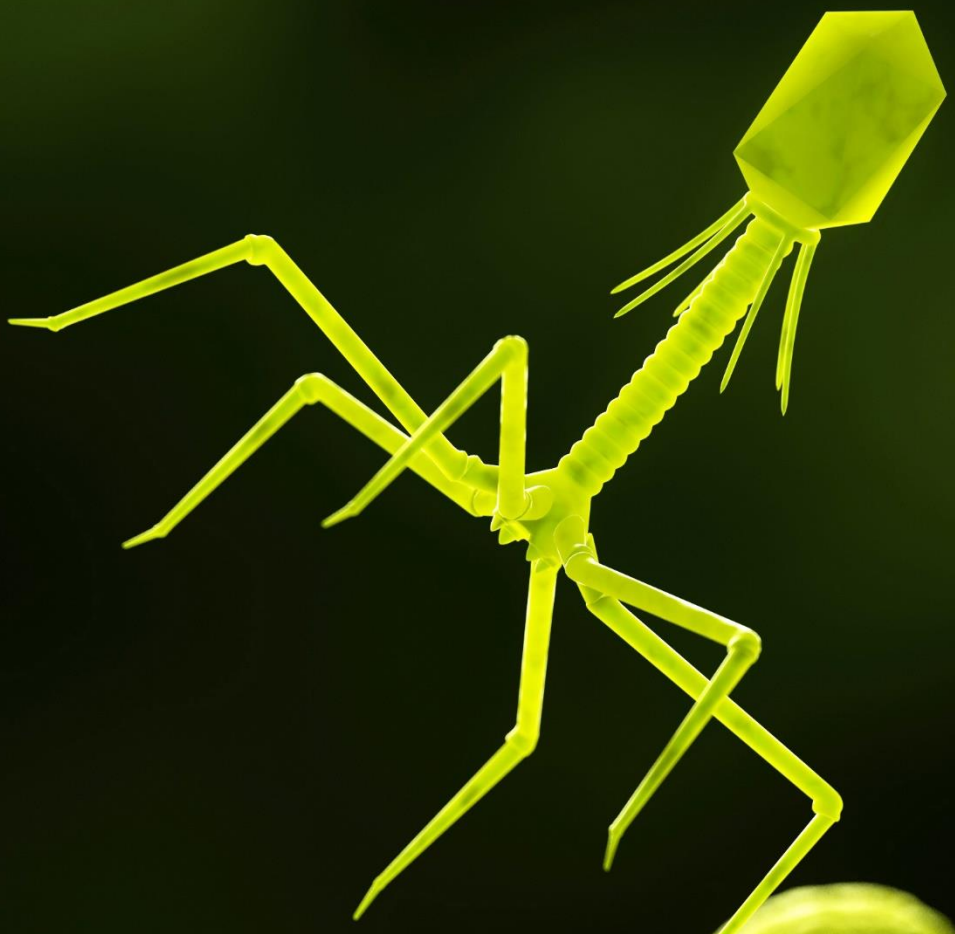
Bacteriophage 2017



ABSTRACTS

17th - 19th January 2017
Location: Online

EuroSciCon

This annual event will discuss emerging research relating to bacteriophage structure and mechanism of action, and their application in medical and industrial biotechnologies.

This event has CPD accreditation

This abstract book will be finalised two weeks before the event

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Invited Speakers Abstracts

Coat to Protect or Print to Detect: Phage-based Smart paper to Enhance Food Safety

Dr Hany Anany, Canadian Research Institute for Food Safety, University of Guelph, Guelph, Canada

Recent years have witnessed a large number of foodborne outbreaks and food product recalls in many countries. The ability of the pathogen to survive and/or grow under unfavorable conditions and the development of resistant strains with new virulence factors, represent a formidable challenge to food processing industries in marketing safe food products. The use of bacteriophages has been emerged as a promising technology to enhance food safety. For biocontrol in the food industry, phages are currently being delivered by spraying the product, which has some limitations. To reduce dissemination of bacteriophage in the food processing environment, which may ultimately give rise to phage resistant host cells, oriented immobilization of phage on low cost, solid substrates like paper may be a good alternative to provide persistent and effective control of potential pathogens. Immobilization would also help to enhance the sensitivity of detection of the target pathogen using phage-based detection approaches. In this context, this presentation will discuss briefly our trials of using immobilized phages to develop phage-based bioactive paper for detection and control of foodborne pathogens

