Poster presentations

Session I. Bacteriophages and plasmids

I.P.1

Isolation and characterization of *Pseudomonas aeruginosa* bacteriophages

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Pseudomonas aeruginosa is a pathogenic bacterium that causes infections in humans and animals. These infections are a world-wide problem because *P. aeruginosa* is naturally resistant to multiple antibiotics. Moreover, currently, antibiotic therapy against bacterial infections is becoming less effective. Therefore, the interest in phage therapy as an alternative to antibiotics has re-arisen.

The subject of this study was the isolation of a bacteriophage capable of destroying *Pseudomonas aeruginosa* and characterization of the phage in terms of its potential use in the treatment of animal infections.

In this study, we isolated a bacteriophage specific to *Pseudomonas aeruginosa* strain obtained from an animal infection. Bacteriophage stock was prepared by enrichment of a sewage sample with bacterial culture. Then, the detection of plaques on the dual-layer plates was carried out. Propagated bacteriophages in liquid culture were concentrated with the use of polyethylene glycol 8000. Capsid morphology and phage taxonomy were preliminarily analyzed by transmission electron microscopy. Next, bacteriophages were tested for their resistance to chloroform, temperature and pH. One-step growth experiments were carried out and latent period, burst size and adsorption kinetics were determined. Additionally, we examined the phage host range in other *Pseudomonas aeruginosa* strains obtained from diseased animals.

This preliminary analysis indicates that this bacteriophage has a potential as a effective treatment of animal infections and is a promising candidate for further studies.

I.P.2

Development of recombinant lambdoid phages with deletions of particular open reading frames and genes from the *exo*-xis region

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Enterohemorrhagic *Escherichia coli* (EHEC) strains are carriers of genes coding for Shiga toxins, which are located in genomes of lambdoid prophages. Their genomic organization and life cycle are similar to bacteriophage λ. Pathogenicity of EHEC depends on lambdoid prophage induction and subsequent production of toxins. This process may be either spontaneous or caused by various agents (e.g. H_2O_2, mitomycin C or UV-irradiation).

In this report, we want to particularly highlight the region between *exo* and *xis* genes of bacteriophage λ and *stx*-carrying $\Phi24_B$ phage, contains highly conserved genes and open reading frames of largely unknown functions. Our previous results presented evidence for the relation between the *exo-xis* region and phage development. So far, the impact of expression of particular components of the *exo-xis* region on lambdoid phage development has been investigated in the presence of additional copies of this region. Now, we propose a new approach based on the deletions in the frame of *exo-xis* regions of lambdoid prophages. The recombinant prophages (λ and $\Phi24_B$) lacking one of the open reading frames *orf61, orf73* or genes *ea22, ea8.5* were constructed by homologous recombination using the λRed system. We observed that deletion of *orf61* or *orf73* result in more efficient lysogenization of *E. coli* bacteria by phage $\Phi24_B$. Interestingly, the deletion of gene *ea8.5* resulted in earlier induction, while *orf61* and *orf73* delayed induction of λ prophage after treatment with mitomycin C. At the same conditions, the deletion of *orf61* caused earlier induction of recombinant $\Phi24_B$ prophage. Surprisingly, different results were obtained after induction of analyzed recombinant prophages by other agents such as H_2O_2 and UV-irradiation.

Key words: phage λ, Shiga toxin-converting bacteriophages, region *exo-xis*, prophage induction, inducing agents

I.P.3

Isolation and characterisation of a bacteriophage specific for *Enterococcus faecalis* – a novel, potential agent to treat infections

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Members of the genus *Enterococcus* are the most prevalent strains cultured from humans and account for more than 90% of clinical isolates. *Enterococcus faecalis* shows a natural resistance to many commonly used antibiotics, such as cephalosporins and semisynthetic penicillins. Its ability to survive in harsh environmental conditions enabled this organism to evolve to become one of the leading factors in urinary tract infections, infective endocarditis and bacteremia.
The aims of this work were the isolation of a bacteriophage specific for *Enterococcus faecalis* and its characterisation as a candidate for phage therapy.
Enterococcus faecalis was isolated from the animal infection in a collaborating vet clinic. The bacteriophage PRA88 specific for *Enterococcus faecalis* was isolated from sewage obtained from the Prabuty's sewage plant. Propagated and purified bacteriophages were analyzed by transmision electron microscopy to preliminarily determine the phage taxonomy as well as to evaluate the length of the phage tail and the shape of the phage head. In the next step, plaque morphology was characterized. Kinetics of adsorption, as the first stage of phage developmental cycle, was analyzed. To determine parameters of PRA88 development, we also performed one-step growth experiments, which enabled us to calculate latent and eclipse periods and the burst size.
The bacteriophage isolated in this work is a candidate for further experimental analyses with respect to its potential use in phage therapy.

