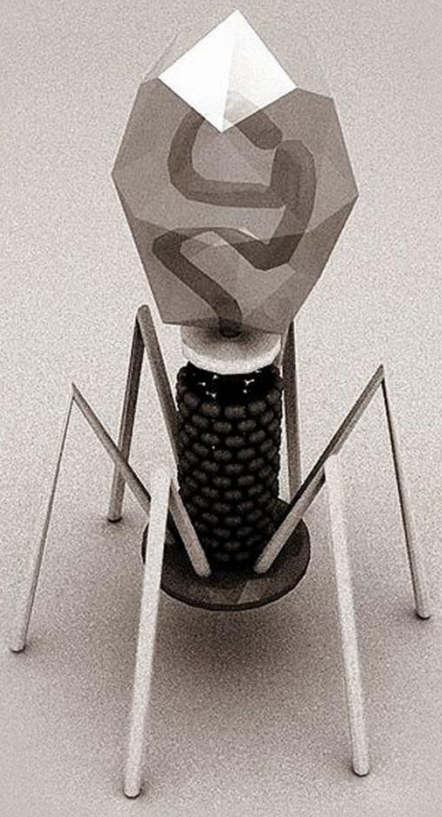
BACTERIOPHAGES 2015

ABSTRACTS



27th Jan - 29 Jan
London, UK

EuroSciCon

This event will discuss the many roles of bacteriophages, ranging from fundamental biological research to their use in medical and industrial biotechnologies. Topics will include uses of phages for therapeutics, food manufacture, delivery vectors, drivers of microbiota structure reporters of microbiome diversity, and their role on the environment

This event has CPD accreditation

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Day 1:

Invited Speakers Abstracts

Regulation of replication of Shiga toxin-converting bacteriophages

Professor Grzegorz Wegrzyn, Department of Molecular Biology, University of Gdansk, Poland

Pathogenicity of Shiga toxin-producing Escherichia coli (STEC) depends on effective synthesis of this toxin which is encoded in genomes of lambdoid prophages. Prophage induction and phage replication are necessary for expression of stx genes (coding for Shiga toxins). Mechanisms of regulation of Shiga toxin-converting bacteriophages' replication will be discussed and compared to those of the closely related phage lambda. Moreover, attempts to develop methods for inhibition of development of these bacteriophages will be presented. Such procedure appears desirable as various antibiotics may stimulate lambdoid prophage induction, thus, the use of these drugs is not recommended for treatment of STEC infections.

Ecology, evolution and applications of bacteriophages in bacterial pathogens with environmental reservoirs

Dr Martha Clokie, Reader in Microbiology, Department of Infection, Immunity and Inflammation, Medical Sciences Building, University of Leicester

Bacteriophages (phages) have been shown to be present in the genomes of most bacteria that have had their genomes sequenced. Phages capable of propagation using the lytic cycle have also been found for most bacterial species. However, these 'lytic' phages are reported far less for some bacterial species than for others. This often reflects technical difficulties of working with certain bacteria, but the net result is that phages for many species have received little research attention. These understudied phages include those that infect many serious pathogens and the exploitation of such phages could play a vital role for their future diagnosis and treatment. Understanding the biology of new phage groups is key to their subsequent development. *Clostridium difficile* is one such 'difficult' pathogen, and we have made significant progress with understanding the biology of phages that infect it. We also have research programmes to exploit their phages as therapeutic and diagnostic agents. In a separate research programme we have characterised the understudied phages of the tropical pathogen *Burkholderia pseudomallei* and shown that the phages we isolated from the environment are distinct from those seen in their host bacterial genomes. Data will be presented on the biology and exploitation of *C. difficile* and *B. pseudomallei* phages. Information will also be shown on how we have used the success of these programmes to inform our work on new projects to investigate phages that infect *S. pneumoniae* and *Borrelia*.

Working collaboratively to jump-start phage therapy

Dr. Elizabeth Martin Kutter, Lab I, The Evergreen State College, Olympia, WA, USA

Phage are being seriously considered for helping solve the serious worldwide antibiotic crisis, but progress towards governmental approval remains frustratingly slow. Our Phagebiotics Research Foundation is exploring a collaborative public-private-academic initiative adding various already-tested Staph aureus phages to the treatment of diabetic foot infections in military and civilian wound-care centers, where standardized protocols and monitoring facilitate data collection for double-blind trials with no significant change from current practice. Compassionate-use case studies adding sequenced commercial Eliava phage to treatment of intractable diabetic toe ulcers where amputation was the prime alternative are drawing encouraging interest from US podiatrists and wound-care physicians.

Bacteriophages for healthy living

Dr Alexander Sulakvelidze, Intralytix, Inc, Baltimore, USA

Bacteriophages or phages are bacterial viruses that are the most ubiquitous organisms on this planet. Phages have remarkable antimicrobial activity against their specific bacterial hosts. The presentation will review the history of phage therapy and will discuss the pros and cons of this approach. It will further focus on the review of recent data on the use of phages in agricultural and clinical settings (including use of phages as a novel class of probiotics or phagebiotics) and the regulatory strategies required for such applications.

Bacteriophage-Derived Biomaterials for the Targeting of Pathogenic Bacteria

Professor. Stephane Evoy, University of Alberta, Canada

Foodborne diseases continue to cause a high level of morbidity and mortality, specifically for infants, young children, elderly and immunocompromised individuals. Development of innovative strategies for food, water, and livestock monitoring are thus necessary. Antibodies have been exploited as molecular probes in order to impart the required specificity to sensing platforms. The production an antibody is however time consuming and is not particularly cost effective. Antibodies also suffer from degradation and reliability issues, severely inhibiting

their prospects. The high level of specificity of phages offers a potent alternative to the use of antibodies for the recognition of pathogens. We previously demonstrated the use of immobilized bacteriophage particles for the detection of Escherichia coli. This being said, our work concluded that recombinant phage receptor binding proteins (RBPs), responsible for the phage-host specificity, can be used as biological probes and present numerous advantages over the use of whole phage particles. We have reported the use of RBPs as novel probes for the capture of several foodborne pathogenic bacteria. Phage RBPs immobilized onto magnetic particles were successfully used for rapid isolation (in less than 3 hours) of bacterial cells from food samples spiked with Campylobacter jejuni cells. Alternatively, lysins represent another class of cell envelope binding phage proteins that degrade the host cell walls and promote the release of newly formed phage particles. We demonstrated that the immobilized recombinant lysin Gp10 from the mycobacteriophage L5 was able to capture Mycobacteria cells from saline solutions as well as the use of recombinant Gp10 for the capture of Mycobacteria from complex natural matrices. This talk will review our overall body of work on the topic.

Crystal structures of the fibre proteins of bacteriophages T4, T5 and T7

Dr Mark van Raaij, Centro Nacional de Biotecnologia-CSIC, Madrid, Spain

Many bacteriophages use trimeric fibre proteins for host cell recognition, binding their receptors with their distal, C-terminal ends. The crystal structures of the receptor-binding domains of the tail fibres of the myovirus T4, the siphovirus T5 and the podovirus T7 will be presented. These structures may provide a framework for rational modification of bacteriophage fibre proteins to detect pathogenic bacteria or even direct chimeric phages towards them.

Control of recombination directionality by serine integrases

Dr Paul CM Fogg, University of York, Department of Biology, Wentworth Way, York, UK

Serine integrases are important tools for synthetic biology and their simple requirements have allowed a diverse array of applications to be developed. Integrase alone can recognize simple attachment sites (≤50 bp) and catalyse unidirectional recombination. When supplemented with recombination directionality factor (RDF) the reaction is efficiently reversed. Recombination requires interaction between the CTDs of 4 integrase monomers and this domain is also integral to directionality control. Mutational analysis was used to provide insights into Int:RDF binding as well as the role of a conserved coiled-coil domain, crucial for efficient integrase function.

A Flood of Bacillus phages: discovering new science and new scientists

Professor Louise Temple, James Madison University, Harrisonburg, Virginia, USA

A consortium of four schools is training undergraduate scientists through hunting for bacteriohages that infect several species of the genus Bacillus. Over four years, our students have isolated 120 viruses of B. pumilus, 150 of B. thuingiensis kurstaki, and 20 of B. cereus. Of these, several isolates infect multiple species of Bacillus. Twenty genomes have been analyzed and 13 submitted to GenBank. A holin/lysin cassette was cloned, expressed, and shown to cause lysis of E. coli. Interesting genomic features, such as introns and large non-coding regions are observed. Hundreds of students experience the scientific process first-hand through the excitement of discovery.

Developing bacteriophage-based assays to detect viable Mycobacteria

Professor Catherine E.D. Rees, School of Biosciences, University of Nottingham, Loughborough, UK

Resolution of branched DNA structures by phage endonucleases

Dr Gary Sharples, Fellow of the Wolfson Research Institute for Health and Wellbeing, Visiting Senior Lecturer in the Department of Chemistry, Senior Lecturer in the School of Biological and Biomedical Sciences, Durham University, UK

Genetic exchange reactions generate four-stranded DNA joints. In most cellular organisms these DNA structures are resolved by symmetrically-related paired incisions. This process needs to be highly accurate as unwarranted cleavage of replication forks could be potentially disastrous for dividing cells. In contrast, phage resolving endonucleases tend to be more promiscuous in the substrates they target. This appears to be an evolutionary adaptation to help remove any branches that might impede genome packaging. This talk will summarize the known phage resolution systems and describe an example where a bacterial X-structure resolvase has been modified to suit the requirements of phage replication.

Leveraging bacteriophages for high speed, enrichment-free environmental monitoring

Dr Michael Koeris, Founder, VP Business Development & Operations, Sample6, Boston, US

Environmental testing for Listeria is an essential and growing component of food safety. However, existing Listeria environmental tests require between 24 and 48 hours of enrichment after sample collection, followed by sample preparation and assay.

Sample6 has developed and commercialized a new technology for the detection of environmental Listeria just six hours after sample collection and without enrichment. The Sample6 technology demonstrates comparable sensitivity and specificity to existing culture-based methods of Listeria detection.
With our simple and accurate test, customers are able to receive the results of the test and take action in less than a single 8-hour work shift.
Here we detail the basic science that underlies the assay - the bacteriophages and the engineering thereof.

Oral Presentation Abstracts

BACTERIOPHAGE FOR DETECTION OF ANTIBIOTIC-RESISTANT BACTERIA

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Emergence of antibiotic-resistant bacteria is one of the most serious threats for healthcare and public safety worldwide. According to CDC information, more than two million people in the USA are sickened and 23,000 dying every year as a result of antibiotic-resistant infections. Real times, sensitive methods of detecting antibiotic-resistant pathogens are needed to start adequate therapy and prevent the spread of infection. Bacteriophages are highly specific to the bacterial host and stable in a wide range of environmental conditions and thus can be effectively used in biosensors as recognition elements for detection of antibiotic-resistant pathogens. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium accountable for a number of difficult-to-treat diseases in humans. Recently, cases of MRSA have increased in livestock animals from which the infection can be transmitted to humans. The rapid and reliable technics for recognition of MRSA are needed. The overall goal of our research was to discriminate methicillin resistant (MRSA) and sensitive (MSSA) strains of *Staphylococcus aureus,* by the specially selected and modified lytic bacteriophage with a wide host range of *S. aureus* strains and a penicillin-binding protein (PBP 2a) specific antibody, using a quartz crystal microbalance. The phages were immobilized by a Langmuir-Blodgett method onto a surface of a quartz crystal microbalance sensor and worked as broad range staphylococcus probes. In this important step, *S. aureus* are separated from the other bacteria on the crystal surface. Finally, to discriminate MRSA and MSSA, the suspension of beads with PBP 2a specific antibody are added. The beads bind only MRSA, that contain PBP 2a protein and do not bind MSSA, which missing this protein. When beads bind MRSA, the signal is generated indicating the presence of methicillin resistant strains of *Staphylococcus aureus.*A total time-to-answer for this assay is about 16 minutes per sample. This time can be dramatically shortened by using QCM devices with a large number of chambers. The anticipated shelf life for the phage sensors is about of 3-4 months at room temperature. With a biopolymer protection it could be prolong up to a few years. The detection limit of *S. aureus* that was measured for this phage was found to be 10^4 CFU/ml. Commonly used methods for detection of MRSA require from 3 to 24 hours carrying out the test. PCR or DNA hybridization of the mecA gene is a relatively fast and accurate method but requires purified DNA and is extremely sensitive impurities. In contrast, the method described in this work is rapid, does not need DNA extraction, and it is not sensitive to admixtures.

INVESTIGATION OF BACTERIAL RESISTANCE TO *PSEUDOMONAS AERUGINOSA* PHAGE AB26, A NOVEL PA73-LIKE PHAGE

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Pseudomonas aeruginosa virulent bacteriophages have been used to prepare cocktails for phage therapy. Among caudoviridae, the large majority of *P. aeruginosa* podoviruses and myoviruses are virulent phages, with rare exceptions resulting from recombination, such as phage Ab31 (Latino, Essoh et al. 2014). On the contrary, siphoviruses are majoritarily temperate phages as shown by their capacity to form stable lysogens. Among them some have not been assigned to a genus and their biology remains undefined. Phage 73, a siphovirus belonging to the Lindberg typing set is closely related to phage Kakheti-25 and phage KL1 of *Burkholderia cenocepacia*. These phages have different behaviors in terms of plaque morphology and host range. Phage Kakheti-25, has a large host range and is used for phage therapy, whereas phage 73 infects a limited number of strains.

We have isolated a new phage in Abidjan (Côte d'Ivoire), Ab26, which forms irregular size clear plaques with a halo, specifically on a clinical strain, PaRD80, from a cystic fibrosis patient. Turbid plaques are seen on a second strain out of more than 20 genetically different strains. The sequence of its genome is highly similar to that of Kakheti-25 and 73, with the same gene arrangement. The main differences between Ab26 and Kakheti-25 lay in tail associated genes. No integrase or excisionase were identified but there were two recombinase genes. RecA may be used for cyclization of the genome.

