Session VII. Medical Microbiology

Oral presentations

VII.OP.1

In vitro infection model reveals restricted intracellular activity of antibiotics against uropathogenic *Escherichia coli*

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Urinary tract infections are among the most common bacterial infections worldwide, and uropathogenic *Escherichia coli* (UPEC) is one of the major causative agents. Even though UPEC were considered as an extracellular pathogen, *in vivo* mouse studies showed that bacteria are also able to invade, multiply and persist within bladder epithelial cells. Internalized bacteria can become more protected from both, host immune system and antibiotic effect. Their eradication is complicated and surviving bacteria might lead to development of chronic and relapsing infections.

In our current work we established the model of UPEC *in vitro* intracellular infection which allows us to examine the effects of different antibiotics against intra- and extracellular bacteria. In addition, intracellular bacterial subpopulation, which survive antibiotic treatment, can be further analysed to determine the precise localisation and metabolic activity of bacterial cells. As model cells we used mouse macrophage-like cells which were infected with UPEC strain CFT073, isolated from the blood of patients with pyelonephritis.

Our results showed that among the tested antibiotics the best effect against intracellular bacteria, was shown by fluoroquinolones (ciprofloxacin and finafloxacin), while gentamicin and sulfamethoxazole were less or no efficient. Small fractions of intracellular bacteria were able to survive treatment with really high concentrations of antibiotics, which was not the case with extracellular bacteria. The reason for that might be lower antibiotic penetrability though eukaryotic membrane or formation of bacterial persisters, which should be determined in the future experiments.

Keywords: Urinary tract, pharmacodynamics, in vitro models, antibiotics

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VII.OP.2

Widespread distribution of soricid- and talpid-borne hantaviruses in Poland

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With the discovery of genetically distinct hantaviruses (family Hantaviridae) in multiple species of shrews and moles (order Eulipotyphla, family Soricidae and Talpidae), the long-held conventional view that rodents (order Rodentia) serve as the exclusive reservoir hosts is no longer tenable. Previously, in an exploratory study of soricine shrews in central Poland, we reported a novel hantavirus, designated Boginia virus (BOGV), in the Eurasian water shrew (*Neomys fodiens*). To ascertain the geographic distribution of non-rodent-borne hantaviruses in central and southeastern Poland, lung tissues from 53 Eurasian common shrews (*Sorex araneus*), 52 Eurasian pygmy shrews (*Sorex minutus*), 12 Mediterranean water shrews (*Neomys anomalus*), 16 Eurasian water shrews, and 69 European moles (*Talpa europaea*), captured during 2010–2013, were analyzed for hantavirus RNA by RT-PCR. As verified by DNA sequencing, Seewis virus (SWSV) was found in six of 18 Eurasian common shrews from Chmiel, BOGV in four of 14 Eurasian water shrews from Boginia, Kurowice and Huta Dłutowska, and Nova virus (NVAV) in 38 European moles from Huta Dłutowska. SWSV was also detected in two of 17 Eurasian pygmy shrews from Kurowice and in one of 12 Mediterranean water shrews from Chmiel. In addition, NVAV RNA was detected in heart, lung, liver, kidney, spleen and intestine of European moles. The nucleotide and amino acid sequence variation of the L segment among the SWSV strains was 0–18.8% and 0–5.4%, respectively. And for the NVAV strains, the L-segment genetic similarity ranged from 94.1–100% at the nucleotide level and 96.3–100% at the amino acid level. Phylogenetic analyses showed geographic-specific lineages of SWSV and NVAV in Poland, suggesting long-standing host-specific adaptation. The cocirculation of BOGV, SWSV and NVAV in Poland parallels findings of multiple hantavirus species co-existing in their respective rodent reservoir species. (Poland National Science Centre grant 2015/19/B/NZ4/03234)

Keywords: hantavirus, shrew, mole, Poland

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VII.OP.3

Assessment of cell surface properties and hydrocarbons degradation potential of three bacterial strains

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Microbial surface is responsible for adsorption and transport of nutrients inside the cell. Its properties depends on many environmental factors and can be modified to enable bacteria to better substrates adsorption. Thus, cell surface hydrophobicity, zeta potential as well as membrane permeability and fluidity can be modified in a wide range. The most frequent factors affecting modification of bacterial surface include: temperature, pH, humidity, oxygenation and bioavailability of nutrients. It should be emphasized that, the rapid cellular response to environmental factors variations determines the survival of microorganisms in a given environment. Taking this into account, the main objective of this research was to determine the impact of microbial exposure on hydrophobic substrates on the modifications of cell surface properties. For this purpose, *Raoultella planticola* M01, *Raoultella ornithinolytica* M03 and *Acinetobacter calcoaceticus* M1B were isolated from different soil samples and subjected to a 12-month exposure to aliphatic and aromatic hydrocarbons. Then, changes in cell surface hydrophobicity, zeta potential and membrane permeability were analyzed and compared to those obtained for control strains. The results of the study showed significant changes in the properties of cells exposed to hydrocarbon contaminants compared to control samples. Observed higher cell surface hydrophobicity and membrane permeability, as well as lower surface zeta potential, allow cells to more effective adsorption and degradation of hydrophobic metabolic substrates. The alternations of cell surface properties also enhanced effective hydrocarbons degradation.

