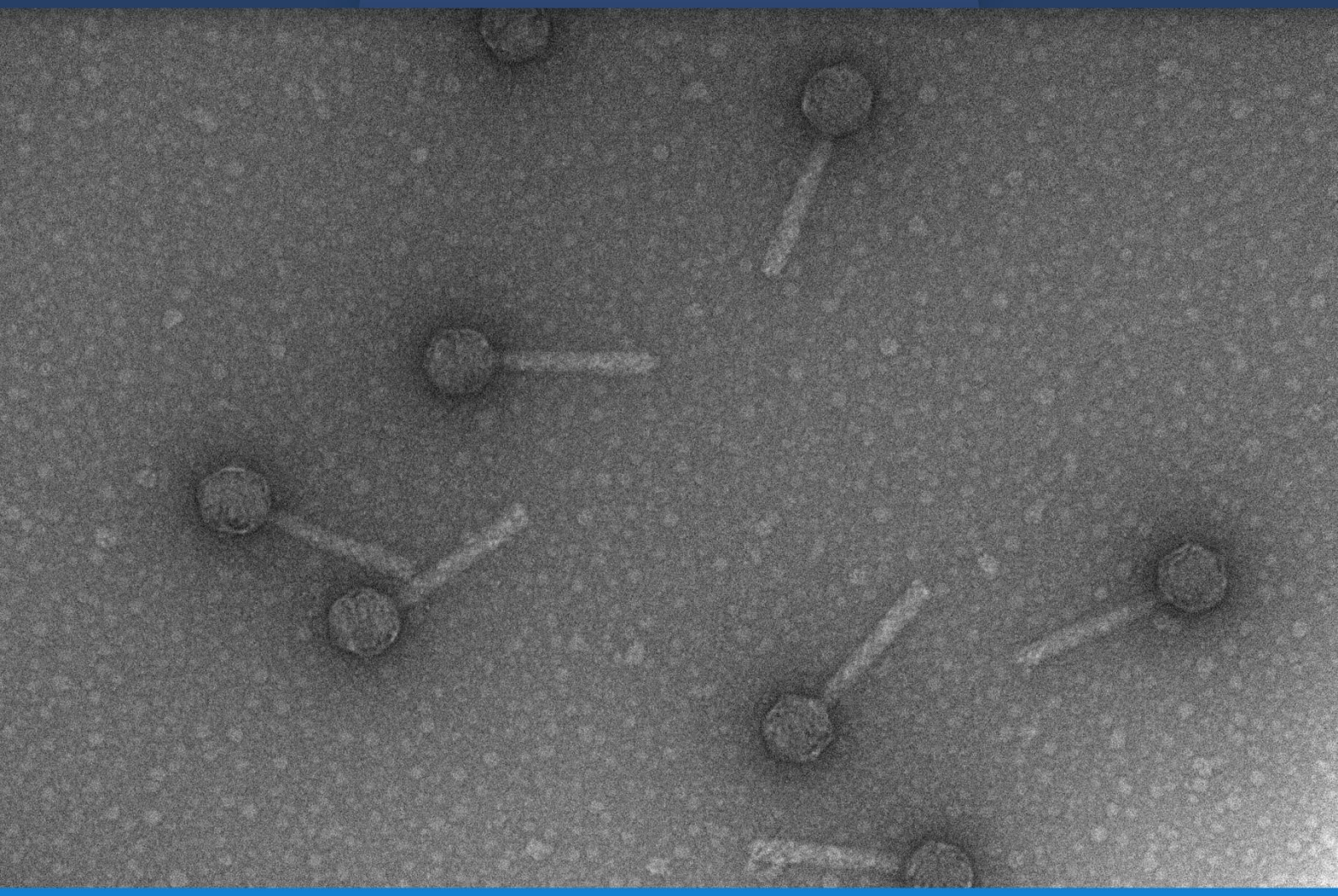
Bacteriophage 2016

ABSTRACTS



19TH - 21ST JANUARY 2016
LONDON, UK

EuroSciCon

Following on from last year's successful event this event will discuss the roles of bacteriophages, ranging from fundamental biological research to their use in medical and industrial biotechnologies.

This event has CPD accreditation

This abstract book will be finalised two weeks before the event
www.phage2016.com

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Invited Speakers Abstracts

Structural Insights into Functional Roles of Phage Coat Protein Accessory Domains.

Professor Andrei Alexandrescu, University of Connecticut, Storrs, CT, United States

The capsids that enclose nucleic acid genomes of viruses and phages are assembled from multiple copies of coat proteins with conserved folds, such as the HK97-motif. Functional versatility is often augmented by accessory domains. In collaboration with Carolyn Teschke's laboratory we determined the NMR structure of the non-conserved I-domain, a genetic insertion within the phage P22 coat protein. The I-domain adopts a 6-stranded beta-barrel fold previously unobserved for phage insertion domains. Functional roles for the I-domain include serving as a folding nucleus, stabilizing the coat protein, and participating in capsid assembly. More recently, we extended NMR structural studies to the I-domains of distantly related phage families represented by CUS3 and Sf-6. We have also initiated NMR structural studies of the decorator protein encoded by phage L, which non-covalently stabilizes phage L and P22 capsids. Accessory domains have the potential to serve as platforms for phage-based nanoparticle design, and we anticipate our structural studies will aid in these efforts.

Location of the unique integration site on an Escherichia coli chromosome by bacteriophage lambda DNA in vivo

Dr. Rinat Arbel-Goren, Weizmann Institute of Science, Israel

After entering an Escherichia coli cell, bacteriophage λ DNA must locate a unique site among about five million possible sites on the bacterial genome, with high efficiency and within physiological times, to integrate and establish lysogeny. We show that λ DNA does not carry out an active search. Instead, it remains confined at its entry point where it undergoes limited diffusion, while the process of bacterial DNA replication conveys the bacterial site close to the λ DNA. This mechanism adds to the list of profligate use of host functions by λ, brought about by coevolution of host–phage processes.

GMP production of bacteriophages

Dr. Laurent Bretaudeau, Clean Cells, Boufféré , France

Bacteriophages have re-gained interest in many countries, facing the emergence of difficult-to-threat bacterial infections. In the European Union, bacteriophages are not of common use, and, in turns, do not have a specific regulatory framework, for both the use and the production of clinical-grade phages.
Under the frame of EU-funded project “PhagoBurn”, Clean Cells has been in charge with the GMP production of two phage cocktails, used in a clinical trial for infected burn patients, in 3 countries (France, Belgium, and Switzerland). The results and experience constitute a contribution for the forthcoming evolutions of the regulatory pathway in the UE.

The E.coli phage shock protein (psp) response-what does it sense and do?

Professor Martin Buck, Dept Life Sciences, Imperial College London, UK

Phage shock protein A (PspA), the central component of the Psp response, and Vesicle-inducting protein in plastids 1 (Vipp1), responsible for thylakoid biogenesis in plants are similar peripheral inner-membrane (IM) binding proteins. Their homologous. N-terminal amphipathic helices (AH) are required for membrane binding. However the membrane features recognized and required for expressing their functionalities have remained largely uncharacterized. PspA apparently binds preferentially to membranes in a stored curvature elastic stress specific manner via insertion of it’s AH, so reducing the energy status of the membrane. Assays probing the transcriptional regulatory function of PspA in the presence of vesicles show a relief of transcription inhibition occurs in a stored curvature elastic stress specific manner. This in vitro recapitulation of membrane stress dependent transcription control implies that the psp response is mounted in vivo when the cells inner membrane accumulates lateral stress.

Interaction of lysis protein E from bacteriophage phiX174 with translocase MraY on the bacterial peptidoglycan biosynthetic pathway

Professor Timothy DH Bugg, Department of Chemistry, University of Warwick, UK

The lysis protein E of bacteriophage phiX174 is known to target translocase MraY on the bacterial peptidolycan biosynthesis pathway, but the mechanism of inhibition is not known. We have identified an RWxxW motif in protein E that interacts with Phe-288 and Glu-287 of MraY. Synthetic peptides based on this motif inhibit MraY, and site-directed mutants of MraY were investigated to verify this hypothesis. This motif is also found in some cationic antimicrobial peptides, that also target MraY. The E-MraY interaction site represents a possible new site for antimicrobial action.

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Artilysins, a novel class of enzyme-based antibacterials

Dr Yves Briers, Department Applied Biosciences, Ghent University – Campus Schoonmeersen, Gent, Belgium

Artilysin®s represent a novel, promising class of antibacterials, combining the lytic power of bacteriophage-encoded endolysins and outer membrane penetrating peptides. Selected peptides that locally destabilize the outer membrane of Gram-negative bacteria have been covalently fused to endolysins. These peptides promote the transfer of the fused endolysin to the peptidoglycan layer across the outer membrane. Time-lapse microscopy has shown that cells are killed within seconds due to active peptidoglycan degradation and subsequent cell lysis. Artilysin®s are active against multidrug-resistant bacteria and persisters.