By picking bacteria inside lysis zones, we have isolated different resistant mutants, some of which contained viral DNA as seen by PCR amplification, after 4 rounds of purification. The mutants were all strongly inhibited for their capacity to swarm. Two putative lysogens, clones SCH-11 and SCH-18 were incubated during log phase growth with mytomycin, and phages could be induced at a high titer. However upon complete genome sequencing of SCH-11, the viral DNA could be found at a very low level corresponding to one out of 20 genomes, and there was no evidence of its insertion into the chromosome. In this mutant a single SNP could be detected inside a gene of unknown function (PA4656 of PAO1), encoding a protein with similarity to different epimerases. These enzymes have been shown to participate in the synthesis of LPS O-antigen.

Thus it appears that Ab26 is a virulent phage with a narrow host range, capable of maintaining a pseudo-lysogenic stage for many generations. It could be used for phage therapy on selected strains.

Latino, L., C. Essoh, et al. (2014). "A novel *Pseudomonas aeruginosa* Bacteriophage, Ab31, a Chimera Formed from Temperate Phage PAJU2 and *P. putida* Lytic Phage AF: Characteristics and Mechanism of Bacterial Resistance." PLoS One **9**(4): e93777.

A CHANGE IN PHAGE TYPE DURING AN OUTBREAK OF ESCHERICHIA COLI O157 IS COUPLED WITH A BROAD RANGE OF GENOMIC CHANGES DURING A SHORT TIME FRAME

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Background
Shiga toxin producing *Escherichia coli* O157 (STEC) can cause severe bloody diarrhoea, haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Phage typing (PT) of STEC facilitates public health surveillance and outbreak investigations and certain PTs are more likely to occupy specific niches and are associated with specific age groups and disease severity. The aim of this study was to analyse the genome sequences of two phylogenetically related strains, one belonging to PT8 and the other to PT54, associated with two concurrent outbreaks that occurred at the same location within a short time frame, to determine the genetic background responsible for the change in phage type and elucidate the mechanisms of host-phage interactions between the typing phages and specific phage types of STEC O157. The two outbreak strains were sequenced using Illumina and PacBio technology and bioinformatic techniques were used to analyse them. The illumina sequencing results showed that the strains were closely related (2-4 core genome SNPs apart) but the PacBio sequencing results also revealed a broad range of genomic changes during a short-time frame that was higher than previously observed during outbreaks; this is due to the more habitual use of short read sequencing(Illumina) that does not resolve prophage regions unlike the use of longer read sequencing (PacBio) that will reveal the changes in prophage regions. A high rate of prophage gain and loss was revealed by PacBio sequencing as well as a large number of gene differences that have changed within the accessory genome.

SELECTIVE PRESSURE IMPOSED ON PSEUDOMONAS AERUGINOSA BY BACTERIOPHAGES

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Pseudomonas aeruginosa is an opportunistic pathogen possessing a large genome with a high level of plasticity, allowing the bacteria to adapt to hostile environments. As a result of changes in the environmental conditions *P. aeruginosa* diversifies into a range of morphologically distinct phenotypic variants through processes such as phase variation and adaptive mutations. A typical feature of clinical isolates of *P. aeruginosa* causing infections difficult to eradicate, and becoming persistent or even chronic, is their high degree of phenotypic diversity. Bacteriophages have been shown to drive the emergence of *P. aeruginosa* variants playing a significant role in bacterial survival, activity, and evolution. Although phage therapy has proven to be promising in several animal case studies, as well as in human clinical trials, it has always been considered with skepticism by the medical community because of the emergence of the phage-resistant variants.

The purpose of this study was to investigate the effect of development of phage resistance on selected phenotypic traits and virulence determinants of *P. aeruginosa,* as this aspect is of utmost importance for their successful use as therapeutic agents. Different kind of infections were performed using phages belonging to four genera in order to lower the possibility of phage cross-resistance. Phage Ab05, a podovirus (ΦKMV-like), phage Ab09, a podovirus (N4-like), phage Ab27, a myovirus (PB1-like) and phage Ab17, a myovirus (KPP10-like), isolated from waste water in Abidjan (Yvory Coast), were used in this study. Single phage infection (M.O.I. of 0.1) or multiple phage infection (Ab09+Ab17, Ab05+Ab27, Ab05+Ab27+Ab17+Ab09) of *P. aeruginosa* strain PAO1, were performed, and a collection of phage resistant variants were isolated and phenotypically studied.

Several phenotypic alterations were observed when the resistant variants were compared with the non-infected strain. Alterations in the swimming and swarming motility associated sometimes with a reduction in the biofilm formation were specifically selected by some phages alone or as a component of a cocktail; whereas others were responsible for emergence of mucoid variants. The growth rate and the phenazynes production of most of the phage-resistant variants did not significantly change compared to the non-infected PAO1.

The genome of sixteen phage-resistant variants was fully sequenced and mutations were identified. Part of the mutants are affected in genes involved in the biogenesis of the type IV pilus, which could explain the reduction in their capability to produce biofilm. Mutations in genes involved in the LPS production, O-antigen synthesis and in the alginate regulation were also identified. In two sequenced genomes, the presence of free phage DNA was detected and there was no mutation. Replating these variants several times it was possible to establish that the phage genome, although able to produce functional phage particles, was not stably kept.

Biofilm and phenazynes production, swarming and swimming motility and mucoidy are bacterial characteristics that make *P. aeruginosa* a pathogen. If phage predation selects for variants with alterations in any of the genes that are involved in biogenesis or regulation of these virulence determinants, the resulting phage-resistant variants could potentially exhibit altered levels of virulence. It is therefore essential to further investigate bacterial resistance to phage to anticipate the potential effects of phage therapy.

We thank Direction Générale de l'Armement (DGA) and ANR "Resisphage" for financial support to this project.

P100 INSENSITIVE *L.MONOCYTOGENES* PLANT ISOLATES AND INFLUENCE OF ENVIRONMENTAL FACTORS ON PHAGE-BACTERIA INTERACTION, ON EFFICACY AND SURVIVAL OF THE PHAGE P100

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The phage P100, which is commercially available, is frequently used in food production to combat *L. monocyotgenes.* However, there is not much known about resistance against it until now. In generally it is known that host-virus interactions, adsorption and replication of phages are depended on the growth rate of the host and the physical and chemical environment. Incorrect used of bacteriophages can enhance the development of resistances and render treatment with phages less effective.

Therefore we screened 501 *L.monocytogens* isolates from Austrian dairy plants for P100 insensitive isolates and examined frequency of occurring resistances before and after P100 treatments. Resistance was confirmed by CFU determinations and measurements of optical density and insensitive isolates were characterized using PFGE. Moreover the influence of temperature, salt concentrations, pH and detergents on the survival of the P100 phage and on host-virus interactions was monitored using plaque assays, adsorption test and growth curves. Additionally the survival of P100 in smear water from cheese production was tested at 4°C and 10°C and the growth of infected bacteria was examined over > 100 days at different temperatures.

We found 13 insensitive plant isolates (serotype 1/2a or b) which derived from dairies or ripening cellars which (experimentally) used the phage P100. No insensitive isolates were detected before P100 treatments. The efficacy of the phages was best at 4°C and high phage concentrations. At 10°C and 20°C re-growth of bacteria was observed. Re-grown isolates were P100 insensitive. Phage titre was decreasing 1.5log10 in SM puffer and 3-4log10 in smear water with in the first 37 days. Phage infectivity was lost at pH 2 and 12 within one hour, but not at high salt or detergent concentration. Attachment of phages was observed under all tested conditions.

INFERRING BACTERIOPHAGE INFECTION STRATEGIES FROM GENOME SEQUENCE: ANALYSIS OF BACTERIOPHAGE 7-11 AND RELATED PHAGES

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Analyzing regulation of bacteriophage gene expression historically led to establishing major paradigms of molecular biology, and may provide important medical applications in the future. Temporal regulation of bacteriophage transcription is commonly analyzed through a labor-intensive combination of biochemical and bioinformatic approaches and macroarray measurements. We here investigate to what extent one can

understand gene expression strategies of lytic phages, by directly analyzing their genomes through bioinformatic methods. We address this question on a recently sequenced lytic bacteriophage 7 – 11 that infects bacterium *Salmonella enterica*.

We identify novel promoters for the bacteriophage-encoded sigma factor, and verify the predictions through homology with another bacteriophage (phiEco32) that has been experimentally characterized in detail. Interestingly, standard approach based on multiple local sequence alignment (MLSA) fails to correctly identify the promoters, but a simpler procedure that is based on pairwise alignment of intergenic regions correctly identifies the desired motifs; we argue that such search strategy is more effective for promoters of bacteriophage-encoded sigma factors that are typically well conserved but appear in low copy numbers, which we also verify on two additional bacteriophage genomes. Identifying promoters for bacteriophage encoded sigma factors together with a more straightforward identification of promoters for bacterial encoded sigma factor, allows clustering the genes in putative early, middle and late class, and consequently revealing the temporal regulation of bacteriophage gene expression. Therefore, direct analysis of bacteriophage genome sequences is a plausible first-line approach for efficiently inferring phage transcription strategies and may provide a wealth of information on transcription initiation by diverse sigma factors/RNA polymerases.

OPTIMIZING PROTOCOLS FOR EXTRACTION OF BACTERIOPHAGES PRIOR METAGENOMIC ANALYSES OF VIRAL COMMUNITIES IN THE HUMAN GUT

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Characterisation of the gut microbiome (GM) has received massive interest during the last decade. Focus has mainly been on the prokaryotic members of the GM, while characterisation of the diversity and structure of the viral communities has received limited attention. Recent advances in next-generation technologies have opened new opportunities for mapping the diversity, evolution and influence of bacteriophages on the bacterial communities of the human gut. However, little attention has been given to the efficiency of current protocols dealing with extraction of faecal bacteriophages prior to viral metagenomic studies. We have optimized two methods for extraction based on tangential-flow filtration (TFF) and polyethylene glycol (PEG) approaches using an adapted method from published protocols (LIT) as reference. We analysed their efficiency to up-concentrate viral-like particles (VPL's) and recovery of spiked phages as well as their impact in the viral community structure.

Compared with LIT, our optimized protocols TFF and PEG yielded up to 20 times more VPL's. Likewise, their efficiency to up-concentrate spiked populations of different tailed-phage representatives of the *Myoviridae*, *Podoviridae* and *Siphoviridae* families, was at least 286, 13 and 8 times more efficient than LIT, respectively. Virions extracted with TFF and PEG yielded 17-60 times more viral DNA per volume compared to LIT. Metagenomic analysis of viromes showed that double-stranded DNA viruses constituted 96.6, 95.6 and 94.5% of the relative distribution in viromes extracted with LIT, PEG and TFF, respectively. Compared with LIT protocol, PEG and TFF had 12% higher relative abundance of reads identified as *Caudovirales*. A separate assessment of the *Caudovirales* sequences showed that the distribution of *Myoviridae* was up to 11% higher in viromes extracted with LIT. Interestingly in TFF and PEG, this shift was found to be compensated by an increase in the distribution of unclassified *Caudovirales* and *Siphoviridae* (mostly those assigned as unclassified *Siphoviridae* phages), remarkably this latter group has been previously considered as one of the most abundant and prominent bacteriophage family found in the human gut.

Through this study we intended to share an efficient way to extract, quantify and recover bacteriophages from stool samples. Due to the directness of these protocols, suitable adaptations can also be applied in order to perform studies that could eventually be compromised by small volumes of faecal matter collected (e.g. mice studies). The optimization of these protocols was focused on minimizing the loss of phages and VPL's at each step of extraction, thereby increasing DNA yields prior to libraries preparation, reducing alterations in the distribution of phage communities and consequently the viral DNA extracted.

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PROPHAGES DETERMINE PHENOTYPIC PLASTICITY OF *LACTOCOCCUS LACTIS* HOST.

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L. lactis IL1403 is the first sequenced prototype strain of *L. lactis* subsp. *lactis* largely used for fundamental and applied research. Similar to all other lactococcal strains, its genome contains prophage related sequences which encompass approximately 6.7% of the chromosome and represents a significant part of genomic differences with other lactococcal strains. Both transcription and proteomic analysis of *L. lactis* IL1403 show that the number of prophage related genes are expressed in various growth conditions. However, dispute general knowledge that genes expressed from prophages can provide the host cell with increased fitness, resistance against infecting phages and modify cellular metabolism, the impact of resident prophages on the different aspects of *L. lactis* physiology was unknown. This is mainly due to the absence of an experimental model such as a *L. lactis* strain cured of their prophages. Here we report construction and characterization of prophage-free derivative of *L. lactis* IL1403 denoted IL6288. We examine the impact of prophage contents and role of individual prophages on different aspects of cell physiology important for industrial and therapeutic applications. Test data clearly show that resident prophages considerably modify growth and survival of host strain at different temperatures *under aerobic and respiration conditions, its autolytic properties and* susceptibility/resistance to cell wall-active antimicrobials, such as lysozyme and virulent bacteriophages. All these phenotypes are not an addition of the effects of the individual prophage genes but the result of the complex genetic interactions.
Presented results demonstrate diverse and significant contributions of resident prophages to *L. lactis* cell physiology.

ANTIBIOTIC RESISTANCE GENES FOUND IN VIRAL METAGENOMES COME FROM BACTERIA, NOT VIRUSES

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The widespread (and often inappropriate) use of antibiotics has led to antibiotic resistance (AbR) genes being pervasive in gut microbiota, and now constitutes a major issue for human and animal healthcare. Traditionally, the rapid spread of AbR within and between bacterial populations is presumed to be due to horizontal transfer mediated essentially by conjugative elements, and to a lesser extent by generalized transduction, a process where bacteriophages encapsidate mistakenly bacterial segments instead of their own genome. Recently, however, viral metagenome analyses of mice feces have suggested that bacteriophages themselves contain AbR genes, a situation that would speed up considerably the spread of AbR. I will bring three lines of evidence suggesting on the contrary that the AbR genes found in the published virome samples of mice feces derive from bacteria, rather than phages: (i) analysis of virome reads shows that the amount of AbR hits is correlated to the amount of 'typically bacterial' hits, in proportions expected for bacterial sequences. (ii) Evaluation of the in silico method used to recover AbR hits, using the thresholds usually applied in virome studies, but on completely sequenced phage genomes, reveals that only 3% of the 430 hits detected by Blast with an E value below 10^{-3} can confidently be considered AbR genes. (iii) Assembly of the virome reads combined with the use of more stringent thresholds to count AbR hits allows recognizing that the putative AbR genes are in a bacterial rather than viral context. These three observations are fully compatible with the generalized transduction process and do not support the view that phages carry AbR genes. A larger analysis following the same three steps, on all available human-associated viromes, confirms this view. The human microbiome field is a rapidly advancing, exciting, and important new science, but as highlighted previously and again here, this science needs to adhere to rigorous standards.

