.00001$. However, no significant differences between genotype prevalence in both loci -31 and -511 in the patients and the control group were observed, ($p = 0.812$, $p = 0.680$, respectively). Also, no significant difference between these genotypes and the levels of inflammatory markers (CRP and LBP) was shown. **CONCLUSION.** The markers such as CRP and LBP are appropriate to demonstrate the severity of the inflammatory response linked to CHD and Hp-associated CHD. However IL-1 β gene polymorphisms (-31 and -511, C>T) are not useful for prediction of inflammatory response in CHD patients.

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AN INTERACTION OF H-FICOLIN WITH LIPOPOLYSACCHARIDE CONTRIBUTES TO AGGREGATION, PHAGOCYTOSIS AND KILLING OF *HAFNIA ALVEI* 1200 BACTERIAL CELLS

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INTRODUCTION. H-ficolin (also called ficolin-3 or Hakata antigen) is a complement-activating pattern recognition molecule, possessing the fibrinogen-like domain involved in carbohydrate binding. Amongst ficolins, H-ficolin has the highest concentration in human serum, however its physiological function remains unclear. Its deficiency has been suggested to increase the susceptibility to infections. The specificity of H-ficolin is poorly characterized and currently limited to a few ligands only. **OBJECTIVES.** We previously presented an interaction of this protein with lipopolysaccharides (LPS) of *Hafnia alvei*, being an opportunistic pathogen. To gain further insight in the physiological role of ficolin-3, we used interaction between LPS and H-ficolin and investigated the influence of H-ficolin on agglutination, phagocytosis and killing of microbes. **MATERIALS AND METHODS.** Phagocytosis of the GFP-expressing bacteria by PMA-differentiated U-937 and agglutination was performed using various experimental approaches includ-

ing flow cytometry, fluorescent microscopy and confocal microscopy. Bactericidal activity was measured by Alamar Blue assay. **RESULTS.** We observed strong agglutination of GFP-expressing bacteria, in the presence of recombinant H-ficolin and calcium. H-ficolin augmented phagocytosis of these microorganisms by macrophages. The phagocytosis rate was estimated by flow cytometry and fluorescence microscopy. More than 40% of cells contained phagocytosed bacteria, compared with less than 15% in the case of control (non-opsonized bacteria). These data were confirmed by confocal microscopy. Moreover, we showed much higher bactericidal activity of normal than H-ficolin-depleted human serum. **CONCLUSION.** To conclude, H-ficolin, in effect of enhancing of phagocytosis and microbicidal activity may be protective from or influence an outcome of the infection with some bacteria. This work was supported by the Polish Ministry of Science and Higher Education, Grant N N401 267339.D

III-P 12

DETERMINANTS OF PATHOLOGICAL INFLAMMATORY RESPONSE IN GUINEA PIGS INFECTED WITH *HELICOBACTER PYLORI*

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INTRODUCTION. *Helicobacter pylori* (Hp) causes chronic gastritis in humans resulting with pathological processes: ulcers and gastric cancers. Inflammation is manifested by the infiltration of gastric epithelium with the immune cells, the activity of which is associated with the production of cytokines. These mediators can have both local and systemic effects. **AIM.** The guinea pig animal model gives the opportunity to investigate and characterize the nature of inflammatory process as well as the course of immune responses towards induced Hp infection. We focused on the moni-

toring of the nature and the intensity of inflammatory response in the gastric epithelium of guinea pigs infected with Hp as compared to uninfected animals. We estimated the *in vivo* proliferative activity of lymphocytes of both groups animals by the evaluation of lymphocyte proliferation driven by the Hp antigens in cell cultures *in vitro*. **MATERIALS AND METHODS.** In animals inoculated *per os* with Hp or 0.85% NaCl, after 4 weeks, the histopathological analysis of gastric epithelium was performed concerning inflammatory response, metaplasia and the presence of Helicobacter like organ-

isms (HLO) according to Sydney System classification. The proliferative activity of lymphocytes isolated from mesenteric lymph nodes, in response to Hp antigens: glycine extract (GE) or lipopolysaccharide (LPS), was assessed *in vitro* by [3H]-thymidine incorporation assay. **RESULTS.** The gastric epithelium of Hp infected animals was colonized by these bacteria and this was accompanied by chronic, active inflammation with symptoms of atrophy and metaplasia. The local inflammatory response was correlated with

the *in vitro* proliferative response of lymphocytes in the milieu of Hp GE but not Hp LPS. **CONCLUSIONS.** The results obtained in this study showed the parallel development of local, inflammatory response and the immune processes in Hp infected guinea pigs. However both positive (GE) and negative (LPS) immunomodulatory activity of Hp antigens has been shown.

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HELICOBACTER PYLORI – DEPENDENT MODULATION OF LYMPHOCYTE CYTOTOXIC ACTIVITY IN A GUINEA PIG MODEL

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INTRODUCTION. *Helicobacter pylori* (Hp) is a causative agent of chronic gastritis and it is involved in the development of gastric cancer. Several virulence factors of Hp may modulate the course of host immune response-towards tolerance or disease development. **AIM.** In the previous study we have shown that Hp lipopolysaccharide (LPS) modulated negatively, whereas Hp glycine acid extract (GE) or *E. coli* LPS positively stimulated lymphocyte cytotoxic activity. Here we made an attempt to establish a guinea pig model for the evaluation of *H. pylori*-driven cytotoxic activity of lymphocytes and to evaluate its sufficiency by the comparison with the results obtained previously on human blood samples. **MATERIALS AND METHODS.** The lymph nodes isolated from Hp infected animals (4 weeks post Hp infection *per os*) and from control animals (inoculated with 0.85% NaCl) were homogenized, washed and cultured for 24 h, 37°C, 5% CO₂ in the milieu of Hp GE, Hp LPS or the LPS of *E. coli* and were further used as effector cells in

the cytotoxicity assay towards guinea pig fibroblasts. The cytotoxic effect was evaluated in MTT reduction assay and expressed as percent of dead target cells (RnD Systems). **RESULTS.** Lymphocyte cytotoxic activity dependent on the type of Hp antigen used for stimulation. Similarly to the LPS of *E. coli*, *H. pylori* GE enhanced cytotoxic potential of lymphocytes, whereas *H. pylori* LPS down-regulated their cytotoxicity. **CONCLUSION.** We demonstrated on guinea pig model that Hp GE stimulate cytotoxic activity, whereas Hp LPS does not possess such potential and thus might be involved in the persistence of infection and the development of chronic, gastric pathologies. These results stays in line with our previous findings carried out on human peripheral blood lymphocytes, what may suggest that guinea pig is an optimal animal model to study the interactions of Hp antigens with the host immune cells.

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RECOMBINANT ENDOSPORES OF *BACILLUS SUBTILIS* CAN ELICIT IMMUNE RESPONSE AGAINST *CLOSTRIDIUM DIFFICILE*

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INTRODUCTION. *Clostridium difficile* is one of the most common infectious agents in nosocomial infections especially associated with antibiotic therapies. In the last years the increasing incidence of *Clostridium difficile* infections leads to necessity of development of alternative therapy. **AIM.** Assessment of induction of immune response against a *C. difficile* antigen exposed on *B. subtilis* endospores. **METHODS.** *Clostridium difficile* flagellar cap protein, FliD, was selected as the antigen. The most immunogenic fragment of FliD was selected with Antigen software to be exposed on the spore surface. To obtain recombinant spores that efficiently express FliD fragment, four coat proteins (CotB, CotC, CotG and CotZ) were used as carriers and the efficiency of display of obtained fusion proteins was tested by western blot analysis. Immunofluorescence microscopy was carried out in order to assess exposition on the spore surface. A quantitative determination of the amount of FliD present on *B. subtilis* spores was obtained

by dot blot analysis. Immunization of the laboratory animals was performed and ELISA tests were performed in order to verify the presence of antigen-specific antibodies in sera. Cytokines secretion was assessed by ELISpot in lymphocytes isolated from the spleen. **RESULTS.** Fusion proteins were successfully obtained for all selected Coat proteins. The higher amount of recombinant protein was observed in strains carrying the fusion with CotG coat protein, while the strongest fluorescent signal was observed in strain carrying the fusion with CotB-Linker. Upon immunization with all recombinant strains, the presence of IgG antibodies was verified in sera obtained from immunized mice. Moreover, the secretion of IL-4 and IFN- γ in splenocytes was assessed. **CONCLUSIONS.** Performed analyses assessed the induction of immune response against selected FliD antigenic fragment in laboratory animals. This led us to claim that recombinant *B. subtilis* endospores represent a good candidate for being used as a vaccine against *C. difficile* infections.

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CREATION OF THE VACCINE AGAINST *CLOSTRIDIUM DIFFICILE* BY ADSORPTION OF FLID TO NON-RECOMBINANT *BACILLUS SUBTILIS* SPORES

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INTRODUCTION. *Bacillus subtilis* is considered to be a non-pathogenic soil bacterium. Usage of its endospores as a surface display system has been widely described. Recently, a new method of presentation of bioactive compounds on the spore surface was reported. Heterogeneous proteins can be adsorbed to the outermost layers of the spores and protected against extracellular conditions.

AIM OF THE STUDY. In this study fragment of FliD – *Clostridium difficile* flagellin – was adsorbed on the surface of *B. subtilis* spores in order to create immunologically active particles, that can serve as an edible vaccine against *C. difficile* infection. **MATERIALS AND METHODS.** The most immunogenic fragment of FliD was selected and its gene was cloned into *Escherichia coli* expression system. Protein was overproduced, purified and adsorbed on the surface of *B. subtilis* spores. Various conditions of the adsorption reaction were tested. The amount of the adsorbed protein was

measured and its location on the surface was proved. Mice were immunized with created constructs in order to elicit immunological reaction. Parameters of the immune response towards FliD were assessed. **RESULTS.** Clostridial FliD was efficiently presented on the surface of non-recombinant *B. subtilis* endospores. The amount of the protein was sufficient to induce strong immune response towards the antigen after administration to mice. Adsorbed proteins were more resistant to harsh conditions in a mouse gastrointestinal tract. **CONCLUSIONS.** Spores as antigen carriers not only can elicit specific immune reaction towards the antigen, but also increase its half life and potency. Adsorption seems to be an effective method to introduce the antigen to the surface of the spore. Non-recombinant spores of *B. subtilis* with the adsorbed clostridial FliD are promising candidates for a vaccine against *C. difficile* infections.

III-P 16

CD25 EXPRESSION AS A MOLECULAR MARKER OF NK CELL CYTOTOXIC ACTIVITY

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INTRODUCTION. Proper cytotoxic capacity of NK cells is critical for adequate innate immune responses. Activated NK cells, besides the production of effector molecules which determine their cytotoxic function, may also overexpress IL-2 receptor (IL-2R, CD25). If so, CD25 could be considered a molecular marker of NK cell activation. **AIM.** The purpose of this study was to evaluate the utility of CD25 molecule as a potential marker of NK cell cytotoxic activity in comparison to well established methods based on the quantification of soluble cytotoxicity markers. **MATERIALS AND METHODS.** Peripheral blood mononuclear cells from 40 donors were isolated and cultured overnight with or without the standard *E. coli* lipopolysaccharide (LPS) O:55 (Sigma) in a final concentration of 25 ng/ml. Non-adherent leukocyte fractions were 1) stained (30 min, 4°C) with murine anti-CD3-Cy-5, anti-CD25-PE and anti-CD56-FITC monoclonal antibodies (eBioscience) and analyzed by flow cytometry (LSR2, Becton Dickinson) using FlowJo Software or 2) used as effector cytotoxic cells (E) towards HeLa target (T) cells in 4-hour

mixed cultures (E:T) : 100 : 1. Cytotoxic effect was measured by the MTT reduction assay by targets (RnD Systems), quantification of soluble cytotoxicity markers in culture supernatants by ELISA: granzyme B (GenProbe), FasL (GenProbe), IFN- γ and IL-2 (RnD Systems) and by the evaluation of CD3⁺CD56⁺CD25⁺ NK cell population. **RESULTS.** Our results revealed that upon *E. coli* LPS stimulation there was a significant enhancement of lymphocyte cytotoxic activity ($p = 0.02$) accompanied by a higher granzyme B ($p = 0.0003$), IFN- γ ($p = 0.004$) and IL-2 ($p = 0.0009$) release. This LPS-driven upregulation of cytotoxicity was positively correlated with an increase in CD3⁺CD56⁺CD25⁺ NK cells: $9.4 \pm 3.1\%$ vs $33.2 \pm 7.9\%$. **Conclusion.** Our results showed that the upregulation of lymphocyte cytotoxicity was associated with the enhancement of soluble cytotoxicity markers release (excluding FasL) and with the expansion of CD3⁺CD56⁺CD25⁺ NK cells ($p < 0.05$, $r < 0.5$). This phenomenon is a result of LPS-induced NK cell activation, and may serve as a surface marker of microbial-activated NK cells.

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NK AND NKT CELL RESPONSES TO *H. PYLORI* LIPOPOLYSACCHARIDE IN RELATION TO LYMPHOCYTE CYTOTOXIC ACTIVITY AND *H. PYLORI* STATUS

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INTRODUCTION. *Helicobacter pylori* (Hp) is a causative agent of peptic ulceration that may promote gastric cancer development. The Hp lipopolysaccharide (LPS) possesses weak endotoxic and

immunogenic properties and strong immunomodulatory capacity. **AIM.** We examined the influence of Hp LPS on the lymphocyte cytotoxic activity and expansion of NK (CD3⁺CD56⁺) and NKT

(CD3⁺CD56⁺) cells from *H. pylori* infected (Hp⁺) and uninfected (Hp⁻) individuals. **MATERIAL AND METHODS.** Peripheral blood mononuclear cells obtained from 22 donors previously classified as Hp⁺ or Hp⁻ (UBT and serology) were cultured overnight with or without the Hp LPS or standard *E. coli* LPS O:55 (Sigma) in a final concentration of 25 ng/ml. Non-adherent fractions were 1) stained with murine anti-CD3-Cy-5 and anti-CD56-FITC monoclonal antibodies (eBioscience) and analyzed by flow cytometry (LSR2, Becton Dickinson) using FlowJo Software or 2) used as effector cells towards HeLa targets in a cytotoxic assay by the MTT reduction method (RnD Systems). **RESULTS.** We showed that the Hp LPS does not induce the propagation of CD3⁻CD56⁺ NK cells, whereas the standard LPS of *E. coli* promotes their expansion in Hp⁻ (p = 0.03) and Hp⁺ (p = 0.0003) donors. The lack of Hp

LPS-driven NK cell expansion was associated with the decrease in lymphocyte cytotoxic activity (Hp⁺, p = 0.04). By comparison, *E. coli* LPS-driven propagation of NK cells was accompanied by the enhancement of lymphocyte cytotoxicity (Hp⁻, p = 0.01; Hp⁺, p = 0.02). However, Hp LPS, as opposed to the LPS of *E. coli*, induced a significant expansion of NKT cells, observed only in the leukocyte cultures from Hp⁺ donors (p = 0.009). **CONCLUSION.** The Hp LPS-driven downregulation of lymphocyte cytotoxic activity accompanied by the expansion of NKT cells, exclusively in Hp⁺ donors, might be explained by the previous *in vivo* stimulation of the immune cells from Hp⁺ individuals with Hp antigens and the adaptive-like properties of iNKT. Further experiments are required to study the possible role of NKT lymphocytes in the course of Hp infections.

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IMMUNOPHENOTYPIC IDENTIFICATION AND CHARACTERIZATION OF PERIPHERAL BLOOD NK CELL SUBSETS BY FLOW CYTOMETRY

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INTRODUCTION. Natural Killer (NK) cells are large granular lymphocytes that belong to the innate immune system and constitute a very heterogeneous cell population, which varies greatly depending on their activation, function and maturity. Still there is no single, universal phenotypic marker for all human NK cells. Thus NK cells are now characterized by the lack of CD3 molecule, and the expression of over 40 surface molecules including CD56, CD16, CD161, CD94 or Nkp46. **AIM.** The aim of this study was to obtain optimized algorithm for NK cell flow cytometric analysis in relation to CD16, CD56 and Nkp46 molecules. **MATERIALS AND METHODS.** Peripheral blood mononuclear cells isolated (Histopaque 1077, Sigma) from 22 donors were stained (30 min, 4°C) with murine anti-CD3-Cy-5, anti-CD56-FITC, anti-CD16-PE and anti-Nkp46-PE monoclonal antibodies (eBioscience) and analyzed by flow cytometry (LSR2, Becton Dickinson) using FlowJo Software. **RESULTS.** Our results showed that in basic morphological analysis (size vs granularity) all leukocyte fractions: mono-

cytes (M), granulocytes (G) and lymphocytes (L) expressed CD16 and CD56 molecules, with various intensity: CD16 (4.6 ± 1.8% M, 3.9 ± 2.4% G, 15.4 ± 2.7% L); CD56 (1.2 ± 0.8% M, 3.6 ± 2.4% G, 11.7 ± 3.9% L). Interestingly, the expression of Nkp46 molecule on cells other than lymphocytes was barely detectable (0.3 ± 0.2% M, 0.1 ± 0.4% G, 10.4 ± 1.8% L). We further analyzed CD3⁻ fraction for the CD56/CD16 or CD56/Nkp46 expression and we found that among CD3⁻CD16⁺ cells there were 5,6% CD16⁺ cells not expressing CD56 molecules, whereas in CD3⁻Nkp46⁺ there were only 0,6% cells without CD56 molecule. **CONCLUSION.** We showed that cells other than NKs may express CD16 and CD56 receptor, but not Nkp46 molecule, which allow direct identification of NK cells in leukocyte fraction as Nkp46⁺ cells. The CD56 and CD16 based NK cell identification requires sequential analysis in which first CD3⁻ cells are gated and further analyzed for the simultaneous presence of CD56 and Nkp46 or CD16 molecules.

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THE EFFECT OF GALLERIA MELLONELLA LYSOZYME AND ANIONIC PEPTIDE 2 ON CANDIDA ALBICANS CELLS

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INTRODUCTION. Lysozyme and antimicrobial peptides play an important role in insect innate immunity against invading pathogens. Antimicrobial activity of defense peptides has been studied extensively, and many peptides have a broad spectrum of activity against Gram-negative and Gram-positive bacteria including antibiotic-resistant ones, fungi, viruses, and parasites. Antimicrobial peptides can also act in synergy with other pep-

tides and proteins, e.g. lysozyme, to kill microbes. The results of our research indicated the synergistic action of *Galleria mellonella* lysozyme and anionic peptide 2 against Gram-negative bacteria. **AIM.** We explored the effect of *G. mellonella* lysozyme and anionic peptide 2 on *Candida albicans* cells. **MATERIALS AND METHODS.** Antifungal activity of *G. mellonella* lysozyme and anionic peptide 2 was determined using the colony counting method on

solid media plates, and the metabolic activity of *C. albicans* cells after incubation with these agents was tested using the LIVE/DEAD staining. Changes on the surface of *C. albicans* cells were imaged and analyzed by atomic force microscopy. **RESULTS.** *G. mellonella* lysozyme used in the concentrations 0.5 μM and 1 μM inhibited *C. albicans* growth by 21% and 45%, respectively, whereas in the concentration 2.5 μM killed nearly 100% of the cells. In contrast, in the case of anionic peptide 2 in concentrations 5 μM and 10 μM

C. albicans growth inhibition was not observed. LIVE/DEAD staining revealed that lysozyme changed cell morphology and decreased metabolic activity of *C. albicans* cells. **CONCLUSIONS.** Lysozyme effectively inhibits the growth of *Candida albicans* at very low concentrations. However, anionic peptide 2 shows no activity against *Candida albicans*.

