

2014 - Exploiting bacteriophages for bioscience, biotechnology and medicine (the 5th in a biennial series)

23rd January 2014

Cineworld: The O2, London, SE10 0DX, UK

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

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

9:00 – 9:40 **Registration**

9:40 – 9:45 **Introduction by the Chair:** *Professor George Salmond, University of Cambridge, UK*

9:45 – 10:10 **Bench to bedside with phage-derived antibodies**

Professor Kerry Chester, UCL Cancer Institute, UK

10:10 – 10:35 **Ecological Aspects of Bacteriophage in the Wider Environment**

Dr Brian Reavy, The James Hutton Institute, Scotland UK

High throughput sequencing technologies and metagenomic approaches are revolutionising our understanding of the composition of bacteriophage populations and their potential roles in the wider environment. Much of this work has concentrated on viruses in marine environments and only recently have studies been extended to examine in detail the viruses present in soils. Recent advances will be discussed with an emphasis on how this is framing our view of the roles of bacteriophages as reservoirs of metabolically important host genes specific to distinct environments, and how they potentially reflect and affect microbial ecosystem functioning.

10:35 – 11:00 **Speakers' photo then mid-morning break and poster exhibition and trade show**

Please try to visit all the exhibition stands during your day at this event. Not only do our sponsors enable Euroscicon to keep the registration fees competitive, but they are also here specifically to talk to you.

11:00 – 11:25 **Purification of bacteriophages using methacrylate based monolithic columns**

Dr. Lidija Urbas, Head of Marketing, BIA Separations, Slovenia

Methacrylate based monolithic columns were developed for efficient isolation and purification of large biomolecules like proteins (IgG, IgM), virus-like-particles, viruses and DNA. Monolithic columns consist of a stationary phase that is a solid single piece continuous bed with internal porosity of 60% and a specially designed housing. The mobile phase and sample to be separated are driven by a pressure force through large, highly interconnected and easily accessible channels that are covered with appropriate ligands. This results in a convective flow enhanced mass transfer which enables highly efficient and flow independent chromatographic separation. The purification of the target molecule can therefore be achieved in a much shorter time in comparison to traditional particle based chromatographic media, without compromising the efficiency of the purification process. In this lecture we will present how to develop purification processes for various bacteriophages based on their properties and characteristics. A basic approach on how to start phage purification on a small scale and then transfer it to large scale will be shown. Additionally the ability of monolithic columns to monitor phage content during different phage production steps (in-process control) in nearly real time will be presented. The "snap shot" can help process developers determine the appropriate time for product extraction, but it also serves as an integration tool between upstream and downstream aspects of the process.

11:25 – 12:55 **Oral Presentations:**

11:25 – 11:40 **ISOLATION OF A BACTERIOPHAGE COCKTAIL EFFECTIVE ON THE ERADICATION OF STAPHYLOCOCCUS AUREUS BIOFILMS**

D. R. Alves, M. C. Enright, A.T.A. Jenkins

Department of Chemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK

11:40 – 11:55 **REGULATION OF BACTERIAL TRANSCRIPTION BY NON-BACTERIAL TRANSCRIPTION FACTORS**

Wigneshweraraj, S

MRC Centre for Molecular Bacteriology and Infection, Flowers Building, Imperial College London, London SW7 2AZ

11:55 – 12:10 **USE OF BACTERIOPHAGE TO DETECT VIABLE MYCOBACTERIA IN BLOOD WITHIN 48 H**

B. Swift, E. Denton, S. Mahendren, J. Huxley, C. Rees

School of Biosciences, Sutton Bonington Campus, University of Nottingham, Nr Loughborough, Leics, LE12 5RD

12:10 – 12:25 **ENHANCEMENT OF THE ANTIMICROBIAL PROPERTIES OF BACTERIOPHAGE K VIA STABILISATION IN OIL-IN-WATER NANO-EMULSIONS**

Patricia Perez Esteban^{1, 2*}, A.T.A. Jenkins¹, Thomas Arnot²

¹Department of Chemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK, ²Department of Chemical Engineering, University of Bath, Claverton Down, Bath, BA2 7AY, UK

12:25 – 12:40 **GENOMIC AND BIOLOGICAL ANALYSES OF BACTERIOPHAGES: A MODEL FOR PSEUDOMONAS SYRINGAE PHAGE-THERAPY**

D Frezza, P D'Addabbo, R Braglia, M Evangelisti, M Scortichini, M C Thaller, G Di Lallo,

Department of Biology, University of Tor Vergata, viale della ricerca scientifica, Roma Italy

12:40 – 12:55 **THE PERFECT VIRUS: NOVEL SALMONELLA PHAGE S16 FEATURES A BROAD HOST RANGE AND TAIL FIBER TOOLS FOR RAPID AND SPECIFIC DETECTION OF ITS TARGET CELLS**

Martin J. Loessner

Institute of Food, Nutrition and Health, ETH Zurich, 8092 Zurich, Switzerland

12:55 – 13:45 **Lunch, poster exhibition and trade show**

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13:45 – 14:15 **Discussion session**

This discussion session is an informal question and answer session. This is an ideal opportunity to get advice and opinion from experts in this area. This session is not for questions about specific talks, which can be asked after the speakers session, but for discussing either general topics or specific issues.

There are three ways you can ask questions:

1. Before the session you can *submit your question to Euroscicon staff* at the registration desk,
2. Before and during the session you can *submit a question or comments, by email*, which will be provided on the day of the event
3. During the session you can *put your hand up* and join in

14:15 – 14:40 **Mining the microbiota for novel phage therapies**

Professor Colin Hill, Professor of Microbial Food Safety, University College Cork, Ireland

The gastrointestinal tract is probably the most densely populated ecosystem in nature, containing as many as 10^{15} bacteriophage particles. We are interested in 'mining' this ecosystem for effective antimicrobials, including bacteriophages. In this talk I will present an example of the isolation of phages against *Pseudomonas aeruginosa* in the lungs of cystic fibrosis patients, which has been validated in cell lines and in murine models.

14:40 – 15:05 Phage nanobiotechnology: from phage display technology to targeted gene delivery

Dr Amin Hajitou, Imperial College Faculty of Medicine, UK

Phage nanobiotechnology has expanded its applications in diverse disciplines. Yet bacteriophages are still considered poor vectors for gene transfer applications as they have no optimised strategies to deliver genes to mammalian cells. We have investigated the barriers to phage-guided gene transfer and found that one of the limitations is phage surface negative charge hindering accessibility to negatively charged eukaryotic cell membranes. Next, we established that the major intracellular obstacle is phage sequestration in endosomes and degradation in lysosomes. To overcome these limitations, we generated a multifunctional bacteriophage with a positive charge and that allows display of strategies to improve gene transfer.