PRIMARY ISOLATION STRAIN DETERMINES BOTH PHAGE TYPE AND RECEPTORS RECOGNISED BY *CAMPYLOBACTER JEJUNI* BACTERIOPHAGES

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In this study we isolated novel bacteriophages, infecting the zoonotic bacterium *Campylobacter jejuni* that may be used in phage therapy of colonized poultry to prevent spreading of the bacteria to meat products causing disease in humans. Many *C. jejuni* phages have been isolated using NCTC12662 as the indicator strain, which may have biased the selection of phages. A large group of *C. jejuni* phages rely on the highly diverse capsular polysaccharide (CPS) for infection and recent work identified the *O*-methyl phosphoramidate modification (Me*O*P*N*) of CPS as a phage receptor. We therefore chose seven *C. jejuni* strains each expressing different CPS structures as indicator strains in a large screening for phages in samples collected from free-range poultry farms. Forty-three phages were isolated using *C. jejuni* NCTC12658, NCTC12662 and RM1221 as host strains and 20 distinct phages were identified based on host range analysis and genome restriction profiles. Most phages were isolated using *C. jejuni* strains NCTC12662 and RM1221 and interestingly phage genome size (140 kb vs. 190 kb),

host range and morphological appearance correlated with the isolation strain. Thus, according to *C. jejuni* phage grouping, NCTC12662 and NCTC12658 selected for CP81-type phages, while RM1221 selected for CP220-type phages. Furthermore, using acapsular Δ*kpsM* mutants we demonstrated that phages isolated on NCTC12658 and NCTC12662 were dependent on the capsule for infection. In contrast, CP220-type phages isolated on RM1221 were unable to infect non-motile Δ*motA* mutants, hence requiring motility for successful infection. Thus, the primary phage isolation strain determines both phage type (CP81 or CP220) as well as receptors (CPS or flagella) recognised by the isolated phages.

Poster Presentation Abstracts

DEVELOPMENT, TESTING AND EVALUATION OF CONVETIONAL AND REAL TIME PCRS FOR DETECTION OF THE PHAGE P100

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The phage P100, which is also commercially available, is frequently used to combat *Listeria* in food production. Routine testing for *L. monocytogenes* in food samples is mostly done by enrichment and microbiological methods therefore the use of phages can lead to false negative results.

The aim of the study was to enable the detection of the phage P100 in food or environmental samples. Therefore the aim the development and establishment of a stable PCR in order to avoid false negative results in routine microbiological testing due to phage contamination.

Altogether eight primer pairs for conventional PCR and four primer pairs for real time PCR with Taqman probes were designed and tested. After design and establishment of the PCRs (testing of primer concentrations, $MgCl_2$ concentrations, temperature and time gradients, etc.), detection limits and specificity was tested. Finally the PCRs were validated and tested using naturally contaminated samples.

All primer pairs were checked bioinformatically for unspecific binding. During testing of the primer several new *Listeria* phage sequences were uploaded to the NCBI database and as a result some of the primer were not specific anymore. Experimental tests revealed that all 8 primer pairs designed for conventional PCR are able to amplify not only the phage P100 but also the *Listeria* phage vB LmoM AG 20 which was isolated in Canada and has a very similar sequence compared with the phage P100. It was not possible to distinguishing the phage P100 from the phage vB LmoM AG 20 in both melting curve and length of the amplicon. Two of four primer and probe combinations designed for real time PCR in contrast were able to discriminate these two phages. These primer were also able to detect the phage P100 in natural contaminated samples.

SCREENING AND CHARACTERISATION OF BACTERIOPHAGE P100 INSENSITIVE LISTERIA MONOCTOGENES ISOLATES IN AUSTRIAN DAIRY PLANTS

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Listeria monocytogenes is one of the most important food-borne pathogens. Although there are different ways for decontamination the use of bacteriophages is an option that becomes more and more popular. A phage to combat *Listeria* is P100 which is also commercially available. P100 is frequently used in food production nowadays but there is not much known about occurrence and the development of resistance against it.
L. monocytogenes isolates obtained from Austrian dairies were screened for P100 resistance and the frequency of occurring resistances before and after P100 treatments was examined. Moreover the efficacy of different phage to bacteria ratios and the development of resistance were investigated and the detected insensitive isolates were molecularbiological subtyped.

Cross-streak-tests (Miller 1998) were used for the screening of phage insensitive isolates. Measurement of optical density and CFU determinations using different phage to bacteria ratios, adsorption tests (Wendlinger et al. 1996) as well as PCR (Rossmanith et al. 2006 and Dourmith et al. 2004) and PFGE were carried out.

Thirteen out of 502 isolates were found to be insensitive to P100. Seven of these isolates, all from 2001, derived from four different plants. All of them shared a ripening cellar in which P100 was used experimentally. The six other insensitive isolates have been found in 2011 and 2012 in one dairy in which P100 was introduced in 2010. Before 2010 no insensitive isolates were found in this facility. None of the insensitive isolates showed significant changes in cell number or in growth curves compared to uninfected controls regardless of bacteria to virus ratio. The phage P100 was not able to attach or replicate in insensitive isolates.

MATURATION OF THE BACTERIOPHAGE SPP1 AND CONFORMATIONAL CHANGES IN THE COAT PROTEIN REVEALED BY FITTING INTO CRYO-EM DENSITY

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Bacteriophages are nanomachines which are sophisticatedly programmed and designed to infect their bacterial hosts with high efficiency and specificity. Phages are a representation of robust biomolecular machines that use powerful ATP driven motors and specialised puncturing devices for the delivery of the viral genome into their host cells. The emergence of many phage structures over the past decade is directing our current understanding that most if not all tailed dsDNA bacteriophages and some eukaryotic viruses have inherited the same capsid fold and other structural components from a common bacteriophage ancestor. They also share a common morphogenetic pathway. Nowadays phages are used as model systems for understanding the processes of DNA packaging.

Capsids of double stranded DNA phages initially assemble into compact globular structures in bacteria known as procapsids. These are precursor assembly intermediates which upon genome packaging undergo expansion. Scaffolding proteins which are not present within the mature virion are initially recruited for the correct assembly of the coat protein envelope. The interaction of the portal protein (required for genome packaging and release) with scaffolding proteins at an early initiating stage of assembly is a critical step in the production of a correctly formed capsid. Structural rearrangements of the capsid coat protein subunits are induced by release of the scaffolding proteins which is replaced by the packaged genome. The final step of phage maturation is accompanied by increased internal pressure within the capsid. This transition is a crucial step for the formation of the mature phages which are primed for bacterial infection.

To understand the structural re-organisation of the major coat protein gp13 in the SPP1 bacteriophage prior to DNA packaging we have determined three-dimensional (3D) reconstructions of the capsid at different stages during its maturation. We have used methods of structural cryo-electron microscopy. Structure-based modelling and bioinformatics comparison with major capsid proteins of other siphoviruses identified the HK97-fold in gp13 allowing to produce an atomic model of the SPP1 major capsid protein and its arrangement in the *T*=7 capsid, revealing contacts between subunits, and the mode of conformational changes.

CHANGES IN PHAGE TYPES OF CLINICAL METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) STRAINS IN THE GDANSK AREA (POLAND) OVER THE YEARS 1997-2013

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MRSA is a significant nosocomial pathogen and a serious therapeutic and epidemic problem in many countries. Several methods have been used to distinguish MRSA strains for the epidemiological purposes in order to limit the occurrence and spread of MRSA clones within and between hospitals, and phage typing is the initial one in such research. For many years, phage typing has been used as the first step in monitoring of the epidemiology of MRSA infection in the main clinical centers in Gdansk area, in the north of Poland. The aim of this study was to asses, retrospectively, the changes in phage types among the clinical MRSA strains isolated in this area over the last years, in relation to antimicrobial resistance patterns. The strains were isolated from various clinics,

wards and clinical materials and typed by the set of ten phages supplied from the Central Public Health Laboratory in London for use on MRSA. Resistance to antimicrobial agents was determined by the disc diffusion method. Resistance to methicillin was confirmed by *Polymerase Chain Reaction* (PCR). Bacteriophages typing showed that in 1997-1998 MR25/M5 type was the prevalent one (56%). It was resistant not only to β-lactam antibiotics, but mostly also resistant to doxycycline, gentamycin, clindamycin, ciprofloxacin, rifampicin and fusidic acid. By contrast, in 2005-2007, this type was replaced by the type 56B (28,9%) resistant to β–lactams and erythromycin, clindamycin, ciprofloxacin, trimethoprim/sulfamethoxazole, tetracycline and gentamycin. In 2008-2013, a new type, MR8 type - resistant to β–lactams and erythromycin, clindamycin, ciprofloxacin and chloramphenicol - was prevalent (44,1%), and this trend continued to the end of 2013. It is noticeable that the number of MRSA strains resistant to the additional set of phages for MRSA typing has been significantly changing during the investigation time from 4.5% to 29.5% and 18.2%, respectively.

HORIZONTAL TRANSFER OF PLASMIDS MEDIATED BY THE BACTERIOPHAGE SPP1

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The lytic *Bacillus subtilis* phage SPP1 mediates generalized transduction of host chromosome and plasmids, the latter being packaged in concatemeric form. It was shown that the transduction efficiency of pUB110 and pC194 plasmids (rolling-circle replicating plasmids) is 100-1000 fold increased if there is a DNA-DNA homology between plasmid and SPP1, and that it is independent of the recombination proficiency of donor or recipient cells. In this work, the contribution of phage replication and recombination proteins in pUB110 plasmid transduction was analyzed. SPP1 DNA replication starts by the theta mode when the phage replisome organizer G*38*P binds the origin region *oriL*. Phage helicase loader (G*39*P) and helicase (G*40*P), as well as the host DNA primase, DNA Pol III holoenzyme, and topoisomerases are also required. After one or several rounds of theta replication, it shifts to concatemeric DNA replication in a process driven by recombination. For this shift the phage recombinase (G*35*P) and the 5'-3' exonuclease (G*34.1*P) are essential. In addition, SPP1 codes for two other non-essential proteins involved in DNA replication and recombination: a single-stranded DNA binding protein (G*36*P) and a Holliday junction resolvase (G*44*P). SPP1 mutants lacking genes *35, 36, 38* and *44* were constructed. From the transduction assays and the PFGE analysis of the plasmid DNA produced after infection, it was observed that both the G*35*P and the G*38*P proteins were essential for plasmid transduction, and for the accumulation of a 44Kb large plasmid DNA. In contrast, mutations in genes *36* and *44*, only showed a reduction in plasmid transduction. The contribution of plasmid type of replication (theta or rolling-circle-replication) and copy number to transduction efficiency was also analyzed. Transduction efficiency was enhanced by increasing plasmid copy number. Rolling-circle-replicating plasmids were transferred at higher frequency than theta-type. The presence of any phage homologous region in the theta replicating plasmid also greatly increased the transduction efficiency. Our findings propose one of the mechanisms of generalized transduction by phages and can be used to improve tools for phage mediated gene transfer.

SOLVING THE PUZZLE OF THE IMPROVED P1 BACTERIOPHAGE DEVELOPMENT IN *ESCHERICHIA COLI* DEVOID OF FUNCTIONAL DksA PROTEIN

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The P1 bacteriophage, a temperate phage of model bacteria *Escherichia coli* and other enteriobacteria, is one of the well known viruses in molecular biology, with its genome and life cycle described in details. P1 is also an extensively used tool in molecular laboratory research for transferring of genetic features. Nevertheless, we would like to report here another phenomenon related to P1 phage biology. The bacteriophage P1 lytic development is improved in *E. coli dksA* mutants comparing to the wild type bacterial cells. In early studies, the DksA protein was described and identified in *E. coli* as a multicopy suppressor of temperature sensitivity phenotype of mutations in chaperone-encoding *dnaK* and *dnaJ* genes. Since then, many of its function in transcription regulation have been described. DksA interacts with RNA polymerase and acts as a cofactor for the stringent response alarmon, ppGpp resulting in the synergy in amplification of its effect (negative or positive). DksA is also involved in regulation of transcription dependent on alternative σ subunits. Therefore, it is not surprising that *dksA* mutations exhibit pleiotropic effects, indicating its important role in many cellular processes.

We have analysed many factors that could explain the observed phenomenon of improved development of P1 phage in DksA-deficient cells. More efficient adsorption of virus particles might underlie this observation. However, we showed that the absence of DksA protein had no significant influence on the P1 phage adsorption

to the bacterial cell. Moreover, we studied the effect of DksA deficiency on bacterial membrane stability since the impaired membrane integration might cause the observed increase in phage yield. Yet, experiments performed with compounds known to affect membrane integrity (such as sodium deoxycholate or ethanol) showed no difference between wild type and *dksA* strain. The preliminary results of the analysis of P1 phage gene expression in wild type and DksA-deficient bacteria revealed that one of the potential factors responsible for the phenomenon could be the P1 phage Lpa protein (Late promotor activator) which is involved in regulation of P1 lytic development. We investigated also the replication of P1 phage in *E. coli* wild-type as well as in *dksA* strains using a mini-P1 plasmid as a model. This work revealed significant differences in the replication of P1 minireplicon in *dksA* and wild-type strain suggesting DksA effect on the level of plasmid replication, not only cell lysis.

Molecular mechanisms of these processes are not closely related, therefore our results show that the phenomenon of the improved development of P1 phage in *dksA* mutant possibly involves global regulation networks and it seems to be more complex than we previously anticipated.