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ADJUVANTS AS FACTORS THAT RAISE THE EFFECTIVENESS OF ORAL VACCINE AGAINST *HELICOBACTER PYLORI*

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INTRODUCTION. Development of new methods for oral immunization is an interesting alternative to the currently used vaccines, requiring subcutaneous injections. Spores of the non-pathogenic, probiotic *Bacillus subtilis* can be used as a viable and convenient to use carrier for protein antigens able to induce a specific immune response at the site of administration (oral route). The immunogenicity of the antigen may be increased by giving it together with adjuvants that can enhance the effectiveness of vaccination. By using an appropriate adjuvant or a mixture of adjuvants a certain type of response – a humoral and/or cellular – can be obtained. Designing an oral vaccine against *H. pylori* we are using recombinant *B. subtilis* spores presenting urease with oral adjuvants that stimulate the production of Th1-type responses. These adjuvants are chitosan (a polysaccharide, a derivative of chitin) and saponin – a chemical compound belonging to the glycosides. **AIM.** Evaluation of usability of adjuvants (saponin, chitosan) in stimu-

lation of immune response in mice immunized orally against *H. pylori* using recombinant *B. subtilis* spores. **METHODS.** Mice were immunized orally with a suspension consisting of the tested adjuvant and *B. subtilis* spores expressing UreB in fusion with CotC protein. After a series of immunizations, cells isolated from spleens and mesenteric lymph nodes were tested for the secretion of IFN- γ and IL-4 in response to the antigen in ELISPOT test. In the obtained serum and mucosal extracts from the stomach the levels of specific IgG and sIgA antibodies were measured by ELISA test. **RESULTS.** Increased secretion of IFN- γ by splenocytes derived from immunized mice in response to an antigen was observed. The most significant results were obtained when using chitosan as an adjuvant. **CONCLUSIONS.** ELISPOT assay results indicate that a cellular immune response is stimulated, which may indicate the usefulness of recombinant spores as edible vaccines against *H. pylori* infections.

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THE EFFECTS OF (1 \rightarrow 3)- α -GLUCAN ON *GALLERIA MELLONELLA* IMMUNE SYSTEM

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INTRODUCTION. Fungal infection of an insect body triggers both a cellular and humoral immune response. At the beginning, PAMPs (Pathogen-Associated Molecular Patterns) have to be recognized. (1 \rightarrow 3)- β -glucan is the main fungal cell wall component detected by the host immune system and whose immunomodulatory activity has already been confirmed. A less known polysaccharide is (1 \rightarrow 3)- α -glucan and its role in pathogenicity is still unclear. **AIM** We wanted to check the ability of (1 \rightarrow 3)- α -glucan to activate different mechanisms of an immune response of *Galleria mellonella* larvae. **MATERIALS AND METHODS.** We have used larvae of the greater wax moth *Galleria mellonella* as a model organism. The larvae were immunized using different doses of (1 \rightarrow 3)- α -glucan, which has been isolated from the *Aspergillus niger*. The hemolymph of the larvae was obtained at a different time after the immunization. The α -glucan effect on melanization was evaluated using a phenoloxidase-activating assay. Antibacterial and antifungal activity

of hemolymph was determined with the use of inhibition zone assay on agar plates with *Escherichia coli* and *Aspergillus niger*, respectively. Electrophoretical methods let us show differences in hemolymph protein profiles. **RESULTS.** In the hemolymph of the α -glucan-challenged larvae, the phenoloxidase system was activated or suppressed, depending on the dose of α -glucan and on the time after the injection. Antibacterial properties of hemolymph increased together with time after the immunization and reached maximum 8 h after the challenge. It was also correlated with the dose of α -glucan, the larger the dose, the higher the antibacterial activity. The antifungal activity was detected 24 h after immunization and remained at a steady level for the next two days. These observations were confirmed and explained by tricine-SDS/PAGE showing the presence of antimicrobial peptide bands. **CONCLUSIONS.** (1 \rightarrow 3)- α -glucan as the fungal cell wall component triggered insect immune reactions and could be considered as PAMP.

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INHIBITION OF TLR SIGNALING PATHWAYS BY ECTROMELIA VIRUS IN RAW 264.7 MOUSE MACROPHAGES

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INTRODUCTION. Ectromelia virus (ECTV) belongs to the genus *Orthopoxvirus* whose members, such as variola virus (VARV) and vaccinia virus (VACV) are well known for their abilities to influence both innate and adaptive immune responses. Toll-like receptors (TLRs) are the elements of innate immune response. TLR signaling pathways involve the activation of nuclear factor κ B (NF- κ B) as well as the production of proinflammatory cytokines. The presence of orthologs of well-known VACV genes influencing such events in ECTV genome suggests similar properties of ECTV-encoded proteins. **AIM.** The aim of this study was to investigate the influence of ECTV on intracellular distribution of p65 subunit of NF- κ B transcription factor as well as the production of proinflammatory cytokines in RAW 264.7 macrophages. **METHODS.** *Immunofluorescence analysis.* Cells were infected with highly virulent Moscow strain of ECTV (ECTV-MOS) and stimulated through TLR3 using a synthetic polyriboinosinic: polyribocytidylic acid (poly(I:C); 50 μ g/ml), or TLR 4 using lipopolysaccharide (LPS from *Escherichia coli* O55:B5; 1 μ g/ml) at different times post infection. Immunofluorescent analysis was performed using FITC-conjugated poly-

clonal antibodies against viral antigens, rabbit monoclonal anti-p65 antibodies and donkey anti-rabbit secondary antibodies conjugated with Rhodamine Red-X. DNA was stained with Hoechst 33342. Enzyme-linked immunosorbent assay. After 3 hours post infection (hpi) cells were stimulated with poly(I:C); 50 μ g/ml, or LPS (1 μ g/ml). Concentrations of interleukin (IL)-6 and tumor necrosis factor (TNF)- α in culture supernatants were determined using OPTEDIA ELISA KITS. **RESULTS.** Our studies showed that in ECTV-MOS-infected RAW 264.7 cells nuclear translocation of NF- κ B transcription factor as well as the secretion of IL-6 and TNF- α by infected macrophages were significantly decreased. **CONCLUSIONS.** We conclude that ECTV-MOS interferes with TLR3 and TLR4 signaling pathways, which involve NF- κ B activation and induce the production of proinflammatory cytokines. ECTV-encoded proteins enable evasion of host response, which is typical for well-known mammalian poxviruses, such as VACV.

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THE ROLE OF IL-18 IN DENDRITIC CELL INTERACTIONS WITH *M. BOVIS* BACILLE CALMETTE-GUÉRIN (BCG)Piotr Szpakowski^{1*}, Magdalena Kowalewicz-Kulbat¹, Franck Biet², Joël Pestel³, Wiesława Rudnicka¹¹ Department of Immunology and Infectious Biology, University in Lodz, Poland² UMR ISP1282, INRA Centre de Tours, Nouzilly, France; ³ UMR 8576, IFR 147 Université Lille 1 Sciences et Technologies, France

INTRODUCTION. The initiation and maintenance of specific responses to infective agents depend on dendritic cells (DC) playing the role of antigen presenting cells. IFN- γ has been demonstrated to be crucial to antimycobacterial immunity. The production of this cytokine by Th1 cells is effectively enhanced by IL-18, however, the role of IL-18 in human responsiveness to *M. bovis* BCG vaccine remains poorly understood. **AIM.** We asked a question whether human IL-18 released by the recombinant BCG strain (rBCGhIL-18) influences DC driven response to mycobacterial antigens in healthy humans who were vaccinated with BCG in childhood. **MATERIALS AND METHODS.** Immature DC were obtained from CD14⁺ monocytes, separated by magnetic sorting and stimulated for 6-days with IL-4 and GM-CSF. Dendritic cell response to live rBCGhIL-18 and BCG strains was estimated on the level of DC receptors (flow cytometry) and cytokines (ELISA)

in 24-h cultures. **RESULTS.** Both mycobacterial strains caused a significant decrease in specific dendritic cell DC-SIGN receptor and an increase in CD86 co-receptor of the immune synapse. Neither rBCGhIL-18 nor BCG changed the expression of CD40 and HLA-DR on DC. Recombinant rBCGhIL-18 but not nonrecombinant BCG enhanced the DC expression of CD80 co-receptor and production of IP-10 chemokine, which promotes T cell migration to lymph nodes. Recombinant rBCGhIL-18 stimulated the production of T cell activating IL-23 and regulatory IL-10 by DC more intensively than nonrecombinant BCG. **CONCLUSIONS.** The obtained results suggest that IL-18 can positively modulate adaptive immune response to BCG bacilli by the intensification of antigen presentation and regulatory functions of dendritic cells.

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III-P 24

DEFECTIVE T CELLS RESPONSE TO MACROPHAGE SIGNALING IN TUBERCULOSIS

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INTRODUCTION. Immune recognition of mycobacteria leads to the cellular activation, which is manifested in the increase in expression of receptors and production of pro- and anti-inflammatory cytokines. These molecules recruit immune cells to the areas of infection, coordinate the inflammatory and adaptive immune

response and control the outcome of the infection, from a latent *M.tb* occupation to active tuberculosis (TB). However, a biomarker of TB protection remains unknown. **AIM.** The aim of the study was an attempt to better understand the macrophage – T cell interaction in active tuberculosis. **MATERIALS AND METHODS.** In total,

123 adults were enrolled in the study: 43 patients with active tuberculosis (TB), 40 patients with nonmycobacterial lung diseases (NMLD) and 40 healthy individuals (HC). In freshly isolated PBML the surface expression of mCD14 and LFA-1 integrin on monocytes and LFA-1 on T cells was measured by flow cytometry. The concentrations of IFN- γ , sFasL, IL-10, were determined in whole blood cultures stimulated for 24 hours with live *M.tb* H₃₇R_v or *M. bovis* BCG. **RESULTS.** The overexpression of mCD14 was observed on monocytes from TB patients as compared to NMLD subjects and HC individuals. Otherwise, a significant increase in monocyte LFA-1 was found in both TB and NMLD groups. The BCG or *M.tb* H₃₇R_v driven

IFN- γ production was significantly lower in TB patients as compared to other groups. **CONCLUSIONS.** The simultaneous increase in monocyte mCD14 and LFA-1 associated with the suppression of IFN- γ and sFasL response to live mycobacteria in TB patients allows suggesting that a T cell defect in responsiveness to mycobacteria driven macrophage signaling can be considered a potent cause of an impairment in *M.tb* resistance leading to active TB.

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III-P 25

FACTORS AFFECTING THE DECISION CONCERNING INFLUENZA VACCINATION AMONG MEDICAL STUDENTS AND STUDENTS OF OTHER MEDICAL FACULTIES

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Influenza is one of the most common cyclic respiratory diseases in humans. Methods of prevention are multidirectional but the most effective and most efficacious way to prevent influenza and its complications are preventive vaccines. This paper is to determine different factors affecting the decision about an influenza vaccine. The percentage of people vaccinated against flu was evaluated, and their knowledge of post-influenza complications, etc. among full-time students and bridging studies of nursing and physiotherapy (full-time and part-time) at the University of Technology and Life Sciences in Radom and students of medicine and pharmacy at the Medical University of Łódź. The research tool was the author's questionnaire with 18 questions. The surveys conducted, consisting of multiple choice questions, were anonymous. Despite the advice of the experts, the popularity of vaccination among the studied group of students was at a low level. In total, the survey involved 241 students. The overall number of persons who had the vaccine against

influenza in the epidemic season 2012/13 was 16 respondents, representing 6.6% of the total group. In each group of students the figure for a group of students of nursing was 6.2%, for physiotherapy students 5.3%, for students of medicine and pharmacy 14.3%. The percentage of respondents who would get vaccinated if it was free of charge was also low. As the most common causes for no vaccination against influenza, future doctors, pharmacists, nurses and physiotherapists said there is no such need, or that they forgot, or that it makes no sense to be vaccinated because one can still get sick, etc. A small percentage of medical students and students of other medical faculties who are regularly vaccinated against influenza and low willingness for individual actions in this area suggest that further efforts should be focused on working out a comprehensive program improving knowledge relating to flu and the effective prevention by vaccination.

III-P 26

DETECTION OF INFLUENZA VIRUS YESTERDAY AND NOW

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The influenza virus is a cause of morbidity and mortality in patients with low immunity but also in healthy persons. Medical diagnostics, due to the similar clinical symptoms in the course of various respiratory infections of viral etiology, is extremely difficult. Influenza, as an acute infectious disease occurring epidemically, was probably known already in antiquity, as may be confirmed by Hippocrates and Livy records of 412 B.C. Between 1173 and 1427 outbreaks were recorded in different European countries. The first documented pandemic influenza was that of 1580. In the nineteenth century the greatest influenza epidemic was the one of 1889. In that time the etiologic factor of influenza was not known. Among the social factors which were conducive to its rapid spread were demographic changes and development of transport. F.H. Parsons, a London physician, while analyzing the course of flu epidemic of 1889–1890 suggested that the influenza

spreads "person to person". It was a very important idea because earlier divergent theories were presented to explain the epidemic of influenza, e.g. miasmatic contra contagious. The isolation of the human influenza virus in 1933 triggered intensive virological and serological tests. Previous methods used in routine tests were based on the detection of influenza virus antigen and its isolation in egg embryos or cell lines. Molecular biology techniques associated with amplification of RNA improved the quality of tests and improved the sensitivity of detection of influenza virus in clinical samples. It became possible to detect mixed infections caused by influenza type A and B and to conduct subtypes within the type A. The development of virological diagnostics for influenza at this time enables to eliminate the use of antibiotics, which has no medical justification, as well as reduce the cost of medical care associated with the flu in the population.

MICROBIAL VIRULENCE FACTORS IN HUMAN, ANIMAL AND PLANT INFECTIONS

PLENARY LECTURES

IV-PL 1

GRAM-NEGATIVE BACTERIA AND LPS – SOME ASPECTS OF 65 YEARS OF RESEARCH

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Endotoxin was identified by Richard Pfeiffer in the year 1892, but it took some additional 60 years to find the appropriate extraction protocol by which the isolation of quite purified endotoxin could be performed. Soon after it became clear that endotoxin consisted of sugars, phosphates and fatty acids, and was thus termed a lipopolysaccharide (LPS). Purified LPS from *Salmonella* displayed endotoxic and antigenic activities, and the first feature could be assigned to the lipid A and the second to the polysaccharide, which was called the O-antigen. In the early 1960ies it had become clear from analyses of a number of LPS obtained from *Salmonella* that LPS possessed common sugars and those that occurred only in particular LPS. Consequently, it was claimed that all *Salmonella* LPS should possess a common core substituted by the O-antigen. In 1971, a general LPS architecture was published which is still valid for most LPS today. Data had indicated that lipid A was a phosphorylated and acylated

GlcN disaccharide however, phosphodiester bridges between these units could still not be excluded. This was also the case concerning the core region which was identified as oligosaccharide, based on the availability of a variety of rough(R)-type mutants, first obtained from *Salmonella*. Also, a novel method for the extraction of in particular R-type LPS was invented. Later, additional mutants obtained from other bacteria, like *Escherichia coli* and *Citrobacter* were obtained and their LPS investigated, and so-called SR-mutants were available which possessed in their LPS a complete core region plus one or few repeating unit(s). From the mid-1980ies on, owing to improved analytical tools and methods including modern NMR spectroscopy and mass spectrometry, and to the development of protocols which e.g. brought about the isolation of a complete lipid A-core backbone, not only many O-antigen structures but also a lot of lipid A-core structures from a range of bacterial species have been identified.

IV-PL 2

HOW TO TURN A BACTERIAL PATHOGEN INTO A PROBIOTIC-LIKE ORGANISM

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The interactions of bacteria with the human host can have different outcomes, ranging from severe and deadly disease with pathogenic bacteria on one extreme of the spectrum to health benefit with probiotic bacteria on the other end. *Bordetella pertussis* is the main causative agent of whooping cough, a severe childhood disease that still causes 200,000 to 300,000 deaths every year, despite the wide use of efficacious vaccines. Most deaths occur in children too young to be protected by current vaccine programs. Based on the observation that natural infection of very young infants with *B. pertussis* is able to trigger strong B and T cell responses and to promote the development of Th1-type immunity, and based on the fact that natural infection usually results in long-lasting immunity of convalescent patients, we have developed a live attenuated *B. pertussis* vaccine strain for nasal administration. This strain, named BPZE1, was attenuated by the genetic removal or detoxification of

three major toxins. It was found to be safe in several animal models, including severely immunocompromized animals, yet able to colonize transiently the mouse respiratory tract and to induce rapid and long-lasting protection upon a single nasal administration. A phase I study in humans has recently been completed and shown safety and immunogenicity in humans as well. Interestingly, the attenuation not only resulted in a total absence of virulence, but it also conferred probiotic-like properties to BPZE1. As such, nasal application of BPZE1 protected mice against influenza virus- or pneumococcal-induced death by dampening the cytokine storm induced by these agents, without affecting viral or bacterial load. In addition, administration of BPZE1 provided significant protection against experimental asthma and dermatitis, revealing the anti-inflammatory properties of the organism. By genetic attenuation we have thus transformed virulent *B. pertussis* into a probiotic-like organism.

IV-PL 3

EMERGING GLOBAL LANDSCAPE OF THE HOST RANGE AND GENETIC DIVERSITY OF HANTAVIRUSES

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INTRODUCTION. Rodents were suspected as possible reservoirs of the etiological agents of hemorrhagic fever with renal syndrome (HFRS) long before the seminal discovery of Hantaan virus in

the striped field mouse (*Apodemus agrarius*) in Korea. Although Thottapalayam virus (TPMV), a previously unclassified virus isolated from an Asian house shrew (*Suncus murinus*) captured in

India a decade earlier, was shown to be a hantavirus, it was assumed to represent a spillover event from a rodent reservoir. Whole genome analysis, however, demonstrated that TPMV formed an entirely separate clade, suggestive of an early evolutionary divergence from rodent-borne hantaviruses. **AIM.** Guided by these data and the long-forgotten reports of HFRS antigens in shrews and moles and empowered by the generosity of mammalogists and museum curators who provided access to archival tissue collections, an aggressive search was conducted to ascertain the host range, genetic diversity, phylogeography and evolution of hantaviruses. **MATERIALS AND METHODS.** Frozen, ethanol-fixed or RNAlater®-preserved tissues from 1,546 shrews (9 genera, 47 species), 281 moles (8 genera, 10 species) and 520 bats (26 genera and 53 species), collected in Europe, Asia, Africa and North America during 1981–2012, were analyzed for hantavirus RNA using

RT-PCR. **RESULTS.** Sequence analysis indicated 23 novel genetically distinct hantavirus species in shrews, moles and bats. Phylogenetic analysis, using maximum-likelihood and Bayesian methods, showed five distinct lineages and evidence of co-divergence and host switching. **CONCLUSIONS.** Newfound hantaviruses detected in Soricomorpha and Chiroptera are genetically more diverse than those harbored by rodents, suggesting that the evolutionary origins of hantaviruses is more ancient and complex than previously conjectured and that ancestral rodents may not have been the original mammalian hosts of primordial hantaviruses. Rapid acquisition of new knowledge about the spatio-temporal distribution and host range of hantaviruses provides strong justification for the expansion and long-term maintenance of tissue repositories.

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IV-PL 4

PREVENTING NOSOCOMIAL WATER-BORNE INFECTION BY CONTROLLING WATER AND WATER SYSTEMS

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Nosocomial infections with *Legionella pneumophila* and *Pseudomonas aeruginosa* is a problem in may hospitals. In Denmark, 25% of all nosocomial *L. pneumophila* cases were observed in the Rigshospitalet before year 2000 and there was an increased incidence rate of *P. aeruginosa* bacteremia/septicemia. Thus, there was focus on nosocomial water-borne diseases. **WATER SUPPLY.** The water in a hospital is not better than what is delivered. The material of the pipes and the flow of water the main and side pipes are the most important factors in biofilm formation and bacterial overgrowth. The public supply cold water whereas hot water is produced in the hospital. **HOT WATER.** The temperature of hot water has to be sufficient high as many bacteria including *L. pneumophila* and *P. aeruginosa* can adapt to relative high temperatures. Pipes with mixed cold and hot water increase the risk for biofilm. **TAPS.** Taps should be simple constructed with separate hot and cold water.

Combined taps and electronic activated taps contain many components and different materials that may increase the risk of biofilm formation. **IRRADIATORS.** Several different types of irradiators are available. Irradiators have large surfaces with risk for biofilm formation. It is important to have a cleaning program. **SHOWERS.** Keeping the water running for 10 min before use reduces the bacteria count in less than 50% of the showers. Heat decontamination of shower head and pipe should be done daily as biofilm occur within two days. Culture of water samples detects 5–10% of the bacteria in a water sample. The strategy for prevention will depend on the degree of the problem. By controlling water systems, nosocomial *L. pneumophila* has almost been eliminated and the incidence rate of nosocomial *P. aeruginosa* bacteremia/septicemia has been reduced 75% at Rigshospitalet.

