Targeting Phage & Antibiotic Resistance

May 17-18, 2018 – Florence, Italy

Local Organizing Committee

Prof. Domenico Frezza

Chairmen of the Scientific Committee

Prof. Domenico Frezza

Prof. Marvin Edeas

Members of the Scientific Committee

Dr. Marco Maria d'Andrea

Prof. Gustavo di Lallo

Prof. Brian Jones

Prof. Gian Maria Rossolini

Prof. Maria Cristina Thaller

Welcome to the Targeting Phage & Antibiotic resistance 2018

Dear Colleagues,

It is an honor to host the 5th World Congress on Targeting Infectious Diseases: Targeting Phage & Antibiotic Resistance 2018 to be held on May 17-18, 2018 in Florence, Italy, the town of Francesco Redi, Leonardo da Vinci and Galileo Galilei. Francesco Redi was the first scientist who adversed the "Spontaneous generation theory" showing with brilliant experiments the inconsistence of that theory which Louis Pasteur disproved with new techniques in 19th century. Leonardo was a multiform mind curious of the structure of "Nature" and tried to answer to many fundamental questions not yet formalizing the scientific method that Galilei stated not much later once for all. Targeting Phage & Antibiotic Resistance 2018 is organized under the hospices of the International Society of Microbiota (ISM).

The interest on new methods and strategies in the struggle against infectious diseases had a strong advancement taking advantage of innovative technics and new strategies with the outcome of emergent bio-tech research. Application of these new tools not always was without hazardous consequences and even more dangerous are the unknown consequences or under estimated risks. The aim of Targeting Phage & Antibiotic Resistance 2018 is to suggest the new possible applications that can avoid negative consequences considering the past experience.

The year 2017 was the 100 anniversary of the discovery by Felix d'Herelle of the Bacteriophage and the occasion is a strong alarm about the delay in the usefulness of this tool. We wonder if it is true that well applied Phage Therapy can be a strong weapon against bacteria. There is a general doubt not well explained about the efficiency of Phage to be clarified. This point has to be scientifically demonstrated and not left as a "guess". Until now the trials had always a bug in the planning. We have to change paradigm and consider the phage not as a pill of antibiotic. The general effort to control bacteria with new techniques is growing and the number of publications in this direction is growing but not enough to resolve the even stronger risk for super bugs that can escape the control with the classic methods. A large part of the efforts is still in the path with old lines with a paradigm that is still considering the microbial world not with it's complete environment and in the equilibrium that even d'Herelle at that time considered. He hypothesized that the illness depending from a bacterial infection of our body derived from the disequilibrium upon a bacterium and the phages controlling it.

During Targeting Phage & Antibiotic Resistance 2018, we want to stimulate discussion among investigators and medical operators about new strategies to develop new tools for the struggle against dangerous bacteria whose risk is incremented by our involuntary thoughtless use of antibiotics and other classic methods that can cause resistance. The maximum efficient tools are also the maximum economical if we think that the town of Florence with the plague of 1348 reduced of 2/3 his citizens and the economy being one of the first of the occidental world dropped and recovered only after one century. There is no comparison with that time but the economic investment to carry the research necessary to develop new methodologies is anyway too low in this phase at least in Europe considering the risk we are exposed to.

During two days, stakeholders from the academic, regulatory and industrial sectors will be gathered to discuss many hot topics. The major aim of the congress is to present the strategies to overcome antibiotic resistance.

Among the strategies will be presented the recent advances of phage therapy, the different innovations to fight antibiotic resistance (peptides, enzymes, new generation of antibiotics …), the strategic role of microbiota and many other strategies.

A round table discussion will be organized between scientists, clinicians, pharmaceutical industrials and regulatory authorities to answer the following questions:

- Antibiotic resistance and phagotherapy in 2018: What is next?
- Considerations on clinical trials and regulatory aspects

We would like to thank all speakers for their contribution. Their breadth of knowledge and expertise has helped make this conference as extraordinary as it is:

Marco Maria D'Andrea, Università di Siena, Italy
Mariagrazia Di Luca, Charité-Universitätsmedizin Berlin Hospital, Germany
Brendan Gilmore, Queen's University Belfast, United Kingdom
Brian Jones, Queen Victoria Hospital NHS Foundation Trust, United Kingdom
Danish Malik, Loughborough University, United Kingdom
Scarlet Milo, Queen Victoria Hospital NHS Foundation Trust, United Kingdom
Richard Novick, New York University, USA
Aoife Rodgers, Queen's University Belfast, United Kingdom
Robert T. Schooley, University of California, USA
Alexander Sulakvelidze, Intralytix, USA
Minmin Yen, Tufts University, USA

We hope that you will join us for this dynamic and strategic program and look forward to welcoming you in Florence.