NUCLEOTIDE SEQUENCE OF CONJUGATIVE PROPHAGE Φ1207.3 (FORMERLY TN*1207.3*) CARRYING THE *MEF*(A)/*MSR*(D) GENES FOR EFFLUX RESISTANCE TO MACROLIDES IN *STREPTOCOCCUS PYOGENES*

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Genetic element Φ1207.3 (formerly Tn*1207.3*) is a prophage of *Streptococcus pyogenes* which carries the macrolide efflux resistance genes *mef*(A)/*msr*(D) and is capable of conjugal transfer among streptococci. Complete nucleotide sequence showed that Φ1207.3 is 52,491 bp in length and contained 58 ORFs. A manual homology-based annotation with functional prediction of the hypothetical gene product was possible only for 34 out of 58 ORFs. Φ1207.3 codes for two different C-methylation systems, several phage structural genes, a lysis cassette (composed by a holin and a peptidoglycan hydrolase), and three site-specific resolvases of the serine recombinase family. Φ1207.3 could be transferred among streptococcal species (*S. pyogenes*, *Streptococcus pneumoniae*, *Streptococcus gordonii*) by a mechanism fitting the operational definition of conjugation. Φ1207.3 always integrated in *S. pyogenes* chromosome at a unique dinucleotide GA target site located in *comEC*/*spy1408* with integration leading to GA duplication, and integration in other streptococcal species still occurred in *comEC* homologous genes. Φ1207.3 may represent a novel class of genetic elements with a molecular mechanism of transfer that still needs to be elucidated.

INVESTIGATION OF STRUCTURES INFLUENCING BACTERIOPHAGE INFECTION OF *CAMPYLOBACTER* SPP.

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The gram-negative, microaerobic microorganism *Campylobacter jejuni* is a major food borne pathogen. The European Food Safety Authority (EFSA) rated Campylobacteriosis as the most frequently reported zoonosis with approximately 214,000 human cases in 2012. Furthermore an increasing trend for recorded outbreaks was observed over five years (2008 - 2012). One of the major vehicles for the transmission of *Campylobacter* is fresh broiler meat with pork, unpasteurized milk or contaminated water also serving as routes of infection. The application of bacteriophages to reduce *Campylobacter* levels on goods destined for consumption has been discussed and tested in several studies. The aim of this study was to investigate structures influencing bacteriophage infection of *Campylobacter* cells. This may help to identify cell-related factors that might affect the efficiency of phage application in biocontrol, as a preparation for challenge trials on skin matrices under storage conditions.

Site-specific knock-out mutations targeting *Campylobacter* colonization and virulence structures were introduced into *C. jejuni* NCTC 12662 phage type 14 (PT14), a phage susceptible strain. Furthermore, bacteriophages have been isolated from different sources and tested for their virulence on these mutant strains. So far capsule- and flagellotropic bacteriophage types have been observed. Preliminary experiments with a bacteriophage cocktail composed of three bacteriophages demonstrated, under meat storage conditions, an approximate 1 $\log_{10}$ reduction of *C. jejuni* on chicken skin at 3 different contamination levels. Further experiments with single bacteriophage approaches targeting different cell surface structures will help investigating which structures have an influence on biocontrol and whether synergetic effects occur through cocktail applications.

EVALUATION OF LYTIC ACTIVITY OF PYO AND INTESTI PHAGE COCKTAILS AGAINST CLINICAL STRAINS OF *PSEUDOMONAS AERUGINOSA* ISOLATED FROM PATIENTS IN TURKEY

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Antibiotic resistance in many bacterial species has become a major challenge in combatting infectious disease. In addition, nosocomial infections due to multi-drug resistant bacteria are becoming a serious problem in hospital environment in many countries.

Pseudomonas aeruginosa is widely spread causative agents of these infections. This gram-negative bacteria is most common multidrug-resistant (MDR) in hospitalized patients, besides one of the main pathogens to colonies skin injuries and cause wound infections. The bacteria also causes other diseases such as pneumonia, bacteremia, meningitis and urinary tract infection in immunocompromised individuals. These evidences have triggered searching alternative therapeutic methods and strategies for P. aeruginosa.

Bacteriophages that are specific for pathogenic bacteria are becoming a potential solution for the treatment of infections caused by antibiotic resistant strains of *P. aeruginosa*. Species-specific bacteriophages are increasingly utilized either alone or in combination with other bacteriophages to combat many bacterial infections that are resistant to several antibiotics. Currently, some bacteriophage cocktails are produced commercially and employed for therapeutic purposes in many countries especially in Georgia and surrounding geography. George Eliava Institute of Bacteriophages, Microbiology and Virology (GEIBMV) was the first center in the world to develop therapeutic phage cocktails against various pathogenic bacteria.

In this study, PYO and INTESTI bacteriophage cocktails, produced by GEIBMV were used to evaluate their lytic activity on *P. aeruginosa* strains that were isolated from patients in Turkey. Fifteen strains of *P. aeruginosa* were used in the study. Initial identification and antibiotic susceptibility profile of the strains were performed by Vitec 2 system (BioMerieux, GN 21341, USA) and identification of the strains were confirmed by Polymerase Chain Reaction (PCR) using *P. aeruginosa*-specific primers. Spot test and method was used to evaluate lytic activities of the phage cocktails on the bacteria strains

As a result, the PYO and INTESTI phage cocktails were found to have lytic activities on 13 and 12 *P. aeruginosa* strains out of the 15 tested, respectively.

These results show that the PYO and INTESTI cocktails are very effective in clinical strains of *P. aeruginosa* isolated in Turkey.

EVALUATION OF LYTIC ACTIVITY OF COMMERCIAL PHAGE COCKTAILS AGAINST CLINICAL ISOLATES OF *STAPHLOCOCCUS AUREUS* İSOLATED FROM PATIENTS IN TURKEY

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Antibiotics are frequently used to treat bacterial infections but resistance to antibiotics in bacteria are gradually increasing world-wide. Therefore, and there is a growing requirement for effective treatment of bacterial infections resistant to the currently available antimicrobial agents. Therefore the researches are focusing to explore more effective therapeutic options and alternative ways to the antibiotics. Bacteriophages are species-specific viruses of bacterial species and capable of infecting and killing bacteria, which is one of the important alternative methods to combat bacterial infections due to antibiotic-resistant bacteria. *Staphlococcus aureus* is a causative agent of a number of infections such as pneumonia, wound infections and osteomyelitis as being primarily an opportunistic human pathogen and includes some strains with antibitoic resistance. Especially methicillin-resistant *Staphlococcus aureus* (MRSA) strains and the isolates with diminished susceptibilities to vancomycin is an imperative problem for nosocomial infections. The introduction of phage treatment was first started in George Eliava Institute of Bacteriophages, Microbiology and Virology (GEIBMV) which was founded in 1923 in Georgia, and the phage treatment was used successfully in treating many infectious diseases.

The aim of this study is to evaluate the lytic activities of a number of commercially available mono and multivalent phage cocktails including STAPHYLOCOCCAL, ENKO, FERSISI, SES, PYO and INTESTI, which were obtained from the GEIBMV, Georgia.

The study comprised 10 different *S. aureus* strains that were isolated in hospitalized human patients in Turkey. The identification and antibiotic susceptibility of the isolates were performed at Sifa University Bornova Education and Research Hospital using Vitec 2 system (BioMerieux, GP 21342, USA). The identity of the isolates were confirmed by a species-specific Polymerase Chain Reaction (PCR) assay. Lytic activity of the bacteriophage cocktails on bacteria was determined by spot test method.

As a result, we found that STAPHYLOCOCCAL monophage and SES cocktail had lytic activities on all the 10 strains of the *S. aureus* tested; The FERSISI and INTESTI phage cocktails had lytic activities on the 9 isolates, and the PYO and ENKO were found to have lytic activities on the 8 and 5 isolates, respectively,
It is concluded the phage cocktails used for S. aureus were highly effective and they may be a good alternative for treatment of infections caused by particularly resistant strains of *S. aureus* in Turkey.

CHARACTERISATION THE HIGHLY LYTIC ΦR4 *STAPHYLOCOCCUS AUREUS* BACTERIOPHAGE AND REVEAL ENVIRONMENTAL FACTORS AFFECTING ITS PROPAGATION EFFICIENCY

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Our aim with this study was to investigate factors influencing host-phage interaction and by this affects rate of phage propagation during production. For this purpose bacteriophages lytic against methicillin-resistant *Staphylococcus aureus* (MRSA) strains were isolated from different environmental samples. Based on their stable phage-host interaction the newly isolated ΦR4 and the human isolate MRSA strain 5 was studied in detail. We have revealed that spectacular differences in the propagation efficiencies could be revealed when different laboratory media were used. With a slightly better efficacy, beside Luria-Bertani, Mueller-Hinton II and TSB broth, the Mueller-Hinton I broth prooved to be the most effective for phage production. Compared to them Tripton and Brain Heart Infusion brothes resulted 3-5 magnitude less ΦR4 plaques. Morphologic analysis of the newly isolated ΦR4 phage by transmission electron microscopy has revealed that this phage belongs to *Siphoviridae*. By using restriction fragment polymorphism we have divided it from other *Siphoviridae* phages from our strain collection able to infect *S. aureus* strains. Annotation the sequence of ΦR4 revealed the genomic structure of this newly isolated phage and differenciated it from 20 other *S. aureus* phages. Host range studies have made it clear that the newly isolated ϕR4 has a narrow host range and by this its is not appropriate for therapeutic uses, but our results flashes the importance of proper media usage for phage production.

A METHOD OF FABRICATION OF UNIVERSAL SUBSTRATE TO SERS AND LSPR DETECTION OF BACTERIOPHAGES

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We are presenting an easy, cheap and reproducible method of electrochemical fabrication of silver nanostructures suitable for detection with both Surface Enhanced Raman Spectroscopy (SERS) and Localized Surface Plasmon Resonance (LSPR). The electrochemical procedure is consistent with the principles of the green chemistry. The substrate of choice is tin-doped indium oxide covered glass (ITO) because of its transparency in visible region of light which enables the LSPR measurements. The protocol of electrodeposition and detection of neurotransmitters with SERS was described in Siek *et al.* Electrochimica Acta 89 (2013) 284. The confirmation of LSPR suitability of presented substrates is shown. Moreover examples of detection of bacteriophages with both techniques, LSPR and SERS, are presented.

BACTERIOPHAGE DETECTION BY ANTIBODY MODIFIED GOLD NANOPARTICLES

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Rapid detection of bacteriophages in biotechnology processes with sensitivity of 10^5 pfu/ml or lower is still a challenge [1]. Therefore looking for new methods, techniques and innovative approaches is needed. Among the various bacteriophage families we choose to concentrate on T1 because they contaminate stocks of other bacteriophages as well as pollute E. coli cultures commonly used in biotechnology.

In our work we focused on detection and determination of the T1 bacteriphage with the help of noble metal nanoparticles exhibiting localized surface plasmon resonance. Gold nanoparticles were modified with polyclonal antibody (IgG-antiT1) [2] and were used for detection of T1 bacteriophages present on the surfaces by following the changes in absorbance intensity the visible light spectrum.

References

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IMPRINTING BACTERIOPHAGES BY ELECTRODEPOSITION OF POLYDOPAMINE

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Virus detection is an important subject that encompasses the detection of human infections, animal diseases and plant viruses. There are many ways to detect viruses like the oldest plaque assays, the most used polymerase chain reaction (PCR) and enzyme-like immunosorbent assay (ELISA), nucleic acid sequence-based amplification, electron microscopy, atomic force microscopy, fluorescence microscopy, surface plasmon resonance [1-3] and surface enhanced Raman spectroscopy. [4] There is still a need for simple and versatile techniques that are capable of differentiating between viruses and selectively detecting a specific strain in a mixture of several virus types. Due to the importance of rapid pathogen identification in implementing proper medical treatment, rapid, sensitive, label free, cheap and easy to use methods are necessary [5].

Here we present a novel approach for a virus sensor based on molecular imprinting to create specific recognition sites for template molecules – T1 bacteriophages. This process involves electropolymerization of a dopamine (DA) monomer [6] in the presence of T1 bacteriophages in an aqueous solution. The polymer (pDA) formation in the presence of the template molecule and without was followed by infrared spectroscopy on the flat ITO electrode and electrode preliminary modified with gold nanoparticles (AuNP). The obtained films were also characterised by SEM and AFM.

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CHARACTERIZATION OF A HOST RANGE VARIANT OF THERAPEUTIC *Staphylococcus aureus* BACTERIOPHAGE Sb-1

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Bacteriophage Sb-1 was isolated in the Georgian Republic of the USSR in 1977 from therapeutic phage preparations and has been used extensively for therapy of various human *Staphylococcus aureus* infections in the Former Soviet Union. Sb-1 has a very broad host range within *S. aureus* that includes drug-resistant strains (Kvachadze *et al*., 2011; Microb. Biotechnol. 4: 643-650). In this work, we tested the efficiency of Sb-1 plating on a panel of 25 representative methicillin-resistant *S. aureus* (MRSA) isolates from US military personnel, including from wound infections. The phage was active against 22/25 (88%) strains. High titer Sb-1 was then plated on a mixed culture of the three phage-resistant strains, an isolated plaque selected and the resulting host range mutant, Sb-1M, characterized in comparison with the parental phage. The efficiency of Sb-1 and Sb-1M plating was tested on 114 diverse global MRSA isolates. Ninety-six of these (84.2%) were susceptible to Sb-1, while nine additional strains (105/114 = 92.1%) were susceptible to Sb-1M. The full genomes of Sb-1 and Sb-1M were then sequenced to look into mutations that could contribute to this marked broadening of host range. A Sb-1 genome sequence determined earlier (NCBI Accession No. HQ163896, Kvachadze *et al*., 2011) was used as the reference sequence. The only differences between genomes of the parental Sb-1 phage and its host range variant were single nucleotide replacements in two genes encoding for AEJ79678.1 and AEJ79753.1, hypothetical proteins that are highly conserved among phages of Gram-positive bacteria.

