Oral presentations

Session I. Bacteriophages and plasmids

I.O.1

New ways to combat pathogenic bacteria – bacteriophage lytic enzymes as an alternative to antibiotic therapy

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Despite constantly growing number of novel beta-lactams, for decades successfully used as antibacterial agents, the dramatic rise of antibiotic resistance is observed. Hence there is an urgent need to develop new alternative approaches to combat pathogenic bacteria. Prokaryotic lytic enzymes can cause bacterial cell lysis by degrading peptidoglycan (PGN), the unique component of bacterial cell wall. The crucial importance of PGN for bacteria makes it an ideal target for antimicrobial drugs.

In our laboratory, based on *in silico* analysis two lytic enzymes derived from thermophilic bacteriophages were discovered: Ph2119 and Ts2631 endolysins. Both endolysins show amino acid sequence similarity to eukaryotic peptidoglycan recognition proteins (PGRPs), of which lytic PGRPs possess strong antibacterial activity. We used this resemblance for further search of novel prokaryotic lytic enzymes that are similar to lytic PGRPs. For analysis we have chosen four lytic enzymes derived from pathogenic, anaerobic bacteria: *Clostridium perfringens* NCTC 8239, *Clostridium perfringens* ATCC 13124, *Clostridium intestinale* URNW and *Clostridium botulinum* E3 str. Alaska E43. Recombinant proteins had specific activity against *Clostridium sporogenes* DSM767 and *Clostridium intestinale* DSM6191.

The specificity is an important criterion for selection of a good antibacterial agent. Novel lytic enzyme should target pathogenic bacteria without elimination of other beneficial bacteria such as a commensal flora in the gut. Using Ts2631 endolysin as a model we defined a protein motif responsible for enzyme specificity of phage lytic enzymes similar to PGRPs. Based on bioinformatics analysis we performed site-directed mutagenesis and functional studies of Ts2631 endolysin substitution variants: Pro54Arg, double mutant Gly52Asn and Trp53Phe and triple mutant Gly52Asn, Trp53Phe and Pro54Val. The results indicate a role of GWP triad of PGRP-like lytic enzymes in PGN degradation. Activity analysis of the variants clearly shows differences in bacterial peptidoglycan degradation rate when compared to native lytic enzyme activity.

Key words: lytic enzymes, PGRPs, anaerobic bacteria, endolysins

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I.O.2

From sewage-treatment plants to phage therapy: step-by-step phage analysis and application

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Bacterial infections that are difficult or impossible to combat have been recently observed with increasing frequency. Drug resistance of bacteria or inaccessibility of the infection location (ears, snout or skin wounds) are the most common reasons for ineffectivity of antibiotics. Thus, after 100 years from its discovery, phage therapy once again appears as an promising weapon against pathogenic bacteria. This presentation will explore the phage analysis pathway, from their isolation to the development of a phage-based treatment for testing in an animal model. We focused on isolation and characterization of bacteriophages capable of developing in, and killing, the bacteria isolated from domestic and farm animal infections.

Firstly, with the use of MALDI-TOF, we identified bacterial strains (of the *Bordetella, Escherichia, Klebsiella, Pasteurella, Proteus, Pseudomonas, Staphylococcus* and *Streptococcus* genera), which had been isolated from animal infections at a collaborating vet clinic. In the next step, we isolated bacteriophages infecting the pathogenic strains. Samples for phage isolation were collected in sewage farms, rivers and canals in the northern Poland. Phage plaque morphology and the time of lysis of bacterial cultures were analyzed. Electron microscopy was used to analyze capsid morphology and assign the phages to *Myo-*, *Podo-* or *Siphoviridae* families. Analysis of kinetics of adsorption, one step growth experiments in a chemostat system and determination of phage host range were also performed. From the phage collection created, we chose a group of phages for the whole-genome *de novo* sequencing. Detailed analysis of the genomes was focused on the presence of genes encoding toxins and lysins. The characterized phages were then purified with the use of HPLC with an ultimate goal of analyzing them in animal models.

In conclusion, we have created a collection of phages capable of killing the bacterial strains causing animal infections that are otherwise difficult to treat.

Key words: bacteriophages, phage therapy, bacterial infections

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I.O.3

Staphylococcal Twort-like phages encode a homolog of bacterial virulence determinants. Can we use these phages for therapeutic purposes?