Interaction of bacteriophages with sessile bacteria

Professor Joana Azeredo, Universidade do Minho, Campus de Gualtar, Braga, Portugal

Biofilms constitute an important survival strategy for microorganisms, and therefore are ubiquitous in the environment, being involved in many chronic and difficult to treat infections. Bacteriophages (phages), as natural predators of bacteria, have evolved and found routes to successfully reach and kill their hosts within the biofilm structure. This talk is about the exploitation of phages and derived enzymes (endolysins/depolymerases) to control infectious biofilms

Ecology, Applied and Otherwise, of Phage-Biofilm Interactions

Dr Stephen T. Abedon, The Ohio State University, Mansfield, United States

Appreciation of virus-biofilm ecological interactions remains poorly developed, as too is our understanding of the applied ecology of bacteriophage-biofilm interactions during phage therapy. A major issue is that biofilms tend to be easily cleared using phages in the laboratory, mostly readily if not necessarily trivially cleared using phages in the clinic, and so far as we know little impacted by phages in the environment. Here I consider both the ecology and applied ecology of phage exploitation and/or eradication of biofilms, exploring in particular a combination of the population biological, physiological, genetical, and clinical susceptibility of biofilms to phages.

The transcriptional battle between phage and host in the Pseudomonas phage infected cell

Mr.Bob G Blasdel, KU Leuven, Leuven, Belgium

Detailed knowledge of phage lifecycles, have been limited to few model bacteriophages mostly infecting Escherichia coli as well as the results of increasingly antiquated methods. However, next-generation techniques now make it possible to explore how phage affect host transcription and metabolism, now re-sparked by modern therapeutic and biotechnological potential, as well as to ask deeper more comprehensive questions. By performing RNA-Seq on synchronously infected cells we are able to compare host transcript data for early, middle and late transcription with six fundamentally different lytic phages: N4likevirus, Phikzlikevirus, Yualikevirus, Pbunalikevirus, Phikmvlikevirus, and novel F7-like viruses infecting P. aeruginosa PAO1.

The phage T4 DNA and protein packaging machine – Old, New, Widely true, useful too

Professor Lindsay W. Black, Department of Biochemistry and Molecular Biology, University of Maryland, Baltimore, USA

Viral genome packaging into capsids is powered by ATP driven high-force-generating motor proteins. In vitro the phage T4TerL alone packages any linear DNA into portal containing proheads by "crunching" it transiently from B- to A-form. But In vivo we show that a twin ring form of T4 TerS is required to gauge DNA synthesis adequate for packaging by "synapsing" two homologous pac sites. The Protein And Nucleic Acid Targeting System Using T4 Phage (PANTSUP) viral nanocontainer can be used for highly efficient and specific transfer of active proteins and corrected sequence DNA into genetically-deficient cell lines. Refs:PMID:25728298

Exploiting Bacteriophage for rapid detection of Mycobacteria

Dr Catherine E.D. Rees, University of Nottingham, United Kingdom

Mycobacterial pathogens are particularly difficult to detect with due to their slow growth, making routine culture impractical, and their mycolic-rich cell wall which results in inefficient DNA extraction for PCR-based detection methods. We have developed a phage-based method that allows the rapid detection of a variety of Mycobacteria in clinical samples. In addition the phage-based assay can be used to monitor the inactivation of microbes and can be used to investigate the physiological state of the cells. Most recently we have reformatted the assay to both reduce the time taken to detection and improve the sensitivity of the technique. In this talk I will outline the basis of our method and show how it has been applied to a variety of studies of these difficult to work with pathogens.

The bacteriophage carrier state of Campylobacter

Professor Ian Connerton, Northern Foods Chair of Food Safety, Division of Food Sciences, School of Biosciences, Sutton Bonington Campus, Loughborough, United Kingdom

Campylobacters are a common cause of human diarrhoeal disease worldwide. Amongst campylobacters recovered from biofilms after phage infection we recovered bacteria that had established a relationship with the bacteriophage typical of the carrier state life cycle (CSLC). In CSLC cultures bacteria and bacteriophages remain associated in equilibrium upon serial propagation (Siringan et al., 2014 Open Biology DOI: 10.1098/rsob.130200). The association confers a survival advantage in extra-intestinal environments to the bacteria but a lack of motility results in an inability to colonise chickens. However, CSLC cultures can release phage particles to prospect for new host bacteria.