Day 2:

Invited Speakers Abstracts

Recognition is the first step - harnessing phage-host interaction for rapid diagnostics

Professor. Dr. Martin J. Loessner, ETH Zurich, Institute of Food, Nutrition and Health, Zurich, Switzerland

Harnessing the properties of phage is not only about using the virus as an antimicrobial. Phage encodes peptidoglycan hydrolases, where N-terminal domains (EAD) harbors the peptidoglycan hydrolase activity, and C-terminal cell-wall binding domain (CBD) direct the enzyme to its substrate with high affinity and specificity. Fluorescent CBD reporter proteins are useful for instant labeling and detection of bacterial cells, and magnetic beads coated with CBD allow efficient recovery of target cells. Phage-encoded tail fibers have properties similar to CBDs; they also enable specific recognition and binding of bacterial cells and are particularly useful for application on Gram-negative bacterial cells.

Extensive bacteriophage mediated horizontal gene transfer during Staphylococcus aureus colonization in vivo

Dr Jodi A Lindsay, PhD, St George's, University of London, London, UK

European Implementation of Bacteriophage Therapy: Impact of Medicinal Product Legislation on Tailored Hospital Care

Dr Gilbert Verbeken, Human Cell- and Tissue Banks, Brussels, Belguim

Since 1987, the Burn Wound Centre (LabMCT) of the Queen Astrid Military Hospital (Brussels, Belgium) cultures human epithelial cells (keratinocytes) for grafting on its critically burnt patients. National legislation and quality guidelines defined the production environment as well as other topics relevant to the safe use of these cultured cells on patients. Production units and cell banks were licensed by national health authorities and inspected by national hospital inspection structures. This system worked for years, without major problems. In 2004, European Directive 2004/23/EC was published and translated into Belgian Law. This European Directive defined standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. For the burn wound centre, the implementation of this Directive 2004/23/EC meant more costly productions without increasing the quality and/or safety of the final products. Later again, in 2007, Europe published the Regulation (EC) No 1394/2007 on Advanced Therapy Medicinal Products (ATMP), amending Directive 2004/23/EC. This ATMP Regulation also covered the above mentioned

keratinocyte productions. Consequently our 27 years old keratinocyte cultures became “Advanced” (?) Therapy Medicinal Products to be produced in a ‘pharma’ environment, to be documented as human medicinal products and to be inspected by the medicinal products agency. The impact is huge and not always in the interest of the patients. We see parallelism in what is happening today when different stakeholders are trying to regulate the (re-) introduction of (natural) bacteriophage therapy into Western Medicine. Therapeutic natural bacteriophages are in use for almost 100 years. But today, regulators are classifying therapeutic (not modified, hence naturally) bacteriophages as human medicinal products. To be regulated through the regular human medicinal product frame. We propose an alternative solution such as a ‘hospital exemption’ for e.g. hospitals who want to implement phage therapy inside their own hospital without the ambition to go to the European market with ‘off-the-shelf’ bacteriophage products.

Developing bacteriophage-based products for bio-control of unwanted bacteria in foodstuffs, animal health and human applications - possibilities and challenges

Dr Steven Hagens, PhD, Chief Scientific Officer, The Netherlands

Phage and phage-derived products hold tremendous potential in various fields to selectively control pathogenic or other harmful bacteria. However, developing successful products requires overcoming numerous hurdles and taking the right steps. These steps include target choice, generating a solution that works beyond the laboratory bench, dealing with regulatory issues and lastly successfully bringing such a product to market. The presentation will focus mainly on principles of target choice and creating the solutions that actually work based on examples from three products that target Listeria, Salmonella and Staphylococcus aureus respectively.

Translating Bacteriophage and Lytic enzymes to uses for control of bacteria in environmental, human and animal applications

Dr David P Trudil, NHDetect Corp & Eliava Institute of Bacteriophage, Reisterstown, MD, USA

Bacteriophage has been applied in treatment and prevention of disease in human and animals since the 1920’s. More recently the concern for antibiotic resistance has spurred increased research in bacteriophage and related lytic proteins. Some are focusing on pharmaceutical applications but the more rapid translational applications may be in the areas of animal and environmental use as well as rapid detection methods.

This presentation will review some of the recent efforts by the NHD and Eliava team including Sulfate Reducing Bacteria, group A strep, mastitis, various detection efforts and others.

Prophage maintenance in Enterobacteria

Dr Mireille Ansaldi, Aix Marseille Université, CNRS – Marseille, France

Prophages represent a large fraction of prokaryotic genomes and often provide new functions to their hosts, in particular virulence and fitness. How prokaryotic cells maintain such gene providers is central for understanding bacterial genome evolution by horizontal transfer. Looking for host factors that play a role in prophage maintenance, we identified the transcription termination factor Rho as involved in prophage maintenance through the control of excisive recombination. Thus, besides its role in the silencing of horizontally acquired genes, Rho also maintains lysogeny. Genetic screens identified a number of other regulators of this process in E. coli and Salmonella species, and are under investigation.

Assessment of the bacteriophage ΦCD6356 and its biologically-active endolysin as novel antimicrobials targeting Clostridium difficile

Dr Olivia McAuliffe, Teagasc Food Research Centre, Cork, Ireland

Clostridium difficile infection is typically associated with the use of broad-spectrum antibiotics. Consequently, there is a need for the development of specific antimicrobials which target C. difficile but do not result in collateral effects on the microbiota. We have identified the C. difficile bacteriophage ΦCD6356, with activity against viable C. difficile. Here, we outline our assessment of ΦCD6356 and its recombinant endolysin, LysCD as potential treatments for CDI.

Novel polymerases from marine phages

Dr. Bin Zhu, Research Fellow, Harvard Medical School Boston, MA, USA

Phages in the ocean harbor the greatest diversity of protein. Since a large portion of these proteins must play roles in nucleic acids metabolism, one would expect numerous novel mechanisms underlying the fundamental processes and thus abundant molecular reagents therein. We recently characterized the single subunit RNA polymerase from marine cyanophage Syn5 and identified its promoter sequence. This marine enzyme catalyzes RNA synthesis over a wide range of temperature and salinity. Its processivity is greater than 30,000 nt and it produces precise transcripts with homogeneous 3’ termini. Therefore, the enzyme is advantageous for the production of RNAs in vitro.

Oral Presentation Abstracts

CHARACTERIZATION OF THE CYSTEINE- HISTIDINE-DEPENDENT AMIDOHYDROLASE/PEPTIDASE DERIVED FROM THE STAPHYLOCOCCAL PHAGE K ENDOLYSIN.

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Staphylococcus aureus is a major cause of infection in humans and animals causing a wide variety of conditions from local inflammations to fatal sepsis. The bacterium is commonly multi-drug resistant and thus many front-line antibiotics have been rendered practically useless for treating human infections, thus bacteriophage technology was explored as a potential for eliminating this bacterium. The genome of the anti-staphylococcal phage K was sequenced and the endolysin was cloned in E.coli.. This enzyme, named LysK has a modular organisation with three domains, a cysteine/histidine-dependent amido hydrolase peptidase (CHAPk), an amidase, and thirdly a cell-wall binding domain. The latter facilitates attachment of the enzyme to the bacterial cell wall, while former two domains catalyse the degradation of the peptidoglycan, mediating rapid bacterial cell death. Deletion analysis of the enzyme showed that full lytic activity against live antibiotic-resistant staphylococci was retained when the endolysin was truncated to its CHAPk (peptidase) domain. The enzyme was purified by ion-exchange chromatography and characterized in detail including elucidation of its 3-D structure. Addition of the enzyme to a turbid bacterial MRSA culture resulted in elimination of turbidity. The peptidase was used in in-vivo studies in mouse models where it successfully eliminated MRSA colonization without adverse effects on the animals; and furthermore, ex-vivo studies confirmed a low immunogenicity. X-ray crystallography studies confirmed the 3-D structure of the enzyme and also indicated the presence of zinc and calcium co-ordination atoms facilitating enzymatic activity.

CHARACTERIZATION OF *BACILLUS SUBTILIS* PROPHAGE SPβ GENE FOR PHAGE ABORTIVE INFECTION

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Bacteriophages (phages) are ubiquitous in most environments where they outnumber bacteria by a factor of about 10-fold. Bacteria are thus frequently attacked by phages and have evolved various mechanisms to evade phage predation. One such mechanism is abortive infection in which a specific gene affects a crucial step in the phage infection cycle that prevents phage multiplication and dissemination. However, details remain elusive. The *Bacillus subtilis* Marburg strain is resistant to bacteriophage SP10 through involvement of phage-resistance *nonA* and *nonB* genes. The *nonB* gene is located in the prophage3 region of the *B. subtilis* genome where it encodes the restriction enzyme, BsuMR, which cleaves phage genomic DNA and inhibits phage growth. The n*onA* gene is located in the prophage SPβ region where it encodes a membrane protein comprising 72 amino acids. We analyzed how NonA functions to achieve abortive infection in *Bacillus subtilis*.

We initially investigated which phase in the SP10 infection process is inhibited by NonA. Southern and Northern blotting revealed that NonA did not inhibit either SP10 phage DNA replication or the transcription of SP10 phage genes. We found that *nonA* transcription is regulated by a sigma factor encoded in the SP10 phage genome during the late stage of the SP10 infection process. The expression of NonA inhibited the accumulation of SP10 phage proteins such as capsid and sheath proteins. Therefore, we proposed that *nonA* functions as an abortive infection gene. We constructed a strain harboring IPTG-inducible *nonA*, and found that artificial NonA expression halted *B. subtilis* cell growth and reduced cellular respiration activity, suggesting that NonA is harmful to *B. subtilis*. We also isolated NonA-resistant mutants that could grow under NonA expression and were permissive for SP10 phage growth. These data suggest that NonA does not specifically inhibit SP10 gene translation but rather inhibits the growth of infected cells to cause abortive infection.
Reference

Tatsuya Yamamoto *et al*. 2014. SP10 infectivity is aborted after bacteriophage SP10 infection induces *nonA* transcription on the prophage SPβ region of the *Bacillus subtilis* genome. J. Bacteriol.

CLINICAL APPLICATION OF AN OPTIMIZED BACTERIOPHAGE COCKTAIL TARGETING MULTI-DRUG RESISTANT *PSEUDOMONAS AERUGINOSA*

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Pseudomonas aeruginosa is an opportunistic pathogen frequently associated with recalcitrant infections refractory to antibiotics. These infections often present as biofilms which, in addition to intrinsic antibiotic resistance, further complicate and limit therapeutic options. These difficult to manage infections represent cases in which the utility of phage therapy could be examined. While approved clinical trials are limited in Western medicine, the few that have been conducted have not reported any harmful side effects. Thus, it appears that the therapeutic application of bacteriophages is safe for human use in select cases.

Here, we present the case of a 75 year old male with a chronic bacterial infection associated with an aortic arch replacement with a Dacron graft. Long term (i.e. 1.5 years) colonization of the graft by *P. aeruginosa* resulted in multiple hospitalizations and surgeries to debride infected tissues. Consistent failure to clear infection by traditional means required the exploration of an alternative therapy and we determined that the patient could benefit from phage therapy. We characterized the microbiome of the infection site, created, and optimized a bacteriophage cocktail from over 50 candidates isolated from the environment. Individual phages were evolved on the naïve host and the resistant mutants that arose for several passages to select for higher virulence of phage and a reduced likelihood of cocktail resistance. Furthermore, particular attention was paid to antibiotic synergy and anti-biofilm potential of the cocktail. Clinical and laboratory data will be presented as will the challenges associated with oversight, creation, and application of a therapeutic bacteriophage cocktail in unique cases such as this.

As more alternative therapies are considered to combat the rise of antibiotic resistant infections, we hope that clinical cases such as this provide useful data for guidelines in the development of approved bacteriophage-based therapeutics in the future.

PHAGE SELECTION RESTORES ANTIBIOTIC SENSITIVITY IN A DRUG-RESISTANT BACTERIAL PATHOGEN

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Pathogenic bacteria contribute greatly to worldwide morbidity and mortality in humans. But there is increasing failure of traditional antibiotics to halt previously controllable bacterial infections, due mostly to the evolution of antibiotic resistance in pathogen populations. Therefore, it is critical that the medical research community devise new strategies for combating bacterial disease. An excellent alternative to traditional antibiotics may be phage therapy. Although this approach has its merits, one concern is that phage resistance can evolve as easily as antibiotic resistance, and in both cases there is no guarantee that the resistance would cause a decline in pathogen virulence. That is, resistant bacteria can flourish because the underlying mutations for resistance do not interfere with the mechanisms of virulence. The solution is to develop therapies that force the desired tradeoff between resistance and virulence. Our approach is to develop Virulence Targeted Antibiotics (VTAs), a new generation of therapeutic phage that specifically bind to the virulence factors of their bacterial hosts. When the inevitable evolution of resistance to VTAs occurs, the bacteria must either lose or modify the targeted virulence factor, likely decreasing their ability to cause disease. Our initial target bacterium for VTA development is *Pseudomonas aeruginosa*, an opportunistic pathogen frequently associated with lung infections in cystic fibrosis patients, and infections in burn victims and immuno-compromised individuals. Current management strategies for these infections rely on chemical antibiotics of increasingly poor effectiveness. Though numerous mechanisms are responsible for this intrinsic antibiotic resistance, a mechanism responsible for resistance to multiple classes of antibiotic is drug efflux by the chromosomally encoded and often redundant Multi-drug efflux pump (Mex) systems. Here we demonstrate that a naturally-isolated phage (family *Myoviridae*) acts as a VTA when attacking *P. aeruginosa*. That is, the phage utilizes the outer membrane porin M (OprM) of the tripartite MexXY-OprM efflux system as a receptor binding site. This mode of infection forces a trade-off in *P. aeruginosa*, whereby the evolution of resistance to phage attack leads to a change in the efflux pump mechanism, causing the bacteria to evolve sensitivity to traditional antibiotics. Moreover, we show that combination therapy involving the phage and traditional antibiotics is synergistic, and especially useful for decreasing bacterial population size. Our approach shows that phage binding to known virulence factors can force an evolutionary trade-off, prolonging the ability to use antibiotics of waning effectiveness.