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One of the major challenges of the contemporary medicine is to accelerate the development of methods of fight with multidrug resistance bacterial pathogens, *Staphylococcus aureus* among them. *Staphylococcal* phages that represent the Twort-like genus *of Myoviridae* family, have a number of properties that make them compelling alternatives to antibiotics. They are obligatorily lytic, cannot transfer genetic material between bacteria by transduction and have a broad host range. However, functions of nearly half of their genes still need to be elucidated, to make sure that they can neither contribute to the virulence of their host bacteria nor to be an environmental source of virulence determinants. Thus, we searched the genomes of seven staphylococcal Twort-like phages, whose virion DNA sequences were characterized by us previously [1], and whose representatives are used in experimental phage therapy of infections at the Institute of Immunlogy and Experimental Therapy, PAN, in Wroclaw, Poland [2], for possible homologs of bacterial virulence associated genes. Surprisingly, a gene, conserved in all these phages, and designated by us as *tgl,* appeared to encode a protein, which was slightly homologous to major surface antigens of *S. aureus* cells, SceD and IsaA – autolysins, which are involved in the *S. aureus* virulence and nasal carriage [3]. In the genomes of staphylococcal Twort-like phages the *tgl* gene is located in the proximity of the phage lytic module, suggesting that it may be a component of this module. The expression of cloned *tgl* in bacteria caused their lysis, indicating that Tgl protein may function as a phage endolysin that can cross the cytoplasmic membrane in the absence of holins. Thus, the presence of *tgl* gene in the genomes of staphylococcal Twort-like phages does not preclude the use of these phages for therapeutic purposes. Additionally, the strong bactericidal activity of Tgl protein makes this protein by itself a good candidate for an antistaphylococcal agent.

Keywords: bacteriophage; phage therapy; *Staphylococcus aureus*

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I.O.4

Construction and characterization of T4-like phages lacking head proteins gp24 orgpSoc

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T4-like phages are common components of human and animal gut flora. They play a role in microbial balance and have been shown as potential antibacterials that are able to combat pathogenic enterobacteria. Intrinsic factors that determine phage survival in various conditions of either mammalian gastrointestinal tract or external environment are dependent on proteins that build phage capsid. Here we present molecular engineering of a T4-like phage to obtain phage mutants deprived of surface proteins gpSoc or gp24 and further testing of these mutants survival in the gastrointestinal tract.

T4-like phage HAP1, previously identified as lacking gpHoc, was used to select mutants with no gp24 or gpSoc (NOP1 and JOM1, respectively). To test phage survival in gastrointestinal tract, a mouse *in vivo* model was applied. Mice were treated with preparations of T4, HAP1, NOP1 or JOM1 5×10^8 pfu/ml in drinking water mixed with PBS. After 20 hours, segments of GI tract (stomach, large intestine, duodenum and distal small intestine) and their content were analyzed. These segments were dissected and thoroughly homogenized in PBS. Then, phage concentrations in the samples were determined.

As a result, NOP1 titers in the stomach were found to be lower in comparison to the other phages, which suggests a higher sensitivity of this mutant to low pH. Interestingly, the phage normalized its concentration in further segments of the GI tract. Additionally, general differences of phage concentrations throughout the GI tract were observed, with lowest phage titers noted in the duodenal samples, the highest in distal part of the tract (in feces). These results suggest that gp24, gpHoc and gpSoc do not play a major role in phage survival in mammalian GI tract, however, to some extent, phage head composition may decide on phage ability to survive in its sections.

Key words: T4 phage, capsid proteins, gastrointestinal tract

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I.O.5

Diversity of bacteriophages in urban sewage

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Phages – the viruses infecting prokaryotes – are the most abundant biological objects on Earth. One of their biggest reservoir is urban sewage, which consist in domestic and industrial wastewater together with surface runoff. The big diversity of bacteria in sewage influences the diversity of bacteriophages in this environment. The aim of this work was to analyze the diversity of sewage phages in the terms of their morphology, physiology, genomics and metagenomics. We created the collection of 83 phages infecting laboratory, clinical and environmental bacterial strains of various species. The phage isolates showed a great morphological and physiological diversity. The electron microscope analysis allowed the classification of all these viruses to the order *Caudovirales*; *Siphoviridae* were the most abundant. Isolated viruses presented narrow host ranges and varied in tolerance against tested physical-chemical factors (temperature, pH, osmotic shock, the presence of the detergents or organic solvents). Podoviruses were the most sensitive and most of the myoviruses were the least sensitive to tested conditions. The individual characteristics of each phage enabled the selection of viruses for further genomic research that involved sequencing and the analysis of their genomes. Pyro sequencing and the bioinformatics analysis resulted in receiving the whole genome sequences for eight phages from the collection. Only one of them was not highly similar to previously known phage genomes. The metagenomic approach used in the study resulted in the creation of metagenomic libraries that consisted of 1 500 clones. Partial sequencing of the libraries showed that most of the inserts were of phage origin. The collection of bacteriophages created in this work, could be a good base for further research on diversity and ecology of bacterial viruses. Better understanding of complex problem of phage sensitivity to external factors could be useful in the context of their application in the industry, agriculture or phage therapy.