Bacteriophages and their endolysins for the biocontrol of Staphylococcus aureus and Clostridium difficile

Dr Aidan Coffey, Cork Institute of Technology, Ireland

Engineering Thermal Stability to Phage-encoded Bacteriolytic Enzymes

Associate Professor Daniel C. Nelson, Ph.D., Institute for Bioscience and Biotechnology Research and Department of Veterinary Medicine, University of Maryland, Rockville, MD, United State

Bacteriophage-derived peptidoglycan degrading enzymes, termed endolysins, have been successfully exploited as novel therapeutics. The modest thermolability of these enzymes limits their antimicrobial potential. To overcome this challenge, directed evolution was applied to the heat-sensitive catalytic subunit, PlyCA, of the streptococcal-specific PlyC endolysin to improve its thermal stability. After screening a total of 18,000 mutants, the lead candidate identified was the point mutant PlyC(PlyCAN211H). When the mutation PlyCAT406R, a stabilizing mutation elucidated during an independent computational screen of PlyC, was combined with PlyCAN211H, the augmentation in thermal stability was additive. PlyC(PlyCAN211H,T406R) displayed a 7.46°C enhancement in thermodynamic stability when compared to wild-type, which represents one of the most thermostable endolysins described to date.

The enemy insight: tectiviruses preying on the Bacillus cereus group

Dr Annika Gillis, Université catholique de Louvain-Earth and Life Institute, Laboratory of Food and Environmental Microbiology, Belgium

Tectiviridae is a rare but interesting phage family comprising non-enveloped tail-less phages, with a linear dsDNA located within a lipid-containing membrane, covered by a rigid icosahedral protein capsid. Several tectiviruses have been found infecting the Bacillus cereus group and they display the unique characteristic of behaving as linear plasmids during their lysogenic cycle. Despite the significant contributions of mobile genetic elements to the evolution of this bacterial group, little is known about the dealings taking place between tectiviruses and their B. cereus sensu lato hosts. Therefore, this work focuses on characterizing the interactions between tectiviruses and the B. cereus group.

Biology and genetics of telomere phages

Dr Stefan Hertwig, Diagnostik, Genetik und Erregercharakterisierung, Federal Institute for Risk Assessment, Bundesinstitut für Risikobewertung, Berlin, Germany

Telomere phages are a small group of temperate phages, whose prophages are linear plasmids with covalently closed ends (telomeres). To date eight telomere phages have been described. In spite of the fact that the phages were isolated from different hosts (Enterobacteriaceae and marine bacteria) they share specific properties and possess a similar genome organization. This talk will give an overview of particular characteristics of these phages and will highlight some striking differences.

Recombinant antibodies displayed on filamentous bacteriophage for single cell proteomics

Dr Peter Kristensen, Aarhus University, Department of Engineering, Denmark

IIn recent years the importance of cellular heterogeneity has become increasingly clear. In developing therapies for important diseases, such as cancer, the ability to isolate and characterize rare cell populations will be important, allowing targeting of minor tumor cell populations such as the tumor stem cells or circulating tumor cells.

We have advanced the phage display technology, thus allowing the isolation of specific antibodies binding to one identified cell in a heterogeneous mixture of cells. In this presentation the technology for single cell analysis using phage antibody technology will be described, building on examples from model systems, endothelial progenitor cells and other pathological situations.

Selective pressure imposed on Pseudomonas aeruginosa by virulent bacteriophages: the importance of pseudolysogeny

Mrs Libera Latino, Institut de Biologie Intégrative de la Cellule - CEA-CNRS-UPSud, Université Paris-Sud, Orsay cedex, France

To resist virulent bacteriophages, bacteria develop different strategies which can have transient or permanent effect on their genome or result in long-term coevolution between the host and the prey. We show for the first time that pseudolysogeny is a frequent outcome of infection by virulent phages of Pseudomonas aeruginosa belonging to different genera, resulting in a long term process of phage production and selection of mutants inside a single colony.

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

Phage-host interactions of lactococcal bacteriophages

Dr Jennifer Mahony, School of Microbiology, University College Cork, Ireland

Lactococcal bacteriophages pose a serious economic and biotechnological threat to dairy fermentations. Consequently, they have been the subject of intense research scrutiny in recent years. These studies have yielded the development of working models of phage assembly and phage-host interactions. This presentation will assess the major developments in the area of lactococcal phage-host interactions and the implications for future research.

Bacteriophages and antibiotic resistance genes in the environment

Dr. Maite Muniesa, Department of Microbiology, Faculty of Biology, University of Barcelona, Spain

Antibiotic resistance is a major concern for society because it threatens the effective prevention of infectious diseases. While some bacterial strains display intrinsic resistance, others achieve antibiotic resistance by mutation, by the recombination of foreign DNA into the chromosome or by horizontal gene acquisition. Several mobile genetic elements (MGEs) have been reported to mobilize different types of resistance genes. Bacteriophages and phage-related particles have recently been highlighted as MGEs that transfer antibiotic resistance. This talk focuses on phages, phage-related elements and on composite MGEs (phages-MGEs), involved in antibiotic resistance mobility and spread in nature.

Phage-like chromosomal islands alter global transcription patterns in Streptococci

Dr Michael McShan, HSC College of Pharmacy, University of Oklahoma, Oklahoma City, United States

The Streptococcus pyogenes phage-like chromosomal islands (SpyCI) confer a complex mutator phenotype on their host cell through growth-dependent regulation of the DNA mismatch repair operon. Related CI are found in other streptococci, including group C and Milleri species. These CI have small genomes (<20 kB) and no identifiable capsid genes. Global transcriptional analysis shows that SpyCI alters the expression of multiple host genes, including many virulence factors. Biological and phylogenetic analysis of the SpyCI and related CI provide important clues to their contribution to streptococcal persistence in human populations in spite of antibiotic therapy and immune challenges.

Structure of the SPP1 bacteriophage and its function.

Professor Elena Orlova, Crystallography, Institute for Structural and Molecular Biology, Department of Biological Sciences, Birkbeck, University of London , London, UK

Bacteriophages are large macromolecular assemblies and rely on a complex program of structural rearrangements to accomplish their biological functions such as genome replication, viral particle self-assembly, disruption of the host cell, and infection of other bacteria. The most abundant group of known phages (~95%) is the tailed phages with dsDNA genome packed in an icosahedral capsid. We have studied structural organisation of the SPP1 phage and its major components. Analysis of the head-to-tail interface before and after genome ejection revealed important conformational changes in the genome gatekeepers that opens reversibly for DNA release from the viral capsid.

Through a viral membrane: genome delivery and packaging in the double-stranded DNA phage PRD1

Dr Hanna M Oksanen, University of Helsinki, Institute of Biotechnology and Department of Biosciences, Helsinki, Finland

Two fundamental steps in the virus life cycle are genome encapsidation and genome delivery. Icosahedral viruses with an internal membrane possess additional complexity as the genome must enter and exit through the viral membrane. Using genetics, virus mutant particles and different electron microscopy techniques, we have solved the structure the viral genome packaging complex embedded in the internal membrane of the tailless icosahedral virus PRD1. We have also depicted how the virus uses its internal membrane for DNA delivery by developing a tail-tube which penetrates the bacterial cell envelope. I will summarize the current molecular models for PRD1 entry and assembly.

Pseudomonas aeruginosa Mu-like bacteriophages: genomic diversity and mechanism of replicative transposition

Dr Christine Pourcel, GPMS, Institut de Biologie Intégrative de la Cellule I2BC, Université Paris-Saclay, France

The genome of transposable Mu-like phages is multiplied by replicative transposition at 50 to 100 sites per bacterial genome. At packaging, phages genomes are released by a nuclease together with bacterial DNA, 33bp on one side and a few hundred bp up to 2kb on the other side. We have isolated and characterized two Pseudomonas aeruginosa Mu-like bacteriophages (Ab30 and 2P1, related to phage D3112), and have used Illumina high-throughput sequencing to analyze bacterial DNA carried by phages. We will report new information on the transposition mechanism, and discuss the existence of preferential insertion sites.

Phagonaute: a tool to predict phage protein functions by distant homology searches

Dr Marie-Agnès Petit, Research Director, INRA, France

Distant homology search tools such as HHpred are a great help to predict phage protein functions, but as they rely on databases of pre-computed protein profiles that are not dedicated to phages, they can lack sensitivity. We developped a database storing the results of pre-computed, all-versus-all, HHsearch phage proteins results, as well as an interface named "Phagonaute", to help predict phage protein functions. Distant homology results are displayed together with their genetic context, to help secure the prediction. Application examples will be presented.