Key words: phage therapy, bacteriophage, *Enterococcus faecalis*

I.P.4

Therapeutic potential of a new *Klebsiella pneumoniae* bacteriophage: isolation, genetic characterization and phage development in slowly growing bacteria

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Klebsiella pneumoniae is a facultative anaerobic Gram-negative bacterium associated with pyoderma in animals. *K. pneumoniae* can acquire resistance to carbapenems, thus becoming insensitive to almost all available antimicrobial agents. Phage therapy has the potential to replace antibiotic treatment. Lytic bacteriophages and/or their gene products such as lysins, can be easily used as therapeutic agents against bacteria as they are host specific and generally show no side effects.
We focused on isolation and characterization of new bacteriophages against *Klebsiella pneumoniae,* which can be potentially used in phage therapy in animals.
We collected *K. pneumoniae* swab samples from the infected animals that could not have been treated with a standard antibiotic therapy. Afterwards, new lytic phage PRA33 was isolated from sewage and, with the use of electron microscopy analysis, it was structurally defined as a member of the *Siphoviridae* family. Phage plaque morphology analysis (the plaque clarity and the ability to increase its diameter in time) and determination of phage host range were carried out. Then, analysis of kinetics of adsorption and one-step growth curves were determined in bacteria growing with slow growth rates in a chemostat system. We also performed phage stability tests in various liquid media. Phage genome was sequenced *de novo* and the identification of putative ORFs was undertaken.
Based on the results of our experiments, we propose PRA33 phage to be a promising candidate for application in phage therapy against *K. pneumoniae* causing infections in animals that are otherwise difficult to treat.

Key words: phage therapy, bacteriophage, *Klebsiella pneumonia*

I.P.5

Effects of *exo-xis* deletions in genomes of λ and Shiga toxin-converting $\Phi 24_B$ bacteriophages on the development of these phages

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The *exo-xis* region is located in the central part of lambdoid bacteriophages' genomes and contains highly conserved genes of largely unknown functions. This region participates in modulating host genome functions but physiological significance of the regulation in phage development has remained unknown. We present the results of the influence of the removal the whole *exo-xis* region or only highly conserved open reading frames (ORFs: *orf60a, orf, 63, orf61, orf73*) on the development bacteriophage λ and Shiga toxin-converting bacteriophage (Stx phage) $\Phi 24_B$. We constructed recombinant phages devoid of either all genes between *exo and xis* (called Δ*exo-xis*) or four ORFs (*orf60a*, *orf63*, *orf61* and *orf73*; called Δ*orfs*). The absence of genes from the *exo-xis* region caused delayed induction of both prophages following stimulation by various agents (mitomicyn C, hydrogen peroxide and UV irradiation). Both types of deletions in this region resulted in increased efficiency of lysogenization by λ and $\Phi 24_B$ mutants. Moreover, survival of cells after phage infection differed between Δ*exo-xis* and Δ*orfs* bacteriophages. Our results demonstrated that the *exo-xis* region played an important role in the regulation of development of lambdoid bacteriophages, including Stx phages.

Key words: Shiga toxin bacteriophages, lysogenization, prophage induction, *exo-xis* region

I.P.6

Isolation and characterization of *Bordetella bronchiseptica* bacteriophage PRA2

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Bordetella bronchiseptica is a respiratory tract pathogen of mammals, able to cause multidrug-resistant infections. Nowadays, the bacterial resistance to antibiotics has started to be a severe therapeutic problem worldwide. One of the methods that can be used instead of antibiotic-based treatment is bacteriophage (phage) therapy.

The aim of the study was to isolate and characterize a bacteriophage infecting a multidrug resistant *B. bronchiseptica* strain and to analyze the potential application of the phage as an antibacterial agent.

The bacterial strain used in this study was isolated from diseased rabbit with nasal infection. The bacteriophage PRA2 infecting the *B. bronchiseptica* strain was isolated from wastewater collected in a sewage treatment plant in Prabuty, Poland. Bacteriophage PRA2 was propagated and purified by centrifugation, filtration and PEG precipitation. With the use of transmission electron microscopy (TEM) it was possible to characterize the capsid morphology, which enabled us to preliminarily characterize the phage taxonomically. We also analyzed the plaque morphology and described phage PRA2 stability in terms of temperature, pH range and osmotic conditions. In order to determine the growth curve and burst size,we used the one-step growth method. We determined the kinetic model of phage adsorption and described the adsorption rate and efficiency.