Bacteriophage; the future cure to treat antibiotic resistant bacteria in Egypt

Professor Ayman El-Shibiny, Zewail City of Science and Technology, Giza, Egypt

Dr. Ayman El-Shibiny is currently an associate professor of biomedical sciences at the University of Science and Technology, Egypt. He completed his Ph.D degree in food microbiology (food safety) from Nottingham University, UK. His main research area is phage therapy and his current research interests include the therapeutic use of bacteriophages. Prior to joining Zewail City, El-Shibiny worked as a postdoctoral fellow at Nottingham University in the U.K., Cardiff University in the U.K., and The Evergreen State College in the U.S. He also served as an associate professor and head of the food sciences department in Suez Canal University (Egypt).

Lambda Display Phage as a Mucosal Vaccine delivery Vehicle for Peptide Antigens

Professor Sidney Hayes and Dr Philip J. Griebel, University of Saskatchewan, Saskatoon, Canada

There is increasing interest in using bacteriophage as protein/peptide and DNA vaccine delivery vehicles. Little isn known, however, about their capacity to induce mucosal immune responses in the small intestine. Targetted delivery of of lambda phage (LP) to specific sites in the small intestine of newborn calves confirmed LP were immunpgenic and we re specifically taken up by mucosa-associated lymphoid tissues, including Peyer's patches. A lambda display phage (LDP) was then used to confirm that specific IgA antibody responses were induced to the display peptide. This specific antibody response was observed in the absence of an adjuvant or exogenous immune stimulating compound. Therefore, we conclude that LDP may be used as oral vaccine delivery vehicle.

Application of bacteriophages in commercial broiler houses- results and population dynamics in field trials

Dr. Sophie Kittler, University of Veterinary Medicine Hannover, Hannover, Germany

Because Bacteriophages are safe, selective and effective in killing pathogenic bacteria without changing the characteristics of food products, they are a suitable measure for the prevention of foodborne zoonoses. Using bacteriophages in primary production for the reduction of pathogenic bacteria aims at reducing the target bacteria in the intestina flora of commercial livestock. First field trials were carried out to reduce Campylobacter in commercial broiler flocks and among practical considerations, population dynamics of the intestinal flora have to be considered for such application strategies. We will present results from field trials and experiences on these issues of phage application.

The effect of phage on modifying the genome of Clostridium difficile

Prof Peter Mullany, UCL Eastman Dental Institute, London, United Kingdom

Clostridium difficile is an important human pathogen, phage have been investigated as alternative forma of treatment for the diseases it cases. However, phages also have an important role in the modification of bacterial genomes. In the case of C. difficile phages have been shown to modulate expression of virulence factors and have been implicated in transducing antibiotic resistance genes. In this talk I will review these properties and how phage interact with the host genome.

Computational biology and tools for uncovering hidden information from the viral dark matter

Professor Alejandro Reyes Munoz, Universidad de los Andes, Bogota, Colombia

With recent advances in DNA sequencing, the possibility of characterizing different microbial environments is a reality. Most studies have shown how Eukaryotic and prokaryotic microorganisms co-exist and thrive in almost any environment; however, very few studies have focused on the viral component of such communities. This lack of studies is not due to lack of interest, rather by the lack of comprehensive genetic information in public databases from viral genomes. However, as more viral metagenomic projects are generated, the opportunity of mining those datasets with novel computational strategies constitute an important approach for further understanding of such diverse communities

Phages and CRISPR-Cas in the classroom

Professor Sylvain Moineau, Universite Laval, Quebec, Canada

Thanks to the genome-editing tool, CRISPR-Cas systems have caught the eye of politicians, the media, the general public, and our students. The field of CRISPR-Cas provides a fantastic opportunity for microbiology education. It offers a learning experience that illustrates the interconnectedness of multiple curriculum areas. Students must use knowledge gained in various fields to fully grasp the activities of CRISPR-Cas systems. For the past four years, we have integrated CRISPR-Cas into our Microbiology program in both lecture and laboratory environments. This seminar will present the laboratory course where students have studied CRISPR-Cas as an defense system against phage infections.

A novel role for phage P22's scaffolding protein: triggering portal ring oligomerization and incorporation during procapsid assembly

Dr. Tina Motwani, University of Connecticut, Storrs, United States

The majority of DNA viruses package their genomes into preformed precursor capsids through a portal protein complex incorporated in the capsid at a single vertex. The existence of a unique portal complex is crucial to the formation of mature infectious virions, but how only one portal assembly is selectively incorporated at a special vertex is unclear. Here, we show incorporation of bacteriophage

P22 portal protein into PC in vitro as pre-assembled dodecameric rings. A novel role for the scaffolding protein in triggering portal ring formation from portal monomers was also found. Thus, new details are revealed about this critical step in the assembly of icosahedral viruses that use a portal protein in conjunction with a genome-packaging motor to encapsulate their genetic material.