15:05 – 15:30 Afternoon Tea, last poster session and trade show

15:30 – 15:55 Blocking Cell Signalling with Recombinant Antibodies

Dr John McCafferty, University of Cambridge, UK

15:55 – 16:20 Phages can be the problem and targeting them might be the solution

Professor David Gally, Division of Infection and Immunity, The Roslin Institute, University of Edinburgh, UK

For a number of bacterial pathogens key virulence factors are encoded on lysogenic bacteriophages. This is certainly the case for enterohaemorrhagic *E. coli* (EHEC) strains for which the serious pathology in humans is associated with phage-encoded Shiga toxins (Stx). Our collaborative research has demonstrated that specific Stx-encoding phages are also associated with higher EHEC excretion levels from cattle, the main reservoir host, increasing the risk of human infection. Moreover, recent research indicates that phage-encoded Stx can potentially be expressed in eukaryotic cells and therefore targeting phage production and phage trafficking during animal or human infection may offer novel therapeutic options to prevent EHEC and other 'bacterial' diseases.

16:20 - 16:45 Temperate phages infecting *Pseudomonas aeruginosa* in chronic lung infections

Dr Darren Smith, Senior Lecturer in Biology, Northumbria University, UK

Pseudomonas aeruginosa is associated with lowered lung function in genetic disorders such as Cystic Fibrosis (CF), non-CF Bronchiectasis and COPD. There are pathophysiological similarities between these conditions where we will present the differences in phage communities/biology even though bacterial communities present are deemed to be almost identical.

16:45 - 17:00 Chairman's Summing Up and Close of Meeting

Registration Website: <http://www.regonline.co.uk/bacteriophage2014>

Key Words: Abortive, Infection, Bacteriophage, Resistance, Toxin, Purification Monolith, Yield, Scaleable, cGMP, clinical trials, regulation, FDA, EMA, therapeutic use, lung, infection, Campylobacter, phage therapy, poultry, biofilms, Clostridium difficile, bacteriophages, genomes, diagnostic, therapeutic, Metagenomics, ecology, microbial functioning, bacteriophages, Genomics, Metabolism, Clostridium difficile, Pseudomonas aeruginosa, gastrointestinal, Bacteriophage, gene delivery, targeted gene therapy, phage nanobiotechnology.

Meeting reports from this event will be published by HONNAO publishing, Expert Reviews in Anti-infective Therapy and Pharmaphorum.

About the Chair

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

About the Speakers

Brian Reavy is a molecular biologist with an extensive background in virology. After his doctoral studies on viruses of insects at Oxford he worked on foot-and-mouth disease virus genetics at the Animal Virus Research Institute (now Pirbright laboratory). Following a spell in the pharmaceutical industry he moved to the Scottish Crop Research Institute (now incorporated into The James Hutton Institute) where he has studied plant viruses with an emphasis on vectored transmission mechanisms. In the last few years he has started to apply modern molecular techniques to examine ecological aspects of viruses, particularly bacteriophage in soils.

Lidija Urbas studied chemistry at the University of Ljubljana, Faculty of Chemistry and Chemical Technology where she graduated in 2005. She then joined the company BIA Separations, where she was working as a research scientist for 6 years. Her main task and a part of her PhD study was the development of downstream processes for the purification of viruses and virus like particles and the development of analytical monolithic column and methods for PAT. After her PhD she became a Project Manager, leading projects dealing with purification and analytics of biomolecules and in 2013 she became Head of Marketing.

Colin Hill has a Ph.D in molecular microbiology and is Professor of Microbial Food Safety in the Microbiology Department of University College Cork, Ireland. His main interests are in infectious disease, particularly in defining the mechanisms of virulence of foodborne pathogens and in developing strategies to prevent and limit the consequences of microbial infections in the gastrointestinal tract. He has published more than 375 peer-reviewed papers and has served on the Scientific Committee of the Food Safety Authority of Ireland for many years. In 2005 Prof. Hill was awarded a D.Sc by the National University of Ireland in recognition of his contributions to research. In 2009 he was elected to the Royal Irish Academy and in 2010 he received the Metchnikoff Prize in Microbiology.

Amin Hajitou obtained his BSc from the University of Fes, Morocco, then Master and PhD from the University of Liège, Belgium. His PhD involved use of retroviral vectors for oncogene transfer. Next, he enhanced his skills in vector technologies using viral vectors to systemically deliver inhibitors of tumour angiogenesis *in vivo*. In 2002, Dr Hajitou joined the MD-Anderson Cancer Center of the University of Texas. There, he acquired substantial expertise in phage technologies and designed new generation of hybrid phage nanoparticles for targeted systemic gene delivery. Since 2007, Dr Hajitou established his group, as a Lecturer, at Imperial College London and became Senior Lecturer in 2013.

Darren Smith's postgraduate research has spanned 12.5 yr and has targeted the biology of temperate bacterial viruses and the nano therapy of HIV. Currently he is a Senior Lecturer leading a new but active research group studying the dynamic interplay between *Pseudomonas aeruginosa* phages and their bacterial host, elucidating their involvement and influence on chronic lung disease. He is the PI of a sequencing facility focusing on viral genomics and host subversion.

PRESENTATIONS

ISOLATION OF A BACTERIOPHAGE COCKTAIL EFFECTIVE ON THE ERADICATION OF *STAPHYLOCOCCUS AUREUS* BIOFILMS

D. R. Alves, M. C. Enright, A.T.A. Jenkins

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The skin – our body largest barrier from invading microorganisms – can be vulnerable to environmental threats and when a breach occurs (e.g., burn wound) it becomes an ideal environment for bacteria to establish. A poor inflammatory response can lead the initial bacterial infection developing to a complex community – a biofilm. Wound biofilms are one of the major reasons in impairment of wound healing and deterioration of patient clinical condition. Bacteria in biofilms are highly resilient to antibiotic drugs and to the immune system action making its eradication challenging. One of the most common and aggressive pathogens in burn infections is *Staphylococcus aureus*. These gram-positive bacteria display a large repertoire of virulence factors that promote host invasion and is also commonly associated with antibiotic resistance determinants (such as MRSA), leading to systemic disease. One of the most critical virulence factors in *S. aureus* is the exotoxin TSST-1. This superantigen toxin can lead to massive overstimulation of the host immune system leading to organ failure and death. It is strongly associated with mortality in burn wounds. Lack of effective and versatile drug therapeutics in alternative to antibiotics is of huge concern. Bacteriophage therapy is a revived bacterial infection-therapeutics and can play an important role in the infectious disease's therapeutic area. Bacteriophages (or phages) are obligate intracellular parasites of bacteria. They infect bacteria through specific cell-wall receptors and once inside they multiply using the host machinery. New phage particles are then released into the environment causing cell lysis and subsequent rounds of replication in new hosts take place as long as they are present. In the present work a group of phages were isolated, from environmental sources, showing a strong lytic activity against a wide range of *S. aureus* strains. A phage cocktail was formulated in order to get the best bacterial infectivity coverage and its efficacy assessed. The phage cocktail was tested against *S. aureus* 48 hours-old biofilms (microtitre plate-model) produced by two different clinical isolates – we observed a significant decrease of the biofilm biomass over 24 and 48 hours of treatment. The phage cocktail established and investigated in the present work so far can be seen as a good candidate to treat bacterial infections under biofilm conditions responsible for the high number of complications after a burn injury.