Keywords: microorganisms; hydrocarbons; cell surface properties

Acknowledgment:

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VII.OP.4

Role of the peroxisomes and pentose-phosphate pathway in xylose alcoholic fermentation of conventional and non-conventional yeasts

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The role of peroxisomes in xylose catabolism and fermentation remained unknown. In this work, we studied effects of the lack of peroxisomes on xylose growth and alcoholic fermentation in the thermotolerant methylotrophic yeast *Ogataea polymorpha* versus non-methylotrophic yeast *Scheffersomyces stipitis* and xylose-utilizing strain of *Saccharomyces cerevisiae*. It was found that strains of *O. polymorpha* deficient in peroxisome biogenesis *pex3*⊿ and *pex6*⊿ are characterized by normal growth on xylose whereas alcoholic fermentation of this pentose was almost totally suppressed. Strains of *O. polymorpha* with overexpressed key peroxisomal enzyme alcohol oxidase were constructed, they show increased ethanol production from xylose relative to the wild-type strain. It was found that overexpression of alcohol oxidase leads to an increase in expression of *DAS1* and *TAL2* coding for peroxisomal transketolase (dihydroxyacetone synthase) and transaldolase, respectively.
We found that peroxisomal transketolase and transaldolase in *O. polymorpha* are required for xylose alcoholic fermentation but not for growth on this pentose. Mutants with knock out of *DAS1* and *TAL2* normally grew on xylose though were defective in its conversion to ethanol. The mutants of *O. polymorpha* with defects of *TAL1* and *TKL1* coding for cytosolic transaldolase and transketolase, respectively, did not grow on xylose; these lesions were complemented by overexpression of the genes of their peroxisomal counterparts *TAL2* and *DAS1*. Overexpression of *TKL1, TAL1, DAS1* and *TAL2* in the wild-type strain increased ethanol synthesis from xylose 2-4 times with no effect on glucose alcoholic fermentation. Finally, co-overexpression of *DAS1* and *TAL2* in the best isolated *O. polymorpha* ethanol producer from xylose led to an elevated level of accumulated ethanol up to 16.5 g/L at 45 °C or 30-40 times more than is produced by the wild-type strain.

Keywords: alcoholic fermentation; peroxisomes, peroxins; pentose-phosphate pathway

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VII.OP.5

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

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Introduction: *Mycobacterium smegmatis* is used as a model organism to study mycobacterial physiology and gene regulation. The genome of this organism contains the MSMEG_4305 gene coding for a two-domain protein. This gene is present across *Mycobacterium* but absent in other species of bacteria. Sequence similarity analysis indicates that the C 'terminal domain is the CobC domain, predicted to be involved in the synthesis of vitamin B12, while the N'terminal domain encodes an RNase H. The function of RNase H domain has been confirmed *in vivo*, however the function of CobC domain remains elusive. **Aim:** The aim of this project is to determine the possible role of MSMEG_4305 protein in the process of vitamin B12 biosynthesis in *M. smegmatis* cells.
Materials and Methods: The ability to synthesize the B12 molecule by *M. smegmatis* was investigated thru analysing the formation of methylfolate trap resulting in hypersensitivity to sulfonamide and thru phenotypic analysis. We compared the growth of three gene deficient mutants MSMEG4305, MSMEG3873 (*cobIJ*, gene with confirmed role in vitamin B12 synthesis) and MSMEG4305/3873 with wild type *M. smegmatis* mc² 155. We used flow cytometry to count bacterial cells and compared this data with the number of colony forming units (CFU). Flow cytometry was used also to determine cell size.
Results: We observed a hypersensitivity to sulfonamide of all of the analysed mutants on the medium enriched with sulfonamide. We noticed also significant differences between the cell length of all analysed strains of *M. smegmatis* compared with wild type. Supplementation of the medium with vitamin B12 restored the phenotype of the mutants to the wild type *M. smegmatis*.
Conclusions: Our results suggest that MSMEG4305 is involved in vitamin B12 synthesis in *M. smegmatis*, though further research is needed to confirm our hypothesis.