Filamentous phage - applications from nano to metagenomic scale

Dr Jasna Rakonjac, Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand

Filamentous phage are secreted from their hosts and they have important roles in virulence. Besides uses in phage display technology, filaments of Ff (f1, fd or M13) page have also been adapted for nano-applications. Using rods rather than filaments in nano-applications, however, can be advantageous. We developed a system for production of functionalized nanorods “Ff nano” (50 x 6 nm) and used them as diagnostic particles. On meta-scale, we developed selective display of surface proteins from complex microbial communities and combined it with metagenomics. Using this approach we identified plethora of proteins forming cellulose-degrading structures, cellulosomes, in the rumen microbial community.

Insights into protein-primed genome replication of temperate phage Bam35

Dr Modesto Redrejo-Rodríguez, Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Spain

Protein-primed replication constitutes a generalized mechanism to initiate DNA or RNA synthesis in a number of linear genomes of viruses, gram-positive bacteria, linear plasmids and mobile elements. By this mechanism a specific amino acid of the so-called terminal protein (TP) primes replication and becomes covalently linked to the genome ends. Bam35 belongs to a group of temperate phages infecting B. cereus sensu latto group, predicted to replicate their genomes by a protein-primed mechanism. Here, we show the biochemical characterization of Bam35 DNA polymerase (B35DNAP) as well as the role of protein p4 as a genuine TP. Replication differences in lytic and lysogenic viral cycles are also discussed.

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We're all in this together: bacterial population defence against viral predators through suicidal abortive infection systems

Professor George Salmond, Department of Biochemistry, University of Cambridge, UK

Bacterial populations are susceptible to attack by bacteriophages, which outnumber their hosts globally by ten-to-one. Despite being outnumbered by such lethal parasites the bacteria survive because they have evolved diverse strategies to circumvent the potentially lethal impacts of viral attack. One type of defence mechanism is that of abortive infection (Abi). Abi systems act post-infection and function by activating a suicidal mechanism arguably akin to a prokaryotic apoptosis. We have studied the Type III class of Abi systems and they have toxin-antitoxin (TA) functionality. Type III TA systems are composed of protein:RNA quaternary complexes in which the protein components are toxic ribonucleases held in caged structures by short pseudoknot RNAs which act to suppress nuclease function. Specific phages can destabilise the TA system leading to bacterial suicide – which also terminates viral replication, thereby preventing productive lysis that would lead to further phage invasion – and potential elimination - of the bacterial population. Thus the Type III TA mechanism may have evolved as a primitive altruistic system operating to defend clonal bacterial populations from phage predation. Bioinformatic interrogation shows that the Type III TA systems are widespread in bacteria, although little is known of their physiological, ecological or evolutionary functions. Some phage mutants can evolve the capacity to evade such abortive infection systems and they escape these Abi mechanisms by different routes.

Bacteriphages infecting the fish pathogen Flavobacterium columnare in aquaculture

Dr Lotta-Riina Sundberg, Centre of Excellence in Biological Interactions, Department of Biological and Environmental Science and Nanoscience Center, University of Jyvaskyla, Finland

Importance and volume of aquaculture is growing steadily, but the productivity of the industry is threatened by a wide range of bacterial diseases. Especially Flavobacterial infections cause persistent infections and high mortality in fry production. However, 30 - 80% of antibiotics used in aquaculture may pass directly into the environment. Addition of bacteriophages infecting Flavobacterium columnare significantly improve rainbow trout and zebra fish survival during bacterial exposure, and phage can persist in flow-through tanks for up to 48 hours. These results warrant for further studies in phage-host interactions in F. columnare and development of phage therapy applications against the disease.

Endolysin-based antimicrobials for control of bacterial pathogens

Dr Mathias Schmelcher, Institute of Food, Nutrition and Health ETH Zurich, Zurich, Switzerland

In the light of an increasing prevalence of multi-drug resistant bacterial strains in both clinical and agricultural settings, bacteriophage endolyins have gained attention as promising antimicrobial agents. These enzymes rapidly and specifically kill Gram-positive pathogens such as Staphylococcus aureus, Listeria monocytogenes, and streptococci and are refractory to resistance development due to their extracellular and highly conserved target sites. In our work, we have demonstrated the efficacy of various parental endolysins and engineered cell wall-lytic enzymes against planktonic cells and biofilms of their respective target bacteria, employing multiple in vitro activity assays and different animal models of bacterial infection.

China, challenges and opportunities for the use of Bacteriophage and Derived Proteins

Dr David Trudil, International Phage Research Center, China

The International Phage Research Center (IPRC), is a not for profit Institute located in the Jiangsu Academy of Agricultural Sciences (JAAS), Nanjing.It's mission is focused on using bacteriophage as well as derivatives for human, animal, agricultural, and environmental applications.

The IPRC has projects with institutes globally covering treatment, prevention and detection. These include Masititis, MRSA and others. The projects will be reviewed with updates provided.

The Institute welcomes new collaborations on a broad range of phage and related areas. The IPRC will also have International meetings every two years. Participation is encouraged from all areas of the world.

Phages limited dependence on host non-essential functions: the bacteriophage SPP1 case

Dr Paulo Tavares, I2BC, Department of Virology, Campus CNRS de Gif-sur-Yvette, Gif-sur-Yvette, France

All viruses are obligate intracellular parasites that depend on cellular functions for multiplication. This dependence offers the host opportunities to develop resistance to infection. Virginija-Cvirkaite Krupovic in our team screened a collection of 2514 single-gene knock-outs of non-essential genes from the Gram-positive bacterium Bacillus subtilis for yielding resistance to infection by siphophage SPP1. Only genes of the yuk operon, coding for the receptor YueB, were necessary for SPP1 multiplication while lack of two other genes was detrimental. The work suggests, together with results obtained for other viruses-host

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systems, that bacteriophages have evolved towards limited dependence on the non-essential host functions.

Bacteriophage-host interaction at the cell surface of S aureus - an essential role for the baseplate protein Gp45 in phi11 adsorption

Dr Guoqing Xia, Institute of Inflammation & Repair, Faculty of Medical and Human Sciences, The University of Manchester, Manchester, United Kingdom

Despite the importance of phages in driving horizontal gene transfer (HGT) among pathogenic bacteria, it remains mysterious in terms of the molecular interactions mediating phage adsorption to S. aureus. Phage phi11 was known as a siphovirus with high transducing efficiency. Here, we show that the minor tail protein Gp45 clearly localized within phi11 baseplate. phi11 was efficiently neutralized by anti-Gp45 serum and its adsorption was inhibited dose-dependently either by the serum or the recombinant Gp45. Flow cytometry analysis demonstrated that biotin-labelled Gp45 efficiently stained the wild-type S. aureus cell, but not a mutant lacking GlcNAc residues on the wall teichoic acids(WTA). Additionally, adsorption assays suggested that GlcNAc residues on WTAs and O-acetyl groups at the 6-position of muramic acid residues in peptidoglycan are essential components of the phi11 receptor. The elucidation of Gp45-involved molecular interactions not only deepen our understanding of siphovirus mediated HGT, but also establish a solid basis for the development of sensitive affinity-based diagnostics and therapeutics for S. aureus infection.

Research activities at the DSMZ - more than a collection

Dr Johannes Wittmann, Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH, Germany

Besides bacterial strains, plant cells, fungi, plant viruses and different cell lines, the Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures also holds a collection of bacteriophages against various bacterial hosts and currently tries to enlarge it with new phages particularly against human pathogens. Apart from collecting and preserving new phages, different research projects are focussed, e.g. the genomic and taxonomic characterisation of putative therapeutic phages or phages from extreme habitats.

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Day 1:

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

EXPLORATION OF PHAGE THERAPY FOR THE CONTROL OF PHYTOPATHOGENIC BACTERIA

Duraisamy Nivas and Velu Rajesh Kannan
Rhizosphere Biology Research Group, Department of Microbiology, Bharathidasan University, Tiruchirappalli – 620 024, Tamil Nadu, India.
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Agriculture is one of the most important sectors for economy in the world and the bacterial diseases are major threat to the agriculture food production. These bacterial diseases are more difficult to control because bactericides in present-day use are not as effective as they have been in the past. Bacteriophages (Phages) represent innovative biological control agents because they are highly specific viruses with extreme host specificity and ability to self-replicate within host cells and destroying the disease-causing bacteria. The goal of the present study was to isolate and characterize efficient bacteriophage for the control of *Xanthomonas* sp. causing bacterial spot in tomato. In this present study, soil and infected leaves samples were collected from Trichy, Madurai and Dindugal districts of Tamil Nadu, India and 11 bacteriophages were isolated form the both samples. All the bacteriophages were checked for its lytic property. Form the 11 bacteriophages TXP1, DXP2 and MXP2 were selected for further study. These 3 phages were further characterized for its bacterial reduction, host range and one step growth curve. These three phages shows good lytic property up to 24h, having broad host range within its host bacteria and burst size of theses phages were calculated as 39,46 and 40 phages/cell. Efficacy of phage in different pH, and temperature was performed and all the three phages show good lytic activity at pH8 with some minor difference at other low and high range pH. These three phages are stable in their lytic spectrum at the temperature of 36°C to 38°C. Phages TXP1, DXP2 and MXP2 genomic DNA and structural proteins were analyzed and size of the genomic DNA of phages are 23kb in size. Further, major structural proteins are observed at 160kDa, 60kDa and 55kDa. Disease control efficiency was evaluated in nursery trails, phages TXP1, DXP2 and MXP2 were formulated with 0.5% sucrose and starch. Disease assessment was calculated by disease severity and disease intensity. Phages shows considerable disease reduction when compare with the standard bio-pesticides.