IV-PL 5

STRUCTURE, SEROLOGY, AND GENETICS OF *PROVIDENCIA* O-ANTIGENS

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INTRODUCTION. Medically important *Providencia* species, *P. alcalifaciens*, *P. stuartii*, and *P. rustigianii*, are mostly associated with urinary tract infections and enteric diseases. Their combined serotyping scheme is based on lipopolysaccharide (LPS) O-antigens and includes 63 O-serogroups. **AIM.** The present studies aim at creation of chemical and genetic basis for serological classification and molecular identification of *Providencia* strains and substantiation of their antigenic relationships to other bacteria. **MATERIALS AND METHODS.** O-antigen structures were analyzed by chemical methods, NMR spectroscopy, and mass spectrometry. A putative O-antigen gene cluster (OGC) was sequenced in

a number of serogroups and gene functions were predicted *in silico*. The functions of genes involved in GDP-colitose and dTDP-D-Qui4Nfo synthesis were verified *in vitro*. **RESULTS.** The unique O-antigen structures of 36 O-serogroups were established, and many of them were found to contain unusual monosaccharides and non-carbohydrate components. Structural data enable substantiation on the molecular level of serological relationships within the genus *Providencia* and between strains of *Providencia* and bacteria *Proteus*, *Escherichia*, and *Salmonella*. OGC was found in the chromosomal region between the housekeeping genes *cpxA* and *yibK* and characterized in 10 *Providencia* O-serogroups.

A peculiar feature of the bacteria studied is the occurrence of genes for synthesis and expression of capsular polysaccharide in the OGC. **CONCLUSIONS.** The data of the O-antigens obtained

validates the serological classification of *Providencia* isolates and opens the way for development of a PCR typing method for their identification.

ORAL PRESENTATIONS

IV-O 1

CHEMISTRY AND IMMUNITY OF LPSs ISOLATED FROM CYSTIC FIBROSIS PATHOGENS

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Persistent bacterial infection is a hallmark of the cystic fibrosis disease with episodic worsening “exacerbations” leading to multiple courses of antibiotics, hospitalization and premature death. Characteristic CF pathogens include *Burkholderia cenocepacia* and a newly identified organism *Pandoraea pulmonicola*. The role of abovementioned pathogens in the course of CF lung disease merits further study at the molecular level. Several potential virulence factors have been defined for CF pathogens connected with bacterial pathogenicity. The most important virulence factor is the lipopolysaccharide (LPS) molecule, that has an important immunostimulatory activity. We have elucidated the O-chain moiety of LPS produced

by the emerging clinical isolate *Pandoraea pulmonicola*; this strain showed to possess a novel structure of O-chain which consists of a particular trisaccharide repeating unit. One common mechanism of resistance against antimicrobial peptides in Gram-negative bacteria is the addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) to the LPS. *Burkholderia cenocepacia* is extraordinarily resistant to antimicrobial peptides but the molecular basis of this resistance is not well understood. We demonstrated that L-Ara4N modification of LPS is the most critical determinant for the resistance of these bacteria to antimicrobial peptides.

IV-O 2

ACTIVITY AND PRELIMINARY IDENTIFICATION OF THE ANTIMYCOBACTERIAL COMPOUND ISOLATED FROM METABOLITES OF THE EARTHWORM GUT BACTERIUM *RAOULTELLA ORNITHINOLYTICA*

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INTRODUCTION. The medical value of earthworms has been widely known in the history of Asian medicine. These invertebrates are a vital component of traditional Chinese medicine. The earthworm extract has been described to treat over 80 diseases. **AIM.** The aim of the study was to analyse the action and identification of the antimycobacterial compound obtained from metabolites of the *R. ornithinolytica* bacteria associated with the gut of *Dendrobaena veneta* earthworms. The bacteria showed lytic activity against various strains of *Mycobacterium*, both saprophytic and pathogenic. **MATERIALS AND METHODS.** The viability and morphological changes were analysed using confocal (test Live-Dead) scanning, transmission and atomic force microscopes. For chemical identification of the compound the Fourier Transform-Infrared Spectroscopy (FTIR) was used. The changes in the chemical composition of the cell wall were determined using the Surface Enhanced

Raman Spectroscopy (SERS). The elemental composition of the obtained compound was performed by EDX (Energy Dispersive X-ray). Using LC-MS the antimycobacterial compound was compared to the already known peptide – nisin. The cytotoxic effect against human skin fibroblasts was determined using the BrdU assay (Roche). **RESULTS.** The results of our research indicated that the obtained compound significantly decreased the viability of *Mycobacterium smegmatis* at the concentration of 12.5 µg/ml. The wall of the cells was damaged and the morphological changes were observed. Chemical identification by FTIR demonstrated high similarity to nisin. The obtained compound possesses minimal (5% after 72 hours) cytotoxicity against human fibroblasts. **CONCLUSIONS.** The EDX, LC-MS and SERS analyses showed differences to nisin and proved that the antimycobacterial compound has a complex structure.

IV-O 3

DOES THE CONCENTRATION OF GLUCOSE AFFECT THE QUANTITY AND CHEMICAL STRUCTURE OF THE *P. AERUGINOSA* BIOFILM FORMED IN PATIENTS WITH CHRONIC DIABETIC FOOT WOUNDS?Agnieszka Machul^{1*}, Diana Mikołajczyk¹, Piotr B. Heczko¹, Sabina Górską², Magdalena Strus¹¹Department of Microbiology, Jagiellonian University Medical College, Cracow, Poland²Institute of Immunology and Experimental Therapy, National Academy of Sciences, Wrocław, Poland

INTRODUCTION. The team is hard to heal wounds in diabetic patients is a growing problem in Poland, due to the increase in the number of patients treated for metabolic deficiencies. Lack of an effective method for inhibiting biofilm which is composed of microcolonies of bacteria immersed in a three-dimensional matrix called EPS (Extracellular Polymeric Substances), weakens targeted antibiotic therapy. Elevated blood glucose level directly affects the growth of the population of all bacterial pathogens, in particular, *P. aeruginosa*. Development of a biological model will be effective in overcoming barriers to biofilm. **AIM.** The aim of this study was to investigate differences in the quantity and biochemical structure of biofilm depending on the level of glucose in patients with diabetes. **MATERIALS AND METHODS.** Strains belonging to the family *Pseudomonas*, which were used in the experiments came from diabetic patients (n = 6) treated for chronic wounds feet. Quantitative changes in the biochemical structure of the biofilm produced in a minimal medium containing 70 mg% and 300 mg% glucose was

tested according to a modified procedure described by Allison et al. (1984). Isolation exopolysaccharide done by extraction with 10% trichloroacetic acid (TCA) and the biochemical characterization was carried out using mass spectrometry MALDI-TOF. **RESULTS.** The study showed a significant difference between the production of a biofilm by the same *Pseudomonas aeruginosa* strains (n = 6) in medium supplemented and not supplemented with glucose. Analysis showed that the exopolysaccharide biochemical structure in both medium is the same and containing: arabinose, rhamnose, mannose, galactose, galactosamine, glucose, glucosamine and uronic acids. **CONCLUSIONS.** In patients with metabolic diseases increased levels of glucose significantly modifies the number of secreted exopolysaccharide, but doesn't change its structure. With these proposals we will be able to approach the development of a biological model of biofilm formation in patients with diabetes. The research carried out under the grant N N401 547040 and statutory grant: K/ZDS/002861.

IV-O 4

EFFECT OF STRESS FACTORS ON EXTRACELLULAR POLYSACCHARIDES SYNTHESIS IN *PROTEUS MIRABILIS* BIOFILMMagdalena Moryl^{*}, Aleksandra Kaleta, Kacper Strzelecki, Antoni Rozalski

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INTRODUCTION. Matrix (EPS) is a basic component of bacterial biofilm, it consists of water (97%), proteins, polysaccharides, nucleic acids and phospholipids. Polysaccharides synthesis is influenced by many factors e.g. environment conditions or carbon source. **AIM.** Analysis of differences in levels of extracellular polysaccharides in *P. mirabilis* biofilms after cultivation in stress conditions. **METHODS.** 22 *P. mirabilis* strains were isolated from biofilms on urinary catheters collected from long term catheterized patients. Biofilms of *P. mirabilis* strains were cultivated on polystyrene plates for 24, 48, 72 hours, 1 and 2 weeks in tryptone soya broth or in modified media containing: extra glucose (500 mM), albumin (1.54 mg/ml), cefotaxime (32 µg/ml) or in medium with reduced nutrient content or with reduced pH (4). Protein levels were studied by Lowry's method. Total polysaccharides were estimated by the sulfuric-phenol method modified by Masuko. N-Acetylglucosamine and N-Acetylgalactosamine levels were calculated by the

ELLA test with the use of selected lectins (WGA and *Helix pomatia*). **RESULTS.** All tested strains formed biofilm on polystyrene plates and the amount of detected protein levels was 5–25 µg/ml. In optimal growth conditions the levels of polysaccharides ranged from 0 to 0.19 µg/mg of proteins. Among all studied components, cefotaxime, stimulated the highest production of polysaccharides by tested *Proteus mirabilis* strains. Its addition in the subinhibitory concentration caused an almost 3.5-fold increase in the level of polysaccharides in the 15 tested strains. After biofilm cultivation in media supplemented with glucose or albumin, a 3-fold increase in the polysaccharide level was detected in the case of 17 or 13 tested strains, respectively. **CONCLUSIONS.** The agents used in this study have a significant impact on the level of polysaccharides in the biofilm of the tested *Proteus mirabilis* strains. The highest levels of polysaccharides were detected after one week incubation in the case of most tested strains.

IV-O 5

DERIVATIVES OF 5-ARYLIDENEIMIDAZOLONE – NEW TOOLS TO COMBAT MDR BACTERIA

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INTRODUCTION. According to World Health Organization (WHO) infectious diseases are involved in more than 15 million deaths each year. One of the reasons for this situation is the

increasing resistance of pathogenic bacteria to available antibiotics. Microbial efflux pumps play a key role in multidrug resistant (MDR) strains. One of the ways to combat MDR is search for efflux

pump inhibitors (EPIs) able to inhibit pump system responsible for drug efflux. **AIM.** Evaluation of EPIs properties among new 5-arylideneimidazolone derivatives. **MATERIALS AND METHODS.** Compounds were tested on strains of *Enterobacter aerogenes* with different expression of AcrAB-TolC pump, using following microbiological assays: (i) evaluating intrinsic antibacterial activity of compounds, (ii) chemosensitizing antibiotic efficacy, (iii) using isobolograms to show cooperation between compounds and antibiotics, (iv) blocking the expelling of fluorescent dye in the real time efflux assays. **RESULTS.** Compounds with free piperazine moiety at position 2 decreased the value of antibiotics MIC in strains overexpressed the AcrAB-TolC pump. The highest activity

possessed derivatives with p-chlorobenzylidene and naphthylmethylidene substituent at position 5. Tested compounds cooperated with chloramphenicol, doxycycline and nalidixic acid in synergistic way. Results obtained for erythromycin indicated additive effect. The highest activity in the real time efflux test was observed for 5-fluorenelidene, 5-naphthylmethylidene and 5-p-chlorobenzylidene derivatives. **CONCLUSIONS.** 5-arylideneimidazolone derivatives with free piperazine fragment at position 2 seemed to be the most potential "adjuvants" among tested compounds. The results allowed us to perform structure-activity relationship (SAR) studies that were important in the design of new lead structures required to improve the compound activity against bacterial efflux mechanism.

POSTERS

IV-P 1

DETECTION OF RHD (RABBIT HAEMORRHAGIC DISEASE) VIRUS IN LIVER CELLS BY REAL-TIME PCR

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INTRODUCTION. RHDV causing rabbit hemorrhagic disease is a virus, which so far could not be multiplied in vitro, therefore its rapid diagnosis is difficult, and the emergence of real-time PCR has created new possibilities for the identification of the virus. **AIM.** The aim of this study was the application of real-time PCR to qualitative determination of RHDV in liver cells of rabbits infected with 10 strains of the RHDV from different European countries, with different biological features (hemagglutination capacity and antigenic variants). **MATERIALS AND METHODS.** The study was performed on liver tissue derived from rabbits infected with 10 tested strains of the RHDV. Liver tissue was collected after the fall of the animals (24, 36 h p.i.). Then, it was homogenized in a 1:3 ratio with PBS and genetic material was isolated. Then the complementary strand of the nucleic acid (cDNA) was obtained in the reverse transcription reaction. cDNA obtained after reverse

transcription was amplified using the technique of real-time PCR with a fluorescent intercalating dye SYBR[®]GreenI. **RESULTS.** As it appears from the results, all samples confirmed the presence of the RHDV in the material with high accuracy, as indicated by both the Ct (cycle number of the reaction, at which the sample fluorescence reached logarithmic growth phase), but also the melting point of the product, which was measured to eliminate the possibility of a non-specific products as a result of fluorescent dye SYBRGreen. **CONCLUSIONS.** It should be noted that the application of real-time PCR in the virological diagnosis of the RHDV, seems to be highly appropriate and specific, as the technique of real-time PCR enables to shorten the reaction time, due to the elimination of a postamplification processing step, and because of the nature of performing this analysis (one tube-capillary) the risk of contamination is minimized. Financed from research grant no. N308 03832/3662.

IV-P 2

ANALYSIS OF CIPROFLOXACIN DISTRIBUTION IN *PROTEUS MIRABILIS* BIOFILM BY LASER INTERFEROMETRY

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INTRODUCTION. Currently, studies of antibiotics diffusion in bacterial biofilms have been limited. These studies based on, for example, antibiotic indicators might change mass, structure or their hydrophilic properties in comparison to native forms of testing substances. **AIM.** We propose a novel application of the laser interferometry method in analysis of antibiotics diffusion through *P. mirabilis* O18 biofilm and its distribution in biofilm structure. This method, based on the phenomenon of wave interference, enables quantitative substance assays by light refractive index changing in real time. **MATERIALS AND METHODS.** Biofilm of *P. mirabilis* O18 with a pDsRed2 prokaryotic expression vector that encodes red fluorescence protein, was formed for 96 h in stationary conditions on sterile nucleopore membrane with pores diameter 0.9 μm, as element of membrane system from laser interferometry equipment. After bacteria growth, the microscopy images of stained membranes

(by crystal violet) or obtained by fluorescence microscopy were collected and the % degree of covering nucleopore membrane by biofilm was determined. The measurements of ciprofloxacin diffusion through *P. mirabilis* O18 biofilm as well as in biofilm structure were measured by laser interferometry system. **RESULTS.** The amount of transported ciprofloxacin through *P. mirabilis* O18 biofilm after 40 min was 30.156% of the initial concentration 1.5 mmol/L. and covered nucleopore membrane surface ($7.0 \times 10^{-5} \text{ m}^2$) in 78.281%. Additionally, the amount of antibiotic in biofilm was increased from $1.28 \times 10^{-8} \text{ mol}$ to $1.86 \times 10^{-8} \text{ mol}$ for times 2 min. and 40 min., respectively. **CONCLUSIONS.** We suggested that laser interferometry method is useful for in real time determination of antibacterial agents diffusion through bacterial biofilms.

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IV-P 3

PHENOTYPE, TOXOTYPE AND GENOTYPE OF *BACILLUS CEREUS* FOOD ISOLATESAgnieszka Bednarczyk-Draż^{1*}, Elżbieta Daczowska-Kozon¹, Ewa Mosiej², Ewa Augustynowicz²¹Department of Applied Microbiology and Biotechnology, West Pomeranian University of Szczecin, Poland²Department of Sera and Vaccines Evaluation, National Institute of Hygiene, National Institute of Public Health, Warsaw, Poland

INTRODUCTION. *B. cereus* group species are genetically closely related and differ in their pathogenicity and host specificity. It has been speculated that emetic strains of *B. cereus* sensu lato form a more phenotypically and genetically homogenous group than other group representatives. **AIM.** The aim of the study was to compare toxic potential, phenotype and genetic profiles of emetic and non-emetic *B. cereus* group strains of food origin. **MATERIALS AND METHODS.** The analyses were performed on 42 emetic and 46 non-emetic food isolates. On the basis of 72 phenotypic features tested (morphology, hemolytic, amyolytic and phospholipase activity, growth at different temperatures, API 50CHB + API20E tests) phenotypic similarity of the strains was assessed using UPGMA method (Statistica 8.0, StatSoft). Toxic potential of the strains was determined by testing the presence of 13 gene fragments encoding virulence factors (enterotoxins, haemolysins, phospholipases and cereulide). Strains genotypic diversity was tested with AFLP assay of DNA digested with *EcoRI* and *MseI* and RAPD-PCR.

Cluster analysis of RAPD profiles was performed with BioGene (Vilber Lourmat) using UPGMA with 3% Dice coefficient. Analysis of AFLP profiles was performed with GelCompar II (Applied Maths) using 1% Pearson coefficient. **RESULTS.** The majority of the emetic *B. cereus* strains tested differed phenotypically from non-emetic ones with two emetic strains (from cereals and muesli) significantly phenotypically different. The analyses revealed broad distribution of the genes encoding virulence factors among *B. cereus* group strains with no significant difference between emetic and non-emetic strains. Several RAPD-PCR profiles did not clearly distinguish emetic strains from other representatives of the group, although most of the emetic strains were closely related. AFLP analysis showed relatively high diversity of emetic *B. cereus* food isolates with most strains divided into four clusters. The research was funded by the National Science Center, Poland, during the years 2010–2012 as a research project NN312234538.

IV-P 4

PATHOGENIC POTENTIAL OF COMMENSAL *ESCHERICHIA COLI* DERIVED FROM HEALTHY HUMANS

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INTRODUCTION. Majority of *E. coli* strains are harmless commensals, but others might cause intestinal or extra-intestinal disease. Epidemiological studies suggest close relationship between the presence of virulence genes (Vgs) and pathogenicity of the strains. Accumulation of appropriate set of virulence genes can transform the commensal strain into a pathogen. **AIM.** The aim of this study was to evaluate the prevalence of Vgs associated with intestinal and extra-intestinal pathotypes of *E. coli* among strains from healthy humans. **MATERIALS AND METHODS.** This study focused on the group of 85 nonidentical commensal *E. coli* strains collected from healthy humans. All strains were screened for the presence of 21 Vgs belonging to 5 functional groups: toxins (*stx1*, *stx2*, *elt*, *estI*, *estII*, *east1*, *ehxA*, *hlyA*), adhesins (*escV*, *bfpB*, *fimH*, *papA*, *sfaS*), iron uptake (*chuA*, *fyuA*, *ireA*, *iroN*, *iutA*), protectins (*kpsMTII*, *kpsMTIII*), related to biofilm formation (*agn43*), using PCR method. **RESULTS.** 13 Vgs were identified among tested strains. In the 5 above-mentioned functional groups the following genes were detected: toxins (*east1* with frequency 3.5%; *hlyA*

– 14.1%), adhesins (*fimH* – 96.5%; *papA* – 37.7%; *sfaS* – 2.4%), iron uptake (*chuA* – 65.9%; *fyuA* – 77.7%; *ireA* – 37.7%; *iroN* – 35.3%; *iutA* – 64.7%), protectins (*kpsMTII* – 72.9%; *kpsMTIII* – 8.2%), related to biofilm formation (*agn43* – 78.8%). Analysis revealed the presence of only two Vgs related to intestinal pathotypes: EaggEC (*east1*) and EHEC (*chuA*), but 11 connected with extra-intestinal ExPEC pathotypes (*hlyA*, *fimH*, *papA*, *sfaS*, *fyuA*, *ireA*, *iroN*, *iutA*, *kpsMTII*, *kpsMTIII*, *agn43*). **CONCLUSIONS.** These results demonstrated that human's commensal *E. coli* are not a reservoir of Vgs characteristic for intestinal pathotypes, but the examined strains carry all tested Vgs related to extra-intestinal pathotypes, responsible for pathogenesis outside the gastrointestinal tract. These genes are necessary to attach to and invade host tissues for toxin production, iron uptake, overcome host defense and biofilm formation. The studies on *E. coli* commensal strains can provide insights into the evolution of virulence.

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IV-P 5

ESSENTIAL OILS (EO) REDUCE INVASIVE PROPERTIES OF *CANDIDA ALBICANS*. STUDY ON SELECTED EO

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INTRODUCTION. Despite the discovery of potent antimycotics, most types of systemic and local *Candida* infections still remain a serious medical problem. In recent years, anti-infective drugs that

target virulence factors, are considered as the most promising. Various virulence factors have been characterized in *C. albicans* such as germ tube formation, adherence, production of enzymes, and

formation of biofilms. In the past few decades interest in natural products has increased, and medicinal plants have been investigated for various biological activities and therapeutic potentials including those mentioned above. **AIM.** To investigate the effects of selected essential oils used at biocidal sub-inhibitory concentrations on the inhibition of germ tube formation/filamentation of *C. albicans*. **MATERIALS.** Reference strain: *C. albicans* ATCC 10231; essential oils (EO) of: *Melissa citrata indica* (Lemon balm oil), *Cymbopogon citratus* (Citronella oil), *Pelargonium graveolens* (Geranium oil), *Eugenia caryophyllata* (Clove oil). **METHODS.** The germ tube and filamentation were induced in a RPMI-1640 medium containing calf serum – FCS (10% v/v). *Candida* suspensions were exposed to EOs at MIC for 1h or at ½ MIC for 24 h. Then, the cell number with germ tubes and filaments were counted microscopically.