REGULATION OF BACTERIAL TRANSCRIPTION BY NON-BACTERIAL TRANSCRIPTION FACTORS

Wigneshweraraj, S

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Many bacteriophages encode transcription factor-like proteins that specifically interact with the host bacterial RNA polymerase (RNAP), the central enzyme responsible for all bacterial transcription, and modulate bacterial transcription to shift host resources to support bacteriophage gene expression and production of viral progeny. Thus, bacteriophage encoded transcription factors, hereafter referred to as pTF, can be considered as non-bacterial regulators of bacterial transcription. Some pTF are low-molecular weight and potent inhibitors of the bacterial RNAP. I will describe the *modus operandii* of one such pTF – Gp2 from the *Escherichia coli* infecting T7 bacteriophage - and provide novel insights into the regulation of bacterial transcription by a pTF and present data showing how pTFs like Gp2 can serve as an inspiration for the development of new antibacterial compounds.

USE OF BACTERIOPHAGE TO DETECT VIABLE MYCOBACTERIA IN BLOOD WITHIN 48 H

B. Swift, E. Denton, S. Mahendren, J. Huxley, C. Rees

School of Biosciences, Sutton Bonington Campus, University of Nottingham, Nr Loughborough, Leics, LE12 5RD

Phage detection methods have been under development for quite some time as a simple rapid method for the detection of pathogens. A commercial phage-based test was developed for the detection of the pathogen *Mycobacteria tuberculosis* (the FastPlaque assay). In this assay, to overcome the limitation of the extreme slow growth rate of these organisms (colonies take weeks to form on solid media), phage are used to indicate the presence of the host strain. However the human diagnostic application was not a commercial success and we have been developing new veterinary applications. *Mycobacterium avium* subsp. *paratuberculosis* (MAP) causes a chronic wasting disease that affects ruminants, called Johne's disease. Our research shown that bacteriophage can be used to detect MAP in the milk of infected animals. We have also successfully developed the assay by combining the phage test with immuno magnetic separation to detect MAP in blood of clinically infected cattle and sheep. Further work is now being carried out to extend the organisms tested to *M. bovis* the causative agent in bovine TB. In addition a new proprietary one day test format has been developed that allows detection and identification of pathogenic Mycobacteria within 6 hours.

ENHANCEMENT OF THE ANTIMICROBIAL PROPERTIES OF BACTERIOPHAGE K VIA STABILISATION IN OIL-IN-WATER NANO-EMULSIONS

Patricia Perez Esteban^{1,2*}, A.T.A. Jenkins¹, Thomas Arnot²

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The rise in antibiotic-resistant bacteria has received much attention over recent years, but the rate of development of new antibiotics to treat these emerging “superbugs” is very slow. The skin represents the primary defence mechanism against infection hence injuries or burns are a significant pathway for bacterial infection. Burns are especially susceptible to colonisers such as *Staphylococcus aureus*, with 10% of all cases becoming infected. The consequences include greatly increased patient morbidity and mortality, and cost of treatment. The absence of new antibiotics has led to interest in alternatives to classical antibiotics, including the use of bacteriophages. The aim is to identify and deliver a suitable phage or “phage-cocktail” to the point of infection without losing efficacy, during delivery or in prior storage. This work evaluates the anti-microbial efficacy of Bacteriophage-K (specific for *S. aureus*) when stabilised in an oil-in-water nano-emulsion, compared to delivery in a simple aqueous dispersion. The emulsion will form the basis for the development of a wound dressing or topical cream, containing stabilised phage. Therefore, the influence of emulsion components, and its effectiveness in phage delivery were evaluated in vitro against different strains of *S. aureus*. Bacterial growth was challenged with emulsion combined with Bacteriophage-K, and the influence of storage time of the preparation on phage infectivity was assessed by standard colony counting together with turbidity measurements. The results show that newly prepared Bacteriophage-K/nano-emulsions have much greater anti-microbial activity than freely-suspended bacteriophage, causing more rapid bacterial death. The same effect was observed for preparations that were either stored at room temperature, or chilled at 4°C, for up to 15 days of storage. In conclusion, we show that nano-emulsions enhance the activity of bacteriophages, which prompts further investigation of these kinds of formulations with respect to the development of novel anti-microbial wound management strategies. Additionally, the influence of emulsions on growth and infection parameters is being investigated, in order to develop a modelling approach for the analysis of the involved mechanisms, which are not yet well understood.

GENOMIC AND BIOLOGICAL ANALYSES OF BACTERIOPHAGES: A MODEL FOR *PSEUDOMONAS SYRINGAE* PHAGE-THERAPY.

D Frezza, P D'Addabbo, R Braglia, M Evangelisti, M Scortichini, M C Thaller, G Di Lallo,

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The actual number of bacteriophage complete genomes in the Genbank database is 2410 and is constantly increasing with the optimization of the sequencing facilities, time saving and lower costs. Nevertheless it is without doubt that a good knowledge of phage genomics is far to be completed since the enormous quantities of phage species, estimated as exceeding 10 times the number of bacterial species (ref 1). It was argued the difficulty of taxonomic and phylogenetic proper description based on genomic similarities since the large number of phage subject to horizontal genetic transfer, among different phages or with the bacterial host. Our aim is to compare each other the available phage genomes clustering them at different level, i.e. performing similarity searches among strains, species, families. Moreover, we are screening phages to enrich the database of peculiar strains to be utilized for possible phage therapy against bacteria of human risk or for industrial application (aquaculture, food conservation etc.). Our analyses investigated the genes for the main viral component/features, like receptors, tail, capsid, lytic and lysogenic adaptation, toxins and resistance selection of bacteria, traces of bona fide bacterial genes orthologous. We would like to define the frequency of horizontal spread of host genes using the actual genomes available (ref 2, 3). The application of bacteriophages for phage-driven therapy could be largely ameliorate improving the knowledge of genomic characterizations with similarities and differences. The number of phage families identifiable in the NCBI Taxonomy database (<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cg>) as fully sequenced is 26, beside a large number of unclassified species. A limited number (19) of archeal are present. *Pseudomonas*, *Staphylococcus*, *Vibrio*, *Lactococcus*, and *Lactobacillus* phage sequenced strains are 85, 77, 54, 34, and 19, respectively. We consider these bacterial species relevant in the applications of new phage therapies. One model approach that we developed is the study of lytic phages specific for the phytopathogen *Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of the bacterial canker of kiwifruit. Phage therapy may constitute a realistic and safe response to the urgent need for novel antibacterial agents for the control of plant bacterial pathogens like the Psa. We already isolated and characterized two lytic phages, as well as a lysogenic one. The two found lytic strains are sufficiently different to be used in a cocktail for the prevention and therapeutic treatment of the plant disease. The lytic nature of these phages was confirmed after the selection of resistant bacteria and treatment with mitomycin C. The efficacy of treatment with the phages selected for the lytic mandatory cycle was studied on in vitro tissue culture derived from kiwi undifferentiated cells. In order to evaluate its potential as biocontrol agent, the phages cocktail was utilized successfully on whole plants pre-infected with Psa (strain 8.43) in greenhouse. The efficacy of phage therapy is dependent on the bacterial strains present in the environment and it will be different in the continents where the infections take place. As a general rule the efficacy of phages should be performed on environmental sources and assayed on bacterial strains responsible for the infectivity in organisms of interest, so we intend replicate the described approach on different strains isolated from the environment or from samples of acute or endemic bacterial infections. Reference: 1) Marine Henry, Laurent Debarbieux; Virology 434 (2012) 151-161. 2) Graham F Hatfull; Curr. Opin. Microbiol 11 (2008) 447-453. 3) Patrick Deschavanne et al.; Virology J. 7 (2010) 163-175.