Keywords: Bacterial spot, Phage therapy, Host range, Biocontrol agent.

BIOCHEMICAL CHARACTERIZATION OF THE GLOBULAR ENDOLYSINS FROM BACTERIOPHAGES INFECTING BACTERIA OF THE GENUS THERMUS AND CLOSTRIDIUM.

*M Plotka**, A Morzywolek, AK Kaczorowska and T Kaczorowski
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Advances in DNA sequencing technology allowed for more and more bacteriophages/bacterial genomes to be sequenced and thus easier identification of novel lytic enzymes. Lytic enzymes due to their ability to lyse bacteria are considered to be good candidates as novel anti-infective agents. In our laboratory, based on homology analysis several lytic enzymes were discovered and then biochemically characterized. Two are derived from highly thermophilic bacteriophages that infect highly thermophilic bacteria Thermus scotoductus. Four lytic enzymes are derived from pathogenic, anaerobes: Clostridium perfringens NCTC 8239, Clostridium perfringens ATCC 13124, Clostridium intestinale URNW and Clostridium botulinum E3 str. Alaska E43.

DEVELOPING GALLERIA MODEL FOR ASSESSING PHAGE THERAPY IN PSEUDOMONAS AERUGINOSA INFECTION

M. Chutia, J. Sahota, J.Y. Nale, M.R.J. Clokie
Department of Infection, Immunity and Inflammation, University and Leicester, UK

ABSTRACT
Phage therapy against clinical strains of Pseudomonas aeruginosa was assessed in Galleria mellonella infection model. Experiments to simulate prophylaxis and remedial treatments were conducted using cocktail of 4 lytic phages on P. aeruginosa including multidrug-resistant strains. An initial infection via the oral cavity was established using 10, 100 and 1000 cells/larvae. Phages were administered via the same route at multiplicities of infection (MOI) of 1, 10 and 100 in the remedial treatment. In the prophylaxis treatment, a single dose of phages was given at 12 h before infection. Survival rate was recorded at 12 h interval up to 3 days of post treatments and haemolymphs of infected and phage-treated larvae were extracted at the same time points for the enumeration of bacteria and phages. The remedial treatments were effective with 100 % survival and lower levels of bacterial load compared to the bacterial control at the end of the treatment regime (48 h) using an MOI of 10. Conversely, 20 % survival rate was observed with the remedial treatment at 24 h, but all larvae were dead by the 48th hours with all MOIs. In all cases, phages were recoverable. Our data show that the Galleria model is a useful tool in assessing phage therapy in P. aeruginosa infection and this knowledge could be applied as a robust and cost-effective measure in the control of infection in the rearing of economic insects.

Keywords: Galleria mellonella, infection model, multiplicity of infection, Pseudomonas aeruginosa; phage therapy.

LISTERIA MONOCYTOGENES' PROPHAGE SERVES AS AN ACTIVE REGULATORY SWITCH TO PROMOTE BACTERIAL VIRULENCE

Assistant Professor Anat A. Herskovits, Department of Molecular Microbiology and Biotechnology, Tel-Aviv University, Tel Aviv, Israel

Unlike lytic phages, temperate phages maintain long-term association with their bacterial host through lysogeny. In this context mutual beneficial interactions can evolve that support efficient co-reproduction. We recently identified a new type of bacteria-phage interaction in which a prophage integrated within a critical bacterial gene serves as an active regulatory switch to regulate the gene expression via genome excision, a phenomenon we termed active lysogeny. Regulation of comK gene in the human pathogen Listeria monocytogenes is mediated via phage genome excision and re-integration. ComK itself is the activator of the competence system, which is necessary to promote L. monocytogenes infection of mammalian cells. Interestingly, prophage excision is specifically induced during L. monocytogenes intracellular growth, primarily within the mammalian cells phagosomes, yet, unlike classic prophage induction, progeny virions are not produced. This study evidences a unique adaptation that turns the prophage into a genetic switch, such that it plays a biologically important role in the intracellular lifestyle of its host.

Day 2:

Oral Presentation Abstracts

Day 3:

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

INDUCTION OF PROPHAGE Φ1207.3 IN STREPTOCOCCUS PNEUMONIAE

F. Santoro, G. Pastore, F. Iannelli, G. Pozzi*
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Φ1207.3 is a prophage of Streptococcus pyogenes which carries the macrolide efflux resistance genes mef(A)/msr(D). Complete nucleotide sequence showed that Φ1207.3 is 52,491 bp in length and contained 58 ORFs. Φ1207.3 codes for two different C-methylation systems, several phage structural genes, a lysis cassette, and three site-specific resolvases of the serine recombinase family. The aim of this study was to characterize Φ1207.3 in a prophage-free Streptococcus pneumoniae model strain. S. pneumoniae FP10 and FP11 were used as host strains, while S. pyogenes 2812A served as Φ1207.3 donor. Φ1207.3 lytic cycle was induced in early exponential phase with 100 ng/ml of mitomycin C for 2 hours. Induction was monitored by Real Time-PCR using primers targeting mef(A) and phage particles were prepared from culture supernatants. Phage particles were processed by standard negative stain and imaged by a Tecnai G2 transmission electron microscope (TEM). S. pneumoniae strain FR1 carrying the prophage Φ1207.3 was obtained by conjugation. Culture supernatants, prepared after mitomycin C treatment, were analysed. Induction assays demonstrated that mef(A) gene was slightly increased in culture supernatants after ultracentrifugation of mitomycin treated culture (7.7 x 108 mef(A) copies/ml). Phage particles were obtained from strain FR1 and visualized with TEM, which showed phage particles with an icosahedral head and a noncontractile tail, placing them in the Siphoviridae family. An average of 7.7 x 104 particles/grid were observed. Solid phase dependent transduction was obtained in S. pneumoniae and S. pyogenes at a frequency of 1 x 10-6 transductants/recipient cell. We could not detect plaque formation using S. pneumoniae, S. pyogenes or Streptococcus gordonii as indicator strains. We showed that Φ1207.3 is a phage belonging to the Siphoviridae family. The phage can be transferred from S. pyogenes to S. pneumoniae where it is still functional, albeit producing a small number of viral particles with a limited infection potential. This is the first demonstration of a functional phage carrying an antibiotic resistance determinant which is transferable among different species.

CHARACTERIZATION OF vB_YenM_TG1 AND vB_YenM_phiR1-RT, TWO HIGHLY RELATED BROAD HOST RANGE BACTERIOPHAGES INFECTING YERSINIA ENTEROCOLITICA

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Bacteriophages vB_YenM_TG1 (TG1) and vB_YenM_phiR1-RT (phiR1-RT), isolated from pig manure in Canada and sewage treatment plant in Finland, and their genomes consist of linear double-stranded DNA of 162,101 and 168,809 bp, respectively, with a guanine plus cytosine content of ca. 34.5% containing 262 putative coding sequences and 4 tRNAs genes. These lytic bacteriophages exhibit a host range restricted to Yersinia enterocolitica, and display activity against the epidemiologically significant serotypes O:3, O:5,27, and O:9. They share 91% overall nucleotide identity and 89.7% homologous proteins. Based on phylogenetic analyses of their whole genome sequences and large terminase subunit protein sequences, a genus named Tg1virus within the family Myoviridae is proposed with TG1 and phiR1-RT as member species. The phages were demonstrated to use both the LPS inner core heptosyl residues and the outer membrane protein OmpF as phage receptors. The phiR1-RT was able to enter into pseudolysogenic state.