Prof. Domenico Frezza
University of Roma Tor Vergata, Italy
Local Organizer of Targeting Phage & Antibiotic Resistance

Prof. Marvin Edeas
University Paris Descartes – Institut Cochin, France
Founder & Executive Chaiman of the International Society of Microbiota

Instructions for Participants

The Abstract book contains:

- Speakers' abstracts (the abstracts of the oral presentations follow the order of the program)
- The abstract of posters on display (in alphabetic order of the presenter name)

Conference Staff: Staff at the conference registration desk will be happy to deal with any queries you may have. If we receive any messages for you, they will be announced at the break and can be collected from the desk.

Badges: Upon registration you have received your own personal badge. Please wear this badge during the entire meeting including the coffee breaks and lunch.

Chairpersons: The Chairpersons will be seated at the president's table.

Speakers: Speakers are invited to give their Power Point presentations for downloading on the computer to the technical team **OUTSIDE AND NOT INSIDE THE CONFERENCE HALL.**

The major speakers will have 20 minutes for presentation and 5 minutes for questions.
The short oral speakers will have 7 minutes for presentation and 3 minutes for questions.
As the schedule is rather tight and to allow sufficient time for discussions, we would be very much obliged if the timing requirements were respected.

Poster Contributors: Please ensure that your poster is displayed at the appropriate location and **please respect your poster number**. Poster contributors are invited to stand by their poster during the poster sessions.

Group Photo: A group picture will be realized on Monday before the lunch break with all attendees.

Speakers Dinner: A dinner is organized on May 17 between speakers and attendees. If you are interested to register, please contact the staff on site.

Personal Belongings: Please keep your valuables and working materials with you at all times. We would advise you to keep your name on the conference notes, as we may not be able to replace these if lost. **Neither Targeting Phage & Antibiotic Resistance organizers nor the venue can be held responsible for any loss or damage to your property.**

Targeting Phage & Antibiotic Resistance

May 17-18, 2018

Florence - Italy

Table of Contents

All abstracts are referenced in the Archive of the International Society of Microbiota – Archives as:

Archives of International Society of Microbiota - Archives, Volume 5 – Issue 1, 2018 – Pages 6 to 100

http://dx.doi.org/10.18143/ AISM_V5I1

Targeting Phage & Antibiotic Resistance

Abstracts for Day 1

May 17, 2018

FIGHTING MULTI-DRUG RESISTANT *KLEBSIELLA PNEUMONIAE* BY USING LYTIC PHAGES

D'ANDREA, Marco Maria[1*], MARMO, Pasquale[2], HENRICI DE ANGELIS, Lucia[1], PALMIERI, Mattia[1]
CIACCI, Nagaia[1,2], DI LALLO, Gustavo[2], DEMATTE, Elisa[1], VANUCCINI, Elisa[3], LUPETTI, Pietro[3]
ROSSOLINI, Gian Maria[4] and THALLER, Maria Cristina[2]

[1] Department of Medical Biotechnologies, University of Siena, Siena
[2] Department of Biology, University of Rome "Tor Vergata", Rome
[3] Department of Life Sciences, University of Siena, Siena, Italy
[4] Clinical Microbiology and Virology Unit, Florence Careggi University Hospital, Florence

marco.dandrea@unisi.it

Introduction: The pandemic diffusion of KPC-producing *Klebsiella pneumoniae* (KPC-Kp) represents a major public health problem, given their wide spread in nosocomial environments, their extensive multidrug resistance profiles and the very high mortality rates[1,2]. Here we characterized two phages able to specifically lyse isolates of the pandemic clones of KPC-Kp, and demonstrated their ability in the protection towards death in a *Galleria mellonella* infection model.

Material & Methods: Bacteriophages were isolated from hospital wastewaters. Host specificity was assessed by spot technique. Phages were characterized by TEM and WGS analysis. The ability of phages to protect towards death was assessed by using a *G. mellonella* infection model.

Results: Phages were able to selectively lyse specific KPC-Kp lineages (i.e. CG258-clade I, CG258-clade II)[3,4]. One phage belonged to *Myoviridae*, while the other was of the *Podoviridae* family. Phages were able to protect towards death larvae of *G. mellonella* infected by representatives of the 2 CG258 clades, including one isolate with a hypermucoviscous phenotype.