Knowing that phage PRA2 maybe a potential candidate for phage therapy it is necessary to analyze its genome sequence, which is our future goal. We believe that the PRA2 phage would be an innovative and safe alternative to antibiotic treatment of bacterial infections.

Key words: phage therapy, bacteriophage, *Bordetella bronchiseptica*

I.P.7

Differentiation of bacteriophages specific to uropathogenic *Proteus mirabilis* strains by RFLP method

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P. mirabilis cause urinary tract infections (UTIs), which are difficult to cure due to the increasing drug resistance of these bacteria. An alternative treatment of these infections involves using the bacteriophages. From a practical point of view quick differentiation of phages is very important. Molecular biology techniques e.g. RFLP can be used for this purpose. The RFLP method differentiates microorganisms based on a pattern of DNA fragments cut by specific endonucleases and analyzed on the agarose gel.
The aim of the present study was to examine the utility of the RFLP method for differentiation of 51 bacteriophages specific to uropathogenic *P. mirabilis* strains.
As a result of the preliminary experiments, phages for RFLP analysis were propagated on nutrient broth and phage DNAs were digested with EcoRV enzyme. The genomes of the phages represented 34 different restriction profiles consisting of about 9 to over 20 bands and one consisting of 1 band (phage 58a Pm1220 resistant to EcoRV). On the basis of the restriction profiles, a dendrogram presenting the relationship between phages were prepared. The 33 RFLP-EcoRV profiles showed a Dice similarity index of 38.8%. Twenty two RFLP patterns were presented by a single phage. The remaining 12 restriction profiles consisted of 2 to 4 viruses. The discriminatory index (HGDI) of 0.982 indicated that EcoRV-RFLP had high differentiating power of *Proteus* specific phage genomes.
To conclude, the results of the present study proved that RFLP is a good method for quick and proper assessment of the genetic diversity among bacteriophages infecting *P. mirabilis* strains. Genomic fingerprints obtained from different *P. mirabilis* specific phages allowed differentiating the collection and determining the relationships between the phages

Key words: *P. mirabilis*, bacteriophage, RFLP

I.P.8

Isolation and identification of a novel *Streptococcus canis* bacteriophage DG11

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Bacteriophages (phages) are bacterial viruses that interact with bacterial cells. During lytic development they disturb bacterial metabolism and cause bacterial cell lysis. As natural predators of bacteria, phages can be used as a substitute for antibiotics, especially in the treatment of infections caused by drug-resistant *Streptococcus* strains. Streptococci are important opportunistic pathogens of dogs and cats and cause various infections, including those in the respiratory tract, skin, genitourinary system, eyes, bones, and the cardiovascular system.
The aims of this study were: 1) the isolation of a bacteriophage specific for *Streptococcus canis* from environmental samples and 2) its characterization.
Bacteriophage DG11 was isolated from a water sample collected in a sewage treatment plant Gdynia Dębogórze, Poland. DG11 isolate was obtained by plaque assay in top agar and purified by centrifugation, filtration, PEG precipitation and HPLC using Monolithic Columns. DG11 morphology was characterized using transmission electron microscopy. To investigate phage physiology and to evaluate its potential to be exploited in therapy, we performed one-step growth experiments, and explored the phage thermal stability and its tolerance to chloroform and pH range.
The results of our study indicate that the bacteriophage DG11 might serve as a candidate for further studies of its potential application as an antimicrobial agent in phage therapy of *Streptococcus canis* infections.

Key words: bacteriophage, *Streptococcus canis,* drug-resistant, phage therapy

I.P.9

Role of the open reading frames *orf60a* and *orf63* located within *exo*-xis region in development of bacteriophage λ and Shiga toxin-converting phage $\Phi 24_B$