Phage Sf6 ejection mechanisms

Professor Kristin N. Parent, Michigan State University, East Lansing, United States

Outer membrane proteins A and C mediate Sf6 infection by dramatically increasing the rate and efficiency of genome ejection. I will describe a combination of in vivo studies with omp null mutants of Shigella flexneri, including classic phage assays and time-lapse fluorescence microscopy to monitor genome ejection at the single virion level, and cryo-electron tomography of phage "infecting" outer membrane vesicles. In addition, mutagenesis studies have demonstrated key regions in OmpA that mediate infection. We conclude that Sf6 phage entry utilizes either outer membrane proteins A or C, with outer membrane protein A being the preferred receptor.

Crystal structures of bacteriophage fibre proteins

Dr Mark J van Raaij, Centro Nacional de Biotecnologia (CNB-CSIC), Spanish National Research Council, Madrid, Spain

Mark J. van Raaij is Group Leader in the Department of Macromolecular Structures at CNB. His group research focuses on structural biology of the tail fibres of viruses and bacteriophages. Knowledge of the structures of viral and bacteriophage fibre proteins could lead to a variety of biotechnological applications. Modification of the bacteriophage fibre receptor binding specificities could permit improved detection and elimination of specific bacteria. The skills and expertise of the group cover construction of bacterial expression vectors, protein expression, purification, characterization and crystallisation and crystallographic structure determination by de novo or molecular replacement phasing.

Detection of phage φ1207.3 in Streptococcus pneumoniae by immunoassays targeting the major capsid protein

Dr. Francesco Santoro, University of Siena, LAMMB - Dept of Medical Biotechnologies, Siena, Italy

Φ1207.3 is a prophage found in a clinical strain of Streptococcus pyogenes which carries macrolide resistance genes. We previously transferred the phage in Streptococcus pneumoniae and showed that it is able to produce mature and complete phage particles. We evaluated the presence of phage particles associated to the host in a bacterial culture of S. pneumoniae using polyclonal antibodies directed against the phage major capsid protein. Φ1207.3 major capsid protein was detected both inside and on the surface of the pneumococcus by western-blot, flow cytometry, immunofluorescence and immuno TEM.

Essential head genes in the giant PhiKZ-related phages

Julie Thomas, Rochester Institute of Technology, Rochester, United States

A "giant" phage has a long genome (>200 kb) and a large, structurally complex virion. The head of Salmonella phage SPN3US is composed of about 50 different proteins. Numbers of these proteins, including a multi-subunit RNA polymerase (vRNAP), are packaged inside the head along with the 240 kb dsDNA genome and ejected into the host cell. However, the functions of the majority of head proteins are unknown. In this talk I will discuss how our new collection of SPN3US mutants has facilitated identification of essential head genes and novel features of giant phage head structure, function and assembly.

Comparative genomics and proteomics of paenibacillus larvae bacteriophages

Assistant Professor Philippos Tsourkas, University of Nevada, Las Vegas, Las Vegas, Nevada, United States

Paenibacillus larvae is a gram-positive bacterium that is the causative agent of American Foulbrood Disease, one of the leading causes of the global population decline of honeybees. Since 2013 the number of sequenced bacteriophages that infect P. larvae has increased from zero to 18, and is set to increase further. P. larvae bacteriophages are heterogeneous, falling into several similarity clusters. Their genomes are 38-55 kb long, containing between 68 and 92 coding genes. Several treatments for AFB using P. larvae bacteriophages and P. larvae bacteriophage endolysins have been published, with promising, if not conclusive results.

Genomics approaches for analysing therapeutic bacteriophages

Ms Henrike Zschach, Technical University of Denmark, Kongens Lyngby, Denmark

In light of the developing antibiotic crisis interest in phage therapy has been greatly renewed in the Western scientific community. At the end of the first bacteriophage era there were many problems with phage therapy, most importantly an insufficient understanding of what 'phage' actually was. This time, the advent of the genomic era is giving us a multitude of new tools to characterise phages themselves and the interaction with their hosts.