RESULTS. In the presence of subMIC of tested EOs, germ tubes formation was reduced by approximately 100–98% and aggregate-like structure (mycelium) formation was inhibited when compared with cells cultured in the absence of EO. The most active EO was Lemon balm oil, which in the first four hours of co-incubation inhibited germ tube formation by 100–80%. **CONCLUSION.** On the basis of the obtained results it is suggested that the inhibition of germ tube formation and filamentation is possible by the action of essential oils. This study demonstrated the efficiency of all essential oils tested to inhibit germ tube formation of *C. albicans*, however, the lemon balm was the best. The obtained results encourage for further investigations of their action in various circumstances. The work was supported by the National Research Center, Poland Grant No 2011/01/N/NZ6/00317.

IV-P 6

DIFFERENCES BETWEEN *SALMONELLA* ENTERITIDIS STRAINS RESISTANT AND SENSITIVE TO SERUM BASED ON 2-DIMENSIONAL ELECTROPHORETIC PATTERNS OF OUTER MEMBRANE PROTEINS

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INTRODUCTION. Serovar *Salmonella* Enteritidis is the most frequently isolated in Poland. It can lead to sepsis if *Salmonella* cells enter the bloodstream. The dynamic changes in the expression of the bacterial surface structures, mainly outer membrane proteins (OMP), play a decisive role in generating the resistance of the bacteria to the serum. **AIM.** To determine the difference in the OMP patterns between clinical *S. Enteritidis* strains resistant and sensitive to the bactericidal action of serum. **MATERIALS AND METHODS.** Clinical *S. Enteritidis* strains are derived from patients with the symptoms of diarrhea. *S. Enteritidis* strains were identified using the traditional diagnostic methods and were confirmed with the MALDI-TOF Biotyper method. Human serum (HS) was taken from the healthy volunteers in Regional Center

of Blood Donation and Treatment in Wrocław, Poland. The bactericidal activity was determined using 75% HS. For OMP isolation detergent Zwittergent 3–14[®] was used. For 2-D electrophoresis (2DE) IPG Ready Strips 4–7 were applied with pH gradient and 7 cm long (Bio-Rad). Electrophoresis was performed with constant power current. Material on 2-DE gels was visualized by the silver staining method. Protein spots were analyzed by PDQuest software (Bio-Rad). **RESULTS.** Bactericidal activity of HS against *S. Enteritidis* strains shows that one strain was resistant to 75% NHS. The analysis of 2-DE gels showed differences between the optical density and the OMP presence. **CONCLUSIONS.** These OMPs are potentially responsible for generating the resistance of the bacteria to the serum.

IV-P 7

THE MICROHETEROGENEITY IN THE LPS O-CHAINS LENGTH IN *SALMONELLA* ERLANGEN

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INTRODUCTION. Lipopolysaccharide (LPS) plays a decisive role in Gram-negative bacteria pathogenesis. *Salmonella* Typhimurium can produce 3 lengths of OAg with different number of O-specific units: 1–16, 16–35 (L-OAg) and more than 100 (VL-OAg). L-OAg generates serum resistance of *S. Typhimurium*. There is no data about the length of OAg of *Salmonella* serovars causing reptile associated salmonellosis (RAS), but RAS can lead to death because of sepsis. **AIM.** The aim of this study was the examination if the length of O-Ag, measured as NeuAc/Kdo ratio, before and after passages of *Salmonella* O48 in human serum (HS), remains constant in further generations. **MATERIALS AND METHODS.** Bacterial strain: *Salmonella* Erlangen was passaged in 50% HS. NeuAc/Kdo ratio was measured in bacterial colonies using gas-liquid chromatogra-

phy/tandem mass spectrometry analysis (GC-MS/MS). Inoculum of tested colonies was collected and frozen in LB medium in –70°C for 3 months. After this time, bacteria from each collected colony were sown reductively on agar plates. Again 5 random colonies were chosen to measure the ratio of NeuAc/Kdo. **RESULTS.** Preliminary data indicated differences among % NeuAc/Kdo ratio in the range 138.9–1.25% (before passages in serum) and 123.9–43.9% (after passages in serum). The data showed, that this ratio is not constant and increased after 3 months storage of the bacterial cells in –70°C. Our data can indicate the microheterogeneity in the length of O-chains of *S. Erlangen* LPS. **CONCLUSIONS.** After passages of bacteria in the serum, the NeuAc/Kdo ratio increased, which suggests the participation of VL-OAg in the serum resistance phenomenon.

IV-P 8

DISTRIBUTION OF VIRULENCE MARKERS IN *AEROMONAS* SPP. STRAINS ISOLATED FROM POLISH POND-RAISED FISH

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INTRODUCTION. *Aeromonas* spp. are inhabitants of a wide range of aquatic ecosystems. These Gram-negative, opportunistic bacteria have been associated with human infections such as gastroenteritis caused by contaminated food of animal origin. Pond-raised carp and trout are a popular food fish with annual production in Poland estimated at 33,000 tons. **AIM.** We asked whether strains containing virulence factors associated with human diseases are widespread amongst *Aeromonas* spp. isolated from Polish farm-raised fish. **MATERIALS AND METHODS.** Bacteria isolated from infected pond fish (carp, trout) were identified to a species level (PIWET, Pulawy). PCR assays for detection of virulence-factor genes encoding cytotoxic enterotoxin (*act*), aerolysin/haemolysin (*aerA*), cytotoxic enterotoxins (*ast*, *alt*), serine protease (*ser*), and glycerophospholipid: cholesterol acetyltransferase (*gcaT*) were performed with DNA of the isolates using specific primers. **RESULTS.** PCR amplification demonstrated that all the strains harboured at least two of four toxin genes. The oligonucleotide primers spe-

cific for *act* amplified a 230-bp fragment of the gene from 96% of the isolates. An amplified 430-bp portion of the *aerA* gene was revealed in almost all the isolates. The identity of the amplicon was confirmed by *Sau3AI* digestion. PCR amplification was also attempted for a 330-bp region of *alt* and a 440-bp region of *ast* and revealed that from 27% to 38.5% of the isolates harboured these genes, respectively. Primers specific for the amplification of the 350-bp and 240-bp regions of the *ser* and the *gcaT* genes, respectively, yielded PCR amplicons of extracellular enzymes from 58% to 100% of isolates. **CONCLUSIONS.** *Aeromonas* secrete a variety of toxins that enhance the severity of many infections. The distribution of virulence-related genes amongst isolates from Polish pond-raised fish confirms that *Aeromonas* are genetically heterogeneous and when they harbour aerolysin/hemolysin genes, they are considered potential foodborne pathogens.

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ANTIMICROBIAL SUSCEPTIBILITY OF *RHODOCOCCLUS EQUI* ISOLATED FROM WILD BOARS

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INTRODUCTION. The occurrence of an increased antimicrobial resistance of *Rhodococcus equi* has been well described for human and equine isolates. However, antimicrobial susceptibility of strains isolated from the wildings, such as wild boars, has not been yet investigated. **AIM.** The aim of this study was to evaluate the antimicrobial susceptibility of *R. equi* strains isolated from wild boars, and to compare the results with those obtained from other mammalian's isolates (literature data). **MATERIALS AND METHODS.** A total of 24 strains of *R. equi* was isolated from the submaxillary lymph nodes of wild boars. MIC₅₀ and MIC₉₀ of 8 antimicrobials, i.e. tetracycline (TC), enrofloxacin (EF), cefotaxime (CT), cephalothin (CE), clindamycin (CH), amoxicillin/clavulanate (XL), rifampicin (RI), and erythromycin (EM) were determined using the E-test (ranges for TC, CT, CE, CH, XL, EM were 0.016–256, and for EF, RI were 0.002–32 µg/ml). Results were categorized using CLSI guidelines

for *Staphylococcus* sp in accordance with literature. **RESULTS.** Values of MIC₅₀ for these antibiotics were 1.5 (TC), 0.75 (EF), 3 (CT), >256 (CE), 0.094 (CH), 8 (XL), 0.125 (RI), and 0.25 (EM), whereas values of MIC₉₀ were 3 (TC), 1.5 (EF), >256 (CT), >256 (CE), 0.125 (CH), 16 (XL), 0.38 (RI), 0.25 (EM). Compared with literature data, MICs of tetracycline, clindamycin, and rifampicin were significantly lower for the wild-boar isolates than for isolates of different origin, whereas MICs of enrofloxacin, and erythromycin were quite similar in both cases. In contrast, MICs of cephalothin and amoxicillin/clavulanate were significantly higher in case of the wild-boar isolates. As for cefotaxime, values of susceptibility were too scattered, which made them impossible to compare with the literature data. **CONCLUSIONS.** *R. equi* strains isolated from wild boars demonstrated higher susceptibility to tetracycline, clindamycin, and rifampicin than strains isolated from other sources.

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MOLECULAR TECHNIQUES USED IN IDENTIFICATION OF ZOOPHILIC DERMATOPHYTES ISOLATED FROM PETS

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Infection of the keratinised tissues (skin, hair, nails, claws) in human and animals are caused by pathogenic fungi – dermatophytes and results as a dermatophytosis. These fungi are classified

as anthropophilic, zoophilic or geophilic species which belong to the genera *Epidermophyton*, *Microsporum* or *Trichophyton*. Conventional laboratory methods are based on detection of fungal

elements in microscopic slides and phenotypic characteristics of *in vitro* culture. Those methods are still the gold standard of dermatophyte identification in routine laboratory diagnostic procedure although they are either slow or lack specificity. The application of PCR methods has made rapid, simple and precise detection and identification of dermatophytes possible. The aim of this work was to investigate the possibility of using PCR method for molecular identification of two species of dermatophytes isolated from animals. **METHODS.** Clinical isolates were obtained from the Division of Mycology of Warsaw University of Life Sciences-SGGW. All clinical isolates were identified by observation of macro- and micromorphology. The strains were cultured on Sabouraud agar and incubated for up to 14 days at 30°C. After harvest, from pure

cultures fungal DNA was extracted according to the two-step method proposed by Brillowska-Dabrowska et al. (2007). Subsequently a pan-dermatophyte specific PCR was performed using the appropriate primers. **RESULTS.** Using the pan-dermatophyte PCR and the PCR condition described by Brillowska-Dabrowska (2007) both specific and unspecific products were obtained. **CONCLUSIONS.** Although, the analysis targeting the pan-dermatophyte DNA gave positive results, it needs to be optimized. Only optimization of temperature-time profile allowed obtaining specific products (366 bp). The necessity of optimization was probably the result of using different reagents than in the publication. The reagents contain amplification amplifiers that probably increase the annealing temperature.

IV-P 11

CYTOTOXICITY IN *BACILLUS CEREUS* GROUP IS ASSOCIATED WITH THE SCOPE OF HUMAN ACTIVITY

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INTRODUCTION. *Bacillus cereus*, *Bacillus thuringiensis* and *B. anthracis* belong to the *Bacillus cereus* group and have a huge impact on human health and economy. Although there are many reports of toxin production and diseases caused by *B. cereus* and *B. thuringiensis* strains, little is known about such properties among *B. mycooides* and *B. pseudomycooides*. **AIM.** We decided to investigate genetic structure and cytotoxicity of *B. cereus* strains isolated from environments with different scope of human activity. **MATERIALS AND METHODS.** Using the MLST method, we analyzed 276 isolates from three areas in NE Poland differing in the scope of human activity: the agriculture field in Jasienówka, the buffer zone of the Biebrza National Park, and the protected area of the Białowieża National Park. Isolates were classified into three species: *B. cereus*, *B. thuringiensis* and *B. mycooides/B. pseudomycooides*. Additionally, to

identify potential food poisoning properties, the presence of *cytK* gene was analysed. **RESULTS.** The environmental isolates were divided into four phylogenetic groups, and cluster I included most of soil isolates (74.0%). Gene *cytK* was localized in 32.94% strains from Jasienówka with the majority of *B. mycooides/pseudomycooides*. In the other two areas less related with human activity, the Biebrza marsh and the Białowieża National Park, gen *cytK* was in 4.21% or did not occur, respectively. **CONCLUSION.** Our results showed a high degree of polymorphism in the *B. cereus* group and lack of clear phylogenetic divisions between the species. Environment with a high scope of human activity favors the evolution of cytotoxic strains that could contaminate crops from field and cause serious food poisoning. Interestingly, *B. mycooides* isolates could potentially cause food poisoning.

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STUDIES OF THE POSSIBILITY OF *PROTEUS MIRABILIS* STRAINS TRANSMISSION AMONG PATIENTS FROM ŁÓDŹ REGION

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INTRODUCTION Typing of bacterial isolates is important in epidemiological studies and allows determining the source of infection and detecting the transmission of pathogens. Because of the increasing clinical relevance of *Proteus mirabilis* rods, selection of the effective methods of typing these organisms is essential for epidemiological investigation. **AIM.** The aim of this study was to examine the possibility of *P. mirabilis* strains transmission among patients in Łódź region, based on the relatedness between clinical strains belonging to the serogroups most common in Łódź: O6, O10, O11, O16, O20, O27, O28, O77, and O78. **MATERIALS AND METHODS.** The study included 195 clinical *P. mirabilis* strains obtained from various sources in four Łódź laboratories and classified to the mentioned O serogroups. Research was realized using a phenotypic method – the Dienes test and a molecular typing technique – rep-PCR using the BOX A1R primer and a pair of the REP 1R-Dt and REP 2-Dt primers. **RESULTS.** Based on the simple and effective Dienes test, groups of related strains in each of the tested

serogroups were identified. The rep-PCR technique yielded complex electrophoretic patterns of chromosomal DNA amplicons for the investigated isolates. Based on the electrophoretic picture the dendrograms were prepared, showing the degree of similarity and evolutionary distance between the isolates within the serogroups. The bands common for all the strains were considered to be characteristic for *P. mirabilis* species. To a large extent the discriminatory power of the selected methods was similar, which confirms the reliability and usefulness of the techniques in epidemiological investigations. **CONCLUSIONS.** The obtained results indicate the occurrence of strains transmission among patients. Homologous strains, especially in serogroups O11, O20, O77, and O78, probably originated from highly pathogenic clones, widely occurring in patients. The ecological success of unrelated isolates belonging to the studied serogroups may be due to their O-antigen structure. The work was supported by grant no. N N401 020135 from the Ministry of Science and Higher Education (Poland).

IV-P 13

ANTIBACTERIAL ACTIVITY OF SELECTED THIOSEMICARBAZIDE DERIVATIVES AGAINST *MYCOBACTERIUM SMEGMATIS*

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INTRODUCTION. Increasing antibiotic resistance of bacterial pathogens causes that it is desirable to find new effective antibacterial agents. Thiosemicarbazides are derivatives of acid hydrazides, which according to literature data, exhibit antibacterial, fungicidal, antiparasitic, anti-inflammatory, antitumor, antinociceptive, anti-convulsant, antihypertensive, and antidepressant properties. **AIM.** Recently, we have reported that 30 selected agents from our collection show antibacterial activity against some reference species. In the present study we investigated their activity against *Mycobacterium smegmatis*. **MATERIALS AND METHODS.** 30 thiosemicarbazide derivatives were synthesized at the Department of Organic Chemistry at Medical University of Lublin. The minimal inhibitory concentration (MIC) of these agents against *M. smegmatis* mc²155 was determined using both the alamarBlue assay and the micro-

dilution method recommended by CLSI. Moreover, the minimal bactericidal concentration (MBC) was estimated. **RESULTS.** Our results revealed that 25 of 30 compounds have strong antibacterial activity against *M. smegmatis* ranging from 2 to 64 µg/mL. Five agents showed unsatisfactory, high MIC value (≥ 500 µg/mL). For all tested compounds the MBC values were in the range similar to MICs, suggesting their bactericidal activity (MBC/MIC ≤ 2). **CONCLUSIONS.** The presented results show that examined thiosemicarbazide derivatives apart from antibacterial activity against the reference set of species have also strong activity against *M. smegmatis*. These features make them good candidates in the search for effective antibacterial drugs to combat other mycobacteria such as *M. tuberculosis* etiological agent of tuberculosis – a disease which is still very difficult to treat.

IV-P 14

MOLECULAR DIAGNOSIS OF *BABESIA MICROTI* AND *BARTONELLA* SPP. INFECTIONS IN WILD RODENTS FROM AREAS OF LOWER SILESIA, POLAND

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INTRODUCTION. *Babesia microti* and *Bartonella* are the etiologic agents of so-called emerging diseases, recognized as threats to the public health. Free-living rodents are a potential reservoir of infection. **AIM.** The aim was to analyze the occurrence of *B. microti* and *Bartonella* spp. in the spleen of wild rodents, an organ involved in host response to infection. **MATERIALS AND METHODS.** A total of 85 small wild rodents: *Apodemus flavicollis* (27), *Apodemus agrarius* (41) and *Myodes glareolus* (17) were captured in 2011 in several areas of Lower Silesia. DNA was isolated from the spleen using GeneMATRIX Bio-Trace DNA Purification Kit (EURx). To detect *B. microti*-specific DNA, nested PCR was conducted using an outer pair of primers Bab1, Bab4 (238 bp products) and inner one Bab2, Bab3 (154 bp), which amplify a specific fragment of the gene encoding 18S rRNA. Primer pair BhCS 781, BhCS 1137n, specific for the fragment of citrate synthase (*glcA*)

gene was used for the detection of *Bartonella* spp. (about 380 bp). PCR products were analyzed in 1% agarose gel. **RESULTS.** *Bartonella* spp. DNA was detected in 35.3% of rodents, as follows: 22.2% *A. flavicollis*, 34.1% *A. agrarius* and 58.8% *M. glareolus* were infected. In the case of *B. microti* 43.5% spleen samples were PCR-positive. Protozoan DNA was detected in 40.7% *A. flavicollis*, 43.9% *A. agrarius* and 47.1% *M. glareolus*. Co-infection with two pathogens was found in 17.6% of studied rodents. Due to the small number of animals examined, the study should be continued. **CONCLUSIONS.** *B. microti* and *Bartonella* DNAs were detected in all three species of rodents from Lower Silesia area. The high prevalence of infection and the presence of co-infections highlights the important role of wild rodents as a reservoir of pathogens in the environment. The results also point to the role of the spleen in the course of infection.

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PROTOPORPHYRIN IX-MEDIATED PHOTOINACTIVATION REVEALS OTHER THAN ANTIMICROBIALS RESISTANCE MECHANISMS DIFFERENCES BETWEEN MRSA AND MSSA STRAINS

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INTRODUCTION. Due to the emergence of multidrug resistance among pathogenic microorganisms, the development of alternative antimicrobial strategies seems to be required. The concept of photodynamic inactivation involves cell exposure to appropriate wavelengths light that leads to the excitation of photosensitizer molecules,

resulting in the production of reactive oxygen species responsible for cell inactivation and death. Recently, we have demonstrated strain-dependent response of *Staphylococcus aureus* to photoinactivation, and observed elevated resistance to photodynamic treatment among methicillin-resistant (MRSA) strains. Nevertheless, the mechanism

underlying this phenomenon remains unexplained. **MATERIALS AND METHODS.** Thus, the aim of the current study was to investigate statistically relevant differences in *S. aureus* response to PPIX-mediated photoinactivation applied to 424 MRSA/MSSA isolates. Moreover, with the use of VITEK 2 Advanced Expert System, we aimed to elucidate if the observed variation in strains susceptibility to photodynamic treatment results from antimicrobial resistance

mechanisms and strains susceptibility to conventional antibiotic therapy. **RESULTS.** The data obtained demonstrated that MRSA are significantly more resistant to photoinactivation than MSSA strains, however, the difference observed did not result from antimicrobial susceptibility or resistance mechanisms. Furthermore, regardless of strains origin (surveillance isolates or infections) similar effectiveness of photodynamic inactivation could be reached.