Conclusion: To our best knowledge these are the first characterized lytic phages targeting KPC-Kp strains of this pandemic CG that could be of potential interest to develop new agents for the treatment of KPC-Kp infections and for decolonization purposes.

References:

1. *Munoz-Price, L. S., et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect. Dis. 13, 785-796 (2013).*
2. *Lee, C. R. et al. Global dissemination of carbapenemase-producing Klebsiella pneumonia epidemiology, genetic context, treatment options, and detection methods. Front. Microbiol. 7, 895;10.3389/fmicb.2016.00895 (2016).*
3. *Chen, L., Mathema, B., Pitout, J. D., DeLeo, F. R. & Kreiswirth B. N. Epidemic Klebsiella pneumonia ST258 is a hybrid strain. MBio 5, e01355-14; mBio.01355-14 (2014).*
4. *D'Andrea, M. M. et al. Diversity of capsular polysaccharide gene clusters in KPC-producing Klebsiella pneumoniae clinical isolates of sequence type 258 involved in the Italian epidemic. PLoS One 9,96827. 10.1371/journal.pone.0096827 (2014).*

THERAPEUTIC BACTERIOPHAGES: PROMISE AND CHALLENGES

SCHOOLEY Robert T.[1]; ASLAM Saima[1]; HAMILTON Theron C.[2]; BISWAS Biswajit[2]; BISHOP-LILLY Kimberly[2]; MERRIL Greg[3]; LEHMAN Susan[4], MORALES Sandra[4], LANGLAIS FURR Carrie-Lynn[4], BILINSKY Igor P.[4], GRINT Paul[4]

[1]University of California, San Diego, USA
[2]Biological Defense Research Directorate, Naval Medical Research Center, Frederick, Maryland, USA
[3]Adaptive Phage Therapeutics, Gaithersburg, MD, USA
[4]AmpliPhi Biosciences; San Diego, CA, USA

Introduction: Recently there has been increasing use of therapeutic bacteriophages. Most interventions have been at the single patient level and directed at those with severe infections and limited treatment options.

Material & Methods: We have used fixed and custom phage cocktails to treat six patients at UCSD under provisions of the FDA eIND mechanism.

Results: In the process of this experience, we have gained additional insights into the clinical utility of phage as therapeutic agents related to their safety, tolerability, clinical efficacy, pharmacology and distribution following parenteral administration. We will present an overview of the clinical indications and target for which these phage were administered. Additional details will be presented related to the first two treated patients that provide insights into selected aspects of their pharmacology, distribution and the resistance kinetics of bacterial agents targeted.

Conclusion: Phage therapeutics continue be of interest as global concerns regarding the further emergence of drug resistant bacterial pathogens continues to evolve. Although insights gained may be helpful in planning additional clinical use of phage as therapeutic agents, significant gaps in our knowledge remain that can only be addressed by the design and execution of rigorously planned controlled clinical trials.

References:

1. *Schooley RT, Biswas B, Gill JJ, Hernandez-Morales A, Lancaster J, Lessor L, Barr JJ, Reed SL, Rohwer F, Benler S, Segall AM, Taplitz R, Smith DM, Kerr K, Kumaraswamy M, Nizet V, Lin L, McCauley MD, Strathdee SA, Benson CA, Pope RK, Leroux BM, Picel AC, Mateczun AJ, Cilwa KE, Regeimbal JM, Estrella LA, Wolfe DM, Henry MS, Quinones J, Salka S, Bishop-Lilly KA, Young R, Hamilton T. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant Acinetobacter baumannii infection. Antimicrob Agents Chemother. 2017 Aug 14. pii: AAC.00954-17. doi: 10.1128/AAC.00954-17.*
2. *Chan BK, Turner PE, Kim S, Mojibian HR, Elefteriades JA, Narayan D. Phage treatment of an aortic graft infected with Pseudomonas aeruginosa. Evol Med Public Health. 2018 Mar 8;2018(1):60-66. doi: 10.1093/emph/eoy005. eCollection 2018.*

CONTROL OF CATHETER ASSOCIATED BIOFILMS THROUGH EFFLUX INHIBITION

JONES BV[1], NZAKIZWANAYO JN[1], SCAVONE[1] P, PATEL BA[1], BARNES L[1], KHONDAKER MR[2], WAND M[3], SUTTON M[3].