THE PERFECT VIRUS: NOVEL SALMONELLA PHAGE S16 FEATURES A BROAD HOST RANGE AND TAIL FIBER TOOLS FOR RAPID AND SPECIFIC DETECTION OF ITS TARGET CELLS

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Foodborne disease remains a significant challenge in all countries, causing thousands of illnesses and extensive financial losses. Bacteriophages have been shown to be useful as biocontrol agent for various pathogens, including *Listeria*, *Salmonella* and others. With respect to the need for improved rapid diagnostic procedures, the use of bacteriophage affinity proteins such as endolysin-cell wall binding domains and tail fibers has recently emerged as a superior alternative to antibodies, with respect to specific immobilization on solid surfaces such as magnetic beads. While CBD proteins are suitable for Gram-positive bacteria, the long tail fiber proteins of phages infecting Gram-negative host cells such as *Salmonella* offer the potential to expand this technology platform. We have recently isolated and characterized the novel T-even *Salmonella* phage S16, which features an unusually broad host range among the genus *Salmonella*. The S16 long tail fiber (LTF) consists of a trimer of gp37, with a single copy of gp38 attached to the tip. Fluorescently labeled LTF specifically recognizes *Salmonella* OmpC, and can decorate and label the entire cell envelope. Magnetic nanoparticles functionalized with recombinant LTF in an oriented fashion enable highly efficient magnetic separation and recovery of *Salmonella* cells from foods, even from very dilute suspensions. In conclusion, S16 is an ideal phage - it is perfectly suited not only for biocontrol purposes, but also serves as a molecular toolbox for harnessing the specificity of phage-host cell interactions for rapid diagnostics.

SMALL COLONY VARIANTS AND ITS SUSCEPTIBILITY TO PHAGE 80A AND TRANSDUCTION OF S. AUREUS

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Staphylococcus aureus is the most common cause of hospital-acquired infection and contributes significantly to patient morbidity and mortality. The ability of *S. aureus* to switch to an alternative phenotype in the presence of antimicrobial agents is clearly favourable. One of these alternatives are small colony variants (SCVs). The novel phenotypes include changes to colony morphology, antibiotic susceptibility, haemolytic activity and many other physiological activities. It is now recognised that SCVs have a deficiency in electron transport, owing to mutations affecting its efficacy. This study investigated SCVs susceptibility to phage 80α and transduction of *S. aureus* wild type and their SCVs 1-3 was studied. Wild type strain of *S. aureus* and SCV3 both yielded a high number of lysogens (~68%) the remaining being resistant mutants. SCV 1 and SCV 2 provided a much lower proportion of lysogens (4-10%). There was no obvious relationship between cellular ATP levels and lysogen formation. Consequently the frequency of lysogen formation (or that of resistance mutants) cannot be related to energy status. Transduction of ciprofloxacin resistance (*grlA*) was observed into COL wild type at a 5-10- fold higher frequency than into SCV1. Transduction of rifampicin resistance (*rpoB*) into SCVs was reduced almost 10-fold. As transduction was significantly decreased into SCVs it is hypothesised this process was influenced by ATP levels. The data thus suggests that SCV strains will be less efficient in gene exchange by transduction *in vivo*.

THE PECULIARITIES OF INTERACTIONS OF ANTIBIOTICS AND BACTERIOPHAGES ON BACTERIAL MEMBRANE RECEPTORS. NEW CONSIDERATIONS IN DETERMINATION OF PHAGE TITERS

Tamaz Mdzinarashvili, Irina Papukashvili, Nino Shengelia, Mariam Khvedelidze, I.Javakhishvili

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Continuous real-time observation of bacterial growth has a great advantage for studying the mechanisms of various compounds interactions with the bacterial cell membrane. Using the method of turbidimetry, we showed that bacterial growth pattern is influenced not only by the presence of the antibiotics and phages, but also on the concentration of them into the medium. We also showed that, the pattern and the speed of bacterial growth depends on the concentration of the liquid media. The concentration of antibiotics and bacteriophages in media are not always directly correlated to the inhibition of bacterial growth. Conversely, it is shown, that their very small amount is practically incapable of inhibiting the growth process. According to our results, receptor proteins on the bacterial cell membrane are not saturated with antibiotics or bacteriophages fully and there are free unbound membrane receptors, which we hypothesize to be the reason for uninhibited bacterial growth. Only after majority receptors are occupied, bacteriophage starts the injection process of DNA into the bacterial cytoplasm. According to our research, the biological method for enumeration of viable phage is not equal to the number of phage plaques plated Petri plate and the real quantity is several degrees higher.

LARGE-SCALE PRODUCTION OF dsRNA AND siRNA POOLS FOR RNA INTERFERENCE USING PSEUDOMONAS PHAGE PHI6 RNA-DEPENDENT RNA POLYMERASE

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RNA interference (RNAi)-based gene silencing is widely applied to study gene function and also possesses great potential for inhibiting replication of viruses in plants and animals. Since double-stranded RNA (dsRNA) molecules are required for RNAi applications, there is a need for high-throughput methods for producing and purifying large quantities of high quality dsRNA. We have setup two, flexible virus-based systems for the efficient production of dsRNA: 1) an *in vitro* system utilizing the combination of T7 RNA polymerase and RNA-dependent RNA polymerase (RdRP) of Pseudomonas phage phi6 to generate dsRNA molecules of practically unlimited length, and 2) an *in vivo* RNA replication system based on carrier state bacterial cells containing the phi6 polymerase complex to produce virtually unlimited amounts of dsRNA. For animal applications the produced dsRNA molecules can be cleaved using Dicer enzyme to produce a target-specific pool of small interfering RNAs (siRNAs). The applicability of this approach has been verified using pathogenic human viruses.