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AN ATTEMPT TO USE BACTERIAL TOXIN LISTERIOLYSIN O (LLO) WITH MONOCLONAL ANTIBODY ANTI-CD20 IN THERAPY

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INTRODUCTION. Bacterial toxins are among the most potent naturally occurring substances with anti-cancer activity. Our attention was paid to listeriolysin O, the main virulence factor of intracellular pathogen *Listeria monocytogenes*. Listeriolysin O (LLO) belongs to a family of haemolysins. It is characterized by cytolytic activity to biological membranes containing cholesterol. Rituximab (RTX) is a chimeric monoclonal antibody specific for an epitope located at the large loop of CD 20 antigen. **AIM.** Using flow cytometry we investigated the effect of bacterial toxin listeriolysin O (LLO) and monoclonal antibody rituximab (RTX) on a human cell line Raji (B-cells). **MATERIALS AND METHODS.** In the experiment, we used a modified listeriolysin O. Cells

of Raji cell line were incubated for 120 min at 37°C in 5% CO₂ at various concentrations of LLO, RTX, and a mixture of these compounds. The effect of the tested agents was observed in the presence of the complement system of human plasma obtained from healthy volunteers. **RESULTS.** *In vitro* experiment involving complement cytotoxicity observed additive growth of cytotoxicity of the mixture of rituximab and bacterial toxin listeriolysin O on human cell line Raji. **CONCLUSIONS.** Comparing the mechanisms of action and the results of our observations *in vitro* we assume that the combined effect of bacterial toxin listeriolysin O and antibody rituximab may give better therapeutic effect *in vivo*.

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VIRULENCE PROTEINS OF *TOXOPLASMA GONDII* LOCATED IN THE PARASITE'S RHOPTRIES – BIOSYNTHESIS OF RECOMBINANT FORMS

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Toxoplasma gondii is a common protozoan parasite of human and veterinary medical importance. The parasite can cause a severe and even life-threatening disease in both congenitally-infected and immunocompromised people. Given the above and the high prevalence of toxoplasmosis, there is a clear premise to develop a prophylactic/therapeutic vaccine. The host cell invasion by *T. gondii* is inextricably linked to the discharge of specialized apical organelles, micronemes and rhoptries. Some rhoptry proteins secreted by the parasite are targeted into the host cell nucleus, while the others associate with the parasitophorous vacuole membrane. They are particularly well suited to alter host cell functions. Two rhoptry kinases, ROP5 and ROP18, have recently become an object of special interest due the pivotal role in parasite virulence, which is attributed to them. Remarkably, the pseudokinase ROP5 has been reported to control the activity of ROP18, thereby blocking parasite clearance in macrophages, mediated by immune-related

GTPases. Thus, ROP5 seems to act as a ROP18 enhancer. In this study we cloned and expressed recombinant forms of ROP5 and ROP18 proteins to be used as candidates for vaccine development. Gene fragments corresponding to whole proteins, N-termini and C-termini were PCR-amplified, introduced into the pHis.Parallel1 vector and expressed in *E. coli* BL21(DE3) cells in the presence of IPTG inducer. The recombinant proteins were found in inclusion bodies, which were subsequently dissolved under denaturing conditions using urea and/or guanidine hydrochloride. The proteins, purified by affinity chromatography, were eventually analyzed by SDS-PAGE and western blot. Full length recombinant ROP5 and ROP18 proteins have been successfully biosynthesized and will be used in further animal studies on the immunoprotective efficacy as they are considered to be vaccine candidates. This work was supported by the National Science Center in Poland, grant UMO-2011/03/N/NZ6/04655.

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ASSESSMENT OF THE PREVALENCE AND DISTRIBUTION IN THE BREEDING ENVIRONMENT OF VRE AND VSE *ENTEROCOCCUS* SPP. STRAINSKrzysztof Skowron¹, Alicja Jeleńska^{2*}¹Department of Microbiology, Nicolaus Copernicus University, Collegium Medicum of L. Rydygier, Bydgoszcz, Poland²Department of Microbiology and Food Technology, University of Technology and Life Sciences, Bydgoszcz, Poland

INTRODUCTION. Intensive animal production may cause environmental contamination and sanitary-epidemiological threat. The animal excrements may contain pathogenic, antibiotic resistant bacteria. The usage of antibiotics in animal production led to developing resistance mechanisms by animal-borne microorganisms, often transferred to the environmental bacteria. The piggery employees, veterinarians, slaughterers and biogas plant workers are predestined to infections with the strains from animal production. **AIM.** The aim of this study was assessment of *E. faecalis*, *E. faecium*, *E. durans* and *E. hirae* frequency in samples from the chosen swine production sectors, determination of the percentage of vancomycin resistant strains and the type of this resistance. Moreover, the determination of genetic relationship between obtained isolates was also conducted. **MATERIALS AND METHODS.** The swabs from feeding troughs, feeding passages, slurry channels and fecal samples collected from the particular sectors of piggery were obtained. Moreover, samples were gathered from the loading ramp

and platform. Genus identification was conducted with the primers specific to 16S rRNA region and species identification – with primers specific to the sequence of gene *sodA* in Multiplex PCR reaction. Resistance to vancomycin at the concentration $6 \mu\text{g} \times \text{ml}^{-1}$ was tested on Muller-Hinton Agar. To assess the resistance type Multiplex PCR, amplifying products corresponding to genes VanA, VanB and VanC, was conducted. Genotyping was conducted using PCR-RAPD method. **RESULTS.** Among 195 isolates 60% belonged to *E. hirae*, 22% – *E. faecalis*, 12% – *E. durans* and 7% – *E. faecium*. Only 2 strains of *E. hirae* were VRE (VanC₁ phenotype). On the basis of genotyping 60 genetically different strains were determined. **CONCLUSIONS.** The obtained results indicated low percentage of VRE strains in the tested piggery. They also marked the genomic inheritance of natural VanC₁ resistance to low vancomycin concentrations. The possible contamination paths involve mash trolley and spreading of slaughtered fatteners' excrements or on personnel's footwear.

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EXPRESSION OF ENTEROTOXINS GENES IN SOIL *BACILLUS THURINGIENSIS* ISOLATES

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INTRODUCTION. *Bacillus thuringiensis* is an entomopathogenic soil bacterium, classified to the *Bacillus cereus*. This bacterium produces during sporulation produce crystals containing delta-endotoxins of insecticidal properties. Moreover, many strains harbor genes encoding HBL, NHE, and CytK enterotoxins, which increase their virulence and may pose a danger for human health. The expression of these genes in *B. thuringiensis* is poorly known. **AIM.** The aim of the study was estimation of the the hblA, nheA and cytK genes expression among *B. thuringiensis* strains using two alternative methods: the real-time PCR and the Duopath test. **MATERIALS AND METHODS.** About 50 *B. thuringiensis* strains isolated from soil collected in the Biebrza National Park and Lithuania and 30 *B. thuringiensis* reference strains were tested during the investigation. The commercially available Duopath test was applied to assess the production of HBL and NHE enterotoxins in *B. thuringiensis*

strains under study, while the expression of the genes encoding the enterotoxins was assessed in the real-time PCR method. **RESULTS.** The real-time PCR analyses showed different levels of the expression of enterotoxins' genes among examined isolates. Although the majority of the *B. thuringiensis* strains harbored the enterotoxin genes, only a low number of them were found to express the genes. The Duopath tests in most cases confirmed these results. However, in some parts they did not agree with each other, indicating a visible lack of the presence of the product encoded by the examined gene. **CONCLUSIONS.** High occurrence of the enterotoxin genes among *Bacillus thuringiensis* strains implies an increase of potential sanitary and epidemiological threat to people. The comparison of the two methods, real-time PCR and Duopath test, in the assessment of the enterotoxin genes expression, indicated a higher sensitiveness and credibility of the real-time technique.

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GENETIC DIVERSITY *REPA*- AND *REPX*-POSITIVE *BACILLUS CEREUS* ISOLATES FROM RAW AND PROCESSED MILK

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INTRODUCTION. *Bacillus cereus* is a Gram-positive, mobile, aerobic, spore-forming bacterium, classified similarly to *Bacillus anthracis* – anthrax etiologic factor, as *Bacillus cereus sensu lato* group.

Recent reports indicate the occurrence in *Bacillus cereus* repA and repX genes, determining replication of pXO1- and pXO2-like plasmids, appropriate for *B. anthracis*. Due to the common prevalence

of *B. cereus* in foodstuffs, for example in raw and processed milk, the lack of data on the relationships between repA- and repX-positive *Bacillus cereus* isolates with virulent *B. anthracis* strains is dangerous.

AIM. The aim of this study was to assess the genetic similarity of *B. cereus* isolates acquired from raw and processed milk, with repX and repA genes by means of analysis of the nucleotide sequences of housekeeping genes (MLST) and genotyping by REP-PCR.

MATERIALS AND METHODS. The analysis of the sequences of the housekeeping genes (MLST) and genotyping by the REP-PCR for twenty four *Bacillus cereus* isolates from raw and processed milk.

RESULTS. By comparison of the nucleotide sequences of seven

housekeeping genes (*glp, gmk, ilv, pur, pyc, pta, tpi*) a high level of genetic diversity among bacteria under study was revealed. However, isolates having typical genes for pXO1- and pXO2-like plasmids form a clear standing apart branches on the phylogenetic tree. Genotyping by the REP-PCR method confirms the results obtained by MLST technique. **CONCLUSIONS.** Widespread occurrence of *repA* and *repX* genes among different genotype bacilli indicates a high probability of horizontal transfer of pXO1- and pXO2-like plasmids among *B. cereus sensu lato* representatives. Therefore, it is extremely important to make effort to protect food against contamination with these *B. cereus sensu lato* bacilli.

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EFFECT OF THE HOMOSERINE LACTONE ON PROTEUS MIRABILIS O18 BIOFILM

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INTRODUCTION. It is known that chemical substances like derivatives of homoserine lactones have a significant impact on the bacterial biofilm formation process. Derivatives of homoserine lactones are involved in communication between cells of the bacteria. The signaling molecules allow for communication between bacteria, so that they can adapt to changing environmental conditions. Understanding the phenomenon of communication between bacteria may be useful in the treatment of *Proteus* spp. infections. **AIM** of this study was to determine the effects of homoserine lactone on ureolytic activity of *Proteus mirabilis* biofilm. **MATERIALS AND METHODS.** To determine the effective concentration of BHL (N-butanoyl homoserine lactone, Fluka) absorbance of crystal violet associated with the *Proteus mirabilis* O18 biofilm grown in dilutions of the substance was measured at $\lambda = 595$ nm. Bacteria were inoculated into a 6-well plate and then incubated at 37°C. Three wells of

the culture were supplemented with 10 nM homoserine lactone, the remaining wells were control. Urea (2%; w/v) and pH indicator (phenol red) were added to wells with formed biofilm. The absorbance at a wavelength of 560 nm for 30 minutes was measured. **RESULTS.** Studies revealed that 10 nM concentration of BHL is efficient for alternation of *Proteus mirabilis* O18 biofilm thickness. It has been showed by the increase in the thickness of the biofilm was measured by crystal violet. Assays with biofilm treated with BHL revealed that urea hydrolysis was conducted more efficient compared to control without the signaling molecule. BHL increased urea hydrolysis by average 10.8%. **CONCLUSIONS.** It has been proven that the homoserine lactone helps to increase the biofilm layer thickness. The ureolytic activities of cells in the biofilm were increased by homoserine lactone. Despite the fact that we observed an increase in the biofilm layer, urea did not diffuse into the cells in the thickness biofilm.

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ECA EXPRESSION IN YERSINIA ENTEROCOLITICA SEROTYPE O:3 IS REGULATED BY TEMPERATURE

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INTRODUCTION. *Yersinia enterocolitica* O:3 (YeO3) bacteria express on the cell surface lipopolysaccharide (LPS) and enterobacterial common antigen (ECA); LPS more abundantly than ECA. ECA is anchored to the outer membrane either *via* phosphatidylglycerol (ECA_{PG}) or lipid A-core of LPS (ECA_{LPS}). Expression of most virulence factors in YeO3 is temperature-dependent. **AIM.** To elucidate the temperature-dependence of ECA expression in YeO3 by Western blotting (WB) using monovalent anti-ECA antisera. **METHODS.** Polyvalent rabbit antisera against three YeO3 R mutants cultivated at 22°C and 37°C were adsorbed with live isogenic ECA-negative bacteria to remove anti-LPS antibodies. The adsorbed and non-adsorbed antisera were analyzed by WB using as antigens the ECA_{PG} standard preparation, LPS_{PCP} preparations and whole cell lysates from ECA-negative and -positive YeO3 strains grown at 22°C and 37°C. **RESULTS.** The non-adsorbed antisera reacted strongly with all LPS_{PCP} preparations and lysates and with

ECA_{PG} indicating that the antisera contained specific anti-core LPS and anti-ECA antibodies. In contrast, the adsorbed antisera showed no reaction with the ECA-negative strains but a strong immunostaining with ECA_{PG}, suggesting that only anti-ECA antibodies remained in these antisera. Interestingly, lysates of YeO3 R mutants cultivated at 22°C reacted a little stronger with the adsorbed antisera than corresponding samples from 37°C. This indicated higher ECA expression at 22°C than at 37°C, which is consistent with the results reported earlier. However, similar differences were not observed for corresponding LPS_{PCP} preparations. **CONCLUSIONS.** The results obtained for lysates from R mutants of YeO3 suggested that ECA expression is regulated by temperature being more effective at 22°C than at 37°C. Due to difficulties in quantitative control of the many steps of LPS isolation by the PCP extraction the whole cell lysates proved to be more reliable samples to study the influence of the cultivation temperature on ECA expression than the LPS_{PCP} preparations.

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AERATION CONDITIONS AFFECT PHOTOANTIMICROBIAL CHEMOTHERAPY

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INTRODUCTION. The recent report indicates the significance of microorganisms' cultivation conditions and diffusion of oxygen into culture media in experiment outcome and points out the need of considered research design and description (Somerville, Proctor; BMC Microbiology 2013). Aeration conditions seem to be important in photodynamic inactivation (PDI) – an attractive method of multiresistant human pathogen eradication – which is based on the integrated activity of light, photosensitizer and molecular oxygen. **AIM.** Analysis of the effect of aeration conditions in photodynamic inactivation of *Staphylococcus aureus*, a facultative anaerobic research model. **MATERIALS AND METHODS.** Reference *S. aureus* strains: Newman and its isogenic $\Delta katA$ mutant (no catalase activity) and three clinical isolates (1 MRSA, 2 MSSA) were cultivated in aerobic and anaerobic conditions in TSB medium. Photodynamic inactivation was conducted with the use of two types of photosensitizers: toluidine blue O (TBO) and protopor-

phyrin diarginate (PPArg₂). Bacteria were irradiated in a light dose dependent manner (100–500 J/cm²) using red light emitting diode lamp ($\lambda_{max} = 627$ nm) and their survival fractions were calculated and compared. **RESULTS.** Aeration conditions of *S. aureus* culture had a minor influence on TBO-based PDI treatment outcome. In the case of PPArg₂, the weaker photoinactivation effect of four out of five anaerobically cultivated strains was observed. The differences in survival fractions reached up to 2 log₁₀ units at 100 J/cm² light dose and up to 3.5 log₁₀ units at 500 J/cm² light dose. In the case of MRSA isolate, anaerobic cultivation changed its phenotype from highly sensitive to intermediate resistant, with respect to the PDI response. **CONCLUSION.** We report that aeration conditions affect the outcome of PDI. Anaerobically cultivated *S. aureus* is more resistant to porphyrin-based PDI, thus pointing for a validity of a careful optimization of research/treatment conditions.

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ENTEROCOCCUS SPP. RELEASES TO GULF OF GDANSK WITH RIVER WATER AND WASTEWATER TREATMENT PLANT EFFLUENTS

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AIMS. The aim of the study was to assess the importance of continuous input of treated wastewater and the Vistula River in dissemination of enterococci with antimicrobial resistance patterns into the coastal water. **METHODS.** The samples of treated wastewater were obtained from two local wastewater treatment plants: WWTP-W (Gdansk) and WWTP-D (Gdynia), while riverine samples were collected from the Vistula river mouth. A conventional method of membrane filtration was used to evaluate the enterococci number. Then species identification and drug susceptibility against 17 antimicrobial agents were tested. The selected strains were investigated by multilocus sequence typing (MLST) for *E. faecalis* and *E. faecium*, and by multilocus VNTR analysis (MLVA) for *E. faecium*. **RESULTS.** The number of fecal enterococci detected in the effluent of WWTP-W and WWTP-D reached the average values of 4×10^4 CFU/100 mL and 1×10^4 CFU/100 mL, respectively, while in Vistula River reached up to 50 CFU/100 mL. In treated wastewater predominance of *E. faecium* was followed

by *E. faecalis* and *E. hirae*. In the case of the Vistula River, *E. hirae* was observed in major presence. Occasionally *E. casseliflavus/gallinarum*, *E. durans* and *E. avium* occurred. Antimicrobial susceptibility tests showed predominance of nitrofurantoin and erythromycin resistant enterococci regardless of their origin. Resistance to glycopeptides was observed only in WWTP-W samples. High-level aminoglycoside (gentamicin and streptomycin) resistant *E. faecium* and *E. faecalis* were observed in both WWTP effluents. In riverine isolates resistance to high-level streptomycin was detected among four *E. hirae* isolates. Molecular typing revealed divergence among both *E. faecalis* and *E. faecium*. HLAR phenotype was typically associated with clonal groups commonly found in hospitals, such as ST16 for *E. faecalis* and CC17 for *E. faecium*. **CONCLUSIONS.** The obtained data showed that among enterococci of riverine and treated wastewater origin, clinically relevant resistance phenotypes can be detected, and in consequence released into the marine environment.

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THE DETECTION OF CEPHALOTHIN SENSITIVE/RESISTANT AMONG UROPATHOGENIC *ESCHERICHIA COLI* STRAINS COLLECTION BY USING FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) AND ARTIFICIAL NEURAL NETWORKSŁukasz Lechowicz^{1*}, Mariusz Urbaniak², Wioletta Adamus-Białek³, Wiesław Kaca¹¹Department of Microbiology, The Jan Kochanowski University in Kielce, Poland²Organic Chemistry Division, The Jan Kochanowski University in Kielce, Poland³Independent Department of Environmental Protection and Modeling, The Jan Kochanowski University in Kielce, Poland

INTRODUCTION. The increasing bacterial antibiotic resistance is serious medical problem. It is obvious of need of rapid techniques for determination of resistant bacterial strains. **AIM.** The aim of this study was to create the neural networks (ANNs) analyzing FTIR spectra of bacterial colony, for distinguish cephalothin sensitive/resistant among uropathogenic *E. coli* strains collection. **MATERIALS AND METHODS.** The study was performed with 109 uropathogenic *Escherichia coli* strains isolated from patients urine samples. 51% of strains were sensitive to cephalothin, 27% were resistant and 22% were intermediate resistant. At 24 hours of cultivation in 37°C, single *E. coli* colonies were transferred to the ATR/FT-IR spectrometer. For each strain 10 typical colonies were recorded. Each colony was scanned 25 times, and the results were averaged. The first derivatives of the spectra were used as an input data to ANNs. To design of ANNs software STATISTICA 10 was used. Data were divided into three independent subsets: learn-

ing, testing and validation. Due to the limitations of ANNs strains spectra were divided into three categories: resistant, sensitive, intermediate resistant. Finally only two categories sensitive and "other" were applied. The category "other" includes intermediate as well as resistant strains. **RESULTS.** ANNs dividing tested *E. coli* strains to the three categories categorized by IR spectra with the correctness of 94.94% for sensitive strains and 67.5% for resistant strains. ANNs dividing strains using only two categories categorized FTIR spectra with the correctness of 93.26% for sensitive strains and 91.89% for "other" strains. **CONCLUSION.** ATR/FT-IR and ANNs may be used to determine drug sensitivity/resistance of bacteria with high precision and the low levels of errors. The combination of FTIR bacterial spectra with ANNs for their evaluation might be one of methods supporting microbiological diagnostic techniques.