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PHAGEBIOTICS IN PREVENTION AND TREATMENT OF HEALTHCARE-ASSOCIATED INFECTIONS

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We have developed a phagebiotic composition using 8 virulent bacteriophages (two strains of each species) which are able to lyse Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus. The unique character of the developed composition is insured by particular properties of each bacteriophage comprising the preparation, by the range of its lytic activity towards specific bacterial pathogens, morphology of its plaques, cycle of its development, restriction profile of its DNA, specificity of its genome (based on sequencing of complete genome) and other properties. The basic requirements for the preparation were safety and efficacy in prevention and treatment of infections. The safety of the phagebiotic composition was confirmed by the results of the investigation of its toxicity (acute and chronic) performed on white outbred mice. The preparation did not produce any signs of acute or chronic intoxication in experimental animals. The histologic investigation of parenchymatous organs of mice that were treated with bacteriophage cocktail showed no lesions in them. Therapeutic and prophylactic efficiency of the phagebiotic composition was demonstrated in the prevention and treatment of the experimental acute K. pneumoniae infection in mice. The investigations showed that the preparation possesses a high therapeutic efficiency which is highly competitive with that of ciprofloxacin which is very effective against the infective strain K. pneumoniae. As a result of the treatment with the bacteriophage cocktail the mice were cured completely of the highly virulent strain K. pneumoniae. A small-scale clinical trial was aimed to evaluate therapeutic effectiveness of the phagebiotic composition in an epidemiological emergency situation in an intensive care unit, which was caused by multi-resistant strains of Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus. Eighty per cent of the initial samples from 14 patients' enterotracheal aspirate, blood and urine were contaminated. Twenty-four hours after the 3-day phage therapy (20 ml of cocktail at a titer for each phage 108 pfu/ml were introduced intragastrically through a tube once a day) contamination level dropped to 28,6%. Hence the obtained results enabled us to create a new phagebiotic composition that may be used as an alternative to antibiotics to treat these healthcare-associated infections.

THE COMPLEXATION OF BACTERIOPHAGE DNA BY POLY (EPSILON-LYSINE) DENDRONS TOWARDS DEVELOPMENT OF SYNTHETIC BACTERIOPHAGES

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Introduction

Dendrons are hyperbranched macromolecules that can be polymerised starting from a variety of monomers to form hyperbranched molecules with chemically modifiable peripheral groups. Cationic dendrons possess a net positive charge at physiological pH and have been described as potential non-viral gene delivery tools. A common feature of both cationic dendrons and lytic bacteriophages, which are viruses that specifically target and kill bacteria, is their ability to carry DNA. Bacteriophage killing of bacterial cells is dependent on the initial delivery of phage DNA to bacterial cells which occurs when bacteriophages interact with specific cell surface receptors. Current methods of phage-based therapy use whole phages, but these titres can deplete over time, requiring re-isolation/propagation before clinical use. Therefore, this study assessed the role of dendrons in interacting with bacteriophage DNA to form complexes that can be delivered to bacterial cells for phage progeny production.

Method

The interaction between bacteriophage DNA and poly (epsilon-lysine) dendrons (Gen3K) was investigated as a first step in assessing the potential of the dendrons to be used as non-viral vectors for bacteriophage DNA-based therapy. The DNA was extracted from bacteriophage using a phenol-chloroform method, the dendron had been synthesised using an adapted Fmoc-based peptide synthesis method. To probe interactions between the dendron and DNA molecules, dendron-DNA complexes were bioengineered by exploiting the electrostatic interactions. The dendron-DNA complexes were characterised by ethidium-bromide displacement assay and agarose gel electrophoretic mobility assay.

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Results
Based on the reduced electrophoretic mobility, the degree of complexation of the DNA molecules was dependent on the charge ratio of both molecules. Complexation was observed at charge ratios of dendron amino groups to DNA phosphate groups from 132:1 to 0.06:1. The ethidium bromide displacement assay demonstrated that the dendron was capable of displacing the ethidium bromide to form Gen3K-DNA complexes, which verified the interaction between dendron and bacteriophage DNA.

Conclusions
Data obtained in the current work demonstrate that the dendron was capable of binding bacteriophage DNA, to form stable complexes. This study further develops the understanding in the strategic development of poly (epsilon-lysine) dendrons as potential gene delivery tools and serves as a useful starting point towards development of synthetic lytic bacteriophages from transfection of dendron-bacteriophageDNA complexes.

HOW BACTERIA DO NOT FORGET THEIR ENEMIES

Stan J.J. Brouns

The CRISPR immune system protects bacteria and archaea from invading viruses and plasmids. Immunity depends on a protein complex called Cascade, which uses small RNA molecules to find matching viral or plasmid DNA. Apart from giving insight into the unusual structure and function of Cascade, I will show how viruses escape immunity by mutating their DNA. A mechanism called priming takes care of these escaped viruses and will quickly update the memory of the immune system. I will highlight a single molecule FRET approach which has revealed that Cascade differentially flags target DNA for either destruction when the match with the small RNA is perfect, or for an update of the memory of the immune system when the match is imperfect.

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ISOALTION AND IDENTIFICATION OF BACTERIOPHAGE(S) INFECTING BACTERIA WHICH CAUSE SOFT ROT DISEASE

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Abstract
Potato is one of the most economically important crop, and is considered as the fourth main food crop in the world. Potato plants are subjected to numerous pathogens and insects which cause considerable loss in the potato yield. One of the most important serious diseases of potatoes is bacterial soft rot. In our study the main bacteria causing soft rot disease was isolated from two infected potato species (Solanum tuberosum L. and Lady rosetta).The isolated bacterial samples were identified by biochemical tests(gram stain, oxidase, catalase, citrate, starch hydrolysis, gelatin hydrolysis and API test) then confirmed by molecular sequencing. In recent years bacteriophages have been isolated, identified and proposed as biocontrol agents for bacterial diseases in plants that is why two bacteriophages infecting soft rot bacteria in this research were isolated and identified as a biocontrol agents to protect potato crop from this drastic disease. Soil samples were collected from potato rhizosphere from a potato cultivated area. Two bacteriophages (Ph1B, Ph1S) were isolated. Each isolated phage was purified and studied by transmission electron microscope (TEM) to determine their morphology. Ph1B possessed icosahedral head (57 nm), neck (10.65nm) and tail (67.7 nm length, 9.4nm width) while the other phage Ph1S showed spherical heads (23.9nm). Ph1B showed its lysis effect till 10-9 (dilution end point) , while Ph1S showed its lysis effect till 10-7 (dilution end point) , phage isolates were subjected to other physical studies as ageing and both sustained its lysis ability uptill now but showed a noticeable decrease in the percentage of plaques during storage time , other physical studies were done as thermal inactivation point and PH determination.

SMALL COLONY VARIANTS AND SINGLE NUCLEOTIDE VARIATIONS IN PF1 REGION OF PB1 PHAGE-RESISTANT *PSEUDOMONAS AERUGINOSA*

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Phage therapy involves the application of lytic bacteriophages for treatment of clinical infections but the bacteria may develop resistance over time. Isolated from nosocomial infection patients, small colony variants (SCVs) are morphologically distinct, highly virulent bacterial strains resistant to conventional antibiotics. In this study, SCVs was derived from Pseudomonas aeruginosa exposed to the lytic bacteriophage PB1 and these cells were resistant to subsequent phage infection by PB1. To elucidate the mechanism of the SCV phage resistance, we performed phenotypic assays, DNA microarrays and whole-genome sequencing. Compared with wild type P. aeruginosa, the SCV isolate showed reduced biofilm formation, twitching motility and enhanced pyocyanin production. Additionally, the surface hydrophobicity was higher than that of the wild type, indicative of changes to cell surface lipopolysaccharide (LPS) composition. Consistent with these results, transcriptomic studies revealed up-regulation of genes involved in B-band LPS biosynthesis, suggesting the regulation of surface moieties may account for phage resistance. Moreover, genes involved in aromatic and branched chain amino acid catabolism were down-regulated, indicating these specific pathways to be less active in the SCV than wild type. Whole genome sequencing of the SCV revealed multiple single nucleotide variations within the Pf1 prophage region, which is known to play a crucial role in biofilm formation and to provide survival advantage via gene transfer to a subpopulation of cells. Insights into phenotypic and genetic changes in SCV gained here should help direct future studies to elucidate mechanisms underpinning phage resistance, leading to novel counter resistance measures.