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Shiga toxins are the main virulence factor of a group of Shiga toxin-encoding *Escherichia coli* (STEC) strains responsible for human infections that result in bloody diarrhea, often with severe complications. Shiga toxins are encoded by genes *stx1* and *stx2*, located in genomes of a lambdoid bacteriophages that are related to the well-characterized nontoxic coliphage λ. Efficient expression of Shiga toxins is stimulated upon lambdoid prophage induction.
Although lambdoid phages (especially λ phage) and their developmental pathways have been investigated in details, functions of some of their genomic regions are not yet clear. In this report we would like to pay attention to the *exo-xis* region, which is located in the central part of λ phage genome. This region is transcribed from the early λ p_L promoter and consists of several open reading frames (ORFs). It was found that four of the ORFs (*orf60a*, *orf63, orf61 and orf73*) are highly conserved among λ and $\Phi 24_B$ phages. Interestingly, previous studies indicated that presence of the whole *exo-xis* region on multicopy plasmid results in impaired lysogenization of *E. coli* bacteria and more effective induction of λ and $\Phi 24_B$ prophages.
In the light of this, we decided to determine the influence of deletion of particular ORFs from the *exo-xis* region on lambdoid phages development. The selected ORFs were deleted from chromosome of *E. coli* cells lysogenic for λ and $\Phi 24_B$ phages by homologous recombination. We observed that deletions of *orf60a* or *orf63* result in more efficient lysogenization of *E. coli* bacteria by phage $\Phi 24_B$. Interestingly the deletion of *orf60a* results in delayed induction of $\Phi 24_B$ prophage whereas unexpectedly no significant effects was found in the case of λ propahge induction.

Key words: lambdoid bacteriophages, Shiga toxin-converting bacteriophages, region *exo-xis*, prophage induction

I.P.10

The genomic characteristic of *Pseudomonas aeruginosa* Paer4_119 – a strain for the propagation of therapeutic phages

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P. aeruginosa is a widely distributed Gram-negative environmental bacterium. It is also an opportunistic pathogen, which causes human and animal infections. They are difficult to treat due to antibiotic resistance of *P. aeruginosa* strains and the emergence of strains resistant to new generation β-lactams. Thus, phage therapy may be an attractive strategy to treat infections by antibiotic resistant *P. aeruginosa* strains.
Only selected *P. aeruginosa* strains are suitable for the propagation of therapeutic phages. For the safety of phage therapy they have to be well characterized. Thus, we determined the complete genome sequence of the *P. aeruginosa* PAER4_119 strain, which is used at the L. Hirszfeld Institute of Immunology and Experimental Therapy for the propagation of certain therapeutic phages. A hybrid approach, combining Roche Genome Sequencer GS FLX+ Titanium (454 platform) and MiSeq Genome Sequencer (Illumina technology) single reads, that were assembled with the Newbler v.2.9 GS De Novo Assembler, served to obtain the PAER4_119 DNA sequence draft of 61 contigs arranged in one 6 497 439 bp scaffold. The contigs were ordered based on the optical map of Paer4_119 genome. Sequences of the remaining gaps were determined by primer walking using as templates amplicons that were obtained with primers specific for gap-flanking regions.
The genome of Paer4_119 strain appears to be highly homologous to the genome of *P. aeruginosa* YL84, which was isolated from compost in Malaysia, and encodes chitinase and chitin binding proteins. However, it contains several *Pseudomonas* pathogenicity associated genetic elements that are absent from the genome of YL84 strain and its closest relatives, but are present in clinical *P. aeruginosa* isolates. Five prophage regions are among the latter. Possibly, similarities of certain PAER4_119 genome regions to those characteristic of clinical *P. aeruginosa* strains contribute to the suitability of this strain for the propagation of therapeutic phages.

Key words: *Pseudomonas aeruginosa*, virulence factors, genome sequence

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I.P.11

Theoretical model for plasmid stability analysis based on the Monte Carlo method

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Plasmids are extra-chromosomal closed circular mobile genetic elements that are not essential for microbial growth. However, their presence could be beneficial as they play significant role in the adaptation of microbes to the changing environmental conditions. From this point of view stable maintenance of plasmids is of vital importance for the living cells. In our research we focused on plasmid pEC156 that carries EcoVIII restriction-modification system. In our previous report (Werbowy *et al*., 2015) we have shown that pEC156 maintenance relies on three elements: (*i*) *cis*-acting *cer* site involved in resolution of plasmid multimers that are distributed randomly; (*ii*) a gene coding for EcoVIII endonuclease involved in postsegregational killing of plasmid free cells; and (*iii*) plasmid copy number control.
Our primary aim was to construct simple theoretical model to analyze plasmid maintenance in bacteria. The main objective was to make the model simple, with the smallest number of free parameters that could be fitted into the experiment. To analyze plasmid maintenance, we have used the Monte Carlo method.
The only input parameter in our theoretical model is plasmid copy number. Using this model the following parameters can be derived: (*i*) the probability of emergence of plasmid molecules that are defective in DNA replication, (*ii*) the probability of a plasmid loss during the cell division, (*iii*) the variance of plasmid copy number in bacteria cultivated without antibiotic pressure, (*iv*) the ratio of plasmids released during the division to the total number of cells. We found that results obtained experimentally with the use of pEC156 derivatives pIB8 (EcoVIII R^+M^+*cer*$^+$*rom*$^+$$Cm^R$, 5.2-kb), pRB1 (EcoVIIIR$^+$M$^+$*cer rom*$^+$$Cm^R$; 4.6 kb) and pRB2 (EcoVIIIR$^-$M$^-$*cer rom*$^+$$Cm^R$; 2.8 kb) are consistent with those derived from theoretical models based on the Monte Carlo method.