NEW GENOMES OF HALOARCHAEAL VIRUSES

E. Roine, A. Senčilo, D. Jacobs-Sera, D.A. Russell, C.-C. Ko, C. A. Bowman, N.S. Atanasova, E. Österlund, H.M. Oksanen, D.H. Bamford, G.F. Hatfull, R. W. Hendrix

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Archaeal viruses are among the least known members of the viral universe. Yet, among the small amount of viruses described there were several new viral morphotypes reported. The global search for haloarchaeal viruses revealed two most common virion morphotypes: pleomorphic and head-and-tail. Haloarchaeal pleomorphic viruses encompass a recently discovered group of euryarchaeal viruses (Pietilä et al. 2009; Pietilä et al. 2010; Pietilä et al. 2012; Roine et al. 2010; Senčilo et al. 2012). The seven described representatives of this group share the same virion architecture and genome with collinear cluster of four conserved genes. The genomes of these viruses are enclosed into lipid envelopes with two major structural proteins. Despite the shared cluster of conserved genes and overall genome synteny, there are four different types of genomes and at least two different genome replication strategies employed by these viruses. Another set of analyzed viral genomes belongs to haloarchaeal head-and-tail viruses. The studied set includes six siphoviruses and four myoviruses with genome sizes ranging from approximately 36 kbp to 144 kbp. Although two of the genomes are closely related to earlier described halovirus HF2 (Nuttall and Dyall-Smith, 1993; Senčilo et al. 2013), the rest of the viral genomes, at the nucleotide sequence level, show very few matches to sequences in the databases. Myovirus HGTV-1 currently has the largest genome among all described archaeal viruses and it also encodes 36 tRNAs as well as a predicted gene containing a zeta-toxin domain (Senčilo et al. 2013). Nuttall, S. D., & Dyall-Smith, M. L. 1993. HF1 and HF2: novel bacteriophages of halophilic archaea. *Virology* 197: 678-684. Pietilä, M.K., Roine, E. (corresponding auth.), Paulin, L., Kalkkinen, N., and Bamford, D.H. 2009. An ssDNA virus infecting archaea: a new lineage of viruses with a membrane envelope. *Mol. Microbiol.* 72:307-319. Pietilä, M.K., Laurinavičius, S., Sund, J., Roine, E., and Bamford, D. H. 2010. The single-stranded DNA genome of novel archaeal virus *Halorubrum* pleomorphic virus 1 is enclosed in the envelope decorated with glycoprotein spikes. *J. Virol.* 84:788-798. Pietilä, M.K., Atanasova, N.S., Manole, V., Liljeroos, L., Butcher, S.J., Oksanen, H.M., Bamford, D.H. 2012. Virion architecture unifies globally distributed pleolipoviruses infecting halophilic archaea. *J. Virol.* 86: 5067-5079. Roine, E., Kukkaro, P., Paulin, L., Laurinavičius, S., Domanska, A., Somerharju, P. and Bamford, D. H. 2010. New, closely related haloarchaeal viral elements with different nucleic acid types. *J. Virol.* 84:3682-3689. Senčilo, A., Paulin, L., Kellner, S., Helm, M., and Roine, E. 2012. Related haloarchaeal pleomorphic viruses contain different genome types. *Nucleic Acids Res* 40: 5523-5534. Senčilo, A., Jacobs-Sera, D., Russell, D.A., Ko, C.-C., Bowman, C.A., Atanasova, N.S., Österlund, E., Oksanen, H.M., Bamford, D.H., Hatfull, G.F., Roine, E. *, Hendrix, G.W. 2013. Snapshot of haloarchaeal tailed virus genomes. *RNA Biol.* 10:803-816.

HALOCINS OF HALOPHILIC ARCHAEA AND BACTERIA ARE ACTIVE AGAINST A BROAD RANGE OF MICROORGANISMS FROM GEOGRAPHICALLY DISTANT HYPERSALINE ENVIRONMENTS

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Halocins are antimicrobial substances produced by halophilic archaea from the family *Halobacteriaceae*. While bacteriocins and eukaryocins have been studied already for several decades, archaeal antimicrobials (archaeocins) were discovered in 1982 and, to date, remain poorly understood. In addition to halophilic archaea, some of the crenarchaeal *Sulfolobus* strains are known to produce archaeocins known as sulfolobocins. While only a few halocins have been subjected to a more detailed analysis, wide screenings of different haloarchaeal strains indicate that halocin production is a very common feature of extremely halophilic archaea. The described halocins are divided into peptide halocins (microhalocins) with a size range of approximately 3-10 kDa and protein halocins of 30-40 kDa. It has been suggested that the production of halocins might increase species diversity and give transient competitive advantages to the producer strain. We studied 90 halophilic archaeal and bacterial strains isolated from nine geographically distant hypersaline environments for halocin production. More than one third of the strains (including both archaea and bacteria) were identified as halocin producers and two thirds were sensitive to halocins. This is the first time when halocins are described for halophilic bacteria. Interestingly, close to 80 % of the halocin producer-sensitivity interactions were detected among microorganisms from different genera, and in a few cases, even across the domain boundary. In addition, most of these interactions were observed between strains from geographically distant locations indicating that halocin-producing microorganisms are distributed world-wide and can target a broad range of sensitive strains.

PHAGE THERAPY AGAINST THE FISH PATHOGENIC *FLAVOBACTERIUM COLUMNARE*

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Centre of Excellence in Biological Interactions, Department of Biological and Environmental Science and Nanoscience Center, University of Jyväskylä, P.O.Box 35, 40014 University of Jyväskylä, FINLAND Aquaculture is the fastest growing food production industry in the world. High rearing densities of fish provide a favorable environment for pathogens to multiply and transmit. Antibiotics are widely used to treat bacterial infections in fish farming, which causes problems such as environmental leakage and evolution of resistance in bacteria. *Flavobacterium columnare* is the causative agent of columnaris disease in farmed freshwater fish worldwide. In Finland the disease is of great economic importance in salmonid fingerling production, and the general severity of epidemics is increasing. We took the first steps towards controlling the disease with bacteriophages. A phage isolated from a fish farm was characterized and its genome was sequenced. Rainbow trout (*Oncorhynchus mykiss*) fingerlings and zebra fish (*Danio rerio*) were experimentally infected with a virulent *F. columnare* strain. Sterile filtered phage lysate was applied to the experimental aquaria as a treatment in different phage to bacterium ratios. The survival and symptoms of the fish as well as the bacterial and phage counts in the aquarium water were monitored. In both fish species the survival of the phage-treated fish was significantly higher compared to the fish not receiving the phage. Zebra fish that received phage treatment had 60 % higher survival than the untreated fish. In rainbow trout the phage treated fish survived similarly the control groups without bacterial exposure, and had over 40% higher survival than the fish that were not treated with phages. We also observed that the tanks treated with phage were either completely free of *F. columnare* or the survived bacteria expressed the non-virulent phenotype. As columnaris causes external symptoms and the bacterial cells are transmitted in the water, the disease is a convenient candidate for phage therapy. Our data shows that phage can efficiently increase the survival of fish exposed to *F. columnare*.

