

Exploiting bacteriophages for bioscience, biotechnology and medicine

The Penridge Suite, 470 Bowes Road, London N11 1NL

Friday, 20 January 2012

Bacteriophages (phages) are arguably the most abundant biological entities on the planet. They play crucial roles in driving the adaptive evolution of their bacterial hosts, and achieve this both through the predator-prey roles of the phage-bacterium interaction and through the adaptive impacts of lysogeny and lysogenic conversion. Bacteriophages are the source of many biochemical reagents and technologies, indispensable for modern molecular biology. Furthermore, phages are being exploited in other areas of biotechnology, including diagnostics, prophylaxis and other aspects of food microbiology. In recent years there has been a growing interest in developing phages for therapeutic purposes (phage therapy) as natural alternatives to antibiotics. The inexorable rise in the incidence of antibiotic resistance in bacterial pathogens, coupled with the disappointingly low rate of emergence of new, clinically useful antibiotics, has refocused attention on the potential utility of phages for treating human and animal disease. Examples of the roles of phages in fundamental biological research and in medical and industrial biotechnologies will be discussed at this meeting

This event has CPD accreditation and will have a discussion panel session.

On registration you will be able to submit your questions to the panel that will be asked by the chair on the day of the event

Meeting chair - *Professor George Salmond*, University of Cambridge, UK

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9:00 – 9:45 **Registration**

9:45 – 10:00 **Introduction by the Chair:** *Professor George Salmond*, University of Cambridge, UK

10:00 – 10:20 **How bacteria survive viralinfection: anti-phage abortive infection systems**

Tim Blower, University of Cambridge, UK

Bacteria, outnumbered ten-to-one by their viral parasites, bacteriophages, have generated many bacteriophage-resistance mechanisms. One class, the abortive infection systems, induce premature host cell death upon bacteriophage infection. By committing this 'altruistic suicide', the host cell destroys the invading bacteriophage and protects the clonal population. The ToxIN abortive infection system was recently identified on a plasmid of the Gram negative plant pathogen, *Erwinia carotovora*. Crystallographic analysis has shown that ToxIN forms a stable protein-RNA complex, with ToxN acting as a toxic endoribonuclease. Work is ongoing to understand how bacteriophages activate and, in some cases, subvert this novel abortive infection system.

10:20 – 10:40 **Streptomycete phage integrases: from fundamental science to translational exploitation**

Professor Maggie Smith, Institute of Medical Sciences, University of Aberdeen, Scotland

10:40 – 11:00 **High Yield Phage Purification with Monolith Chromatography**

John Creedy, Progressive Research Systems Ltd, Cambridge, UK

Viruses and phages present a challenge for chromatographic purification. They are typically excluded from traditional particulate media, due to their size. Polymeric CIM-monolith materials allow virus particles direct access to internal binding sites by convectional/pumped laminar flow. Avoiding diffusional mass transport, monoliths promote fast, efficient adsorption/desorption to monolith ion exchangers essentially independent of flow rate. Fast, high titre, high yield purifications can be achieved from crude preparations at all scales. Throughpores (mean diameter 1.5µm) allow unimpeded access to large nanoparticles and pore morphology ensures laminar flow with no dead-end pores. This delivers high capacity for large particles with high yield.

11:00 – 11:05 **Speakers' photo**

11:05 – 11:30 **Mid-morning break and Poster Viewing**

11:30 – 11:50 **Nature and exploitation of phages for pathogenic anaerobes**

Dr Martha Clokie, University of Leicester, UK

Clostridium difficile is the major cause of infectious diarrhoea in hospitals and can result in death. It is difficult to diagnose and to treat and one promising source of novel diagnostic agents and anti-microbials may come from bacteriophages. We have isolated a set of 20 bacteriophages which infect *C. difficile*. We have shown that they have diagnostic/therapeutic potential as they infect the dominant *C. difficile* ribotypes found in UK hospitals. We have characterised their morphology, infection parameters, and host ranges. We have also generated whole genome sequences which have revealed that they encode a mosaic of expected and unexpected genes.