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

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VII.OP.6

Link between bacterial DNA uptake and host cytokine sequestering

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Several bacterial species uptake extracellular DNA (eDNA) in biofilms to acquire new genes and nutrients for survival in nutrient-poor environments. Moreover, various pathogens can sequester and uptake host cytokines to modulate their virulence and perturb host defence. Here we report that these two characters are most likely linked in an oral opportunistic pathogen, *Aggregatibacter actinomyctemcomitans*.
In this study, we investigated the interactions of different human cytokines with the extramembranous domains of secretin channel HofQ/ComE (emHofQ), which was earlier shown to bind DNA. The strongest interaction was detected with interleukin (IL)-8, which had the dissociation constants of 43 nM and 2.4 μM, in static and dynamic settings, respectively. We identified the interaction site of IL-8 in emHofQ with crosslinking and confirmed the site with mutational assays. Structural comparison revealed a similar interaction site in a related domain of *Neisseria meningitidis* secretin PilQ, which has previously been shown to be involved in IL-8 uptake. When the *hofQ* gene was deleted from *A. actinomycetemcomitans* genome, the cells did not respond to IL-8 by decreasing the amount of eDNA in the biofilm like their wild type counterparts. In addition, Δ*hofQ* mutants were more susceptible to β-lactams than the wild type cells, and IL-8 interacted with DNA. Thus, in biofilm the IL-8 bound to eDNA could use the same channel, secretin HofQ, as the internalized eDNA to enter the *A. actinomycetemcomitans* cell.
This is the first time when the bacterial internalization of eDNA has been associated with host cytokine uptake. Since the potential molecular machinery of cytokine uptake seems to have several functions, atomic level information about the protein-protein interaction sites are needed in order to be able to disturb them in further studies about the role of cytokine sequestering in bacterial virulence.

Keywords: *Aggregatibacter actinomycetemcomitans*; natural competence; interleukin-8; secretin HofQ/ComE

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VII.OP.7

Characterization of the newly isolated lytic bacteriophages and their efficacy against multi-drug resistant *Staphylococcus aureus* biofilm

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The majority of microorganisms in their natural habitats is attached to a variety of solid surfaces forming groups of bacterial cells which are embedded within an extracellular matrix. These communities of microorganisms are known as biofilms. It has been estimated that biofilms are responsible for approx. 65% of all microbial infections. Treatment of biofilm related infections can be problematic as the structure and composition of the biofilm provides protection against antimicrobial agents. As a consequence, a lot of research effort is currently being spent developing methods to eradicate bacterial biofilm. Much hope is placed in bacteriophages that have been shown to be an effective therapy for antibiotic-resistant infections.

This study characterized two phages isolated from sewage which show lytic activity against clinical isolates of multidrug resistant *Staphylococcus aureus* (MDRSA). Morphology and biological properties, including plaque morphology, host range, adsorption rate, latent time, and phage burst size were studied for the phages. The potential of using these phages to eradicate multidrug-resistant *S. aureus* biofilms was assessed on five multidrug-resistant clinical isolates of *S. aureus*. Biofilms formed in microtiter plates after 24 h were treated with phage lysate. The removal of established biofilms was investigated by crystal violet staining, MTT assay, scanning electron microscopy (SEM) and CFU enumeration.

Both phages tested exhibited high rates of lytic potency on 60-70% of *S. aureus* clinical isolates and suppressed planktonic cells of selected MDRSA strains with multiplicities of infection ranging from 0,01 to 1 for 4 h without apparent regrowth of bacterial populations. Treatment of biofilms resulted in a significant reduction of biomass and a decrease in the metabolic activity of biofilm produced by selected isolates. SEM analysis revealed the reduction of the number of cells in all biofilms treated with phages.

Keywords: bacterial biofilm; bacteriophages; *Staphylococcus aureus*

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VII.OP.8

Interleukin-8 interaction with lipopolysaccharide from *Aggregatibacter actinomycetemcomitans*

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Cytokines play an important role in the progression of an immune response against harmful pathogens during infections. Various Gram-negative species are known to sequester host cytokines using their surface exposed outer membrane proteins, or surface modulation by sulphated polysaccharides. We have previously shown that an outer membrane lipoprotein (bacterial interleukin receptor I, "BilRI") of a periodontal pathogen *Aggregatibacter actinomycetemcomitans* binds several cytokines, including interleukin (IL)-8.