[1]School of Pharmacy and Biomolecular Sciences, University of Brighton, UK
[2]Institute of Pharmaceutical Sciences, Kings College London, UK
[3]Infection Control Service, Public health England, UK

B.V.Jones@bath.ac.uk

Proteus mirabilis poses particular problems in the care of individuals undergoing long-term urethral catheterization. This organism forms extensive crystalline biofilm structures on catheter surfaces that block urine flow, leading to serious complications such as pyelonephritis, septicemia and shock. We have previously shown that efflux systems are important for P. mirabilis biofilm formation on catheters, and mutants defective in particular systems are less able to block catheters, highlighting potential therapeutic targets.

Subsequently, we screened a range of existing drugs already used in human medicine to identify potential efflux pump inhibitors (EPIs). Molecular modelling indicated selected EPIs showed strong interaction with efflux systems related to biofilm formation, and these compounds were also able to attenuate P. mirabilis biofilm formation and catheter blockage in laboratory models of catheter associated UTI.

Overall this suggests efflux inhibition may be a valid approach to control catheter blockage, and existing medicines have the potential to be repurposed for control of bacterial biofilm formation.

LYTIC BACTERIOPHAGES IN THE TREATMENT OF BIOFILM-FORMING BACTERIA INVOLVED IN PROSTHETIC JOINT INFECTIONS

DI LUCA, Mariagrazia

BCRT, Charité - University Medicine Berlin, Berlin, Germany;
NEST, Istituto Nanoscienze-Consiglio Nazionale delle Ricerche, Pisa, Italy

diluca.mariagrazia@gmail.com

Introduction: Prosthetic joint infections (PJI) represent a unique challenge due to the formation of biofilms, in which bacteria are up to a thousand times more resistant to antibiotics than their planktonic counterparts. The use of lytic bacteriophages is considered a promising treatment for PJI.

Material & Methods: Human saliva samples were screened for the presence of *Staphylococcus aureus* (*Sa*) lytic bacteriophages. *In vitro* anti-biofilm activity of phages, alone or in combination with antibiotics, was evaluated against *Sa* by isothermal-microcalorimetry. The phage capability of degrading the matrix and kill persister cells was investigated by confocal microscopy and cell counting, respectively.

Results: Six *Sa* bacteriophages exhibiting a broad lytic host spectrum were isolated from different saliva samples. All the tested phages showed a strong killing activity against both planktonic and biofilm-embedded *Sa*. Pre-treatment with phages followed by the administration of sub-inhibiting concentrations of antibiotic exerted a synergistic effect in eradicating *Sa* biofilm. After phage treatment, a dose-dependent reduction of matrix exopolysaccharide was observed. High phage titers showed direct killing activity on *Sa* persister cells.

Conclusion: The ability of particular phages to exert an anti-biofilm activity makes them suitable for the treatment of sessile bacteria in the context of PJI.

MICROENCAPSULATION OF PURIFIED BACTERIOPHAGES FOR TARGETED THERAPEUTIC APPLICATIONS

MALIK, Danish Javed

Chemical Engineering Deprtment, Loughborough University, Loughborough, United Kingdom

d.j.malik@lboro.ac.uk

Unleashing the therapeutic potential of bacteriophages requires careful consideration of formulation and delivery aspects which heretofore have been largely overlooked (1). Targeted delivery and controlled release of high titres of phages at the site of infection remains a challenge. Oral delivery of unformulated phages could potentially result in inactivation of phages and reduction in phage titre upon exposure to gastric acidity thereby compromising efficacy of phage therapy (2). Targeting multi-drug resistant infections caused by bacteria that lead an intracellular lifestyle is another challenging problem as free phages may not be able to access eukaryotic cells without artificial vectorization approaches.

This talk will present outcomes of recent research from our group on developing scalable microfluidic process technologies allowing precise encapsulation of bacteriophages in microcapsules and nanocapsules to address the aforementioned challenges. Two examples will be presented in depth (i) a *Salmonella* Felix O1 phage (family *myoviridae*) microencapsulated in a pH responsive polymer formulation and (ii) nanoencapsulation of *S. aureus* bacteriophage K (phage family *myoviridae*) in nanoscale liposomes.

References:

1. Malik DJ et al., Adv Colloid Interface Sci. 2017; 249:100–33.
2. Vinner GK et al., PLoS One. 2017;12(10):1–27.

DEVELOPMENT OF INFECTION-RESPONSIVE SURFACE COATINGS FOR BACTERIOPHAGE DELIVERY IN THE CATHETERISED URINARY TRACT

MILO, Scarlet; NZAKIZWANAYO, Jonathan; JONES, Brian; JENKINS, Toby

University of Bath, UK

S.Milo@bath.ac.uk

Introduction: The crystalline biofilms of *Proteus mirabilis* can seriously complicate the care of patients undergoing long-term indwelling urinary catheterisation. Expression of bacterial urease causes a significant increase in urinary pH, leading to the supersaturation and precipitation of struvite and apatite crystals. These crystals become lodged within the biofilm, resulting in the blockage of urine flow. We present an infection-responsive surface coating for urinary catheters, which releases bacteriophage in response to elevated urinary pH, in order to delay catheter blockage.