11:50 – 12:10 Maintenance of Escherichia Coli prophages into the host chromosome

Mireille Ansaldo, Laboratoire de Chimie Bactérienne - CNRS, Aix-Marseille Université, France

In prokaryotic genomes, genomic islands can represent a large amount of the total genomes, and a large part of these islands are prophage remnants that are no longer infectious. Two main questions drive our current research: (i) why are these prophages maintained into the host genome, and (ii) how are they maintained? KpIE1 is a defective prophage of Escherichia coli K12, fully competent for excisive recombination, which genome contains 16 open reading frames, and is used as a model system for site-specific recombination studies. The overlap between attL and the integrase gene promoter leads to a negative autoregulation of the integrase gene, and such a regulatory loop was predicted in the majority of t-RNA inserted prophages found in prokaryotic genomes. As a consequence, the main switch that controls the excision of these prophages is the expression of the recombination directionality factor (RDF) gene which is absolutely required to control the integrase activity. Recent work suggests that host-encoded general regulators are involved in prophage maintenance.

12:10 – 12:30 What have we learned about the biocontrol of campylobacter s with phage?

Professor Ian Connerton, University of Nottingham, UK

Controlling campylobacters in poultry represents one of the greatest challenges to the agriculture and food industries if they are to achieve consumer and governmental demands to reduce human food borne disease. Research into the potential of bacterial viruses to treat bacterial infection (phage therapy) has increased greatly in recent times largely due to the quest to find alternative, sustainable methods to replace antibiotic treatments which no longer perform due to the dramatic rise in multi-drug resistant bacteria

12:30 – 13:30 Lunch and Poster Viewing

13:30 – 14:30 Question and Answer Session

Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day

14:30 - 14:50 Phage-based diagnostics: current and future opportunities

Dr Cath Rees, University of Nottingham, UK

For many years it has been recognised that the specificity of the bacteriophage-host interaction could be exploited to develop rapid methods of bacterial detection. In addition phage are easier to isolate and propagate, and production is certainly simpler and cheaper than the costs associated with production of new antibodies. However achieving a practical test with sufficient specificity and sensitivity has proved challenging. Many test format have been described, and some have been developed into test kits - although these have had limited commercial success. More recently tests using combinations of methods have begun to be developed that have started to solve some of the limitations of solely phage-based methods. In this talk I will review the challenges that need to be met in terms of achieving sufficient selectivity, specificity and sensitivity to meet the needs of the end user and will then describe how each of the commercial tests developed to date have addressed these problems. Finally I will describe some of our recent work developing using a combined methodology approach to develop a phage-based blood test.

14:50 - 15:10 Challenges and Successes in Bacteriophage Formulation for Topical and Transdermal Delivery

Brendan F Gilmore, Biofilm Research Group, School of Pharmacy, Queen's University Belfast

In the past decade significant advances have been made in order to translate bacteriophage therapeutics from the laboratory to the clinic. These have ranged from improved isolation and characterisation, demonstration of synergy with conventional antimicrobials and improved clinical trial design and analysis. Despite this, significant formulation challenges exist in the development of appropriate phage-delivery systems, challenges which will need to be addressed in order to provide delivery of effective phage therapeutics. Current studies typically describe the application of simple aqueous bacteriophage formulations, however, stability and storage of bacteriophages in standard dosage forms has received less attention.

In this presentation, the development of a novel polymeric phage delivery platform for topical and transdermal applications will be discussed. The ongoing studies in our group, using T4 phage as a model bacteriophage, have examined the formulation and long term stability of T4 phage in various polymeric carriers, examining the role of pH, temperature, ionic strength and polymer composition on phage stability and aggregation. Finally, the successful transdermal delivery of viable T4 bacteriophage using novel polymeric hydrogel microneedle arrays in an in vivo rat model will be discussed

15:10 - 15:30 The potential of phage therapy in an animal model of P. aeruginosa pulmonary infection

Dr Marine Henry, Institut Pasteur, France

Using a mouse model of lung infections we previously demonstrated the curative potential of bacteriophages. In order to setup guidelines for choosing the most efficient therapeutic bacteriophages among those isolated from environment, we compared 8 different bacteriophages to determine whether *in vivo* and *in vitro* efficacies could be correlated. Using a bioluminescent strain of *P. aeruginosa*, we found that light quantification in the 8 hours following infection (ie 6 hours post phage treatment) could be used as a predictive tool to evaluate the bacteriophage therapeutic potential.