Poster Presentation Abstracts

DIFFERENTACTION PATHWAYS OF THE PHAGE VB_ASPP-UFV1 IN REDUCING BIOFILMS FORMED BY BACTERIAL ISOLATES FROM FEED WATER OF REVERSE OSMOSE SYSTEM

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Biofilms occur spontaneously in different environments, whether biotic or abiotic, is an important survival strategy of bacteria. Biofilm formation on reverse osmosis (RO) systems represents a drawback in the application of this technology by different industries, including oil refineries. In RO systems the feed water maybe a source of microbial contamination and thus contributes for the formation of biofilm and consequent biofouling. In this work, the influence of non-specific bacteriophage was investigated in the biofilm formed by bacteria. Bacteria isolates of feed water sampled from a RO system that showed greater ability to form biofilm, i.e. higher biomass, were selected and used in the biocontrol assay employing thephagevB_AspP-UFV1 against biofilm formation. A phage suspension of a multiplicity of infection (MOI) of 0.01 was added into 96-well microtiter plate containing bacterial suspension, and biofilm formation was measured using biomass quantification with crystal violet staining (CV).Statistically significant reductions were observed in 7 of the 11 tested bacteria, some showed dose-dependent reduction and others, increasing the MOI used did not cause further reduction in biofilm. After the CV analysis, two bacteria, which responded differently with MOI increasing, were selected and analyzed in MBEC® devices by scanning electron microscopy (SEM) in order to evaluate the influence of phages on biofilm structure. By SEM was possible to observe that phage interferes with the adherence of one isolate and in the stability of biofilm formed by other bacterial isolate. The results suggest that the phage vB_AspP-UFV1 (*Podoviridae*) interfered in biofilm formationby two strategies, by action of enzymes or phage infection, without bacterial lysis. This approach may represent a good alternative in biofilm control on membranes in reverse osmosis systems. Support: Petrobras

TRANSFER OF ANTIMICROBIAL RESISTANCE GENES BETWEEN CLINICAL MRSA BY ENDOGENOUS PROPHAGES.

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Methicillin-resistant Staphylococcus aureus (MRSA) are commensal bacteria of the nose and the most common cause of serious antibiotic-resistant infection. Resistance genes to all antimicrobials have been found in clinical MRSA strains but no strains have accumulated resistance to all. Transduction via bacteriophage is the main mechanism of gene transfer in S. aureus, and all clinical strains harbour at least one prophage. Bacteriophage are thought to be induced by environmental conditions including exposure to UV light or exposure to exogenous agents such as antibiotics, and 30% of hospitalized patients who are carriers are prescribed antibiotics. The aim of this study was to investigate whether free bacteriophage can be isolated from nasal swabs in hospitalised patients and are if they are able to mediate effective transfer of resistance genes between colonizing populations of clinical MRSA strains. The effects of subinhibitory concentration of antimicrobials on phage induction and effectiveness of transfer of antimicrobial resistance were also analyzed. A high-throughput droplet digital PCR (ddPCR) system quantitated the number of transducing phage particles versus virulent phage particles. Sixteen of 38 (42%) of MRSA positive nasal swabs obtained from patients admitted to St Georges' Hospital of London, UK were positive for free phages. Bacteriophages were capable of generalized transduction and successfully transferred antibiotic resistant genes between clinical strains. We detected increased induction of transducing and virulent particles from the donor strain in response to subinhibitory concentration of nine antimicrobials. Interestingly, the ratio of each type of particles differed for each antimicrobial.These findings suggest that exposure of colonizing MRSA strains to antimicrobials can trigger prophage induction and lead to enhanced horizontal transfer of antimicrobial resistance and other genes between strains during colonization, potentially leading to fully resistant MRSA.

A NEW SCREEN TO DETECT HOST FACTORS INVOLVED IN PROPHAGE MAINTENANCE

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Temperate phages have the ability to integrate their genome into the host chromosome, a process called lysogeny. Once integrated as a prophage, the phage genome passively replicates within the host chromosome. However, any stress that threatens the host and thus the prophage survival allows the lytic cycle to resume. During evolution, some prophage genes can be lost, especially those coding for lytic cycle capacity. While some of these defective prophages are perfectly competent for excision, they are maintained in bacterial genomes, suggesting a selective pressure to keep them. This is the case for our model, the defective prophage KplE1 in *Escherichia coli* K12.

To study prophage maintenance in Enterobacteria genomes, we developed a genetic screen that should allow for the identification of host factors involved in prophage maintenance. To detect KplE1 maintenance, we inserted a fluorescent cassette inside the prophage genome. As KplE1 is not able to replicate it is lost by clonal dilution upon excision, therefore leading to the loss of fluorescence. The screen consists in transforming the reporter strain with a plasmid library covering the host genome. Then, we forced excision to occur and looked for plasmids that allowed fluorescence emission, and thus prophage maintenance. We identified several candidate genes involved in general metabolism and stress response. First results show the implication of genes coding for proteins involved in detoxification of nitric oxide (NO) in KplE1 maintenance. Nowadays, we are trying to understand and characterize the link between NO detoxification and prophage maintenance and by extension horizontal gene transfer. In many examples, *p*rophages can bring new skills to the host and thus confer a growth advantage. In these cases phage domestication could be favored.

THE DEVELOPMENT OF A REPORTER PHAGE FOR THE IDENTIFICATION OF *CLOSTRIDIUM DIFFICILE*

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Clostridium difficile (*C. difficile*) is the most common bacterial cause of infectious diarrhoea in health care environments. In 2014 it was responsible for 18,005 infected patients; with an estimated cost of £7,000 per *C. difficile* infection (CDI) in the United Kingdom and $4,846 in the United States of America. The bacterium is highly resistant to many disinfectants and cleaning agents, allowing it to survive on surfaces as spores for months. Therefore, controlling the spread of *C. difficile* is of utmost importance, to limit the risk of new infections. However, there is currently no specific method to monitor *C. difficile* in the environment (health care settings), which would validate the elimination of *C. difficile* following cleaning. Bacteriophages (phages), bind very specifically to their target organism and the aim of our research is to exploit this interaction to produce a phage-based assay to detect the presence of *C. difficile* on health care environmental surfaces. This will be done by constructing a light tagged (reporter) phage assay, which will produce a detectable light signal only when the phage meets its *C. difficile* prey.

Reporter phages (consisting of two genes *luxA* and *luxB* from the bacterium *Vibrio harveyi*), have been successfully developed for many bacterial species including *Yersinia pestis* and *Listeria monocytogenes*. The technology allows specific detection of the target pathogen with minimal processing and the overall cost of the assay is relatively low. No such diagnostic is currently available for *C. difficile*.

The fully sequenced *C. difficile* specific phages K12 and F2 (both lysogens) were chosen to be modified to produce reporter phages. Phages K12 and F2 is able to infect the most common ribotypes (sub-groups) of *C. difficile,* including the prevalent ribotype 014/020. The plasmid pBlueScript Sk+ was initially used to design the reporter genes cassette and was then moved to a *Clostridium* shuttle plasmid which allowed transfer of the construct to the phage via conjugation. To minimise disruption of the phage genome, multiple predicated non-essential genes were targeted for homologous recombination to identify the best site of insertion of the reporter genes for both phages. Following conjugation, transformations were screened to test for insertion of the reporter gene and multiple rounds of plaque purification were used to identify a positive reporter phage.

In principle a *C. difficile* reporter phage assay could aid in limiting and controlling the spread of *C. difficile* in clinical facilities and help to reduce the financial burden of CDI in these sites.

CHARACTERIZATION OF THE HOST-RANGE MUTATIONS IN GENOME OF POLYVALENT STAPHYLOCOCCAL PHAGE 812

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The emergence and alarming increase of multiresistant strains of *Staphylococcus aureus* emphasizes the need for new and innovative antimicrobial strategies. Polyvalent bacteriophages from the family *Myoviridae*, sub-family *Spounavirinae* of genus Twort-like viruses that infect *S. aureus* have potential for use in phage therapy. In this study we characterized genomic sequences of the polyvalent staphylococcal phage 812 and its spontaneous host-range mutants selected on non-susceptible *S. aureus* strains. Bacteriophage 812 is closely related to phage K. The wild type phage 812 was shown to lyse up to 65%, whereas host-range mutants up to 95% of *S. aureus* strains under study. Selected host-range mutants of phage 812 were included in antistaphylococcal phage lysate for topical application STAFAL® produced under GMP by Bohemia Pharmaceuticals s.r.o. in the Czech Republic.

The phage susceptibility testing was estimated on 120 MRSA strains with different genotypes defined by sequence type (ST)/spa type/SCC*mec* type. Genomic DNAs of five phages were sequenced using 454 Genome Sequencer Junior (Roche). Bacteriophage DNA sequences were assembled by software GS De novo Assembler and GS Reference Mapper (Roche). Genome of phage K was chosen as the reference.

Detailed analysis of obtained sequence data showed that the phage genome consists of linear double-stranded DNA without cohesive ends, with 35-bp tandem terminal repeat and repeat count 5. Genome size of the wild type phage is 142,096 bp and molar GC content was 30.42%. In the genome, 220 putative genes were identified, out of which for 148 genes their function can be predicted. Three genes for tRNA were found. Neither toxins nor superantigens nor genes for lysogenic phage cycle were detected which confirms suitability of the bacteriophage for clinical purposes. The sequences of the host-range mutants involve SNPs, short (5 bp or 9 bp) substitutions and long deletions. The major deletions concern 2,012-bp deletion associated with rearrangement of tail tube gene sequence, 456-bp deletion in gene for hypothetical virion structural protein, 77-bp deletion in gene for putative tail appendices protein, 33-bp deletion in DNA ligase gene, 46-bp deletion in DNA primase gene, and 1,509-bp deletion in the endolysin gene.

The possible defense mechanisms of the strains which can result in their insensitivity to phages are unknown. Different phage resistance mechanisms such as phage adsorption, SauI restriction-modification system, presence of specific prophage types and CRISPRs were examined. No correlation with the host-range mutations and characteristics of non-susceptible host strains was found yet. The completely resistant *S. aureus* strains fell to sequence types ST 7, ST 45, ST 80 and ST 239.

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BACTERIOPHAGES FROM *STAPHYLOCOCCUS AUREUS* ST398 PACKAGE DIFFERENT TYPES OF MOBILE GENETIC ELEMENTS

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It is widely accepted that temperate bacteriophages play a major role in horizontal gene transfer and contribute to spread of mobile genetic elements (MGE) including SCC*mec* element in *Staphylococcus aureus*. The newly acquired genes responsible for virulence and antibiotic resistance are thus rapidly disseminated in the staphylococcal population. It is supposed that serological group B bacteriophages of *S. aureus* are capable to package different parts of bacterial DNA to their capsids and transferred bacterial genes by generalized transduction. The clinical methicillin-resistant *S. aureus* (MRSA) isolate UMCG-M4 with the sequence type 398 studied in this work contains prophages belonging to the serological groups A, B and Fa. The *S. aureus* strain UMCG-M4 carries the SCC*mec* type V (5C2&5) element responsible for methicillin resistance (Chlebowicz *et al*., Int. J. Med. Microbiol. 2014, 304:764-774). While SCC*mec* is generally regarded as a mobile genetic element, the precise mechanisms by which large SCC*mec* elements are exchanged between staphylococci have remained enigmatic. The main aim of this work was to study horizontal transfer of SCC*mec* and another MGE by staphylococcal bacteriophages induced from clinical strain and detection various regions of SCC*mec* in bacteriophages particles by PCR and long PCR.
The plasmid pT181 was transferred by electroporation to clinical strain UMCG-M4 and modified strain was used for our transduction experiments. The natural occurring phages were induced by UV irradiation from strain UMCG-M4 (pT181) and lysate was used for transduction experiments and for phage DNA isolation. The phage particles were purified by CsCl density gradient centrifugation and were treated with DNase I and RNase to remove non-encapsulated bacterial DNA (Maslanova *et al*., Environ. Microbiol. Rep. 2013; 5:66-73). DNA was extracted from the phages by pronase treatment and phenol-chloroform extraction. The transductants were selected on tetracycline plates and verified by PCR. The parts of SCC*mec* were detected in phage particles by PCR and long PCR.
Our results show that some of induced phages from strain UMCG-M4 can transduce plasmid DNA to another recipient strain. We proved transfer of plasmid pT181 to the recipient *S. aureus* strain RN4220. We optimized transduction conditions for clinical strain UMCG-M4. The possible involvement of induced phages in the transmission of the large SCC*mec* type V (5C2&5) element of *S. aureus* UMCG-M4 was investigated. While no transduction of the complete SCC*mec* element was observed, we were able to demonstrate that purified phage particles did contain large parts of the SCC*mec* element of the donor strain, including the methicillin resistance gene *mecA*.

The staphylococcal phages can encapsulate different types of mobile genetic elements including the resistance determinant *mecA* of a large SCC*mec* type V (5C2&5) element, which may lead to its transfer to other staphylococci.
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APPLICATION OF PHAGE COCKTAILS AGAINST *CAMPYLOBACTER JEJUNI* ON ARTIFICIALLY CONTAMINATED CHICKEN SKIN

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Campylobacter jejuni is a major cause of foodborne human enteritis. Poultry meat has been reported to be the main sources of contamination. The lack of effective control measures against these pathogens makes *Campylobacter spp.* a serious public health threat and new control methods need to emerge. The application of phages as biocontrol agents against *Campylobacter spp*. in food has previously been investigated in a limited number of studies using single phages. However, the use of cocktails of phages with complementing lytic activity has not been tested against these pathogens in food matrices yet.
The aim of this study was to find the most efficient phage combination to combat *C. jejuni* in food. A total of nineteen phages, that had been isolated from free-range poultry farms located all over Denmark and characterized by Sørensen et *al*. (2015), were used in our experiments. Seven of these phages were dependent on the bacterial flagella for infection, whereas the remaining twelve bind to the capsular polysaccharide (CPS) of *C. jejuni* in order to infect these pathogens. The efficiency of single phages and phage cocktails was tested against *C. jejuni* NCTC12662 *in vitro* and *in vivo* at 5°C under anaerobic conditions for 24 hours.
In vitro experiments showed that two CPS phages isolated from the same farm were the most effective against *C. jejuni* resulting in 68% and 76% reduction, respectively. Application of a cocktail of these two phages led to 1 log reduction of *C. jejuni*. Artificially contaminated chicken neck skin was used for the *in vivo* experiments. Frozen chicken skins supplied from a Danish slaughterhouse were defrosted the day of the experiments. After application of the bacteria and the cocktail of these two phages, chicken skin pieces were incubated in Modified Atmosphere Packaging (MAP) conditions at 5^{o}C for 24 hours. 1-log reduction of the recoverable Campylobacter was observed, an outcome that was in accordance with the results from the *in vitro* experiments. Further investigation is required for the elucidation of the interactions between *C. jejuni* and cocktails of phages.