MOLECULAR AND PHYSIOLOGICAL CHARACTERIZATION OF A NEW LYTIC BACTERIOPHAGE AGAINST KPC-POSITIVE *KLEBSIELLA PPNEUMONIAE*

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ABSTRACT
Resistance to carbapenems represents an important clinical problem and KPC (*Klebsiella pneumoniae* carbapenemases) producing bacteria constitute a very serious challenge. Bacteriophages could provide a valuable tool to control the dissemination of KPC-producing isolates. In this work we have characterized one lytic bacteriophage, called φF48, specific for KPC-Kp (KPC-positive *Klebsiella pneumoniae*) strain 12C47, belonging to the Sequence Type (ST) 101 highly widespread in European countries and currently emerging in the Italian territory. TEM, burst size, host range and sensitivity to temperature and pH were used to characterize phage φF48. Molecular characterization through High Throughput DNA Sequencing and direct sequencing by Sanger method was employed to define the phage genomic sequence. Genome annotation was performed by RAST analysis. Bacteriophage φF48 was classified as a member of *Myoviridae* on the basis of TEM analysis. Physiological characterization demonstrated that φF48 is highly stable to both temperature and pH variations. RAST analysis revealed 284 possible coding regions and an hypothetical function was assigned to 120 (42%). The results obtained suggest that φF48 could represent a valid alternative therapeutic agent. In addition, the phage exhibit a number of proprieties indicative of a potential utility in phage therapy cocktails.

ISOLATION AND ANALYSIS OF ESKAPEE PHAGES FOR PHAGE THERAPY

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The aim of this project is to set up a phage therapy laboratory in Finland. We are currently collecting and analysing therapeutic phages that infect ESKAPEE bacterial species: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Escherichia coli and Enterobacter cloacae. Our goal is to obtain phages that infect 50 – 70% of clinical ESKAPEE strains. Our phage collection contains now 75 phages, out of which 5 infect E. faecium, 2 S. aureus, 17 K. pneumoniae, 2 A. baumannii, 9 P. aeruginosa 26 E. coli, and 14 E. cloacae. The phages have mostly been isolated from waste water samples throughout Finland. The strain infection coverage has been tested with 50 clinical strains of each bacterial species, and varies from 100% of S. aureus to 53% of A. baumannii. The EM analysis of phage morphology shows that 25.7% of phages belong to Myoviridae, 38.6% to Podoviridae and 35.7% to Siphoviridae. We have also sequenced the genomes of most of the phages by NGS, and the genome sizes vary from ~30 kb to ~180 kb.

IDENTIFICATION OF PHAGE RECEPTORS OF TWO BROAD HOST RANGE BACTERIOPHAGES, VB_YENM_TG1 AND VB_YENM_fR1-RT, INFECTING YERSINIA ENTEROCOLITICA

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The genomes of bacteriophages vB_YenM_TG1 (TG1) and vB_YenM_fR1-RT (fR1-RT), isolated from pig manure in Canada and sewage treatment plant in Finland, consist of linear double-stranded DNA of 162,101 and 168,809 bp, respectively. They share 91% overall nucleotide identity and 89.7% homologous proteins. Based on phylogenetic analyses of their whole genome sequences and large terminase subunit protein sequences, a genus named Tg1virus within the family Myoviridae is proposed with TG1 and fR1-RT as member species.

These lytic bacteriophages exhibit a host range restricted to Yersinia enterocolitica, and display activity against the epidemiologically significant serotypes O:3, O:5,27, and O:9. Using transposon mutagenesis we have isolated host mutants and demonstrated that these phages use both the LPS inner core heptosyl residues and the outer membrane protein OmpF as phage receptors. In addition, fR1-RT was able to enter into pseudolysogenic state.

EFFICACY OF BACTERIOPHAGES TO CONTROL LISTERIA MONOCYTOGENES ON SLICED MUSHROOMS RELATIVE TO CHEMICAL OR PHYSICAL INTERVENTIONS

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The following study investigated the potential of bacteriophages to control the growth of Listeria monocytogenes on sliced mushrooms. The phage based treatments were compared to chemical (hydrogen peroxide, peroxyacetic acid, ozonated water, electrolyzed water, chitosan, lactic acid), biological (Listeria bacteriophages), and physical (UV-C, UV–hydrogen peroxide) interventions. None of the treatments achieved more than a 1.2 log CFU reduction in L. monocytogenes levels; bacteriophages at a multiplicity of infection of 100 and 3% (vol/vol) hydrogen peroxide were the most effective of the treatments tested. However, growth of residual L. monocytogenes during post-treatment storage attained levels equal to or greater than levels in the non-treated controls. The growth of L. monocytogenes was inhibited on mushrooms treated with chitosan, electrolyzed water, peroxyacetic acid, or UV. Yet, L. monocytogenes inoculated onto mushrooms and treated with UV–hydrogen peroxide decreased during posttreatment storage, through a combination of sub-lethal injury and dehydration of the mushroom surface. Although mushrooms treated with UV– hydrogen peroxide became darker during storage, the samples were visually acceptable relative to controls. In conclusion, of the treatments evaluated, UV–hydrogen peroxide holds promise to control L. monocytogenes on mushroom surfaces but use of bacteriophages is limited.

INVESTIGATION OF BACTERIOPHAGE AS POTENTIAL SOURCES OF ORAL ANIMICROBIALS

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Introduction: Fusobacterium nucleatum is a member of the human oral flora associated with periodontitis, where Fusobacterium nucleatum acts as a bridging organism between early oxygen-tolerant and late obligate anaerobe colonizers.

Aim: Characterisation of a prophage residing in the genome of Fusobacterium nucleatum polymorphum ATCC 10953 and an assessment of its presence in periodontal patient plaque DNA. In addition we are investigating three potential lysin proteins via recombinant production to assess potential antimicrobial activity as therapeutics.

Materials and methods: Mitomycin C was used to induce the 10953 prophage with visualisation by TEM. Three prophage genes with potential lysis activity were identified using the PHAge Search Tool (PHAST) server: FNP_1707 (amiC), FNP_1699 and FNP_1700. These genes were commercially synthesised for expression in E. coli. Cloning of the genes and overexpression of its protein were attempted in BL21 and C41. Furthermore, plaque samples from chronic periodontitis patients in Sheffield were screened for F. polymorphum and the occurrence of its prophage using PCR primers.

Results: Despite good expression of amiC-pGEX (FNP_1707 protein), it was found to be mainly insoluble. Cloning and overexpression of FNP_1699 and FNP_1700 are ongoing alongside attempts to further improve production AmiC. 23/45 patients tested screened positively for the presence of Fusobacterium nucleatum polymorphum ATCC10953 strain and its prophage, which represent 405 plaque samples, tested. In addition induction of the prophage has proved inconsistent with visualisation of a tailed phage in some samples.

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Disscusion & Conclusions: We have identified that the prophage of FNP10953 is present in a large number of patients, indicating that it is present not only in a lab strain but also in the general population. Attempts to isolate this prophage and its potential lysins are ongoing but present potential novel antimicrobials that would target Fusobacterium nucleatum spp, and might aid in the treatment of gum disease.

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PREVALENCE AND CHARACTERISTIC OF CAMPYLOBACTER BACTERIOPHAGE ISOLATED FROM RETAIL CHICKEN LIVER

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ABSTRACT
Bacteriophages have gained recognition as therapeutic agents to control pathogens in livestock and poultry and represent a potential approach to control campylobacters in livers. However, there was no previous report found on the attempts of isolating Campylobacter phages from poultry liver. In this study, retail chicken livers were screened for the presence of Campylobacter and their phage on the surface and within the internal tissue. Three Campylobacter-specific bacteriophages were isolated from the total of 109 liver samples along with their campylobacter host which was found in 95 and 90 of surface samples and the internal tissue samples, respectively. Campylobacters were present by enrichment in more than a half samples but 47 samples (43%) contained considerably higher counts which ranged from 1.8 - > 3.8 log10 CFU/cm2 for surface samples, and 3.0 – > 3.8 log10 CFU/g for internal tissue samples. The liver phages were able to lyse 12-16% Campylobacter isolated from chicken liver with the Efficiency of plating (EOP) ranging from 0.001 to 3.19. Pulsed Field Gel Electrophoresis (PFGE) analysis showed that all phages were classified into Group III bacteriophage based on their genomic DNA which is approximately 140 kb in size.
Keywords: Bacteriophage, Campylobacter, liver, poultry

TEMPERATE BACTERIOPHAGES IN THE GENUS BRUCELLA: ANALYSIS OF THE BROAD HOST RANGE SIPHOVIRUS BIPBO1 FROM B. INOPINATA

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Brucellae are facultative intracellular pathogens that belong to α-proteobacteria and may cause severe infections (brucellosis) in humans and animals. The genus currently consists of eleven species that can be divided in (I) the classical species (B. melitensis, B. abortus, B. suis, B. canis, B. ovis, & B. neotomae), (II) marine brucellae (B. ceti and B. pinnipedialis) and (III) the atypical species B. microti, B. papionis, and B. inopinata. All Brucella species are genetically highly related with overall genome similarities of >90% at the nucleotide level. Up to now, only little is known about mobile genetic elements of these pathogens. Neither plasmids nor temperate phages have been described in brucellae.