15:30 – 16:00 Afternoon Tea/Coffee and Poster Viewing

16:00 – 16:20 **Counting of bacteriophage by NTA and F-NTA in trivial and complex suspensions**

Dr Patrick Hole, Nanosight, UK

Despite the importance of concentration of viruses and bacteriophage, existing methods for their quantification frequently rely on slow and complex techniques such as plaque assay or estimation of TCID. Nanoparticle Tracking Analysis (NTA) is a new method for the direct analysis of nanoparticles, including viruses, in liquids. Each and every particle is visualised and analysed separately, resulting in a direct number distribution which directly gives virus size and concentration and can distinguish viruses from larger cell debris. In cases where this distinction becomes more complicated it is possible to augment the technique with the use of fluorescence labelling (F-NTA).

The ability of the NanoSight instruments to visualise, count and size viruses and their aggregates is important to manufacturers who are interested in monitoring the purity of the viral preparation at various key stages of the purification process and determine the concentration of virus material present. The total viral titre (i.e. infectious titre plus non infectious viruses) generated by the Nanosight technique can be used in conjunction with the infectious viral titre as provided by infectivity assays to understand what percentage of the total viral titre are infectious. In many cases the infectious viruses may represent as little as 1% of the total virus particles present in a preparation, and such low yield of infectious particles is perhaps indicative of the purification process used. As such, this information can be fed back into the process development to more effectively produce a final product.

The NanoSight technique is rapid gives high resolution data and the systems (of which there are >450 installed worldwide) are inexpensive and easy to use.

16:20 - 16:40 **Biopolymer degrading enzymes from environmental isolates of unknown Bacillus phage – hope for novel tools for biofilm disruption.**

Jakub Barylski, Department of Molecular Virology, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland

During our studies of microflora of lake Góreckie (Wielkopolski National Park Poland) we found bacteriophage infecting one of bacteria inhabiting this ecosystem. This strain was identified later using 16S rRNA gene sequencing. This sequence displayed significant similarity to those of *Bacillus pumilus*. Further investigation was concentrated around revealing of genomic sequence of mentioned phage.

16:40 – 17:00 **Clinical trials for phage therapy and regulatory considerations**

Dr. David R. Harper, AmpliPhi Biosciences Corporation, Bedfordshire, UK

The therapeutic use of bacteriophages has long been eclipsed by chemical antibiotics. A major reason for this is the lack of modern, regulated clinical trials. Many studies have been conducted, but only a very few of these have been carried out to standards acceptable to current EU and US regulators. It is clear that data from fully regulated clinical trials is necessary in order to convince regulators, investors, and potential partners in large pharmaceutical companies that bacteriophages can really achieve what they promise.

17:00 **Chairman's summing up**

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About the chair

Professor Salmond is currently in the Department of Biochemistry at the university of Cambridge. He graduated in microbiology (BSc, Strathclyde) followed by a PhD in bacterial genetics and phage-host interactions (Warwick) and an MA and ScD (Cambridge). He has taught in Kent, Warwick and Cambridge universities. He has multiple research interests in microbiology, including the molecular basis of bacterial virulence in plant and animal pathogens, quorum sensing, biosynthesis and regulation of bioactive secondary metabolites (including antibiotics), protein secretion systems, and the biology and exploitation of bacteriophages - the subject of this meeting. He has long-standing interests in the isolation of novel phages from the natural environment for the development of genetics and functional genomics of diverse bacteria, including plant, animal and human pathogens. He also has current research interests in how bacteria evade the potentially lethal impacts of viral infection via phage abortive infection systems.

About the Speakers

Tim Blower is currently a postdoctoral research associate at the Department of Biochemistry, University of Cambridge. Having obtained his BA and MSci in Biochemistry at the University of Cambridge, he continued on to complete his PhD, examining the activity of a unique form of bacteriophage resistance mechanism by biochemical and structural biological means. He was awarded the Sir Howard Dalton Young Microbiologist of the Year award in 2009 by the Society for General Microbiology, and won the Nat L. Sternberg Thesis Prize in 2010, co-ordinated by Cold Spring Harbor Laboratories, New York, USA.