Key words: phages, sewage, diversity, metagenomics

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I.O.6

Diversity of centromere-like *parS* elements within *repABC* cassettes of *Rhizobium leguminosarum* bv. *trifolii* TA1

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Rhizobia are soil bacteria, which establish symbiosis with legumes providing them with fixed nitrogen and enabling their growth on nitrogen-limited soils. Rhizobial genomes usually comprise circular chromosome and large plasmids. Megaplasmids are equipped with *repABC* cassettes that control both their replication (*repC* gene) and partition process (*repAB* genes and *parS* centromere-like sequences). In *Rhizobium leguminosarum* bv. *trifolii* TA1 (RtTA1) genome, beside chromosome four megaplasmids were identified (pRleTA1a-d), each possessing *repABC* genes. Despite the highly similar genetic organization, individual *rep* cassettes are diverse, particularly with respect to the number, position and sequence of centromere-like elements. Within *repABC* operons of pRleTA1b and pRleTA1c, two almost canonical *parS* centromere-like sequences were identified, while in the *rep* regions of pRleTA1a and pRleTA1d only non-canonical *parS*-resembling elements were found.
The aim of this work was to examine non-canonical *parS* sequences for their function as potential centromere-like elements and to investigate the specificity of binding *parS* elements and RepB proteins in multipartite genome of RtTA1. RepB proteins of four RtTA1 megaplasmids were over-expressed in *E. coli* and purified. *parS* resembling element located within *repB* of pRleTA1d was shown to be the centromere-like element: when introduced *in trans* into RtTA1 genome cloned into broad host range plasmid it exerted incompatibility against pRleTA1d. Recombinant RepB/d bound to the DNA fragment containing the *parS*-D1 sequence. By series of short PCR products overlapping the pRleTA1a*repABC* cassette, DNA fragment was mapped, whose introduction into RtTA1 resulted in incompatibility against pRleTA1a. The results demonstrated substantial sequence diversity of *parS* elements in RtTA1. In series of EMSA the individual RepB proteins were able to bind only to the *parS* elements of cognate plasmid in a very specific manner.

Key words: *Rhizobium*, megaplasmids, *repABC,* RepB protein, *parS*

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I.O.7

Role of the *exo-xis* region in development of bacterial viruses from the family of lambdoid phages

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Lambdoid bacteriophages serve as useful models in microbial and molecular studies on basic biological process. This family of viruses plays an important role in pathogenesis of Shiga toxin-producing *Escherichia coli* (STEC), responsible for human infections that result in bloody diarrhea and hemorrhagic colitis, often with severe complications. The main virulence factors of STEC are Shiga toxins encoded by *stx1* and *stx2* genes, located in genomes of specific lambdoid bacteriophages, called Stx phages. Moreover, efficient expression of these genes is stimulated upon prophage induction and multiplication of the phage genome. Therefore, understanding the mechanisms regulating these processes appears essential for both basic knowledge and potential anti-STEC applications. The *exo-xis* region, present in genomes of lambdoid bacteriophages, contains highly conserved genes and open reading frames of largely unknown functions. It is dispensable for phage lytic development under standard laboratory conditions. We demonstrated that an increased dosage of the whole λ or $\Phi 24_B$*exo-xis* region can significantly influence phage development. Our results indicated that after prophage induction, an increase in phage DNA content in the host cells is more efficient in *E. coli* bearing additional copies of the *exo-xis* region, while survival rate of such bacteria is lower. Importantly, by using quantitative real-time reverse transcription PCR, we have determined patterns of expressions of particular genes from this region. Interestingly, genes' expression patterns differed significantly not only between conditions of phage infection and prophage induction, but also between induction agents (mitomycin C and hydrogen peroxide). This may shed a new light on our understanding of regulation of lambdoid phage development, depending on the mode of lytic cycle initiation. We conclude that the *exo-xis* region may have specific functions in the regulation of lambdoid phage development, especially at the stage of the lysis-vs-lysogenization decision and prophage induction.

Key words: lambdoid bacteriophages, Shiga toxin-producing *Escherichia coli* (STEC), Shiga toxins, gene expression, region *exo-xis*, lysis-vs-lysogenization decision, prophage induction

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