MICROENCAPSULATED BACTERIOPHAGES FOR THE BIOLOGICAL CONTROL OF *Salmonella* spp AND *Escherichia coli* O157:H7 FROM TOMATOES FRUITS SURFACE

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INTRODUCTION: Foodborne pathogens such as nontyphoidal Salmonellae and *Escherichia coli* O157:H7 are highly virulent and are also considered major foodborne pathogens worldwide. Recent antimicrobial and disinfectant bacterial resistance have renewed interest about using bacteriophages as biocontrol against foodborne bacterial pathogens. High specificity, easy handling and low production cost have been considered as phage advantages; however, susceptibility to certain environmental conditions (pH and temperature) can limit its action on fresh produce surface. Solid microencapsulation through spray drying technique has been described to be easy to perform, relative low cost and moreover has demonstrated to offer bacterial and bacteriophage protection against environmental factors, however phage microencapsulation process is very little documented. **AIMS:** To standardize the microencapsulation process of a bacteriophage cocktail for controlling *Salmonella* spp and *E. coli* O157:H7 on tomatoes surface. **METHODS:** Five wild bacteriophages, previously isolated and characterized were propagated to reach high titer by the double agar overlay, and then the bacteriophage cocktail in SM buffer was prepared. This bacteriophage cocktail was deposited with polymers (maltodextrin and modified starch) mixtures at different proportions, and the efficiency and viability of bacteriophage formulations were determined. Later, bacteriophages and polymer mixtures were subjected to microencapsulation by spray drying at different input/output temperatures; after obtaining the microcapsules, efficiency and viability of microencapsulated formulations were determined. A simplex centroid experiment design was applied for determining the optimum polymer mixture, whereas for determining the best spray drying conditions a composed central 2^k was used. **RESULTS:** Five different mixtures were obtained resulting the lineal part with a statistical significance of p=0.003. The efficiency of the five mixtures was above 90 %, however the best efficacy was found to be a binary mixture of modified starch and maltodextrin (99.0891 %). On the other hand, the optimal suitable drying conditions were: 70-85°C input temperature, and 40-45°C outlet temperature, with an efficiency of 85.0292 %. The microencapsulated bacteriophages remained viable for six weeks, and its concentration had a maximum reduction of 0.53 Log₁₀ PFU/g. **CONCLUSION:** A mixture of modified starch and maltodextrin showed to be an excellent encapsulating agent for the microencapsulation of bacteriophages by spray drying, providing an extended shelf life for its use on fresh produce surface for at least six weeks. **Acknowledgement:** The authors thank Fundación Produce Sinaloa A. C. for the financial support.

ANALYSIS OF T4 AND λ VIR PHAGES DEVELOPMENT IN SLOWLY GROWING BACTERIA UNDER DIFFERENT CONDITIONS OF AERATION

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Development of bacteriophages under standard laboratory conditions (a high-density, fast-growing bacterial culture in a rich medium at 37°C with aeration) is a well described process. However, in the natural environment, bacteria very rarely meet conditions that do not limit their growth. Strategies employed by bacteriophages to adjust their development to bacterial hosts in their natural habitats seem to be fundamental to the persistence of phages. Studies on these strategies will enable an effective use of phages as therapeutic factors and will help to prevent their destructive role in the biofermentation industry. Generally, the development of phages in bacteria depends on the physiological status of bacterial cells – especially on the bacterial growth rate (μ). Previous studies revealed that with decreasing μ , the rate of phage T4wt release and the burst size decrease while the eclipse and latent periods increase [1, 2]. However, control of development of phages in conditions with limited aeration, which frequently occur in the natural habitat, is still poorly described. In this work we present preliminary data of the analysis of T4wt and λ vir phages development in slowly growing *Escherichia coli* cultures *under different conditions of aeration*. In our experimental approach we used a chemostat system in which we can control bacterial growth rate and the aeration level. We cultivated *E. coli* with $\mu = 0.2$ (which correlated with the generation time = 3.5 h) *under aerated conditions* and under the limited presence of oxygen. We analyzed efficiency of T4wt and λ vir phages adsorption to bacterial cells. Based on the results of one-step growth experiments, we calculated parameters of T4wt and λ vir development: eclipse time, latent period time and burst size. In conclusion data presented in this poster shed new light on the development of wild-type T4wt and λ vir under conditions which are often found in the natural phage environments. Literature:

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BACTERIOPHAGE INACTIVATION KINETICS WITH PURIFIED EXOPOLYSACCHARIDES AND LIPOPOLYSACCHARIDES.

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Difficulties in the treatment of infections caused by *Pseudomonas aeruginosa* or *Klebsiella pneumoniae* are mainly due to multidrug resistance and high virulence properties of these bacteria. A separate issue is the ability of these species to form a biofilm, which prevents the penetration of drugs and promotes the survival of colonies due to the population of bacterial cells that exhibit low metabolic activity. Lytic bacteriophages specific to multidrug-resistant bacterial strains for many years have been the main subject of interest for clinical microbiologists. Due to the high antibacterial potential of both complete virions and individual enzymatic proteins (particularly endolysins and depolymerases), many scientists believe that research on phages will lead, someday, to the effective antibacterial therapies. Studies conducted on the application of lytic phage as a tool for treatment of infections require in principle to determine the phage specificity to particular host receptors. The outer membrane of gram-negative bacteria contains a variety of structures, enabling the adsorption of phage virions (exopolysaccharides EPS, lipopolysaccharides LPS, structural proteins, fimbriae). The purpose of this study was to determine the role of EPS and LPS as phage receptors for selected bacteriophages specific for *K. pneumoniae* and *P. aeruginosa* from the collection of the Department of Pathogen Biology and Immunology, Institute of Genetics and Microbiology University of Wrocław, Poland. The phage-receptor binding were measured by the virion inactivation kinetics obtained during the application of purified exopolysaccharides or lipopolysaccharides. The LPS-virion binding experiments were performed on five *Pseudomonas* environmental bacteriophages, belonging to the *Myoviridae* family, and pure LPS extracted according to Westphal O. *et. al.* with modifications, from reference *P. aeruginosa* PAO1 strain. The EPS-virion binding experiments were performed on one *Klebsiella* environmental bacteriophage, belonging to the *Siphoviridae* family, and pure *Klebsiella pneumoniae* biofilm-associated EPS extracted according to Bales P.M. *et. al.* method with modifications. According to A.M. Kropinski method, LPS ability to phage inactivation was checked by the treatment of bacteriophages with known titre with series of EPS/LPS concentrations for one hour with gentle mixing. Then the mixture was serially diluted, seeded with the host cells on TSA plates using the double-layer agar technique. The number of plaques were counted after 18 hours of incubation at 37°C. As a result irreversible phages adsorption to purified receptors (LPS or EPS) was suspected. The neutralized phage should be unable to infect bacterial host what is equivalent to inability to plaques forming. In this experiment phages formed no centers of infection on bacterial lawn when appropriate amount of polysaccharide receptor was added. Moreover, serial dilutions of purified receptors resulted in increased number of plaques visible on the bacterial lawn. Results were expressed as the concentration of exopolysaccharides / LPS which caused 50% inhibition of phage infectivity PI_{50} . Reference: 1. Kropinski A.M., Measurement of the Bacteriophage Inactivation Kinetics with Purified Receptors, *Bacteriophages, Methods in Molecular Biology™* Volume 501, 2009, pp 157-160. 2. Westphal O., Jann K., Bacterial lipopolysaccharides. Extraction with phenol-water and further applications of the procedure. In: Whistler RL, Wolfan ML, editors. *Methods in Carbohydrate Chemistry*. New York: Academic press; 1965. pp. 83–91. 3. Bales P.M., Renke E.M., May S.L., Shen Y., Nelson D.C., Purification and Characterization of Biofilm-Associated EPS Exopolysaccharides from ESKAPE Organisms and Other Pathogens, 2013, *Plos One*, Volume, Issue 6, DOI: 10.1371/journal.pone.0067950