John Creedy has worked with LKB Instruments, Pharmacia UK and Tosoh Bioscience and since 1978 has developed a career in Sales and Marketing of separations and purification technology. He established PRS in 1990, introducing perfusion chromatography in Europe alongside other innovative chromatography technologies from various companies. Since 2005 he has compiled the current portfolio of products which bring new enabling technologies to the biopurification sector. He is a keen advocate of innovative technologies such as monolith chromatography, parallel 96-array automated chromatography for process methods development and a new proprietary technology for counting and sizing virus preparations.

Patrick Hole is currently Head of Development at NanoSight and has been with the company five years. Previously he has completed a PhD in optoelectronics at the University of Southampton and a Masters Engineering degree at Oxford University. He has focussed on developing both the hardware and software involved in the Nanoparticle Tracking Analysis (NTA) technique, invented eight years ago, to be an easy to use, accurate and robust system.

Martha Clokie's research is focused on how bacteriophages influence the biology of their bacterial hosts. She works on cyanobacteria and in medical pathogens as in both systems bacteriophages play key roles in controlling the evolution and population dynamics of their hosts. She did a degree in Biology at the University of Dundee, an MSc at Edinburgh and a PhD in Molecular Ecology at the University of Leicester. She did 2 Post-docs with Prof Nick Mann at the University of Warwick and gained a scholarship to work in Scripps in San Diego for 3 months before taking up a position as a lecturer at the University of Leicester in 2006.

David Harper is Chief Scientific Officer of AmpliPhi Biosciences, a US/UK company which holds a leading position in the development of bacteriophage therapeutics. After completing his Ph.D. at the University of Newcastle he carried out post-doctoral work at St. Bartholomew's Medical School in London and at the University of Iowa in the US. He joined the faculty at St. Bartholomew's in 1991 and left for the private sector in 1999. Dr. Harper is also the virology editor for John Wiley's Encyclopedia of Life Sciences, and is the author of "Viruses: Biology, Applications and Control", published in June 2011.

After obtaining her PhD in the Cork Institute of Technology (Republic of Ireland) for her works on mycobacteriophages under the supervision of Dr Jim O'Mahony and Dr Aidan Coffey, **Marine Henry** started as a postdoctoral researcher in the Institut Pasteur in Paris, in the team of Dr Laurent Debarbieux, where she currently works on the potential of phage therapy in an animal model of *P. aeruginosa* pulmonary infection.

Ian Connerton BSc MSc PhD from the University of Warwick where he studied Biochemistry. Three years research Genetics Department at Cambridge University working on the molecular genetics of filamentous fungi. In 1987 joined the Microbiology Department at Reading University. In 1991 to the Institute of Food Research as a Section Leader and graduating to Deputy Head of Food Macromolecular Science with Institute Programme responsibility for Macromolecular Function and Design. From 1998 to date Prof. Connerton was appointed as the first Northern Foods Professor of Food Safety at the University of Nottingham

Registration Web Site:

www.regonline.co.uk/bacteriophage2012

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POSTERS

BIOPOLYMER DEGRADING ENZYMES FROM ENVIRONMENTAL ISOLATES OF UNKNOWN BACILLUS PHAGE – HOPE FOR NOVEL TOOLS FOR BIOFILM DISRUPTION

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During our studies of microflora of lake Góreckie (Wielkopolski National Park Poland) we found bacteriophage infecting one of bacteria inhabiting this ecosystem. This strain was identified later using 16S rRNA gene sequencing. This sequence displayed significant similarity to those of *Bacillus pumilus*. Further investigation was concentrated around revealing of genomic sequence of mentioned phage.

Bioinformatic analysis of partial viral genome sequence turned out to contain two putative genes. Basing on sequence similarity we identified them as two novel enzymes probably involved in biopolymer degradation: poly- γ -glutamate hydrolase (similar to corresponding enzyme from *Bacillus subtilis* phage phiNIT1) and polysaccharides lyase (similar to pectin lyase-like proteins from various *Bacilli* and their phages).

We confirmed that crude protein extracts from phage-infected cultures are able to effectively degrade purified mucoid polymer produced by host cells (which we believe to be poly- γ -glutamate).

We hypothesized that discovered region is probably responsible for degradation of host mucoid capsule, presumably operone involved in freeing of progeny phage particles.