Since IL-8 carries a net positive charge at physiological pH, the aim of this study was to investigate whether it also interacts with the negatively charged lipopolysaccharides (LPS) in the outer membrane of bacteria. LPS was purified from the outer membrane of *A. actinomycetemcomitans* (N=13), *Pseudomonas aeruginosa* (N=1) and *Escherichia coli* (N=1). IL-8 was found to interact with each of the purified LPS-preparations, the K_d values for the interaction varying from 1.2 to 17 μM. The affinity of IL-8 for LPS was dependent neither on the bacterial serotype, nor the amount of negatively charged phosphate present in the LPS-preparations, but it was significantly higher than the affinity of IL-8 for BilRI (K_d >25 μM). IL-8 was also found to interact with outer membrane vesicles isolated from *A. actinomycetemcomitans*. Outer membrane vesicles are released by many bacteria, and they carry many virulence factors and toxins, including LPS.

Although the mode of interaction between IL-8 and LPS remains to be resolved, our findings suggest that LPS might be involved in binding IL-8 to the outer membrane of *A. actinomycetemcomitans*. Thus, our results raise an interesting question whether Gram-negative species may use LPS to sequester IL-8 *in vivo*, and whether this might have an impact on the role that IL-8 plays in the immune response related to bacterial infections.

Keywords: *Aggregatibacter actinomycetemcomitans*, lipopolysaccharide, interleukin-8, serotype

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VII.OP.9

Rapid confirmation of methicillin resistant *Staphylococcus* spp. by MALDI-TOF MS in clinical isolates from Latvia

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Twelve *S. aureus* strains were used for identification result and spectra comparison using three different sample preparation methods. For resistance analyses, 65 *Staphylococcus* spp. strains from clinical and hospital environment isolates with susceptibility to methicillin-37 resistant and 28 sensitive were used to confirm phenol-soluble modulin mec (PSM-mec) peptide presence by 2414±2 Dalton (Da) peak and delta-toxin occurrence examination by 3006±2 Da peak using FlexAnalysis and BioNumerics. Reproducibility of PSM-mec peptide was done with direct method in triplicates of nine spectra total per sample.

The direct methods identification results of 12 samples significantly differed at α level 0.05 compared to extraction and direct method with 70% formic acid. Spectra of samples prepared with three methods varied in peak intensities and presence. PSM-mec peak was not visible in extraction method. From 65 analyzed strains, peak 2414±2 Da specificity was 100%, presented only in methicillin resistant strains of 51% and none in sensitive. Peak reproducibility from five analyzed *S. aureus* strains with the presence of 2414±2 Da was 86.66%. Delta-toxin was observed in 48/65 samples regardless of methicillin susceptibility and in all our samples with PSM-mec peak presence.

The 2414±2 Da peak is specific only in methicillin resistant strains carrying the *mecA* gene but the absence does not exclude resistance presence in the strain. Implementing peak recognition in a routine laboratory work especially within clinical samples would take less time and costs for diagnosis determination.

Keywords: delta-toxin, methicillin resistance, phenol-soluble modulin, *Staphylococcus aureus*

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VII.OP.10

Discovery of the new super-resistant phenotype of *Candida* yeasts grown under the modeled microgravity conditions.

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Colonization of the spaceships by microorganisms is one of the common problems to deal with. During long space-flights astronauts are affected by the various stresses like: ionizing and UV radiation, closed environment thus can lead to the decreased immunity, allergic diseases and increased risk to suffer microorganism caused infections. Microgravity conditions are causing alterations in the pathogenicity of the microorganisms as well as in their resistance to the various treatments. Since the usage of the chemical compounds is strictly limited during the long-term space-flights, alternative methods for the biocontrol of the skin colonizing yeasts are under great relevance. Pulsed electric field could be the alternative for the treatment of *Candida* related diseases.

For the simulation of the microgravity we used rotary cell cultivation system designed by the Synthecon. This system ensures the bubble-free, membrane-based oxygenation and simulates the microgravity by using vertical rotation. After the cultivation in RCCS we analyzed the changes of the *Candida* genera yeast resistance to various chemical compounds as well as physical stress. Cultivation in RCCS led to the extreme increase in the antifungal drug resistance. We showed, that switch to the pseudohyphae morphology leads to the 10 and 20 times increased resistance to amphotericin B in *C. lusitaniae* and *C. guilliermondii* and cultivation in RCCS led to 20 and 32 times increased resistance respectively. RCCS grown *C. lusitaniae* and *C. guilliermondii* showed increased survivability after PEF (pulsed electric field) treatment as well. These findings indicate the new super-resistant phenotype not related to the morphology switching.

Keywords: microgravity, *Candida,* altered morphology, pseudohyphae, super-resistance

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