In this talk, I will detail how we used a combination of sequence analysis tools to characterise the Intesti bacteriophage cocktail, a key commercial product of the Eliava Institute, Georgia, targeting gastrointestinal pathogens.

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

Day 1:

Day 2:

Day 3:

DUAL-REPORTER MYCOBACTERIOPHAGES (Φ2DRMS) REVEAL PRE-EXISTING MYCOBACTERIUM TUBERCULOSIS PERSISTENT CELLS IN HUMAN SPUTUM

*P. Jain**, B. Weinrick, E. Kalivoda, H. Yang, V. Munsamy, C. Vilcheze, T. Weisbrod, M. Larsen, M. O'Donnell, A. Pym, and W. Jacobs Jr.

*Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461.

Persisters are the minor subpopulation of bacterial cells that lack resistance-conferring alleles to a specific bactericidal antibiotic but can survive otherwise lethal concentrations of that antibiotic. In infections with Mycobacterium tuberculosis (Mtb), such persisters underlie the need for long-term antibiotic therapy and contribute to treatment failure in tuberculosis cases. It has been difficult to study persister cells as we have lacked tools to isolate these rare cells. Here, we report the development of mycobacteriophage TM4 derived dual-reporter mycobacteriophages (Φ2DRMs) that encode a green fluorescent marker of viability and and a red fluorescent protein marker to identify

the persisters cells. We demonstrate the value of these novel Φ2DRMs for identifying and characterizing Mtb-persisters.

The addition of isoniazid (INH) to exponentially growing Mtb consistently resulted in a 2- to 3-log decrease in colony-forming units within 4 days, and the remaining ≤1% of cells, which survived despite being INH-sensitive, were INH-tolerant persisters with a distinct transcriptional profile. We fused the promoters of several genes upregulated in persisters to the red fluorescent protein tdTomato gene in Φ2GFP10, a mycobacteriophage constitutively expressing GFP, thus generating Φ2DRMs. A population enriched in INH-persisters exhibited strong red fluorescence, by microscopy and flow cytometry, using a Φ2DRM with tdTomato controlled from the dnaK promoter. Interestingly, we demonstrated that, prior to INH exposure, a population primed for persistence existed in Mtb from both cultures and human sputa and that this population was highly enriched following INH exposure. We conclude that Φ2DRMs provide a new tool to identify and quantitate Mtb-persister cells.

Relevant References:

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Poster Presentation Abstracts

Poster abstracts will be finalised weeks before the event

Comparative Genomic Analysis Of 130 Pseudomonasbacteriophages

Anh D. Ha and Dee R. Denver

Department of Integrative Biology, Oregon State University, Corvallis. Oregon 97333, USA

Bacteriophage, or 'phage', are the most abundant and genetically diverse form of life in the biosphere. To date, about 6,000 phage genomes have been sequenced, approximately 10% of which infect members of the Pseudomonas genus of bacteria. Pseudomonas bacteria live in a wide variety of ecological environments, including water and soil. These bacteria also associate with fungi, plants

and animals. Consistent with their occupation of diverse niches [1], Pseudomonas bacteriophages possess highly variable genetic material [2,3]. Here we examined the diversity and evolution of Pseudomonas-infecting phages by analyzing 130 complete genome sequences available in public sequence databases. We observed extensive variation in genome size, ranging from 3 kb to 316 kb and GC content (from 36.83% to 66.41%). Based on nucleotide similarity and the number of shared gene products, 111/130 genome sequences were grouped into 12 clusters; 19 did not have close relationship with other phages and were defined as 'singletons'. Interestingly, five of these clusters each included phage members isolated from different specific Pseudomonas host species. We focused specifically on Cluster L, as members of this cluster infect five different host species. Despite infecting different host species, they share at least about 60% of sequence identity. The predicted open reading frames (ORFs) identified in these 130 phage genomes were distributed into 4462 related groups ('phamilies'), based on cutoff values of 35% identity in ClustalW alignments, and BLASTP e-values of 1e-50 or smaller. Phamily-level analysis again revealed substantial diversity among Pseudomonas phage genomes analyzed, as the largest gene Phamily had only 39 members and 2992 Phamilies had only one member. Highly conserved regions between sequences and also clear breaks in synteny can be identified in clusters. In a subset of 30 phamilies selected for evolutionary analysis, the majority of phamilies showed signatures of purifying selection. This study provides further insight into the enormous diversity, complexity and the dynamic host-phage relationships in the complex and still vastly under-surveyed universe of Pseudomonas bacteriophages.

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