Poly- γ -glutamate is known as important for formation of biofilms formed by bacteria from genus *Bacillus* and *Staphylococcus epidermidis*. This biofilms play important role in colonization of novel niches and in some cases pathogenesis. It is possible that discovered enzymes may be used to prevent biofilm formation effectively solving problem of unwanted colonization by mentioned bacteria

ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES AGAINST *ACINETOBACTER BAUMANNII*.

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Acinetobacter baumannii is a pathogen, which has an increasing prevalence of multi-drug resistance. *A. baumannii* exists widely in natural environments, and frequently in health-care settings where it has proven difficult to eradicate using antibiotic therapy. A possible alternative to conventional antibiotics is the use of bacteriophages (phages) as antibacterials. Here we present the isolation and characterization of three novel virulent phages against this pathogen. A mixture of three distinct *A. baumannii* strains were used as hosts to isolate phages from sewage and soil samples from different sources in County Cork, Ireland. Three distinct phages were successfully isolated and designated Φ_{me1} , Φ_{me2} , Φ_{me3} . The phages had plaque sizes ranging from pinpoint to 0.5 mm in diameter and all plaques were surrounded by a zone of lysin activity. Their host ranges differed from each other indicating that they may be useful as a cocktail if employed as an antibacterial strategy. Transmission electron microscopy showed that each had an icosahedral head and a contractile tail with tail fibers, thus confirming them to be myoviridae viruses. Protein profiles were constructed for each. One of the phages (Φ_{me3}) was subjected to genome sequence analysis, which revealed that it has a double stranded DNA genome with a length of 234,900 bp and a 31% G+C content. Bioinformatic analysis predicted 334 potential ORFs, which were subsequently annotated using various software programs. The phage is exclusively lytic, which makes it a potential candidate for phage therapy applications.

ISOLATION AND CHARACTERISATION OF CAMPYLOBACTER BACTERIOPHAGES

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Campylobacter jejuni and *C. coli* cause a significant number of gastrointestinal infections in developed countries. Source attribution studies have indicated an overlap between human and chicken strains (EFSA, 2010). Efficient methods for control of contamination would decrease frequency of human campylobacteriosis. Therefore, bacteriophages are being explored to control number of campylobacters in poultry production.

We screened several poultry and pig samples for the presence of Campylobacter bacteriophages. Using two different indicator strains, we isolated eight bacteriophages. They were tested against a set of Campylobacter strains of human, poultry, pig and environmental origin to estimate their diagnostic and therapeutic potential. Isolates were obtained from different European countries. Slovenian isolates of chicken origin tend to be more susceptible for isolated bacteriophages than other strains.

We examined also their morphology and genomic DNA restriction endonuclease profiles. They all show similar morphology and belong to *Myoviridae* family. DNA restriction profiles were obtained for some of the isolates. We noticed that DNA of certain phages coprecipitates together with protein contaminants which may affect result of restriction digestion.

IN SILICO MODELLING OF THE STAPHYLOCOCCAL BACTERIOPHAGE-DERIVED PEPTIDASE CHAP_K

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The aim of this study was to use comparative modelling to predict the three-dimensional structure of the CHAP_K protein (cysteine, histidine-dependent amidohydrolase peptidase domain of LysK). Iterative PSI-BLAST searches against the Protein Data Bank (PDB) and non-redundant databases were used to populate a multiple alignment for analysis using the T-Coffee Espresso server. A consensus Maximum Parsimony phylogenetic tree with a bootstrap analysis setting of 1,000 replicates was constructed using MEGA4. Structural templates relevant to our target (CHAP_K) were identified, processed in Espresso and used to generate a 3D model in the alignment mode of SWISS-MODEL. These templates were also processed in the I-TASSER web server. A *Staphylococcus saprophyticus* CHAP domain protein, 2K3A, was identified as the structural template in both servers. The I-TASSER server generated the CHAP_K model with the best bond geometries when analysed using PROCHECK and the most logical organization of the structure. The predicted 3D model indicates that CHAP_K has a papain-like fold with CATH analysis predicting alpha-beta classification and alpha-beta-alpha architecture. The putative active site maintained a highly conserved Cys54-His117-Glu134 charge relay and an oxyanion hole residue Asn136. The residue triplet, Cys-His-Glu, is known to be a viable proteolytic triad in which we predict the Cys residue is used in a nucleophilic attack on peptide bonds at a specific site in the pentaglycine cross bridge of staphylococcal cell wall peptidoglycan. Comparative modeling can approximate the 3D structure of target proteins for which only the sequence is available and determine valuable information in relation to structure, binding and active sites. Further exploration and investigation of phage lysins and their various domains, particularly at a structural and bioinformatic level, can provide important information for understanding biochemical function and specific substrate interaction mechanisms. This in turn may contribute to specifically engineered designer lysins with diverse and improved applications for development as novel antibacterials.