EXPLOITING BACTERIOPHAGES AS NATURAL ANTIBACTERIALS IN MILK

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Using *Mycobacterium smegmatis* as a rapidly growing host, six mycobacteriophages were isolated from a variety of environmental sources and characterised by restriction endonuclease digestion. Morphologically, all six phages had long, non-contractile tails and isometric heads characteristic of *Siphoviridae*. All phages were relatively heat-stable up to 72°C and generally retained infectivity over a pH range of 4-10 for 60 min. Each individual phage when tested against *M. smegmatis* in both growth media and reconstituted milk typically reduced host numbers by up to 7 logs. When all 6 phages were combined as a cocktail in milk, a strong bactericidal effect on *M. smegmatis* was observed over 96 h as evidenced by a 9-log reduction in host cell numbers. Given that these phages were also capable of lysing *Mycobacterium avium* subsp. *paratuberculosis*, their ability to remain viable after incubation at 72°C for 15 minutes is significant in the context of application for control of this important pathogen, which has been isolated from both raw and pasteurised milk.

IN SILICO ANALYSIS OF HOLINS ENCODED BY KP32 AND KP34 *KLEBSIELLA* PHAGES

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Due to the severity of infections caused by multi-drug-resistant bacteria, interest and in of bacteriophages and phage therapy is steadily increasing. However, phage treatment could be risky because of the phage ability to mutate or transfer of DNA between the hosts. For this reason, utilizing of separate phage enzymes, which are able to destroy bacteria or bacterial envelopes, seems to be more promising potential therapeutic tool, than whole, active phage particles. Antibacterial properties exhibit several phage enzymes as: endolysins (which hydrolyze peptidoglycan), phage depolymerases (which hydrolyze bacterial, exopolysaccharides) and holins (which are able to perforate the cell membrane and are the main subject of this presentation). Holins are small phage proteins collaborating with phage endolysins. Holins accumulate in the bacterial cell membrane at the final step of lytic cycle making holes in it. These pores are required for transport of fully folded endolysins to its target –peptidoglycan. Holin encoding genes have enormous diversity. More than fifty unrelated holin gene families have been already described. Due to the number of transmembrane domains (TMD). Currently characterized holins are divided into three classes (class I – holins with three TMDs; class II – with two TMDs and class III- holins with only one TMD). Phage holins are extremely important because they have the potential application as anti-bacterial agents. They can also be used for destroying bacterial outer membrane which protects Gram-negative bacteria. On the poster, *in silico* comparison analysis of holins encoded by two different *Klebsiella*-specific phages: KP32 and KP34, isolated by our lab, is presented. Through the use of various bio-informatics and immuno-informatics tools (which now provide more sophisticated methods to sequence analysis) chemical and physical parameters, structural analysis with 3D structure, epitope prediction and family classification is investigated.

***IN SILICO* CHARACTERIZATION OF ENDOLYSINS ENCODED BY *KLEBSIELLA*-SPECIFIC PHAGES**

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Drug resistant *Klebsiella pneumoniae* strains cause serious hospital infections. Among these bacteria, many strains demonstrate high resistance to available antibiotics. At present time, one of the methods that hold promise to fight carbapenem-resistant *Klebsiella pneumoniae* (KPC) is phage therapy. Interest of phage research (phages are viruses that invade and lyse bacteria) has increased in recent years - mostly because they could serve as support or alternatives for chemical preservatives and antibiotics. However, after studying bacteriophages more closely the tendency for mutations and possible DNA transfer properties were observed, which could lead to unfavorable consequences for the patients. For these reasons some decided to focus deeply on viral enzymes, which hold great promise in future therapy. The greatest hopes are placed with endolysins (peptidoglycan-hydrolyzing enzymes) which have been already proven to successfully eradicate bacterial infection caused by Gram-positive bacteria, in animal model. Our group have isolated and sequenced five phages specific for *Klebsiella*, among which are: KP15, KP32 and KP34 lytic phages. Endolysins encoded by these phages hold promise in future treatment of resistant *Klebsiella pneumoniae* strains. During our research, high effectiveness of endolysin encoded by phage KP32 on permeabilized *Klebsiella pneumoniae* isolates and other Gram-negative bacteria has been confirmed. Here we present genomic and proteomic characterization of above endolysins. Genes encoding endolysins from KP15, KP32 and KP34 were subjected to *in silico* analysis, with application of available software. As the result, probable 3D structure information as well as physical and chemical data are presented. The type of enzyme (depending on the bond in peptidoglycan, which endolysin is able to hydrolyze), as well as molecular structure and the chemical formula were determined. If examined proteins show any characteristic features, such as guanine-cytosine to adenine-thymine ratio or high occurrence of hydrophobic or hydrophilic clusters, proper data are also provided. We believe that through the comparison of data collected *in silico* and *in vitro* tests, it is possible to predict or presume *in vivo* activity of investigated proteins.

PHAGE-MEDIATED BIOPROCESSING IN DECONTAMINATION OF MEAT AND DAIRY PRODUCTS CONTAMINATED WITH E.COLI

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Introduction. Microbiological safety of food products remains a pivotal problem in nutritional hygiene in the XXI century. The emergence of new technologies of low-temperature storage, film packaging, minimal processing of refrigerated raw food, prolonged transportation, and negligent attitudes towards hygiene basics among food industry workers, all increase the likelihood of growth of intestinal infection agents as Salmonella, Esherichia, Shigella, Campylobacter, Listeria, Staphylococci, and others in food products. Usage of antimicrobial agents in the food industry does not solve this problem. It lowers the degree of ecological purity in food products, thus provoking the emergence of a new category of pathogens – opportunistic bacterial strains resistant to antibiotics. Development of a new decontamination method – bioprocessing based on bacteriophages, will allow to reduce the risks of sporadic cases and outbreak of food-borne infections, and also to retain nutrition value and palatability of food products. This study aims to develop a method for phage-mediated decontamination of ground beef and milk experimentally infected with E. coli strains.