In this work, we analysed genomic sequences of various reference and type strains of the GenBank database (National Center for Biotechnology Information) and identified a number of putative prophages residing within the Brucella chromosomes. By mitomycin C induction, a phage of the family Siphoviridae was isolated from B. inopinata strain BO1. Phage BiPBO1 infects several Brucella species including B. abortus and B. melitensis. Integration of the phage genome occurs adjacent to a tRNA gene in chromosome 1. The bacterial and phage attachment sites comprise an identical sequence of 46 bp. This sequence exists in many Brucella and Ochrobactrum species. The BiPBO1 genome is composed of a 46,877 bp double-stranded DNA. Eighty-seven putative gene products were determined, of which 32 could be functionally

assigned. Strongest similarities were found to a temperate phage residing in the chromosome of O. anthropi ATCC 49188 and to prophages identified in several families belonging to the order rhizobiales. Our data underline the close relationship between pathogenic Brucella and environmental Ochrobactrum species.

GENETIC DIVERSITY AMONG STREPTOCOCCUS THERMOPHILUS BACTERIOPHAGES

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Bacteriophages are the main cause of the fermentation failure during the manufacture of cheese and fermented dairy products. Streptococcus thermophilus bacteriophages found in the dairy environment are considered to be homologous group. In 1997, the classification into two major types was proposed by Le Marrec et al. S. thermophilus phages could be divided into pac- or cos-type, depending on the DNA packaging mechanism. In 2005, additional grouping based on variable region in the antireceptor gene of S. thermophilus phages was proposed (Binetti et al., 2005). The discovery of phage 5093 with genotypic and morphologic features unusual for dairy S. thermophilus phages suggested reevaluation of this taxonomy system (Mills et al., 2011). Our research revealed 3 additional S. thermophilus phages that could not be classified according to the standard grouping. We sequenced the collection of 59 lytic phages covering 42 groups of S. thermophilus strains. The genome analysis and comparison with the sequences available in database showed that two of those phages shared more homology with Lactococcus lactis rather than S. thermophilus phages. One of the phages from our collection was highly similar to phage 5093, which is another representative of this untypical S. thermophilus phage genotype.

ADAPTATION OF GROWTH PARAMETERS OF ENVIRONMENTAL BACTERIOPHAGES TO STANDARD LABORATORY CONDITIONS

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Bacteriophages are seen as agent that can supplement or even substitute antibiotics in prevention and eradication of pathogenic bacteria. However, despite reports of successful treatment and phage-based medicines undergoing clinical trials this method still meets scepticism. Agencies like European Medicines Agency or U.S. Food and Drug Administration are concerned about phages ability to mutate, evolve and adapt their life cycle to growth conditions of their host. Since bacteriophages used for phage therapy are usually isolated from environmental samples, then transferred to laboratory conditions and finally applied to a patient, there is a risk that phage biology may change during therapy development or use.

Various development strategies used by the phages in response to unfavorable growth conditions of their host has been observed and described. For example, some phages are able to halt their development in a host cell without replication of the genome (pseudolysogeny). However, adaptations of phage growth parameters in response to switching from natural environment to laboratory environment, where usually bacteria are cultivated as monocultures in conditions that support high growth rate, haven't been extensively studied yet.

During our research we isolated bacteriophages from wastewater samples and passaged through bacterial cultures in standard laboratory conditions. The data we would like to present show which aspects of phage growth parameters changed in response to transition from natural environment to laboratory conditions. We hope that our research will help to better understand the process of phage adaptation and address concerns regarding this subject.

OBSERVATION OF PHAGE-ANTIBIOTIC SYNERGY IN VARIOUS COMBINATIONS OF BACTERIA, BACTERIOPHAGE, AND ANTIBIOTICS AND ITS UNDERLYING MECHANISMS

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When phages infect bacteria cultured in the presence of sub-lethal doses of antibiotics, sizes of phage plaques significantly increase. This phenomenon is known as phage-antibiotic synergy (PAS). We extended observation of PAS to a wide variety of bacteria-phage pairs with four different classes of antibiotics. PAS was observed in both Gram-positive and Gram-negative bacteria. Cell wall synthesis inhibitors and DNA metabolism inhibitors generally induced PAS. Some protein synthesis inhibitors induced PAS, while others did not. The use of sub-lethal dose of ampicillin, cefotaxime, ciprofloxacin, or mitomycin C allowed formation of highly visible plaques of increased sizes when various bacteriophages infected Gram-positive bacteria such as Staphylococcus aureus, Bacillus cereus, and Enterococcus feacalis. In Gram-negative bacteria, Escherichia coli and Pseudomonas aeruginosa phages showed increased plaques in the presence of sub-lethal dose of cefotaxime, ciprofloxacin, or mitomycin C. We also confirmed that cells stressed with β-lactam and quinolone antibiotics filamented or swelled extensively. Burst sizes of bacteriophages also increased in the presence of antibiotics. Increase of production of phages in the presence of antibiotics was also shown in vivo using Caenorhabditis elegans as a model animal. Induction of SOS response was observed in many cases of PAS, but not all. Enlarged production facility allowed prolonged assembly as well as increased phage DNA replication, transcription, and translation, resulting in PAS.

A NEWLY ISOLATED BACTERIOPHAGE, PBES 02, INFECTING CRONOBACTER SAKAZAKII

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A novel bacteriophage, PBES 02, infecting Cronobacter sakazakii was isolated and characterized. It has a spherical head 90 nm in diameter and a tail 130 nm in length, and belongs to Myoviridae as observed under a transmission electron microscope. The major virion protein appears to be 38 kilodaltons (KDa) in size. The latent period of PBES 02 is 30 min and the burst size is 210. Infectivity of the phage remained intact after exposure to temperatures ranging from 4 to 55oC for 1 h. It was also stable after exposure to pHs ranging from 6 to 10 for 1 h. PBES 02 has a double stranded DNA genome of 149,732 bases. Its GC ratio is 50.7%. Sequence analysis revealed that PBES 02 has 299 open reading frames (ORFs) and 14 tRNA genes. Thirty-nine ORFs were annotated including 24 related to replication and regulation functions, 10 related to structural proteins, and 5 related to DNA packaging. The genome of PBES 02 is closely related to that of two other C. sakazaki phages, CR3 and CR8. Comparison of DNA sequences of genes encoding the major capsid protein revealed a wide geographical distribution of related phages over Asia, Europe, and America.

ANALYSES OF THE USE OF BACTERIOPHAGES ON SULFATE REDUCING BACTERIA BIOFILM CONTROL

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The biogenic production of sulfide is the main cause of the souring process seen on oil industry. This sulfide production occurs due to the action of sulfate reducing bacteria (SRB) which are anaerobic microorganisms that use sulfate as final electrons acceptor , with the formation of hydrogen sulfide that is excreted. The hydrogen sulfide is highly reactive, corrosive and toxic. Bacteriophages emerge as an alternative to the SRB control in reservoirs, because of its capacity to degrade the polysaccharide matrix in biofilms, infect cells and cause an extensive biofilm disruption, which makes them a good strategy on SRB control in different fluids on oil reservoirs. This work aimed to evaluate the use of bacteriophages on biofilm control formed by SRB. The sulfate reducing bacteria Desulfovibrio alaskensis was inoculated on Postgate E medium with a steel coupon and maintained at 30ºC. After 15 days a bacteriophage cocktail was added and the coupons were incubated for 3 more days at 30ºC. A steel coupon on Postgate E medium was used as negative control and ,as positive control, Desulfovibrio alaskensis was inoculated on Postgate E medium containing a steel coupon. The coupons were analyzed on scanning electronic microscopy and optical profilometry. It was observed a reduction on the biofilm formed, with the presence of free bacteria cells, which was not observed on the positive control. There was also a significant reduction on values of

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average roughness (Ra) and quadratic roughness (Rq) of the metal when the coupons were treated with bacteriophages. This results indicates that bacteriophages were able to disrupt the biofilm formed, acting on the biofilm matrix. This demonstrates that the use of bacteriophages on the SRB biofilm control represents a promising alternative on the control of this prokaryotes.

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ANALYSES OF MICROBIAL DIVERSITY ON MIXED CULTURES FROM SAMPLES OF OIL EXTRACTION WELLS

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The process of biogenic souring on oil reservoirs occurs mainly due to the reduction of sulfate to sulfide by sulfate reducing bacteria (SRB). This is a consequence of a sea water prolonged use, with low salinity and high sulfate concentration, on injection for secondary recovery of oil. The sulfide (H2S) SRB production is responsible for toxicity and corrosion of installations bringing security risks on the oil recovering process and reducing the economic value of the oil produced. SRB are a taxonomically varied group which are strictly anaerobic. The obligatory anaerobic and restrict nutrition ensure that SRB are always found as components of a microbial community. These communities are found as biofilms on interfaces or solid substrate like steel surfaces. On this context, the present work aimed to analyze the microbial diversity present on mixed cultures from samples of oil extraction wells. Cultures from oil wells P1, P2, P3, P4, P5, P6, P7 e P8 was cultivated on medium Postgate C, BANHT e Capcis at 30ºC, 55ºC e 80ºC. The analyses of culture diversity were performed through the sequencing of part of 16S gene from the pool culture from the same oil well. The sequencing showed that cultures from wells P4 e P8 had a greater number of genus. In all cultures were identified genus capable of reduce sulfate, elemental sulfur or thiosulfate, with the production of hydrogen sulfide.