THE HUNT FOR GOOD BACTERIOPHAGES TO COMBAT *BURKHOLDERIA PSEUDOMALLEI*, THE CAUSATIVE AGENT OF MELIOIDOSIS

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Burkholderia pseudomallei is a Gram negative bacterium that causes the severe invasive infection melioidosis. It is naturally resistant to many antibiotics and yet there is no vaccine against melioidosis. As an alternative to antibiotics, utilisation of bacteriophages to control bacterial infection has received much academic and commercial attention recently. In this project we aim to isolate and characterise lytic bacteriophages which infect *B. pseudomallei*. Soil samples from North-Eastern Thailand were screened on *B. pseudomallei* strains. Thirteen phages have been isolated and purified. Most of the phages are podoviruses and can infect both *B. pseudomallei* and *B. thailandensis* (a non-pathogenic *B. pseudomallei*-like strain). Four phages with large burst size and short latent period were sequenced. Their genomes are novel, similar to each others, and show limited similarity to a *Ralsonia* phage ϕ RSB1 and a prophage in *B. thailandensis* MSMB43. Detailed genomic analysis is ongoing.

ISOLATION AND CHARACTERIZATION OF SIX NOVEL MYCOBACTERIOPHAGES AND INVESTIGATION OF THEIR ANTIMICROBIAL POTENTIAL IN MILK.

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Using *Mycobacterium smegmatis* as a rapidly-growing non-virulent host, six mycobacteriophages were isolated and characterised. Morphologically, all six phages had long, non-contractile tails and isometric heads characteristic of Siphoviridae. In addition, all six genomes had distinct restriction maps. All phages were relatively heat-stable up to 72°C. Phages generally retained infectivity over a pH range of 4 to 10 for 60 min. Bio-control assays in milk revealed that the phage cocktail exerted a bacteriocidal effect on *M. smegmatis* over 96 h where a 9-log reduction in cell numbers was observed. Individual phages significantly halted growth resulting in a 7-log reduction in mycobacterial cell numbers. While these results were obtained using *M. smegmatis* as a non-virulent target, the data is significant as they suggest that these phages may also have a potential application in the control of other mycobacterial species such as *Mycobacterium avium* subsp. *paratuberculosis* (MAP), which is reported to survive the pasteurization temperature of milk. Preliminary analysis with the latter pathogen, whose cultivation takes several months, indicated that the presence of phage caused a reduction in MAP biomass.

WHOLE GENOME SEQUENCING, CHARACTERISATION AND APPLICATION OF ACTINOPHAGES TO COMBAT ACTIVATED SLUDGE FOAMING

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Foaming is a major operational problem in activated sludge wastewater treatment plants worldwide. Its formation leads to increased maintenance costs, safety issues and most importantly, poor quality effluent. This stable foam formation is caused typically by the proliferation of a group of hydrophobic filamentous bacteria, the Mycolata. These bacteria have cell walls containing hydrophobic long chain mycolic acids, and include species from the genera *Gordonia*, *Tsukamurella*, *Mycobacterium*, *Nocardia*, *Rhodococcus* and *Skermania*.