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ANTIMYCOBACTERIAL ACTION OF THE EXTRACT FROM SEEDS OF *SIDA HERMAPHRODITA* (L.) RUSBY (*MALVACEAE*)Kinga Lewtak^{1,2*}, Marta Fiołka², Ewa Szczuka¹, Jerzy Wydrych³, Radosław Keller⁴, Michał Rawski⁴, Krzysztof Skrzypiec⁴, Ewaryst Mendyk⁴¹Department of Plant Anatomy and Cytology, Institute of Biology and Biochemistry, Maria Curie-Skłodowska University, Lublin, Poland²Department of Immunobiology, Institute of Biology and Biochemistry, Maria Curie-Skłodowska University, Lublin, Poland³Department of Comparative Anatomy and Anthropology, Institute of Biology and Biochemistry, Maria Curie-Skłodowska University, Lublin, Poland⁴Analytical Laboratory, Faculty of Chemistry, Maria Curie-Skłodowska University, Lublin, Poland

INTRODUCTION. *Sida hermaphrodita* (Virginia Mallow) is a tall perennial herb from the mallow family. Currently the plant is a subject of interest because of potential economic importance e.g. as biomass for energy generation, as a source of fibers and cellulose, resins or wax for cellulose industry. **AIM.** The aim of our study was to evaluate the action of the extract from *Sida hermaphrodita* seeds against *Mycobacterium smegmatis* cells. **MATERIALS AND METHODS.** The protein extract from seeds of *S. hermaphrodita* was prepared in Sørensen buffer (pH 6.4) after homogenization of seeds. Action of the extract on the morphology and metabolic activity of mycobacterial cells was analyzed using confocal (test Live/Dead), scanning, transmission and atomic force microscopes. SERS (Surface Enhanced Raman Spectroscopy) technique was used to determine the chemical differences in the cell wall of *M. smegma-*

tis after treatment with *S. hermaphrodita* extract. **RESULTS.** The extract from seeds of *S. hermaphrodita* caused significant decrease in viability of *M. smegmatis* cells at a concentration of 50 µg/ml. After incubation *Mycobacterium* with protein extract morphological changes connected with destruction of cell wall and deformation of cells shape were visible. SERS analysis showed increased number of chemical bonds characteristic for carbohydrates and reduction of chemical bonds of proteins and lipids in the cell wall of the *M. smegmatis* after exposure to the extract from *S. hermaphrodita* seeds in comparison to untreated control culture. **CONCLUSIONS.** The extract from seeds of *Sida hermaphrodita* decreased the viability of the *M. smegmatis* cells and damaged the cell walls resulting in changes in *Mycobacterium* shape.

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INFLUENCE OF DEFERIPRONE ON THE REMOVAL OF BIOFILM OF *ENTEROCOCCUS* SPP. STRAINS

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INTRODUCTION. Enterococci occupy a significant position among bacteria causing nosocomial infection. One of the main factors of enterococci virulence is the biofilm formation. Iron regulation of biofilm formation has been demonstrated in many bacterial species. **AIM.** The aim of this study was to investigate the effect of synthetic iron chelator – deferiprone on the inhibition of synthesis, biofilm removal and recovery of *Enterococcus* spp. strains. **MATERIALS AND METHODS.** We used 24 strains belonging to the species *E. faecalis* and *E. faecium*. All strains were isolated from clinical material. MICs of deferiprone for each strain were determined by serial micro-dilutions of chelator in a liquid medium. To test the ability of the chelator to inhibit the synthesis of biofilm, the bacteria were cultivated in liquid medium supplemented with different concentrations of chelator, amounting to 25%, 50% and 75% MIC. The

next section outlines the chelator role in the disturbing of mature biofilm. For this purpose, mature biofilms prepared earlier in the liquid medium were placed in a saline environment with the addition of 25%, 50% and 75% MIC deferiprone. The last stage checked the biofilm's structure reproduction after removal of deferiprone from the environment for growth. Mature biofilm, previously treated with various concentrations of chelator (25%, 50%, 75% MIC) were re-introduced in the iron-rich liquid media environment. At each stage of research, biofilm was determined quantitatively by MTT reduction. **RESULTS.** None of the used concentrations of deferiprone inhibited the synthesis of biofilm, while destroying mature biofilm, and reduced the intensity of its reproduction. The use of combination therapy with deferiprone and appropriate antibiotic could be a breakthrough in the treatment of infections caused by enterococci.

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THE FREQUENCY OF ISOLATION AND THE ROLE OF VIRULENCE FACTORS IN STRAINS OF *E. FAECALIS* FROM HUMAN CANAL ROOTS

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INTRODUCTION. The eradication of enterococci persisting in the distant areas of the tubular root canal system is a major challenge in treatment regimens, and is crucial for the long term preservation of endodontically treated teeth. *E. faecalis* can proficiently invade dentinal tubules, survive chemomechanical instrumentation and intracanal medication. It may result from the ability to produce externally released factors, like enzymes, enterocins [Kayaoglu, 2004]. **PURPOSE OF THE STUDY.** The purpose of his study is to evaluate the frequency of *E. faecalis* isolation from canal root infections and to characterize these strains phenotypically and genotypically. **METHODS.** Enterococci were isolated from the patients undergoing endodontic treatment. They were identified with rapidID32 STREP and PCR for *ddl* gene. The presence of *agg*, *cylL*, *esp*, *gelE*, *sprE* genes was tested. Sensitivity of enterococcal strains was assessed with disc-diffusion method. Ability to produce enterocins was tested against:

E. coli ATCC35218, *E. faecalis* ATCC51299, *E. faecalis* ATCC29219, *E. faecium* ATCC6569, *S. mutans* ATCC25175. At the same time isolation and identification of anaerobic bacteria was carried out (ATB system). **RESULTS.** During the analysis of fifty six teeth twelve strains of *E. faecalis*, *viridans* streptococci, *S. saccharoliticus* and two *C. albicans* strains were isolated. Among anaerobes several strains of *P. acnes* (n = 7) were obtained, then others, e.g. from *Actinomyces*, *Propionibacterium*, *Clostridium* genus). Resistance to penicillin, tetracycline and erythromycin was observed. Two isolates synthesized enterocins active against *E. faecalis* ATCC29219 and *E. faecium* ATCC6569. **CONCLUSION.** *E. faecalis* remain main facultative anaerobic bacteria invading dentinal tubules. Strains isolated from root canals seem to be rather sensitive to antibiotics and do not possess many virulence factors. However, the ability to produce enterocins may be their important feature.

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EFFECT OF SILVER NANOPARTICLES ON BACTERIAL BIOFILMS

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INTRODUCTION. Antimicrobial efficacy of silver nanoparticles (AgNPs) was proved against bacteria, other microorganisms and also viruses. Despite proposed broad applications of AgNPs, the mechanism of their antibacterial activity still is not entirely resolved. **AIM.** Examination of silver nanoparticles activity on bacterial biofilms. **MATERIALS AND METHODS.** *Pseudomonas aeruginosa* PCM499, *Klebsiella pneumoniae* PCM1, *Listeria monocytogenes* PCM2191 and *Staphylococcus aureus* ATCC29213 were

used in this study. AgNPs colloid was obtained from NanoTech (Poland). Minimal biofilm inhibitory concentration (MBIC) values were measured by a two-fold dilution method. To study the effect of AgNPs on biofilm formation after 6 or 24 h of incubation, the classic method based on the absorbance of crystal violet by cells adhered to microtitre plates was applied. Survival of bacterial cells in biofilms exposed to subinhibitory concentrations of AgNPs was analyzed after staining with dimethyl thiazolyl diphenyl tetrazo-

lium salt (MTT) followed by absorbance signal measurement or by using BacTiter-Glo Microbial Cell Viability Assay (Promega). Disorders in the formation of biofilms on the catheter fragments were visualized by staining urological biomaterials with 2,3,5-triphenyl tetrazolium chloride (TTC). **RESULTS.** MBICs of AgNPs were 8 micrograms mL⁻¹ for Gram-negative bacteria and 12 micrograms mL⁻¹ for Gram-positive strains. 0.5 MBIC of AgNPs diminished bacterial adhesion to polystyrene by 80% for *K. pneumoniae*

and 55% for *P. aeruginosa*. AgNPs influenced the biofilm formation of Gram-negative bacteria after 24 h incubation, no decrease was observed in the case of *L. monocytogenes* and *S. aureus*. Subinhibitory concentrations of AgNPs, 0.1 MBIC and 0.2 MBIC, resulted in bacterial cells survival decrease by 50% and 70% respectively for *P. aeruginosa*. **CONCLUSIONS.** Silver nanoparticles influence biofilm formation and bacterial cell survival. Strong antibiofilm effect was shown only on Gram-negative bacterial strains.

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DETERMINATION OF THE LYTIC ACTIVITY OF BACTERIOPHAGES AGAINST UROPATHOGENIC *MORGANELLA MORGANII* STRAINS

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INTRODUCTION. Bacteriophages are a group of viruses capable of selective killing of bacteria. Nowadays in an era of increasing microorganisms resistance to antibiotics, there is a growing interest in using bacteriophages to treat bacterial infections. Urinary tract infections (UTIs) are among the most common infections in humans and they pose serious clinical and economic problems. One of the etiological agents of catheter-associated UTIs are bacteria *Morganella morganii*. **AIM.** The main aim of this study was to find polysaccharide depolymerases producing polyvalent phages of uropathogenic bacteria *M. morganii*. **MATERIALS AND METHODS.** The research was performed on a collection of bacterial strains belonging to the species: *P. mirabilis* (36), *P. stuartii* (10) and *M. morganii* (39), isolated from people with urinary tract infections, including patients undergoing long-term catheterization. 162 bacteriophages (99 against *M. morganii*, 50 against *P. mirabilis*, 13 against *P. stuartii*) were used. The double-layer plate method

was used to define plaque morphology. The phages host range was determined by the "spot-test". **RESULTS.** All tested phages produced one of 3 types of plaques: spot < 0,5 mm, round (from 0,5 to 15 mm diameter) or plaques with an irregular edge. From 162 tested bacteriophages 66 produced plaques surrounded by a halo, which suggests that these phages produce polysaccharides depolymerases. 28 viruses specific to *M. morganii* demonstrated large lytic activity within the corresponding species (phages lysed ≥ 20 strains). Besides, 11 of these phages against *M. morganii* produced plaques surrounded by a halo. A few bacteriophages able to infect only single bacterial strains outside the species were found in the present study. **CONCLUSIONS.** To sum up, polyvalent bacteriophages producing polysaccharide depolymerases will become a valuable tool for research on the prevention and eradication of infections caused by uropathogenic strains of *M. morganii*.

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INVESTIGATION OF GENETIC DIVERSITY OF *MYCOBACTERIUM TUBERCULOSIS* STRAINS WITH THE USE OF QUB VNTR TANDEM REPEATS

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INTRODUCTION. *M. tuberculosis* is a causative agent of tuberculosis. This disease constitutes a serious health problem worldwide with about one-third of the world's population infected. Combating tuberculosis requires epidemiological analysis. Genotyping is often used in such research, due to insufficient discriminatory abilities of traditional typing methods. IS6110 restriction fragment length polymorphism is the gold standard for genotyping *M. tuberculosis*. However, this reference method is difficult to perform, laborious and time-consuming. Thus, there is a need to develop faster and simpler method(s). **AIM.** To genotype 111 *M. tuberculosis* strains using six selected QUB VNTR (variable number tandem repeats identified at Queens University of Belfast) loci and to determine their discriminatory power. **METHODS.** DNA samples were amplified using the polymorphic chain reaction method. Afterwards nucleic acid electrophoreses were performed in 2% agarose gel.

DNA was stained with ethidium bromide and visualized in UV light. The number of tandem repeats for each loci was determined on the basis of the lengths of the acquired amplicons (167–1525 bp). **RESULTS.** The number of QUB VNTR tandem repeats varied from 1 to 25 copies at the six loci investigated. QUB3232 was the most polymorphic (allelic diversity $h = 0.894$) and QUB1895 was the least polymorphic ($h = 0.557$). Five loci (QUB11a, QUB11b, QUB26, QUB3232, QUB3336) showed high discriminatory power, while locus QUB1895 was moderately discriminating. Discriminatory power (expressed as Hunter-Gaston discriminatory index, HGDI) for QUB VNTR typing equaled 0.984 resulting in 77 different types, of which 63 were unique and 14 were shared by 2–9 strains. **CONCLUSION.** QUB VNTR method possess a high discriminatory power and proved useful for genotyping *M. tuberculosis* strains.

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DETERMINATION OF DIFFERENCES
BETWEEN *PROTEUS MIRABILIS* LIPOPOLYSACCHARIDES (LPSs)
ISOLATED FROM PLANKTONIC AND BIOFILM CULTURES

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INTRODUCTION. *Proteus mirabilis* is a main causative agent of catheter-associated urinary tract infections (CAUTI). Biofilm plays an important role in CAUTI. **AIM.** Determination of differences between *P. mirabilis* LPS isolated from planktonic or biofilm cultures. The differences in the structure and biological activity of LPS were previously reported for *Pseudomonas aeruginosa* strains. **METHODS.** 1. planktonic forms were obtained by cultivation of bacteria in nutrient broth (NB) in aerated conditions; 2. growth of biofilm was performed in glass bioreactor with constant NB flow 1 ml/min; 3. LPS was extracted by Westphal method; 4. SDS PAGE, Western Blot and ELISA with rabbit anti-O serum against plank-

tonic forms were used in serological studies. **RESULTS.** 2 out of 3 LPSs obtained from *P. mirabilis* biofilm reacted to the lower titers with O-specific sera compared to LPSs extracted from planktonic forms. Both SDS PAGE and Western Blot showed differences in LPSs patterns. LPS isolated from biofilm had shorter O-specific part compared to the LPS from planktonic forms. Silver staining also revealed, that LPS obtained from one strain growing in biofilm may also differ in core region. Additional analysis of LPS from biofilms will be performed with NMR spectroscopy. **CONCLUSION.** It is probable that *P. mirabilis* LPSs obtained from biofilm are partially different, comparing to LPSs extracted from planktonic cells.

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INHIBITION OF *STAPHYLOCOCCUS AUREUS* ADHESION TO ECM PROTEINS
IN AN ENVIRONMENT OF PHYTOCHEMICALS

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INTRODUCTION. Infective endocarditis (IE) is a pathology of the endocardium or native/artificial valves. In IE occurring without identifiable pre-existing risk factors, *Staphylococcus aureus* is one of the most frequent etiological agents. Facing the therapeutic challenges, a better understanding of the pathogenic mechanisms of *S. aureus* and development of new medical strategies of IE are urgent. The study of plants as an alternative to other forms of drug discovery has attracted great attention. Well-known antimicrobial activity of plant products (i.e. polyphenols) may be useful in preventing the *S. aureus* colonization of damaged valves and myocardial tissue. **AIM.** To evaluate the effects of plant products (*Leonurus cardiaca* extract and ursolic acid) on the modification of the selected features of *S. aureus* cells, which are involved in interaction with eukaryotic cells/ECM, important for IE pathogenesis. **MATERIALS AND METHODS.** *S. aureus* strains with different expression of virulence factors, essential for the interaction with plasma proteins: *S. aureus* NCTC 8325-4 (α -toxin⁺, SpA⁺, Coa⁺,

ClfA⁺, SAK⁺) and Wood 46 (α -toxin⁺, SpA⁻, Coa⁺, ClfA⁻, SAK⁻); culture plates with immobilized ECM proteins: fibrinogen (Sigma, USA), collagen (Life Technologies, USA), fibronectin (R&D Systems, Inc.); *Leonurus cardiaca* herb extract (original), ursolic acid (Sigma, USA). The adhesion of microorganisms was assessed by using the colorimetric assay (Alamar Blue[®], Trek Diagnostic Systems, Inc.) after 2 h incubation in the presence of plant products (used at 1/2 MIC). **RESULTS.** Both plant preparations caused significant impairment of microbial adhesion to immobilized ECM proteins (all types). The most prominent was effect of ursolic acid which inhibited *S. aureus* binding to immobilized fibrinogen by 78%. **CONCLUSIONS.** Some of natural plant polyphenols, in addition to their biocidal properties, show the desired effect on microbial virulence. Their direct contact with *S. aureus* limited adhesion of bacteria to ECM. Thus, it can be expected that in the presence of the above phytochemicals the degree of tissue colonization by bacteria may be limited.

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ENZYME INHIBITORS EFFECT ON THE BIOFILM FORMATION BY *PSEUDOMONAS AERUGINOSA*
ISOLATED FROM CHRONIC DIABETIC FOOT INFECTION

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INTRODUCTION. In the chronic diabetic foot infection, bacteria *P. aeruginosa* plays a dominant role. These microorganisms have a high capability for biofilm formation by increasing at the same

time its insensitivity to commonly used therapeutics. Two different approaches in biofilm inhibition: tested substances were added on the early stage of biofilm formation or on the already formed,

mature biofilm, which sensitivities are far different. Many research studies have shown, that the biofilm layer effectively hinders the penetration of applied substances preventing them from the bactericidal effect on bacteria. **AIM.** Define the enzyme inhibitors effect, that blocks the biosynthesis of exopolysaccharides, on the biofilm formation of selected strains of *Pseudomonas aeruginosa*. **MATERIALS AND METHODS.** All tests were performed in *Pseudomonas aeruginosa* strains: PAR5, PAR20, PAR50 isolated from a patient with chronic diabetic foot infection. The assessment to check the ability of biofilm formation was carried out using 96-well plates with the adherence surface (Greiner Bio-One). In subsequent steps, in order to analyze how the bacteria *Pseudomonas aeruginosa* respond to tested inhibitors (n = 4 from Olivier R. Martin).

RESULTS. Enzyme inhibitors used on the early biofilm reduced the quality of biofilm formation in average by 70% for the analyzed strains. It might indicate that the tested substances join the exopolysaccharides chain and prevent its further expansion. On the other hand, activation on the generated 48-hour biofilm showed no activity in inhibiting the production of a matrix. **CONCLUSIONS.** It appears that all tested compounds were able to inhibit early biofilm formation by *P. aeruginosa*, which indicates that the synthesis of exopolysaccharides plays an important role in early biofilm formation by this pathogen. Inhibition of glucosyltransferases and other enzymes which are involved in exopolysaccharides synthesis has a direct impact on *P. aeruginosa* biofilm formation and development. Grants: N N401 547 040 and K/ZDS/002861.

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DIVERSITY OF PATHOGENIC PROPERTIES OF RHIZOCTONIA SOLANI AG5 AGAINST SUGAR BEET SEEDLINGS

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INTRODUCTION. *Rhizoctonia solani* is a causal agent of some plant diseases. Within *R. solani* species fourteen anastomosis groups (AG) were distinguished based on hyphal anastomosis reactions. One of them is AG5, mostly nonpathogenic to plants or slightly pathogenic. **AIM.** The aim was to study the pathogenic activity of isolates of *R. solani* AG5 against sugar beet seedlings and to check the influence of preparations Zaprawa nasiennaT and Tachigaren on the development of tested fungi. **MATERIALS AND METHODS.** The isolates of *R. solani* were obtained from sugar beet seedlings with symptoms of damping-off. Determination of AG was made by pairing an unknown isolate to the tester isolate 05R01. Fungal activity against sugar beet seedlings was investigated during the field experiments. The field soil was infected with inocula grown on the sand-corn medium. In the laboratory, the influence of fungicide preparations was tested on the fungal colonies development by the method

routinely used to assess fungicidal activity. **RESULTS.** The results showed evident pathogenic activity of some isolates of *R. solani* AG5 against sugar beet seedlings. The average seedlings' emergence was lower than in the control. Besides this, there were also some isolates with low pathogenic activity. Tested fungi showed different sensitivity against fungicides. Each of the isolates was completely inhibited by tested fungicides. Isolates showed quite good level of tolerance to both fungicides, even an increase of growth was observed in the case of Tachigaren. We have observed some differences in the morphological features of isolates. **CONCLUSIONS.** The results of the study suggest the possibility of pathogenic activity of AG5 isolates more evidently than it is shown in the literature. Our results show that fungicides routinely used as a seed coat, to control soil plant pathogens causing damping off, cannot inhibit *R. solani* AG5 development and therefore seedlings are not properly protected.