PHAGE-ANTIBIOTIC SYNERGY TO MANAGE BURKHOLDERIA CEPACIA COMPLEX INFECTIONS

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Burkholderia cepacia complex (Bcc) is a group of 18 phenotypically similar gram-negative bacteria. Of these, *Burkholderia cenocepacia* is one of two species responsible for chronic infection in Cystic fibrosis (CF) patients. At present, a combination of three or more drugs is used for treatment. Because the Bcc have high innate resistance to antimicrobials, present treatment is not effective. Hence, there is a need for alternate methods of treatment. One such strategy is phage therapy using bacteriophages (or phages), which are viruses that target bacteria. Recently, there have been reports of certain antibiotics stimulating phage growth. This effect is referred to as phage-antibiotic synergy (PAS). We tested Bcc phages in combination with antibiotics for PAS effects. Phages KS12 and KS14 were tested against *B. cenocepacia* K56-2 and C6433, respectively. Several antibiotics were tested, of which ciprofloxacin, meropenem and tetracycline produced the most pronounced PAS effect. Bacterial cells were observed in the presence of low concentrations of antibiotics using electron microscopy. Cells showed different morphologies in the presence of the three antibiotics. In the presence of ciprofloxacin, cells were observed to be present as long filaments, with meropenem, cells appeared to form chains, and with tetracycline, cells appeared to be clustered. Increases in plaque sizes and phage titers were observed with increases in antibiotic concentration. This effect was also observed for cells previously made tetracycline resistant. Clinically relevant antibiotics minocycline, levofloxacin and ceftazidime were also tested for PAS; these antibiotics did not produce significant PAS effects. *B. cenocepacia* K56-2 cells treated with a combination of phages and antibiotics showed increased killing over time in an *in vitro* assay. We also tested treatment of K56-2 infections *in vivo* in a *Galleria mellonella* infection model. K56-2 infected *G. mellonella* larvae showed significantly enhanced survival when treated with a combination of phage KS12 and meropenem or minocycline as compared to those treated with the same dose of phage KS12 or meropenem or minocycline alone. These data show PAS for the Bcc exhibits better results than either antibiotics or phage alone. These results suggest that phages can be combined with certain antibiotics to increase effectiveness of treatment.

BACTERIOPHAGE COCKTAIL ACTIVE AGAINST THREE *Shigella* SPECIES

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Shigellosis, or bacterial dysentery, is a diarrheal illness that afflicts 80–165 million people and results in 600,000 deaths per year globally. A serious public health problem is the rapid development and dissemination of multiple antibiotic resistance among clinical isolates of *Shigella*, especially in South and Southeast Asia (Niyogi, 2007, Clin Microbiol Infect 13:1141-3; Folster *et al.,* 2011, Antimicrob Agents Chemother 55:1758–60). This requires development of alternative antibacterial drugs such as therapeutic bacteriophages (phages). Phages have been successfully used for prophylaxis and treatment of dysentery in the USSR and FSU countries for decades (Kutateladze & Adamia, 2008; Med Mal Infect 38:426-30) but there are no phage preparations against shigellosis licensed for use in the West.

The purpose of this work was to isolate and characterize potentially therapeutic phages active against *Shigella.* Wastewater from five different locations and reactors at the Blue Plains Wastewater Treatment Plant (Washington, DC) was used as the source of phages. The cultures of *Shigella flexneri* (several serovars), *Shigella sonnei*, and *Shigella dysenteriae* were used for phage enrichment. Multiple enrichment procedures allowed for the isolation of 36 different bacteriophages lytic for *Shigella* with genome sizes varying from ~37 to ~160 kb. Three mixes of three phages each were shown to completely sterilize *S. flexneri* liquid culture in repeated 24-h tests, suggesting that this *Shigella* strain does not develop resistance to the phage mixes. Testing host ranges on 97 strains of *Shigella* isolated mostly in Southeast Asia showed that the best 3-phage cocktail was active against 100% of *S. sonnei* isolates, 97.4% of *S. flexneri* (including serotypes 1, 1a, 1b, 1c, 2, 2a, 2b, 2ab, 3a, 3b, 4, 4a, 5, and 6, as well as variants X and Y), 100% of *S. dysenteriae,* serotypes 1 and 2, and 16.7% of *Shigella boydii* strains. Serotypes 9 and 12 of *S. dysenteriae* were resistant to the phage mix. Overall observed activity was 89.9%. Since 94.4% of strains were pigmented on Congo Red medium and thus carried the virulence plasmid, the results suggest that the phage cocktail was successfully working against virulent clinical isolates of *Shigella*.

CHARACTERIZATION OF TEMPERATE BACTERIOPHAGES OF *PASTEURELLA MULTOCIDA* STRAINS FROM DIFFERENT HOST SPECIES

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The diversity of temperate bacteriophages was examined in 40 *Pasteurella multocida* isolates associated with different infections in the major domesticated host species cattle, sheep, pigs and poultry. The strains represented various capsular serotypes, outer membrane protein types, 16S rRNA types and multilocus sequence types. The phage particles were induced with Mitomycin C and characterized morphologically by transmission electron microscopy. Phage particles were identified in 24 *P. multocida* isolates and included *Myoviridae*, *Siphoviridae,* and *Podoviridae*-like phages; in addition, phages representing an unrecognized family were also identified. Structural variation was observed in the tails of *Myoviridae* phages. Interestingly, both *Myoviridae* and *Siphoviridae* phages, as well as *Siphoviridae* and *Podoviridae*-like phages, were induced in the same *P. multocida* isolates, indicating that a single bacterial host may harbour multiple prophages. DNA was isolated from 14 phages and restriction endonuclease (RE) analysis identified 10 different RE types. The *P. multocida* bacteriophages exhibited a narrow host range since plaques were formed on a limited number of indicator strains. However, a subgroup of five ovine serotype D isolates contained *Siphoviridae* phages of identical RE type that possessed similar abilities to infect and form plaques on the same panel of indicator strains. PCR analysis showed that phage DNA from four toxigenic strains contained the *toxA* gene. From our results we conclude that strains of *P. multocida* recovered from different host species carry a diverse range of bacteriophages. These phages are likely to play important roles in the evolution and virulence of this important veterinary pathogen.

CHARACTERISATION OF SHIGA TOXIN 1 PHAGES REVEALS INSIGHTS FOR FORMATION OF NEW PATHOGENS

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Shiga toxin (Stx) producing *Escherichia coli* (STEC), most notably STEC serotype O157, is an important foodborne human pathogen. Production of Stx is a key STEC virulence factor and this is conferred upon the bacteria by Stx-encoding bacteriophages (Stx phages), which infect and integrate into the host bacterial genome. These phages carry either stx_1 or stx_2 genes, which encode two immunologically distinct toxins with similar biological function. *stx* phages have a broad host range and have been found within several members of *Enterobacteriaceae*. Australian STEC O157 strains are associated with lower incidence of clinical disease than strains from other countries, and interestingly have stx_1 phages integrated at a genomic locus not previously associated with stx_1 phages: tRNA-*argW*. This prompted us to investigate these novel stx_1 *argW* phages.

We found that stx_1 phages induced from two clinical Australian STEC O157 isolates, could each infect *E. coli* K-12, where the phages also integrated into tRNA-*argW*. Stx quantification in both STEC O157 and their derived K-12 Stx1 lysogens revealed that host genetic background influences toxin production. Electron microscopy and genome sequencing revealed the two Stx1 phages belong to the *Podoviridae* family and are genomically highly related to each other and to sequenced Stx2 phages. We also found that these phages can potentially induce a translational frameshift with two overlapping tail-coding sequences with different host recognition domains. This is a previously unidentified mechanism in *Podoviridae*, which may account for the broad host range of Stx phages leading to the emergence of new Stx producing pathogens.

SHIGA TOXIN 1 ENCODING CHIMAERIC BACTERIOPHAGE FROM THE ENTEROHEMORRHAGIC *ESCHERICHIA COLI* O157:H7 SAKAI STRAIN

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Shiga toxin-producing *Escherichia coli* (STEC), and especially enterohemorrhagic *E. coli* (EHEC) are important, highly virulent zoonotic and food-borne pathogens. One of their main virulence factors, the Shiga toxin represents a family consisting of two main types (Stx1 and Stx2) encoded by lambdoid prophages. One of the prototypic, fully-sequenced EHEC O157:H7 Sakai strain carries both types of Stx toxin, and is known to harbour several prophages, including Sp5 and Sp15, which carry the genes encoding Stx2 and Stx1, respectively. Sp5 was found inducible in earlier studies, and recombinants of Sp5 and Sp15 were also reported, but only partial sequence data were published.
Our phage induction experiments with the Sakai strain revealed a virulent phage of novel genotype carrying the *stx1* gene cluster. The phage showed *Podoviridae* morphology, and was able to propagate on *E. coli* K-12-derived C600, MG1655 and DH5 alpha strains. Moreover, we isolated several lines of C600 and MG1655 which were lysogenised by the phage. Shiga toxin production of a lysogenised MG1655 line was verified on Vero-cell cultures.
We determined the whole genome sequence of the phage, which is 61,137 bp long and contains 93 open reading frames (ORF). Genome analysis revealed significant recombination between Sp5 and Sp15, representing the first whole genome of such a chimaeric phage originating from the Sakai strain. The majority of the novel *stx1*-encoding recombinant phage genome originated from the *stx2*-encoding prophage Sp5, with major rearrangements in its gene order. The genomic region containing the *stx2* genes in Sp5 was replaced by a region containing six ORFs from prophage Sp15 including the *stx1* genes, forming a chimaera of these two prophages.
In summary, the reported chimaeric genome structure represents a new type of recombinant Stx1 converting phage from the *E. coli* O157:H7 Sakai strain, furthermore, the existence of a novel Stx phage suggests its potential dissemination in nature by phage transduction.

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Day 3:

Invited Speakers Abstracts

Stx prophage control of host phenotypes

Dr Heather E. Allison, University of Liverpool, BioSciences Building, Crown Street, Liverpool, United Kingdom

Shiga toxin encoding phages were first discrovered in 1982 following an E. coli O157:H7 outbreak in 1982. These phages have gone on to acquire a large host range, begging the question, Why? Genome sequencing has revealed that these phages posses, in general, a much larger genome than their archetypal family member, Lambda phage. The use of RNA Seq and standard cloning experiments have elucidated a wealth of phage-controlled host systems that provide substantial fitness advantages to the lysogen host. This provides selectable advantages to Stx phage lysogens and also indicates unquie roles for known as well as hypothetical proteins encoded by these phages.

Modern ideas for combating bacteria

Dr Zuzanna Drulis-Kawa PhD., Institute of Genetics and Microbiology, University of Wroclaw, Poland

The need for novel antibacterial drugs is especially pressing when considering infections caused by multidrug-resistant (MDR) pathogenic bacteria. The great variety of innovative approaches presented in the current literature is astonishing. Antimicrobials, including new antibiotics, β-lactamase and efflux pump inhibitors, quorum quenching molecules and nanoparticles with antibacterial activity are currently being intensively studied. The development of alternative/supportive therapies such as phage/phage based products therapy and photodynamic therapy, in which the mechanism of action is completely different from current antibiotic treatment, is of great importance.

Overview on Yersinia phages and update on phage therapy initiative in Finland

Dr Mikael Skurnik Ph.D., Professor of Bacteriology, University of Helsinki, Finland
Haartman Institute, Department of Bacteriology and Immunology Research Programs Unit, Immunobiology, Helsinki University Central Hospital Laboratory Diagnostics

Genus Yersinia consists of 17 species of which Y. pestis, Y. pseudotuberculosis and Y. enterocolitica are human pathogens. Y. pestis causes bubonic plague while Y. pseudotuberculosis and Y. enterocolitica cause yersiniosis. Bacteriophages infecting Yersinia are introduced and some discussed in detail.
A phage therapy initiative started in Finland 2013 will be described and updated.

Bacteriophage lytic enzymes with a broad antibacterial spectrum.

Dr Magdalena Plotka, Department of Microbiology, University of Gdansk, Gdansk, Poland

Lytic enzymes (endolysins) are highly evolved enzymes produced by bacteriophage to lyse bacterial cell for phage progeny release. Endolysins from Gram-positive background typically utilize a modular design. Generally, they consist of catalytic domain and cell wall binding domain (CBD), the latter responsible for endolysin specificity. Our group is interested in small endolysins derived from thermophilic, Gram-negative bacteria that lack of CBD. Thereby, they have a broad antibacterial spectrum. They also possess highly desirable features, such as high thermostablility and activity at high salt concentration. These unique properties make them good candidates for use in biotechnology and medicine.