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BACTERIOPHAGE HOST RANGE EXPANSION RESULTS IN STRAINS WITH USEFUL INFECTION CHARACTERISTICS AND GENOMIC CHANGES

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Bacteriophage specificity can be problematic when choosing phages to include in treatment cocktails. Thus phages with a broad host range are desired.

Phage discovery from a library of environmental samples was performed against a panel of Staphylococcus aureus strains. At least one phage capable of infecting each isolate was found with the exception of a single isolate. We were interested in expanding the host range of the discovered phages to cover this recalcitrant host. To accomplish this, phages were used to infect a susceptible host in the presence of the resistant host, and those capable of proliferating were further examined.

Of the tested phages, one able to infect the new host was isolated and purified for further analysis. Whole genome sequencing was performed on the parent phage as well as its expanded host progeny to determine any genetic differences possibly responsible for the expansion. 3 SNPs and 1 single base deletion were discovered, but only the deletion and 1 of the SNPs were originally rare mutations, which makes them more likely candidates for overcoming bacterial resistance.

To further test the efficacy of this expanded phage, a bacterial mutant was generated that was resistant to a library of phages, including the parent phage. The expanded phage was able to successfully infect this strain. These results indicate expanded host range can be achieved by evolutionary means, and provides interesting genetic candidates which could be responsible for this phenotype.

NATURAL STRAIN IMPROVEMENT: PHAGE TRANSDUCTION IN L.LACTIS.

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Lactic acid bacteria (LAB) are Gram positive bacteria widely used in the food industry for the production of fermented food like butter milk, yogurt, cheese, sausages etc. L.lactis, together with other bacteria from the genera Lactobacillus, Leuconostoc, Streptococcus and Pediococcus is one of the most used among the LABs as a starter culture in the dairy industry.

In order to constantly meet costumers expectations regarding, for example, the taste, texture and quality of the end products, one of the dairy companies goalsis to develop new starter strains with enhanced fermentation abilities.

One promising way to introduce new desirable genetic traits into L.lactis is exploiting horizontal gene transfer mediated by bacteriophages. Both generalized and specialized transduction can, in fact, give rise to viral particles able to transfer genetic material from a strain to another.

A collection of bacteriophages is at the moment under investigation. A first effort is oriented toward the analysis of the genome sequence of each bacteriophage in order to acquire information about the type of transduction the phages can undergo.

At the same time convenient selectable markers are used to follow the transduction process of phages that display a more broad host range. This allows to test their ability to transfer and integrate genetic material of certain sizes from the genome of one host to the genome of a second one.

JUMBO PHAGES IN THE NORTHERN FRESHWATERS

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A growing number of jumbo phages (i.e. large phages with genome size over 200 kbp) have been identified in recent years. Jumbo phages have been largely overlooked due to their difficult isolation that has hampered their detection. Our studies have focused on the phages infecting Flavobacteria, ubiquitous freshwater bacteria that contribute to water quality in lakes and rivers and have a major role in environmental biochemical cycles. Phages infecting this genus, as well as other freshwater bacteria, are generally unexplored. Our collection of 25 phage isolates includes four large genome phages, all members of the family Myoviridae. Two of these are able to infect a majority of the 31 Flavobacterium-strains in our collection whereas the other two have an intermediate host range. Sequencing revealed genome sizes from 220 up to 380 kbp. For example, in the genome of phage FKo-2, 66 % of the predicted ORF's had no significant homologs in BLAST search. Our collection indicates that large DNA phages could be easily isolated from freshwater environments as no specific methods favoring detection of jumbo phages were used. It remains to be experimentally tested whether the conditions in the boreal freshwaters (low nutrients, low temperature) favor jumbo phage-host systems and whether the interaction of the host and the parasite benefit the survival of both.

IS PHAGE THERAPY FOR LYME DISEASE POSSIBLE?

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Bacteriophages (phages) are viruses that infect and kill bacteria. They are natural antibiotic alternatives, and have enjoyed a recent renaissance due to the increasing problem of antibiotic resistant bacteria. Lyme disease is caused by a group of spirochaetes, collectively referred to as Borrelia burgdorferi sensu lato (s.l.). Among the Lyme disease spirochaetes, three genospecies are predominating: B. burgdorferi sensu stricto (s.s.), B. garinii and B. afzelli. In order to exploit the therapeutic use of phages to combat Borrelia infection, the fundamental biology of Borrelia phages needs to be investigated. So far, no systematic effort has been made to study phages that infect the Lyme Borrelia bacterial species.

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In this project, we aim to identify/characterise phages that infect Lyme Borrelia strains. Two strategies will be adopted. Tick, environmental, and clinical samples will be screened for lytic phages. Meanwhile, temperate phages will be induced from Borrelia strains using low dose of antibiotics. Isolated bacteriphages will be subjected to a series of tests for characterisation, such as morphological study, growth dynamics, genomic study, and phylogentic analysis. Those phages with broad host ranges, large burst sizes, and short latent period will be further analysed in terms of their effectiveness at clearing representative Lyme strains using in vitro models. This will involve testing individual phage as well as a phage cocktail on single and multiple Borrelia strains. In parallel, bioinformatic method will also be adopted to explore the phage-encode enzymes (enzymes to break down the bacterial cell wall). Specifically, those enzymes will be cloned and over-expressed and their 'antibacterial' activity will be tested on Borrelia strains.

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NOVEL BACTERIOPHAGE MIXTURES FOR THE CONTROL OF BACTERIAL PATHOGENS OF CROPS

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Bacterial pathogens of crops are responsible for substantial losses through disease, damage and failure to meet market specifications. An effective, sustainable solution is a priority across the UK and wider European industry. Bacteriophage are ideally placed to tackle this challenge; they are naturally-occurring viruses of bacteria, highly specific and safe. They have the potential to be developed as sustainable and highly effective biocontrols against bacterial pathogens. This presentation will describe the development, characterisation and trial of bacteriophage mixes against key disease pathogens across the growing and production cycle. It will focus on Pectobacterium, spp. infections of potatoes, responsible for the development of 'blackleg' in the growing plant and associated soft rots in harvested tubers. Bacterial rots are the biggest cause of customer complaints to the potato industry, whilst bacterial-induced blackleg is a key disease across all sectors of the UK potato industry. This is particularly the case in the high-value seed sector, in which even minimal blackleg levels can result in significant financial losses through crop downgrade or even disqualification from the seed-classification system.

INDUCTION OF A *LACTOBACILLUS FERMENTUM* PROPHAGE BY AN EXTRACELLULAR PROTEINACEOUS SUBSTANCE DERIVING FROM *LB. FERMENTUM* STRAIN.

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Lactic Acid Bacteria isolated from food have been intensively investigated for their positive role in fermentation technologies and for their antimicrobial activity against pathogens, given by bacteriocin production. During a survey on prophages integrated in lactobacilli of dairy origin, it was noticed that the supernatant of a grown culture of *Lb. fermentum* UMB-LF6 strain, without the evidence of any induced bacteriophage, caused the lysis of *Lb. fermentum* UMB-LF12 strain. This phenomenon was triggered by the induction of a prophage and not by the residual presence of the antibiotic Mitomycin C. Although it was not possible to isolate it through the plaque assay, the induced bacteriophage (named ϕM) was observed by TEM after purification with CsCl density gradient. It was attributed to *Siphoviridae* family, having a 55nm diameter icosahedral head and a non-contractile tail 220nm long with a basal plate of 20nm diameter. The ϕM genome resulted a dsDNA of 39.2 kbp long, assessed by RLPA with different enzymes (EcoRI, BamHI, ClaI, ScaI, BglII, PstI). The hybridization of the genomes of the two bacterial strains with non-radioactive probes made with ϕM genome, confirmed that it had been integrated as a prophage into the UMB-LF12 chromosome. Experimental trials lead to the hypothesis that UMB-LF6 strain produced an extracellular substance causing the induction of ϕM bacteriophage. The UMB-LF6 supernatant was treated with different lytic enzymes (DNAseI, RNAseA, RNAseT1, Pancreatin, Pepsin, Proteinase K, Rennin) and the inducing activity got lost only if Proteinase K treatment had been carried out, suggesting the proteinaceous nature of the substance.

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