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SILVER NANOPARTICLES PREVENT STAPHYLOCOCCUS AUREUS BIOFILM RE-GROWTH AFTER PHOTOANTIMICROBIAL CHEMOTHERAPY

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INTRODUCTION. A number of chronic bacterial infections involve biofilms, making these infections difficult to be eradicated by antibiotic treatment. Photoantimicrobial chemotherapy (PACT) is an alternative way of efficient control of microbial growth. The method uses 3 elements: photosensitizer, light and oxygen, whose simultaneous action leads to generation of reactive oxygen species further causing the death of target cells. One of the problems with PACT is bacterial re-growth after treatment. Therefore, there is a need to strengthen the effect of the PACT by adding agents preventing the re-growth of bacterial cells. **AIM.** We asked whether photoinactivation of staphylococcal biofilm at sublethal doses followed by the action of silver nanoparticles (AgNP) prevents *S. aureus* cells re-growth after treatment. **MATERIALS AND METHODS.** A method to grow cells in biofilm using MBEC High-throughput (HTP) Assay was applied. We used reference *S. aureus* strain Newman. Homogenous mixture of silver nanoparticles (ProChimia

Surfaces Co., Poland) and two photosensitizers were used in the study, namely meso-tetra(N-methyl-4-pyridyl)porphine tetratosylate and New Methylene Blue. The light source used in the study was Q. Light 70 NT phototherapy system (Q.Products AG, Switzerland), with an output power of 100 mW, a fluence of 0.127 J/cm². **RESULTS.** Minimal Biofilm Eradication Concentration (MBEC) values of AgNP alone, and incubated simultaneously with photosensitizers, as well as sublethal and lethal PACT doses against staphylococcal biofilm were determined. PACT treatment allowed for reduction of the number of biofilm-grown bacteria by 4 log₁₀ units (CFU/peg), as measured directly after PACT treatment, however, a re-growth of cells was observed after 24 hrs post-treatment. Combining the action of PACT with AgNP allowed for (i) eradication of bacterial biofilm and (ii) prevented the re-growth of cells 24 hrs post-treatment. **CONCLUSIONS.** Combination of PACT and AgNP is a viable approach to treat staphylococcal infections.

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ECA IMMUNOGENICITY OF *YERSINIA ENTEROCOLITICA* DEEP-ROUGH MUTANTS

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INTRODUCTION. Enterobacterial common antigen (ECA) is immunogenic when present as ECA_{LPS}. *Yersinia enterocolitica* O:3 (YeO3) Rc mutants that have complete inner core in their LPS were ECA immunogenic. It was tempting to study whether the whole Rc core of YeO3 was needed to express ECA_{LPS}. **AIM.** Determination of the presence of anti-ECA antibodies in rabbit antisera against YeO3 deep-rough mutants that produce either chemotype Rd₁, Rd₂ or Re LPS. **METHODS.** Polyvalent antisera against deep-rough mutants were obtained and adsorbed with live ECA-negative strain to remove anti-LPS antibodies. These antisera were analyzed by ELISA and Western blot (WB) techniques using as antigens ECA_{PG} standard, LPS_{PCP} preparations (free from ECA_{PG}, might contain

ECA_{LPS}) and whole cell lysates from the deep-rough mutants. **RESULTS.** In ELISA assay all the antisera reacted with ECA_{PG} and LPS_{PCP} preparations from homologous deep-rough mutants. In WB all the polyvalent antisera reacted with ECA_{PG} as well as the adsorbed anti-Re antiserum. All the antisera reacted with LPS_{PCP} preparations and whole cell lysates from homologous deep-rough mutants. Only the non-adsorbed antisera reacted with the ECA-negative strain. **CONCLUSIONS.** All deep-rough mutants, even Re type were ECA immunogenic. Thus, the Re type LPS was sufficient to express ECA_{LPS}; indicating that the ECA substitution in YeO3 LPS is located in the Kdo region. Structural studies are warranted to confirm this.

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ACTIVITY OF ANTIMICROBIAL PEPTIDES
AND SHORT LIPOPEPTIDES AGAINST PLANCTONIC CELLS
AND MATURE BIOFILM OF *PROVIDENCIA STUARTII* CLINICAL STRAINS

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INTRODUCTION. A constantly increasing number of bacterial multi-drug resistance strains limits the applicability of chemotherapeutic agents and forces to seek new effective antibacterial compounds. **AIM.** Evaluation of the antimicrobial activity of peptides against planktonic cells and mature biofilm of *P. stuartii*. **MATERIAL AND METHODS.** Clinical *P. stuartii* strains and the following peptides: Citropin 1.1, Pexiganan and lipopeptides composed of arginine (R) or lysine (K) conjugated with palmitic acid (Palm): Palm-KK-NH₂, Palm-KKK-NH₂ and Palm-RRR-NH₂, were used in the study. Compounds included in the study were synthesized manually by the solid-phase method using the 9-fluorenylmethoxycarbonyl chemistry (Fmoc). The completeness of each coupling reaction was monitored by the chloranil test. The cleaved lipopeptides were precipitated with diethyl ether and were purified by solid phase extraction and high-performance liquid chromatography (HPLC). Compounds were analyzed also by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-

TOF MS). Susceptibility of planktonic cells at the peptides was examined by broth microdilution method, and two values – MIC (Minimal Inhibitor Concentration) and MBC (Minimal Bactericidal Concentration) were determined. Bacterial biofilms were grown on polystyrene plates with peptides in concentration range 256 µl/ml – 1 µl/ml. The biofilm intensity was measured colorimetrically by the use of staining with MTT and 1% crystal violet. **RESULTS.** Tested peptides exhibit strong bacteriostatic and bactericidal activity against studied *P. stuartii* planktonic cells. The most active were Citropin 1.1 and Pexiganan. These peptides at concentration 128 µl/ml – 256 µl/ml almost totally disrupted transformation of planktonic cells in to a settled form and inhibited biofilm formation in the case of all tested strains. Moreover, application of those peptides at concentration 256 µl/ml resulted in eradication of mature biofilm by over 90% for 65% of tested strains. **CONCLUSION.** Tested peptides had very strong antimicrobial activity against both planktonic cells and mature *Providencia stuartii* biofilm.

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URINE COMPONENT AND EXTERNAL FACTORS AS MODULATORS
OF THE *PROVIDENCIA STUARTII* BIOFILM FORMATION PROCESS

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INTRODUCTION. The environmental conditions of the urinary system, and other external factors modulate the properties of microorganisms, their vitality, hydrophobicity, and a predisposi-

tion to adherence and hence their ability to form biofilm. **AIM.** The goal of this study was to determine the effect of urine components: creatine, magnesium and calcium 2-valent cations, urea

and external factors: sugars – mannose, glucose and protein – albumin bovine serum (BSA), on the process of biofilm formation by *P. stuartii*. **MATERIALS AND METHODS.** 20 clinical strains of *P. stuartii* isolated from biofilms on urological catheters obtained from long catheterized patients were used in the study. Bacterial biofilms were grown on polystyrene plates in tryptic soy broth and in modified media supplemented with 2-valuable calcium and magnesium cations in the concentration 0.65 mg/ml, creatine (1.1 mg/ml), urea (25 mg/ml), sugars – glucose and mannose (500 mM) and bovine serum albumin (BSA) in concentration 1.54 mg/ml, which corresponds to the conditions prevailing in the body of patients with renal impairment. The biofilm intensity was

measured colorimetrically by the use of staining with 1% crystal violet and MTT. **RESULTS.** Extremely strong antagonistic effect on the biofilm formation was observed for urea which caused inhibition of biofilm growth by 70% in the case of 50% of tested strains, and by 50% for 99% of the strains. Addition of glucose in the concentration 500 mM resulted in a decrease in the intensity of biofilm formation by 80% in 65% of the strains. Extremely strong stimulating effect on biofilm development was observed in the case of creatine. Addition of divalent cations as well as BSA had no significant effect on bacterial biofilm formation. **CONCLUSIONS.** Compounds which most strongly inhibit the biofilm formation process are glucose and urea.

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ANTIBIOTIC SUSCEPTIBILITY PROFILES OF *KLEBSIELLA* SP. AND *PROTEUS* SP. ISOLATES

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INTRODUCTION. *Proteus* sp. and *Klebsiella* sp. representatives have a great contribution to nosocomial infections, often caused by multi-drug-resistant strains. These pathogens show many similarities in their surface antigens. **AIM.** We wanted to find out if such similarities also concern the antibiotic susceptibility profiles of isolates from both genera. **MATERIALS AND METHODS.** Minimal Inhibitory Concentrations (MICs) of eight antibiotics and one sulfonamide, officially recommended for *Enterobacteriaceae* testing, were determined for 57 *Proteus* sp. strains (*P. mirabilis* – 16 isolates, *P. vulgaris* – 16, *P. genomospecies* – 17, *P. penneri* – eight) and 41 *Klebsiella* sp. strains (*K. pneumoniae* – 33 isolates, *K. oxytoca* – eight) isolated from patients from Lodz area. The antibiotic susceptibility profiles were analyzed from different points of view. **RESULTS.** The biggest number of antibiotic resistant strains were observed among *K. pneumoniae* and *P. mirabilis* isolates. Only in the case of *Klebsiella* genus, the strains resistant to all thera-

peutics used were noted and the majority of them were extended-spectrum β -lactamases producers. Taromentin appeared to be the most effective medicine against *Proteus* sp. isolates, and norfloxacin – against *K. oxytoca* strains. None of the used antibiotics was distinguished by its effectiveness against *K. pneumoniae* strains. The highest number of strains resistant to antimicrobials was found among *P. mirabilis* and *K. pneumoniae*, and the lowest number – among *P. vulgaris* and *K. oxytoca*. **CONCLUSIONS.** More antimicrobials-resistant isolates observed for *P. mirabilis* and *K. pneumoniae* may result from the higher frequency of these species isolation (within each genus) from the clinical sources, which may lead to their resistance.

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ANTIBIOTICS AND PLANT POLYPHENOLS SYNERGY AGAINST *STAPHYLOCOCCUS AUREUS*

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INTRODUCTION. Classic therapy against microbial infections becomes ineffective as many strains easily develop resistance to antibiotics. This phenomenon is clearly visible in chronic wounds contaminated with biofilm-forming multiple resistant *S. aureus* strains. Thus, there is an urgent need to search for some alternative methods of treatment in those cases. One of the options is using natural plant products such as polyphenols. **AIM.** We investigated the possibility of improving the antibiotics effect on *S. aureus* growth by addition of selected plant derivatives. **MATERIALS AND METHODS.** *S. aureus* strains: 29213 (reference strain), 948/05 (clinical isolate). Antibiotics: oxacillin, vancomycin, linezolid, mupirocin. Phytochemicals: commercial polyphenols (gallic acid, carvacrol, oleanolic acid), natural extracts (*Vaccinium myrtillus* leaves – EL 12, *Rosmarinus officinalis* herb – EZ 2, *Frangula alnus* bark – EO 10). The polyphenols/plant extracts (used at ½ and ¼ MIC) influence on the minimal inhibitory concentrations

(MIC) of antibiotics were determined with microdilution method in Mueller-Hinton Broth. **RESULTS.** We observed positive effect of phytochemicals addition. The majority of tested polyphenols improved the antibiotic effect. Amongst commercial products the strongest synergistic effect (8-fold lower MIC) was demonstrated by carvacrol with two antibiotics: oxacillin and linezolid (strain 948/05) and gallic acid with linezolid (29213). EL 12 was the most effective natural plant extract as it lowered MIC of linezolid (32-fold) and mupirocin (8-fold) for both bacterial strains. Only two phytocompounds: gallic acid (½ and ¼ MIC) and EZ 2 (¼ MIC) impaired the activity of oxacillin and vancomycin, respectively (antagonistic effect). **CONCLUSIONS.** There is a chance to use natural phytochemicals for restoring the activity of classic antibiotics against multiple resistant bacteria. However, more detailed studies on the mode of their action are necessary to select the natural substances most efficient and safe for eukaryotic cells.

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MICROBIOLOGICAL QUALITY, ORGANIC ACIDS AND PATULIN CONTENT
IN DRIED INFLORESCENCES AND FRUITS OF HAWTHORN (*CRATAEGUS* SPP.)
USED AS COMPONENTS OF FUNCTIONAL FOOD PRODUCTS

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INTRODUCTION. Functional foods products (FFP) containing aerial parts of hawthorn (*Crataegus* spp.) exhibiting highly efficient cardiotoxic and cardioprotective properties in humans with aging-related major vascular diseases caused by endothelial dysfunction. However, reliable data on microbial contamination and mycotoxins content in dried hawthorn inflorescences (HI) and fruits (HF) used in Poland for preparation of extracts, infusions, juices and FFP are unsatisfactory, inaccessible and scarce. **AIM.** Representative samples of the HI- and HF-based FFP were examined for numbers of aerobic mesophilic bacteria (APC), numbers of total moulds and yeast along with determination of ascorbic acid, organic acids and patulin content. **MATERIALS AND METHODS.** APC were counted onto standard plate count agar (PCA) and incubated at 30°C for 3 days (PN-EN-ISO4833:2002). Moulds and yeasts were counted onto standard dichloran-glycerol (DG18) agar and incubated at 25°C for 5 days (PN-ISO21527-2:2009). The ascorbic and oxalic acid were determined by redox-titrations while tartaric acid

by colorimetric assay. Enzymatic methods were applied for citric and malic acid. Patulin was determined by selective MWCN-SPE followed by HPTLC. **RESULTS.** APC counts up to 3.6×10^6 and 6.3×10^6 cfu/g were observed in, respectively, HI and HF samples. Highest mould counts reached 5.3×10^2 cfu/g for HI and 1.9×10^4 cfu/g in case of HF. The 60% of HI and 100% of HF samples contained fungi. But *Aspergillus* spp. was recognized in 50% of HF. In HI and HF mean content of ascorbic acid 28.83 mg/100g were comparable. The average amount of organic acids decreased in HI samples as follows: oxalic 1044.07 > citric 449.78 > tartaric 388.38 > malic 286.63 mg/100g. In HF samples this sequence was changed: oxalic 445.04 > malic 251.08 > tartaric 204.65 > citric 84.56 mg/100g. Patulin was below 1.0 µg/100g in all analyzed HI and HF samples. **CONCLUSIONS.** Statistically significant multivariate relationship ($p = 0.004$) was observed between logarithm of mould counts and concentration of citric, malic and tartaric acids in HI and HF samples.

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DOES THE TYPE 1 FIMBRIAE REALLY COMMONLY OCCUR
IN COMMENSAL *ESCHERICHIA COLI*?

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INTRODUCTION. Type 1 fimbrial adhesin plays an important role in the adhesion and pathogenesis of urinary tract infections caused by *Escherichia coli*. Nine *fim* genes are involved in the production of type 1 fimbriae, and *fimH* gene which is commonly used as the virulence marker. **AIM.** The aim of the research was to determine the prevalence and the expression of fimbrial genes responsible for the presence of active type 1 fimbriae. **MATERIALS AND METHODS.** The research material consisted of 85 commensal *E. coli* strains isolated from 31 adult, healthy people. The prevalence of 6 important genes for synthesis of type 1 fimbriae (structural genes: *fimA*, *fimH*, *fimI*; regulator genes: *fimB* and *fimE*; and transporter gene *fimC*) was determined by the PCR method. Subsequently the genes expressions were defined by Real-Time PCR. All strains were tested for the ability to cause MS agglutination of sheep red blood cells which is caused by mannose-sensitive type 1 fimbriae. **RESULTS.** The viru-

lence marker *fimH* gene, was detected with frequency of 96,5%, also the *fimE*, *fimI*, *fimB* and *fimC* genes occurred with the frequency over 90%, but only 54,1% of all isolates were carrying a full set of *fim* genes. Moreover, reverse transcription with Real-Time PCR study revealed that *fimH* and *fimI* genes were not expressed. Also, phenotypic study of MS agglutination assay was negative. **CONCLUSIONS.** The results indicate that active type 1 fimbriae is not present in commensal *E. coli* although the genetic marker in the form of *fimH* gene - in the presented study and literature reports- is detected. This may be due to incomplete set of *fim* genes or the lack of genes expression. The presented data suggest that type 1 fimbriae do not commonly occur in commensal *Escherichia coli* and the *fimH* gene is not suitable marker for these purposes.

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PHOTODYNAMIC ANTIMICROBIAL CHEMOTHERAPY (PACT) IN COMBINATION
WITH ANTIBIOTICS FOR TREATMENT OF *STAPHYLOCOCCUS AUREUS* INFECTION

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INTRODUCTION. The century of antibiotic therapy is getting to the end. Growing multiresistance of pathogenic microorganisms, especially *Staphylococcus aureus*, forces the alternative therapies

development. Photodynamic inactivation (PDI) is a very promising therapeutic option that consists of three elements: photosensitizing agent, appropriate wavelength light and oxygen. The excited

state photosensitizer transfers its energy to ground state molecular oxygen producing reactive oxygen species (ROS) causing bacterial cell death. The current study was aimed to analyze the killing efficacy of protoporphyrin IX-mediated PDI and its synergy with selected antibiotics to determine the most effective conditions for eradication *S. aureus* growing in planktonic and biofilm cultures. **MATERIALS AND METHODS.** The reference and clinical *S. aureus* strains were used in all experiments. PDI studies concerning planktonic and biofilm cultures with different PPIX concentrations up to 50 μM were performed. Cells were illuminated with red (620–780 nm) and blue (385–480 nm) light (light doses from 10 to 400 J/cm^2). In the case of biofilm cultures, after irradiation the bio-

films were homogenized, serially diluted and aliquots were taken to determine the colony forming units (CFU). The synergistic effect of photoinactivation with selected antibiotics (ciprofloxacin, chloramphenicol, tetracycline, trimethoprim or vancomycin) at its MICs concentrations was studied. **RESULTS.** Preliminary results indicate that synergistic effect of photodynamic inactivation with selected antibiotics was observed in the case of ciprofloxacin, vancomycin and tetracycline. PDI with chloramphenicol and trimethoprim gave antagonistic effect. **CONCLUSIONS.** Reaching the aim of these studies would benefit alternative therapeutic option development and confirm that bactericidal effect of photoinactivation could be enhanced when combined with selected antibiotics.

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SEROLOGICAL STUDIES AND STRUCTURE OF THE O POLYSACCHARIDE OF *PROTEUS PENNERI* BR 114 STRAIN ISOLATED FROM A POLISH PATIENT AND CLASSIFIED INTO A NEW *PROTEUS* SEROGROUP O80

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INTRODUCTION. *Proteus penneri* are opportunistic pathogens, isolated from patients less frequently than *P. mirabilis* or *P. vulgaris*, but capable of causing serious infections, most frequently urinary tract and wound infections as well as sepsis. The species is characterized by noticeable serological uniqueness within the genus *Proteus*. **AIM.** The aim of this study was serological classification of *P. penneri* Br 114 strain, isolated from a wound of a patient treated in Barlicki's Hospital in Lodz. **MATERIALS AND METHODS.** The LPS was isolated from *P. penneri* Br 114 strain by phenol/water extraction. Polyclonal O-antiserum was obtained by immunization of a rabbit with heat-killed cells of the strain. The serological studies were performed using ELISA and its inhibition, as well as the Western blot technique. **RESULTS.** The reactions of *P. penneri* Br 114 LPS with rabbit O-antisera directed against strains representing *Pro-*

teus O1 – O79 serogroups as well as the reactivity of the obtained O-antiserum with O1 – O79 LPSs were studied. No cross-reactions were observed, which could indicate the serological uniqueness of the investigated *P. penneri* strain. Chemical research revealed that its O-antigen is structurally distinct from O polysaccharides within the 79 *Proteus* O serotypes described so far. **CONCLUSIONS.** The immunochemical studies of the *P. penneri* Br 114 LPS are the basis for the creation of a new serogroup O80 in the genus *Proteus*, consisting of one *P. penneri* strain so far. This is another *Proteus* O-serogroup formed from strains isolated from Polish patients, thus it may be presumed that many unique variants of the O-antigen still exist in *Proteus* spp. strains in Poland.

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IV-P 46

THE HARMFUL BIOLOGICAL FACTORS IN SELECTED WORK ENVIRONMENTS

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INTRODUCTION. The threat of biological agents is the current issue of occupational medicine and social policy. The places of work particularly affected by this problem include diagnostic laboratories, health care, waste management facilities, museums, archives, libraries, composting plants and tanneries. **AIM.** The aim of this study was a quantitative and qualitative assessment of microbiological hazards at the workplaces in museums, archives, libraries, composting plants and tanneries. Moreover the potentially pathogenic species of microorganisms present at the selected workplaces for producing the virulence factors were characterized. **MATERIALS AND METHODS.** Air samples were taken using the impaction method with MEA and TSA media, samples from surfaces were collected using RODAC plates (with Sabouraud and CASO media). Identification of bacteria was conducted by API-test. Fungi were identified using taxonomic keys. Identification of the harmful to health microorganisms was verified by genetic analysis (for bacteria: 16S rRNA, for fungi: ITS1/ITS2 rDNA). The study of viru-

lence factors included: an analysis of the hemolytic, proteolytic, toxinogenic and cytotoxic properties, production of polysaccharide capsule. **RESULTS.** Quantitative analysis revealed the highest microbial contamination in the air in tested tanneries (at a level of 1.2×10^3 to 3.7×10^3 cfu/m³), and in composting plants (from 1.4×10^3 to 2.5×10^4 cfu/m³) and the least for museums, archives and libraries (from 2.1×10^2 to 7.0×10^3 cfu/m³). The harmful to human health microorganisms belonging to the: *Alternaria*, *Aspergillus*, *Bacillus*, *Candida*, *Cladosporium*, *Corynebacterium*, *Cryptococcus*, *Erysipelothrix*, *Penicillium*, *Staphylococcus*, *Vibrio*, *Trichosporon* were isolated. In the study features of environmental strains such as cytotoxicity, toxinogenic (brevianamid F, fumagillin, meleagrins, roquefortine C, and others), hemolysis and production of polysaccharide capsule were identified. **CONCLUSIONS.** At the studied workplaces microbiological hazard resulting from the increased number of microorganisms and the presence of pathogens capable of producing pathogenic factors was found.