GMP manufacture of phages for clinical trial

Mr Tony Hitchcock, Technology Director, Cobra Biologics, Newcastle, UK

GMP production of novel bacteriophages for first in man clinical studies is a critical and challenging phase in therapeutic product candidate development.
Phage products are being developed for an increasingly wide range of therapeutic applications. Currently, researchers and phage development companies are poorly serviced with viable, scalable and GMP compliant manufacturing solutions suitable for their supply needs. Cobra is working to fulfil this requirement

Monolith chromatography for fast and scalable phage purification

Dr Rok Sekirnik, BIA Separations, Slovenia

Monolith chromatography is a novel technique for efficient isolation and purification of large biomolecules, including phages and other viruses. Monoliths consist of large, highly interconnected channels functionalised with a selected ligand chemistry. They enable convective mass transfer which leads to highly efficient and flow-independent chromatographic separation. The talk will present novel approaches for the development of fast, efficient and selective purification processes for bacteriophages, based on their physicochemical properties, such as charge, shape and size. Additionally, the use of monolithic columns for in-process monitoring of phage production will be presented based on chromatographic “snap shots” of the fermentation broth, serving as an integration tool between upstream and downstream aspects of the process.

Oral Presentation Abstracts

DEVELOPING PHAGE THERAPY TO TREAT CANINE SKIN INFECTION (PYODERMA) CAUSED BY STAPHYLOCOCCUS PSEUDINTERMEDIUS

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Background: The extensive use of antibiotics has led to the emergence of multi-resistant bacteria such as methicillin-resistant *Staphylococcus pseudintermedius*, a coagulase-positive species responsible for skin infection in the dog, and now also causing post-operative infections and urinary tract infections. The administration of bacteriophages (phage therapy) can be an alternative to antibiotics to treat infections. Lytic phages, which lyse their host and release new viruses, are usually regarded as the only appropriate type of phages for phage therapy as opposed to temperate phages, which can survive within their host (lysogeny). However, it is possible to mutate temperate phages so that they are no longer able to establish lysogeny. For example, in the phage λ the cI gene encodes a repressor that binds to an operator that controls the expression of the *cro* gene which itself is involved in the control of the production of phage particles. So-called *vir* mutants have lost the operator to which the phage repressor binds. This means that they are not sensitive to the repressor anymore and constitutively enter the lytic cycle.
Aims:
- Isolation of lytic and temperate phages from different sources (samples from dogs, soil, water, *S. pseudintermedius* strains)
- Genotypic and phenotypic characterisation of the isolated phages, and in the case of temperate phages, isolation of *vir* mutants
- Selection of the best candidates for phage therapy, and test of their efficiency *in vitro* and *in vivo*
Methods: The isolation of phages was performed through screening of a variety of samples (faeces, skin swabs, soil, etc.) or induction of temperate phages through mitomycin C treatment. Characterisation of the isolated phages was performed through Restriction Fragment-Length Polymorphism (RFLP), electron microscopy, whole-genome sequencing and testing the host range. The isolation of *vir* mutants was attempted through exposure to hydroxylamine.
Results: Several temperate phages have been isolated and were characterised phenotypically and genotypically. These phages are diverse on the genetic level and exhibit a rather narrow host range. The mutagenesis of the phages through hydroxylamine to obtain *vir* mutants was not successful. A potential operator region has been identified in the genome of several of these phages providing a target for direct mutagenesis and isolation of *vir* mutants. No naturally lytic phages have been isolated.
Conclusion: the most successful approach to isolate phages against *S. pseudintermedius* was to induce prophages from bacterial strains. Naturally lytic phages against this pathogen seem to be very difficult to find. Insight was gained into the biology of the isolated phages as a step forward towards the development of phage therapy to treat pyoderma.

SYNERGY BETWEEN BACTERIOPHAGE-ENCODED PEPTIDOGLYCAN HYDROLASES AND HIGH PRESSURE ON THE INACTIVATION OF *LISTERIA MONOCYTOGENES* AND *STAPHYLOCOCCUS AUREUS*

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High hydrostatic pressure (HHP) treatment of food products increased in popularity over the last decade because it allows for inactivation of food-borne pathogens while preserving the product's nutritional and organoleptic properties [1]. However, although HHP treatments can markedly decrease the viability of microorganisms directly after the treatment, a large proportion of cells might be only sublethally damaged and able to recover under favorable conditions [2,3]. To ensure the microbiological stability and safety of HHP processed food products, HHP treatments can be combined with other preservative hurdles. In our study, we investigated the effect of a combination of bacteriophage-encoded peptidoglycan hydrolases, i.e. endolysins, with HHP treatments on the inactivation of the important foodborne pathogen *Listeria monocytogenes* and *Staphylococcus aureus*.
Multiple strains of *L. monocytogenes* and *S. aureus* were selected based on their diverse endolysin susceptibility and genetic/proteomic characteristics. Inactivation of late exponential phase cells by endolysins against *L. monocytogenes* (PlyP40, Ply511 or PlyP825) and *S. aureus* (HY-100 or HY133), applied individually or in combination with HHP, was assessed in buffer.
The chosen HHP parameters alone did not inactivate *L. monocytogenes*. However, when cells were first incubated with endolysin PlyP40, Ply511 or PlyP825, even at low concentrations which did not affect viable cell counts, cell morphology or membrane integrity (as assessed by LIVE/DEAD staining), HHP treatment resulted in a

significant inactivation of selected *L. monocytogenes* strains (~2-5 log reduction). We also found a higher inactivation of *S. aureus* by endolysin HY-100 or HY-133 in combination with HHP (compared to individual treatments), but the synergistic effect was less pronounced and dependent on strain and endolysin concentration.
Altogether, our data shows a strong synergistic effect between endolysin and HHP treatment on the inactivation of *L. monocytogenes* and *S. aureus*, which could be utilized in the preservation of food products susceptible to harsh processing conditions (e.g. milk or salmon).

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MATURATION OF THE BACTERIOPHAGE SPP1 AND CONFORMATIONAL CHANGES IN THE COAT PROTEIN REVEALED BY FITTING INTO CRYO-EM DENSITY

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Bacteriophages are nanomachines which are sophisticatedly programmed and designed to infect their bacterial hosts with high efficiency and specificity. Phages are a representation of robust bimolecular machines that use powerful ATP driven motors and specialised puncturing devices for the delivery of the viral genome into their host cells. The emergence of many phage structures over the past decade is directing our current understanding that most if not all tailed dsDNA bacteriophages and some eukaryotic viruses have inherited the same capsid fold and other structural components from a common bacteriophage ancestor. They also share a common morphogenetic pathway. Nowadays phages are used as model systems for understanding the processes of DNA packaging.

Capsids of double stranded DNA phages initially assemble into compact globular structures in bacteria known as procapsids. These are precursor assembly intermediates which upon genome packaging undergo expansion. Scaffolding proteins which are not present within the mature virion are initially recruited for the correct assembly of the coat protein envelope. The interaction of the portal protein (required for genome packaging and release) with scaffolding proteins at an early initiating stage of assembly is a critical step in the production of a correctly formed capsid. Structural rearrangements of the capsid coat protein subunits are induced by release of the scaffolding proteins which is replaced by the packaged genome. The final step of phage maturation is accompanied by increased internal pressure within the capsid. This transition is a crucial step for the formation of the mature phages which are primed for bacterial infection.

To understand the structural re-organisation of major coat protein gp13 in the SPP1 bacteriophage prior to DNA packaging we have determined three-dimensional (3D) reconstructions of the capsid at different states during its maturation (resolution ~4 Å and 7 Å respectively). We have used methods of structural cryo electron microscopy. Structure-based modelling and bioinformatics comparison with major capsid proteins of other siphoviruses identified the HK97-fold in gp13 allowing to produce an atomic model of the SPP1 major capsid protein and its arrangement in the *T*=7 capsid, reveal contacts between subunits, and the mode of conformational changes.

Poster Presentation Abstracts

PHAGE-HOST INTERACTIONS: SPATIAL REFUGE AND QUORUM SENSING

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Vibrio anguillarum is an important pathogen in aquaculture. Phage therapy to control infections as an alternative to antibiotic treatment has drawn interest recently. However, for successful application of phages in pathogen control, a detailed understanding of phage-host interactions is needed. Quorum sensing, known as a

ubiquitous cell-cell communication phenomenon in nature, by using this devise, bacteria coordinate their cell density-dependent gene expression. *N*-acyl homoserine lactones (AHLs) mediated QS circuits have been identified in *V. anguillarum* like many other *Vibrios* species, contains components for multiple QS-phosphoreplay system, and controls QS-regulated genes via the transcription factor VanT, which is activated in response to extracellular autoinducers. The goal of our study is to uncover the roles of spatial refuge and cell-cell signaling in shaping phage-host interactions*. V. anguiilarum* PF430-3 QS mutants, Δ*vanT*-locked in low cell density and Δ*vanO*-locked in high cell density, allowed exploration of the role of QS for protection against phages infection. With Δ*vanT* becoming more susceptible to phage KVP40 by enhancing biofilm formation, providing protection of host against phage infection by creating spatial refuge, whereas, Δ*vanO* became more resistant by down-regulation phage receptor OmpK to reduce its susceptibility to phage infection with no bacterial aggregates. Further, wild type experiment showed a strong negative correlation between AHLs production and ompK expression, which was in consistent with previous mutants' results. Taken together, the data presented here suggest that VanO negatively regulates the expression of VanT, which in turn represses the expression of essential phage receptor-*ompK*.

SEQUENCE AND COMPARATIVE ANALYSIS OF AVIAN PATHOGENIC *ESCHERICHIA COLI* PHAGES

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Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis and significant economic losses in the poultry industry. The goal of this study was to isolate and characterize poultry fecal phages that are active against *Escherichia coli* serogroups O1, O2, and O78; the main causative agents of avian colibacillosis.

Lytic phages (71) were isolated from poultry fecal material, by enrichment using the *Escherichia coli* K514 and *E. coli* C600. Phage lytic spectrum of activity against 61 APEC strains collected from Belgium was determined. The phages with the broadest activity spectrum were characterized by, restriction-fragment length polymorphism (RFLP). Based on their obligate lytic characteristics and broad host spectrum of activity, some were selected for whole genome sequencing using the Nextera XT™ kit (Illumina, San Diego, USA). The morphology of the phages was determined by negative staining and Transmission Electron Microscopy (TEM).

Phages PhK7, PhK10S, PhK10L, PhK12B, PhK21, PhK22, PhK12A, PhK13, PhK26, PhK2, PhK3, PhK6, Ph431, PhC3 and PhK33C were further characterized. Some phages showed great similarity in their genome sequences, however their host spectrum of activity varied e.g. Ph10S (16/61), PhK7 (8/61), PhK10L (7/61), PhK12B (8/61), PhK21 (2/61) and PhK22 (5/61) were identical but with different host spectrum of activity. Another set with similar characteristics were phages PhK12A (8/61), PhK26 (7/61) and PhK13 (0). Phages PhK2 (7/61) and PhK3 (5/61), almost identical phages with few mismatches, had nearly similar host range spectrums.

The most active phages were Ph431 active against 70% (43/61) of the APEC strains, PhC3 against 68% (42/61), PhK33C against 41% (25/61), PhK10S against 26% (16/61), Ph12A 19% against (12/61).

In all samples, particles were observed consisting of a non-enveloped head with icosahedral symmetry and a tail with helical symmetry; characteristics attributing the particles to the bacteriophage order of the Caudovirales and belonged to families Myoviridae (PhC3) and Siphoviridae.

Genome analysis of the phages indicated there were close relatives of Siphoviridae present under the unclassified Tunalikevirus (PhK2, PhK3, PhK6) and unclassified Siphoviridae (PhK10S, PhK7, PhK10L, PhK12B, PhK21, PhK22, Ph12A, PhK26, PhK13). The presence of phage holin and lysis genes confirms that these are lytic phages. The analysis of the annotated proteins of the Siphoviridae viruses, confirmed the structure of these phages as per TEM.

FROM THE UNEXPLORED TO FUTURE THERAPEUTICS – FRESHWATER PHAGES INFECTING FLAVOBACTERIA

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Flavobacteria are ubiquitous freshwater bacteria that have a major role in biochemical cycles and contribute to water quality in lakes and rivers. Few of the members of genus *Flavobacterium* are known pathogens that cause substantial economic losses to the fish farming industry. Phages infecting this genus, and other freshwater

bacteria, are generally unexplored. We have isolated and studied phages infecting *Flavobacterium* in Finnish fresh waters. Genomic studies revealed high variability of phage genome sizes (up to 380 kbp) and long reads of unknown sequence. Also a novel phage type was isolated – a tailless icosahedral (capsid size approx. 45 nm) phage with 10 kbp ssDNA genome. Most importantly, our isolates showed major potential for using phage therapy against the fish pathogenic *F. columnare*. Phage addition significantly increased the survival of two experimentally infected host fish species, rainbow trout and zebra fish. This is promising as the rainbow trout production suffers greatly from columnaris-disease. Indeed, fish-farming industry has shown interest towards phages as a new tool to resist disease outbreaks.

EFFECT OF ANTIBIOTIC TYPE, DOSE AND COMBINATION WITH PHAGES ON THE ADAPTIVE POTENTIAL OF *P. AERUGINOSA*

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With escalating resistance to antibiotics there is an urgent need to develop alternative therapies against bacterial pathogens and pests. One of the most promising is the employment of bacteriophages, which may be highly specific and evolve to counter anti-phage resistance. Despite an increased understanding of how phages interact with bacteria, we know very little about how their interactions may be modified in antibiotic environments and, reciprocally, how phage may affect the evolution of antibiotic resistance. We experimentally evaluated the impacts of single or combined applications of antibiotics (different doses and different types) and phages on *in vitro* populations of the opportunistic pathogen *Pseudomonas aeruginosa* PAO1. We also assessed the effects of treatments on bacterial virulence *in vivo*, employing larvae of *Galleria mellonella*. We find that phages and antibiotics have a negative synergistic effect on bacterial populations, and this is positively correlated with antibiotic dose and independent of the antibiotic type. The results indicate that the main advantages of combining a lytic phage with different antibiotics are to prevent the recovery of bacterial populations and to limit antibiotic resistance levels. Finally, all treatments reduced bacterial virulence in wax moth larvae hosts, but the addition of phages limited the magnitude of the reduction in virulence. From an applied perspective, our results indicate that phages can contribute to managing antibiotic resistance levels, with limited consequences in the evolution of bacterial virulence. We discuss the relevance of our findings for future research aimed at treating bacterial infections in human health care.