Reference:
1. Werbowy R, Boratynski A, Dekowska, Kaczorowski T (2015) Plasmid **77**: 39–50.

I.P.12

Fragile balance between DNA methylation and restriction confers antiviral strength

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The restriction-modification systems (R-M) are generally accepted as antiviral defense system of bacteria. However, there are some limitations for the absolute restriction of foreign DNA. Our studies show that the wild-type and higher than wild type levels of EcoRI R-M genes expression challenged by viral infection demonstrate high and low efficiency of DNA restriction, respectively. These results suggest, that the balance created by R-M components is highly sensitive to any changes in protein concentrations to degrade invading DNA effectively. Our results provide further insight into a type II R-M system maintenance and the potential conflict with the host bacterium.

Key words: restriction and modification DNA, bacterial antiviral system

I.P.13

Chromid-dependent maintenance of bacterial chromosomes

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Current research based on whole-genome sequencing indicates that many bacterial species have multipartite genomes. The newly proposed classification of bacterial replicons includes not only chromosomes and plasmids, but also chromids – extrachromosomal elements sharing some characteristics of both chromosomes and plasmids. Two subclasses of chromids have been distinguished: (i) primary chromids, which are indispensable for host viability (carry genes of the core genome), and (ii) secondary chromids, which are required for survival in the natural environment but are dispensable under optimal laboratory conditions. We present preliminary results of the analysis of chromids occurring in bacteria of the genus *Paracoccus* (*Alphaproteobacteria*). Genome sequence analysis of *Paracoccus denitrificans* Pd1222 (type strain of the genus) showed that a chromid of this strain encodes DnaA protein, which is crucial for the chromosome replication initiation. Interestingly, the chromid, besides the *dnaA* gene, contains a functional *dnaA*-like replication system, typical for primary chromids of *Paracoccus* spp. and for plasmids of bacteria from the *Roseobacter* clade. Partitioning of the components of the chromosome replication systems between different replicons is a unique phenomenon, yet not restricted only to *P. denitrificans*. We found that at least five closely related *Paracoccus* species share such genetic organization profile. This dualism implies that *dnaA* containing chromosomal gene clusters have been transferred into co-residing plasmids, thereby stabilizing them in bacterial population and converting into indispensable replicons. To conclude, our recent data show that chromids may not only determine basic metabolic properties of the host strain, but may be also crucial to chromosome maintenance. Such unique relation provides a novel insight into diversity and evolution of multipartite genomes.

Key words: mutlipartite genomes, chromids, essential genes, *Paracoccus* spp.

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I.P.14

Plasmid-encoded RepA proteins of *Rhizobium leguminosarum* bv. *trifolii* are able to self-associate *in vitro* and differ in the oligomerization pattern

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Rhizobia are soil bacteria able to recognize and establish symbiosis with legumes, providing them with fixed nitrogen and enabling their growth on nitrogen-limited soils. Rhizobia commonly have very complex genomes with a chromosome and several megaplasmids belonging to the *repABC* family. RepA and RepB are members of the ParA, ParB families of partitioning proteins while RepC is crucial for plasmid replication. *Rhizobium leguminosarum* bv. *trifolii* TA1 (RtTA1) genome comprises chromosome and four megaplasmids (pRleTA1a-d) equipped with functional *repABC* genes, which are organized as operons negatively regulated by RepA. The aim of this study was to determine individual RepAs ability to dimerize/oligomerize. In cross-linking experiments with dimethyl pimelimidate (DMP) individual His_6-RepA/a-d formed dimers and, at a higher DMP concentration, the bands corresponding to multimeric fractions were observed. A time course experiment with a fixed concentration of DMP revealed that each of the tested RepA was initially fixed into covalently bound dimers, but yet the multimeric forms were observed very quickly. The proteins differ slightly in the kinetics of the respective dimer/multimer formation. The oligomerization pattern of the His_6-RepA proteins seemed not to be influenced *in vitro* by the specific or non-specific DNA but in the presence of ATP in a cross linking reaction, the bands corresponding to putative multimers were absent or diminished. The RepAs ability to oligomerize seems to be related with operon regulation as well as plasmids partition.

Key words: *Rhizobium*, megaplasmids, *repABC,* RepA protein