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CHARACTERIZATION OF BACTERIAL PROTEASES

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INTRODUCTION. The presentation of documents of the recent progress in structural and functional characterization of diverse bacterial proteases, including SplC and SplF proteases from *Staphylococcus aureus*, Lit protease from *Escherichia coli* and SpoIIIGA from *Bacillus subtilis*. Spl proteases belong to the cluster of six serine protease-like (spl) enzymes located within staphylococcal pathogenicity islands and are hypothetical virulence factors of *S. aureus*. Lit and SpoIIIGA are classified as proteases of “Unknown Catalytic Type” and may potentially function through a novel mechanism of catalysis. Lit functions as a specific “suicide” protease which is activated during bacteriophage infection. It targets elongation factor EF-Tu. SpoIIIGA plays a role in sporulation process of *B. subtilis* and cleaves pro- σ E transcription factor precursor controlling transcription of genes involved in regulation of cell morphology. **AIMS.** The purpose of this study was to develop an efficient method for the expression of said proteases in a recombinant prokaryotic sys-

tem (*E. coli*), purify the enzymes and determine initial conditions for their crystallization. **METHODS.** SplC, SplF, Lit and SpoIIIGA were cloned into suitable expression plasmids. All proteins were heterologously expressed in *E. coli*. Subsequently all enzymes were purified using standard chromatographic procedures (affinity, ion exchange, gel filtration) and crystallization trials were performed. Diffraction data for SplF were collected and the structure was solved. **RESULTS.** Active SplC, SplF, Lit and SpoIIIGA proteases and selected inactive mutants were obtained. SplF was successfully crystallized and the structure was solved. Preliminary crystallization conditions were obtained for SpoIIIGA. Crystallization trials for Lit protease are in progress. **CONCLUSION.** Characterization of substrate specificity of SplC is currently attempted. SplF structure is analogous to chymotrypsin family proteases. Structure of SpoIIIGA will likely reveal its catalytic mechanism in the near future. Further work is needed to obtain crystal structure of Lit protease.

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IDENTIFICATION OF SIX CLINICALLY IMPORTANT CANDIDA SPECIES BASED ON HOMOCITRATE SYNTHASE GENE AND T-RFLP METHOD

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INTRODUCTION. Infections caused by fungi from the genus *Candida* are a very serious problem all over the world. Invasive candidosis are very difficult in treatment and is characterized by high mortality. There is a need for fast and specific diagnostic kits because of different antifungal resistance pattern dependent on yeast species. **AIM.** Construction and optimization of diagnostic kit for molecular identification of 6 clinically important species from the genus *Candida* (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. dubliniensis*). It was assumed that the test must be cheap to use, resistant to contaminations and without toxic reagents (such as ethidium bromide). **MATERIALS AND METHODS.** All experiments were based on PCR (Polymerase Chain Reaction). Fungal strains (reference and clinical) were from the collection of the Department of Microbiology. Genomic DNA was extracted using commercial kits. PCR reactions were carried out using specific oligonucleotide primers projected after analysis of homocitrate synthase gene sequences (GenBank NCBI) within

studied *Candida* species. Primer forward was fluorescently labeled by fluoresceine. Amplicons were digested using two endonucleases (*RsaI* and *AdelI*). Products of digestion were separated by electrophoresis in agarose gel without ethidium bromide (visualization in UV light). **RESULTS.** Specific amplicons with fluoresceine at 5' ends were obtained after PCR reaction. Digestion of PCR products allowed getting 5' terminal fragments of different lengths for each species. After electrophoresis only fragments with labeled forward primers were visualized. Identification of *Candida* species was made on the basis of the specific length of 5' terminal digestion product. **CONCLUSIONS.** Polymorphism of the homocitrate synthase gene is sufficient to differentiate 6 clinically important species from the genus *Candida* using the T-RFLP method. The optimized diagnostic kit allows accurate identification of *Candida* species, eliminates using expensive and toxic reagents, sophisticated equipment and is insensitive to bacterial and other fungal DNA contamination.

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FLOW CONDITIONED BIOFILM – A MODEL TO STUDY CANDIDA ALBICANS ERADICATION IN LABORATORY

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INTRODUCTION. Microbial biofilms cause a large number of chronic infections that are not susceptible to traditional antibiotic treatment. Biofilm-forming microbes are held together by a self-

produced matrix that consists of polysaccharides, proteins and extracellular DNA. *Candida albicans* causes infections, which are health- and life-threatening to patients with chronic diseases or

immunodeficiency. **AIM.** Formation of biofilm by microorganisms is a multifactorial process. The aim of this study was to develop and optimize the method for *C. albicans* biofilm formation with the use of MBEC™ high-throughput assay (a polystyrene lid with 96 identical pegs). **MATERIALS AND METHODS.** The following strains of *C. albicans* were used in the studies: ATCC (10231, 14053, 90028), clinical (151, 166, 257) and bioluminescent strain B311. The MBEC™ assay consists of two parts. The top half of the device is a polystyrene lid with 96 identical pegs which is inserted into the bottom half of the device – a 96 microtiter plate containing an inoculated growth medium. The entire device is placed on a rocking table in an incubator (37°C). Biofilm is washed with PBS

and sonication is performed to release the cells from the biofilm on a peg. Serial dilutions of sonicated cells are obtained and colony forming units/ml (CFU) are determined. **RESULTS.** Different conditions (rocking table speed, time of growth, foetal bovine serum (FBS) pegs pretreatment, sonication time) were studied to obtain the best biofilm form of *C. albicans*. The study showed that biofilm formation is mainly strain dependent (the best biofilm formation was showed by ATCC strain 90028 and clinical strains). Coating the pegs with proteins increased the number of cells in the biofilm. The number of cells in biofilm varied from 10^3 to 10^6 CFU/ml (depending on strain and conditions) which makes this method suitable for studies of *C. albicans* biofilm form grown in flow conditions.

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EDTA-DEPENDENT CHANGES IN *PROTEUS MIRABILIS*-INDUCED URINARY STONES FORMATION

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INTRODUCTION. *Proteus mirabilis* cause urinary tract infections especially in patients with indwelling catheters or structural abnormalities of the urinary tract. Urinary stones are a common complication of these infections. Urease is an essential virulence factor in urolithiasis. Ammonia, produced by the enzymatic hydrolysis of urea, elevates urine pH causing supersaturation and crystallization of magnesium and calcium salts as struvite ($MgNH_4PO_4 \times 6H_2O$) and carbonate apatite ($Ca_{10}(PO_4)_6 \times CO_3$), respectively. Additionally, extracellular polysaccharides of *Proteus sp.* may accelerate crystals growth and calculi formation. This study describes the influence of EDTA on *P. mirabilis* virulence factors involved in urolithiasis and the crystallization process. **MATERIALS AND METHODS.** *P. mirabilis* strain was isolated from urinary stones. Crystallization experiments were performed in synthetic urine infected with bacteria alone or with the addition of EDTA in the concentrations 3,

5, 10 mM. During 24 h incubation at 37°C the number of bacteria (CFU/ml), urease activity and intensity of crystallization were determined. The influence of EDTA on bacterial membrane was also established. **RESULTS.** Insignificant effect of EDTA on bacterial viability and urease activity was observed. However, in the presence of this compound pH of synthetic urine increases slowly and crystallization intensity decreases compared with the control. Additionally, it was observed that the struvite crystals aggregate more quickly and intensely in the presence of EDTA. We suggest that lipopolysaccharide released from the cells by the action of EDTA affects crystals aggregation. **CONCLUSIONS.** Analysis of biomineralization in the presence of EDTA, which chelates divalent cations affecting bacterial physiology and crystallization process, expands knowledge of the formation of urinary stones and can lead to the development of prevention methods and treatment of infectious disease.

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APPLICATION OF CHICKEN EMBRYO MODEL AND NEMATODE MODEL IN STUDIES OF DETERMINANTS RESPONSIBLE FOR HOST-SPECIFIC VIRULENCE OF *STAPHYLOCOCCUS AUREUS*

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INTRODUCTION. *Staphylococcus aureus* infections are of major importance in human and veterinary medicine. Human and zoonotic strains of *S. aureus* have been convincingly demonstrated to be genetically distinct. As the bacterium has a highly conserved core genome, host-specific adaptations, influencing virulence, are probably determined by a small number of genes carried on mobile genetic elements. Studies of the virulence of this bacterium are complicated by inconsistent results obtained in different animal models. **AIM.** The aim of this study was to assess whether there is a correlation between origin as well as the genotype of particular

S. aureus strains and their virulence in chicken embryo and nematode models. **MATERIALS AND METHODS.** The relatedness of twenty one poultry strains was determined using multiple state-of-the-art typing methods including among others multiple locus variable number tandem repeat fingerprinting, pulsed field gel electrophoresis, multilocus sequence typing and *agr* group-specific multiplex PCR. The staphylococcal virulence was evaluated in *Galus gallus* (chicken) embryo and *Caenorhabditis elegans* models. **RESULTS.** The variation in virulence between strains is greater for the chicken model than the nematode model. The cumulative host

survival measured in the embryo model ranged from approximately 90% to 0%, while in the *C. elegans* model it had much lower spread (15–40% among the majority of strains). The similar susceptibilities of nematodes to the bacteria, regardless of the origin and genotype of the *Staphylococcus* strains involved, highlights the poorer ability of this model to respond to genetic variations between strains. The chicken embryo model consistently divided the tested poultry strains into virulent, avirulent, and intermediate groups. In all typ-

ing methods the virulent strains formed distinct clusters located on different branches of the dendrograms from the avirulent strains. **CONCLUSIONS.** Our findings indicate the potential usefulness of the embryo model for future identification of host-specific adaptations and virulence factors in *S. aureus*.

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A PROTEOMIC VIEW OF *STAPHYLOCOCCUS AUREUS* HOST-SPECIFIC VIRULENCE FACTORS

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INTRODUCTION. *Staphylococcus aureus* is an opportunistic human and animal pathogen. The ability of effective host invasion and colonization is provided by the production of a wide range of virulence factors. Previous studies revealed a clear correlation between the virulence of poultry-origin *S. aureus* strains, determined using chicken embryo model, and their genotype. This suggested the presence of host-specific adaptations in *S. aureus*. **AIM.** The aim of the study was to compare extracellular proteomes of well characterized, poultry-originated *S. aureus* strains, previously identified as virulent and non-virulent in a chicken embryo model, in order to identify proteins that potentially act as host-specific virulence factors. **MATERIALS AND METHODS.** Proteins from culture fluids of seven virulent and five non-virulent strains were precipitated with trichloroacetic acid and labeled with fluorescent dyes. Labeled proteins were separated using two-dimensional difference gel electrophoresis (2-D DIGE) in pairs (virulent *versus* non-virulent). The obtained protein profiles were compared. Protein spots

that were unique or more abundant in the virulent strain, were cut out from the gel, trypsin digested and subjected to MicrOTOF-QII mass spectrometry analysis. **RESULTS.** In 16 comparisons 330 spots reflecting proteins (over)produced by a virulent strain, were identified. The majority of the differences were qualitative. Subsequent MS analysis of the protein spots revealed 122 different proteins. Most importantly, among those proteins alpha-hemolysin appeared in all virulent examined strains, bifunctional autolysin and staphopain B were present in six strains, whereas 1-phosphatidylinositol phosphodiesterase and serine-aspartate repeat-containing protein E were identified in five out of seven virulent strains. Multiple other proteins were also found together in the majority of virulent strains. **CONCLUSIONS.** The identified proteins most probably play a significant role in the staphylococcal virulence against poultry hosts, and as such may be considered as host-specific virulence factors. This work was partially supported by the grant N N303 813340 (to BW), from the Ministry of Science and Higher Education.

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ANTIBACTERIAL ACTIVITY OF POLY (PROPYLENE IMINE) DENDRIMERS (PPI) IN THE PRESENCE OF DOXYCYCLINE

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INTRODUCTION. Doxycycline is a semisynthetic tetracycline antibiotic often used in the treatment of various infections. The frequent use of this chemotherapeutic led to the acquisition of drug resistance by microorganisms. As a result, alternative methods of bacterial infections treatment are being searched for. The potential solution seems to be dendrimers – macromolecules with a three-dimensional structure and well-defined physical and chemical properties. Polypropyleneimine dendrimers are repeatedly branched polymers containing on their surface tertiary alkyl ammonium groups. **AIM.** The aim of this study was to evaluate the correlated effects of the antibacterial properties of PPI dendrimers (unmodified) with antibiotics against the reference strains of gram-positive and gram-negative bacteria. **MATERIALS AND METHODS.** The evaluation of antimicrobial activity of the dendrimer and the analyzed antibiotic was performed using the modified broth

microdilution method, according to the standards defined by the National Committee for Clinical Laboratory Standards (NCCLS). The analysis involved incubation of the tested microorganisms with serial dilutions of the analyzed compounds on microtiterplates and spectrometric measurement of cell density at 630 nm. The control sample was a bacteria culture without the addition of the analyzed compounds. **RESULTS.** The analysis of the correlated action of PPI dendrimers with the antibiotic showed enhanced antimicrobial activity, compared to the samples containing the antibiotic or dendrimers only. The results concerning the gram-positive strain in samples containing PPI dendrimers and doxycycline are particularly promising. **CONCLUSIONS.** The applications of the correlated action of PPI dendrimers and doxycycline showed the possibility of using lower doses of pharmaceuticals.

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ANALYSIS OF THE RELATEDNESS BETWEEN CLINICAL *PROTEUS PENNERI*, *PROTEUS VULGARIS* AND *PROTEUS* GENOMOSPECIES STRAINS ISOLATED IN LODZ

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INTRODUCTION. Gram-negative *Proteus* sp. bacilli belong to the *Enterobacteriaceae* family, and are opportunistic pathogens, causing diseases particularly in people with the weakened immune system or with defects in the urinary tract. The most frequent etiologic factor in the genus is *P. mirabilis*, however, other species are also identified. **AIM.** The aim of the study was to determine relatedness among clinical strains belonging to three, uncommon *Proteus* species, and analysis of the frequency of particular strains occurrence in Lodz area patients. **MATERIALS AND METHODS.** 9 *P. penneri*, 34 *P. vulgaris* and 41 *P. genomospecies* strains were used. Genomic DNA was obtained according to the instruction of the manufacturer, and used in rep-PCR with BoxA1R, as well as REP 1R-Dt and REP 2Dt primers. Amplified DNA specimens were run in 1.2% agarose gel, together with the molecular weight marker, in TAE buffer. Computer programme PyEph 1.4 served as a tool for DNA patterns

analysis. **RESULTS.** Based on the band patterns in agarose gel, the presence of a band of about 1300 bp characteristic for *Proteus* sp. was confirmed. No specific bands were found for any species. The investigated strains were assigned to appropriate relatedness groups. Some strains showed 100% homology – these were usually derived from one patient. The remaining strains were genetically diverse. A high degree of similarity between investigated *P. genomospecies* strains in the new O79 serogroup was also shown. **CONCLUSIONS.** 1. The investigated strains demonstrate high genetic variability and seldom undergo transmission in the Lodz region. 2. *Proteus* genomospecies strains are the second (in the genus *Proteus*) in the frequency of species isolation in Lodz, and these which represent O79 *Proteus* serogroup tend to be prevalent in the region. This work was supported by grant no. N N401 020135 from the Ministry of Science and Higher Education (Poland)

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A NEW LM-PCR METHOD FOR DIFFERENTIATION OF *MYCOBACTERIUM TUBERCULOSIS* STRAINSAnna Żaczek^{1*}, Anna Brzostek², Aneta Kuroń², Arkadiusz Wojtasik², Jarosław Dziadek^{1,2}¹Department of Biochemistry and Cell Biology, Rzeszow University, Rzeszow, Poland²Institute of Medical Biology, Polish Academy of Sciences, Lodz, Poland

INTRODUCTION. A fast and inexpensive epidemiological method which could be applied for the analysis of *Mycobacterium tuberculosis* strains needs to be developed. An interesting alternative are methods based on ligation-mediated PCR (LM-PCR) useful in the epidemiological analysis of a number of bacterial species. The LM-PCR methods can be adapted for mycobacteria if based on IS6110 polymorphism. **AIM.** The development of a new LM-PCR method named fast ligation amplification polymorphism (FLAP), and its application to the published reference set of 62 *M. tuberculosis* strains isolated in a single city of Poland. **MATERIALS AND METHODS.** Genomic DNA of strains was extracted from Löwenstein-Jensen slants. The FLAP products were separated in an acrylamide gel and visualized in UV. The specificity of patterns was verified by southern blot hybridization. Dendrograms were generated using the BioNumerics package 5.0. The discriminatory power of FLAP was calculated as the Hunter-Gaston index (HGI). **RESULTS.** FLAP is based on PCR amplification of 5' flanking

region of IS6110, which includes *Sall/PvuII* digestion of chromosomal DNA, ligation of *Sall* linker and differentiation of IS6110 carrying restriction fragments by suppression substrate hybridization. FLAP typing differentiated 32 patterns among the 62 clinical isolates; 14 were unique, whereas the remaining 18 cluster patterns included 48 strains. The same set of strains was previously evaluated using MIRU-VNTR and IS6110-RFLP analysis presenting 28 and 36 clusters, respectively. The discriminatory power was 0.973 for FLAP compared to 0.966 for MIRU-VNTR and 0.971 for IS6110-RFLP. **CONCLUSIONS.** The discriminatory power of FLAP is close to that of the reference method, IS6110-RFLP. FLAP analysis can be applied as a complementary method when typing mycobacteria using methods other than based on IS6110, e.g. MIRU-VNTR. FLAP method is cheap and very fast and could be used as an interesting molecular-epidemiology tool for the analysis of collections with a limited number of strains.

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THE INFLUENCE OF COLLOIDAL SOLUTION OF NANOSILVER AND NANOCOPPER ON CHEMILUMINESCENCE AND VITALITY OF NEUTROPHILS

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INTRODUCTION. Nanotechnology is used in many areas of daily life and industry. One example is using the bactericidal activity of silver and copper nanoparticles. It is important to examine whether these particles have an influence on first line defense immune cells – neutrophils in human body. **AIM.** The goal of this study was to examine whether silver nanoparticles affect the human immune system by stimulating or inhibiting the respiratory burst in neutrophils and if they change neutrophil vitality. **MATERIALS AND METHODS.** Neutrophils were isolated from 14 healthy donors' peripheral blood. To examine the influence of nanoparticles two methods were used. Firstly, the MTT assay which measures cell vitality with a tetrazolium salt reduction to an insoluble formazan. The amount of the reduced MTT dye is proportional to the mitochondrial cell oxidative activity which is connected with the number of metabolically active (live) cells in a population. Secondly, the physiological condition of neutrophils rated by the ability to gener-

ate reactive oxygen forms was measured with chemiluminometry. **RESULTS.** Both incubations of neutrophils with low concentrations – 0.4 ppm and 2 ppm of nanosilver and nanocopper solution did not affect the mitochondrial activity of neutrophils. The use of 10 ppm solution of nanosilver decreases neutrophils activity, contrary to nanocopper solution which does not have a negative effect. The mitochondrial activity of neutrophils is significantly decreased by 50 ppm nanosilver and nanocopper solution. The studies of chemiluminescence showed that silver and copper nano particles do not affect the leverage or the inhibitory effect on human neutrophil. Presence of silver and copper nanoparticles did not change chemiluminescence level of neutrophils activated by standard stimulator. **CONCLUSIONS.** The commercial colloidal nanosilver and nanocopper solutions with high concentrations can decrease mitochondrial neutrophils activity but they do not affect the ability to generate the reactive oxygen forms by neutrophils.

There should be:

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THE INFLUENCE OF COLLOIDAL SOLUTION OF NANOSILVER AND NANOCOPPER ON CHEMILUMINESCENCE AND VITALITY OF NEUTROPHILS

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