Methods. In our course research we isolated from different sources and studied several strains of bacteriophages active against Shiga-toxin producing E. coli. To form the basis of our processing aid, we selected coliphage EcD7 as the most potentially productive, based on its phenotypic and molecular-genetic characteristics. We tested the bacterial host strain chosen to cultivate the phage by a mitomycin C induction to ensure the absence of a prophage. Full genome sequencing and bioinformatical analysis substantiated EcD7 virulent nature and differentiations from the earlier known T-like myoviruses. We confirmed its safety for lab animals in preclinical trials. Preliminarily boiled milk (3 min at 100°C, 10 ml each sample) and sterilized ground beef (1 min in 70% ethanol, 5 g each sample) were contaminated with 18-hour cultures of E. coli K12 C600 strains at 10⁶ CFU/ml. One hour after the contamination the experimental samples were decontaminated with EcD7 in various titres (10⁷, 10⁸ and 10⁹ PFU/ml). Control samples were treated with physiological solution instead of the bacteriophage. The number of bacterial cells in both control and experimental samples (stored at 4±2°C) was estimated before the bacteriophage was introduced, and additionally 2, 4, 6 and 24 hours after phage decontamination. **Results.** The content of E. coli in all of the milk samples one hour after contamination was 10⁶ CFU/ml. Two hours after, elimination in experimental samples was 99.97% when EcD7 phage was used in the titre of 10⁷ PFU/ml and up to 99.99% – 10⁹ PFU/ml. E. coli was completely lysed in 10⁸ and 10⁹ PFU/ml EcD7-treated samples, and it was 4.0×10² CFU/ml when phage was used in the titre of 10⁷ PFU/ml. Meanwhile, the level of E. coli in the control samples continually increased (from 10⁷ after 4 hours to 10⁹ CFU/ml after 24 hours). The number of bacterial cells in all ground beef samples one hour after contamination was also 10⁶ CFU/ml. The total elimination of E. coli was showed in phage-treated ground beef samples 24 hours after decontamination. The content of E. coli in the control samples kept at the initial level. **Conclusions.** This study proves a high specific lytic activity of coliphage EcD7 against E. coli strains able to contaminate milk and ground beef during its harvest and processing, and cause food-borne infections. Using a new method of meat and dairy products decontamination – phage-mediated bioprocessing – completely retains nutrition value and palatability of these products.

CHARACTERISTIC OF *PSEUDOMONAS AERUGINOSA* PB1-LIKE PHAGE PS44 AND GENETIC BASIS OF *P. AERUGINOSA* PS44 RESISTANCE PHENOTYPES

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Pseudomonas PB1-like bacteriophages are representatives of the *Myoviridae* family, which have been described as obligatorily lytic and thus are considered good candidates for therapeutic applications (Essoh *et al.*, 2013; Krylov, 2013). They are components of phage cocktails that are used to treat infections by *Pseudomonas aeruginosa* strains. We characterized one of such phages – PS44, from the therapeutic phage collection of the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, PAS, in Wrocław, Poland. The 66477 bp genome of PS44 contains 95 predicted protein-coding genes. The predicted products of all of them are identical or nearly identical to the predicted gene products of certain prototypical PB1-like phages. The pattern of sequenced PS44 DNA fragments assembly suggests phage DNA packaging by the headful mechanism and the terminal redundancy of virion DNA. PS44 adsorbs quickly to its host cells and has a short propagation time. It forms clear plaques with a turbid halo, on a layer of cells of its propagation strain, indicating the activity of exopolysaccharide depolymerase in the phage tail. Despite clear plaque centers, colonies of PS44 propagation strain cells that survived the infection could be isolated. They represented three different morphological types. Surprisingly, the colonies of one type contained cells of PS44 lysogens. Moreover, the lysogenic relation of PS44 with its host appeared to be relatively stable, what makes therapeutic use of PB1-like phages questionable. Colonies of the second morphological type contained cells with a large insertion in a single locus of their chromosome. However, they occasionally segregated into heterogeneous population of PS44 sensitive and PS44 resistant cells, suggesting that the unstable insertion may be a factor which is responsible for balance between the population of PS44 and its host under certain environmental conditions. The genome of PS44 propagation host contains several mobile genetic elements, six potentially active prophages among them. Which of these elements is responsible for the unstable PS44 resistance phenotype remains to be elucidated. Essoh C, Blouin Y, Loukou G, Cablanmian A, Lathro S, Kutter E, Thien HV, Vergnaud G, Pourcel C. The susceptibility of *Pseudomonas aeruginosa* strains from cystic fibrosis patients to bacteriophages. PLoS One. 2013, 8(4):e60575. Krylov VN. Bacteriophages of *Pseudomonas aeruginosa*: Long-term prospects for use in phage therapy. Adv Virus Res. 2014, 88:227-78. This work was supported by funds from the Operational Program 'Innovative Economy, 2007-2013' (Project No. POIG01.03.01-02-003/08).

A TubZ TUBULIN HOMOLOG IN A PHAGE-ENCODED PARTITION SYSTEM

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Virulence factors of pathogenic bacteria are often carried on mobile extra-chromosomal elements. Partition systems are responsible for the process whereby large and essential plasmids are accurately positioned to daughter cells during bacterial division. They are typically made of three components, a centromere-like DNA sequence, a centromere-binding adaptor protein and an assembling partition motor NTPase. Based on the nature of the protein responsible for the movement during segregation, these systems have been classified into type I (contains an ATPase with a variation of the Walker A-type fold), type II (uses actin-like ATPases) and type III, which utilizes the tubulin/FtsZ-like GTPase TubZ. We present the 2.3Å structure and assembly properties of a new bacteriophage TubZ tubulin homolog, and unravel the *Clostridium botulinum* phage c-st type III segregation system. Phage TubZ has an overall folding related to the plasmid protein, including the characteristic C-termini with a single and long helix and a flexible tail, but shows shorter helix H6 and surface loops. Surprisingly, this structure lacks the structural element (helix H0) that in plasmid TubZ wedges between N- and C-terminal domains and drives the domain rearrangement involved in the filament twisting. However, phage TubZ shows a rotation of the two domains within one subunit and assembles with GTP and Mg²⁺ into double helical filaments. These filaments contain mainly GDP, supporting a microtubule-like end-capping mechanism and showing that the tubulin superfamily of cytomotive proteins spreads into phages. Using biochemical and biophysical approaches we have reconstructed *in vitro* several putative interactions of the prophage segregation machinery. We prove that a gene upstream from *tubZ* encodes the adaptor TubR and we localize the centromeric region (*tubS*), both essential for anchoring phage DNA to the motile TubZ filaments. Finally we describe a conserved fourth type III partition system component, TubY, which modulates the TubZ-R-S interaction.

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