We have isolated, sequenced and characterised a group of phages infective for these foaming Mycolata. These phages are members of the order *Caudovirales* with long, non-contractile tails and isometric capsids characteristic of the family *Siphoviridae*. The majority have a broad host range being infective for multiple species of the Mycolata and in some cases their host ranges extend across multiple genera. An example of one such phage is GTE6 that is infective for several members of the genera *Gordonia* and *Nocardia*. These phages possess dsDNA genomes and 454 genomic sequencing data has revealed that the phages have novel genomes. It has also revealed the presence of numerous gene recombination and horizontal gene transfer events. Lab scale foaming assays show that these phages appear to reduce the stability of the foams stabilised by the Mycolata. It is yet to be determined if these phages can be used in full scale wastewater treatment plants

IDENTIFICATION OF *BRUCELLA* SPECIES BY LYSOTYPING - REVIVAL OF AN OLD TECHNIQUE

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The history of *Brucella* phages and their use as diagnostic tool for the identification of *Brucella* species begins with the discovery of phage Tb (Tbilisi, Russia) in the 1960s. Today several *Brucella* phages (Tb, Fi, Wb, Bk2, R/C, Iz, Np) classified in seven groups based on their host specificities can be used for *Brucella* spp. typing. During the last years several new species within the genus *Brucella* have been discovered. Up to now it has not been clarified whether the phages are able to lyse these new species. Moreover, there is only scarce information available on the biology and genetics of the phages. For a better understanding of *Brucella* phages, further research is mandatory. One aim of this study was to reappraise the usefulness of phages as a diagnostic tool for *Brucella* typing. Therefore, the lytic activity of some selected phages of our phage collection on *Brucella* spp. strains was determined. Here, we present the host range of the investigated *Brucella* phages including the new species *B. microti*, *B. innopinata*, and *B. ceti*. In accord with the observations of Rigby *et al.*, (1989), we also show that all members of the different phage groups exhibit only partial differences in their DNA restriction fragment patterns. This strong similarity suggests that all described *Brucella* phages are host range mutants originating from a common ancestor.

CHARACTERISTICS OF THE *CAMPYLOBACTER JEJUNI* GROUP III PHAGE CP81

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Thermophilic *Campylobacter* species are common agents of human bacterial enteritis worldwide. Majority of infections are caused by the consumption of *C. jejuni*-contaminated poultry meat. Prevalence rates of *Campylobacter*-positive chickens are generally high and transmission of the bacteria from bird to bird occurs rapidly. Intervention strategies mainly focus on biosecurity measurements and postslaughter decontamination of the carcasses. Another strategy for the reduction of *Campylobacter* in poultry is the application of virulent bacteriophages. Some phages have been successfully used to reduce *C. jejuni* colonization of broiler chickens and to combat the bacteria on chicken skin up to several orders of magnitude. Phages intended to control pathogens in food have to be safe in order to avoid any undesired side effect. Therefore, a deep knowledge about the biology and genetics of the phages is mandatory. Here, we report the properties of the virulent, *Campylobacter jejuni*-specific phage CP81 which was recovered from the skin of a chilled chicken portion in Bavaria (Germany). As typical for the majority of described *Campylobacter* phages CP81 electron micrographs also revealed morphological characteristics of the family *Myoviridae*. The virions comprised of an isometric head (96 nm) and a contractile tail (97 x 21 nm) with a baseplate and tail fibers. CP81 exhibited a rather narrow host range and was lytic only on some *C. jejuni* strains of the CNET-strain collection. The CP81 genome analyses revealed a linear genome of 132,454 bp with a G+C content of 26.1% slightly lower as reported for its *C. jejuni* host. Our examinations indicate that CP81 seems to be the prototype of European group III (130-140 kb) *Campylobacter*-phages. Genome analyses revealed some similarities to T4-type phages and suggest that group III *Campylobacter* phages are distantly related to members of the T4-superfamily.

MAINTENANCE OF ESCHERICHIA COLI PROPHAGES INTO THE HOST CHROMOSOME

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In prokaryotic genomes, genomic islands can represent a large amount of the total genomes, and a large part of these islands are prophage remnants that are no longer infectious. Two main questions drive our current research: (i) why are these prophages maintained into the host genome, and (ii) how are they maintained?

KpIE1 is a defective prophage of *Escherichia coli* K12, fully competent for excisive recombination, which genome contains 16 open reading frames, and is used as a model system for site-specific recombination studies. The overlap between attL and the integrase gene promoter leads to a negative autoregulation of the integrase gene, and such a regulatory loop was predicted in the majority of t-RNA inserted prophages found in prokaryotic genomes. As a consequence, the main switch that controls the excision of these prophages is the expression of the recombination directionality factor (RDF) gene which is absolutely required to control the integrase activity. Recent work suggests that host-encoded general regulators are involved in prophage maintenance.