Materials & Methods: The coating employs a dual-layered system comprising of a lower hydrogel 'reservoir' layer impregnated with bacteriophage, capped by a 'trigger' layer of the pH-responsive polymer poly(methyl methacrylateco-methacrylic acid) (EUDRAGIT®S 100). Evaluation of prototype coatings using a clinically reflective *in vitro* bladder model system provides quantitative microbiological and chemical assessment of biofilm formation, as well as qualitative visual analysis of encrustation.

Results: Catheter blockage time was doubled (13 h to 26 h ($P < 0.05$)) under conditions of established infection (10^8 CFU ml^{-1}) in response to a 'burst-release' of bacteriophage (10^8 PFU ml^{-1}). Coatings were stable both in the absence of infection, and in the presence of urease negative bacteria [1].

Conclusion: Quantitative and visual analysis of crystalline biofilm reduction show that bacteriophage constitute a promising strategy for the prevention of catheter blockage, a clinical problem for which there is currently no effective control method.

Reference:
Milo, S. et al, J. Mater. Chem. B, 2017, 5, 5403-5411

IMPACT OF VIRULENT BACTERIOPHAGES ON VIBRIO CHOLERA INFECTION AND THEIR USE IN PREVENTING CHOLERA

YEN, Minmin

minmin.yen@tufts.edu

I'm investigating the population dynamics between Vibrio cholerae, the bacterium that causes cholera, and virulent bacteriophages. My current work shows that a bacteriophage cocktail is successful in preventing cholera disease in two animal models when administered up to 24 hours prior to V. cholerae infection. I will continue my bacteriophage cocktail work in the Camilli Lab as well as pursue a Master's in Public Health at Boston University.

In the future, I hope to combine my background in biological engineering and microbiology to reduce health inequities around the world.

SELECTION AND CHARACTERISATION OF PHAGES ABLE TO DEGRADE BIOFILM PRODUCED BY CLINICAL ISOLATES OF E. FAECALIS

MARMO, Pasquale[1], PERINI Nicoletta[1], KRASOJEVIC Klaudia[1], ROMANO Elena[1], THALLER MC[1], D'ANDREA Marco Maria[2], FREZZA Domenico[2], DI LALLO Gustavo[2]

[1]Dept. Biology, University of Roma Tor Vergata, Rome, Italy
[2]Dept. of Molecular Biotechnologies, University of Siena, Siena, Italy

Pasquale.Marmo@uniroma2.it

Phage therapy is a promising tool against infection sustained by antibiotic resistant bacteria. Recently, a further advantage has been envisaged, in what some phages infect bacteria inside the biofilms, where antibiotics are poorly active.
We evaluated the activity of *E. faecalis* phage Φ1.1, a new *Myoviridae* virus of the *Spounavirinae* subfamily, on 15 isolates from inpatients admitted to the Careggi University Hospital of Florence, Italy.

Results: Genomic analysis showed that Φ1.1 is characterized by a linear dsDNA of 143.5 Kb (%GC=35.8) ending with terminal repeats of 1911 bp. The closest homologue of Φ1.1 is ΦEF24C, a previously described broad-range, lytic phage of *E. faecalis*, to which Φ1.1 display a 98% nucleotide identity. A total of 203 ORFs can be observed in the Φ1.1 genome, with 4 of these that potentially encodes proteins related to digestion of bacterial cell wall. The phage infects 8 of the 15 strains that we tested. These strains were also infected in stationary phase of growth although with a lower efficiency. The production of biofilm by *E.faecalis* isolates was evaluated and its measured by Crystal-Violet staining after phage infection. Phage Φ1.1 was able to reduce the biofilm. This effect was observed also on the 7 strains that were not permissive for phage replication. The confocal microscopy biofilm analysis confirmed these results.

ANALYSIS OF THE CONSERVED GENES PRESENT IN MRSA STRAINS: CAN THEY MAKE PHAGE THERAPY HARDER THAN EXPECTED?

MIR-SANCHIS, Ignacio and RICE, Phoebe

University of Chicago, Chicago, USA

imirsanchis@uchicago.edu

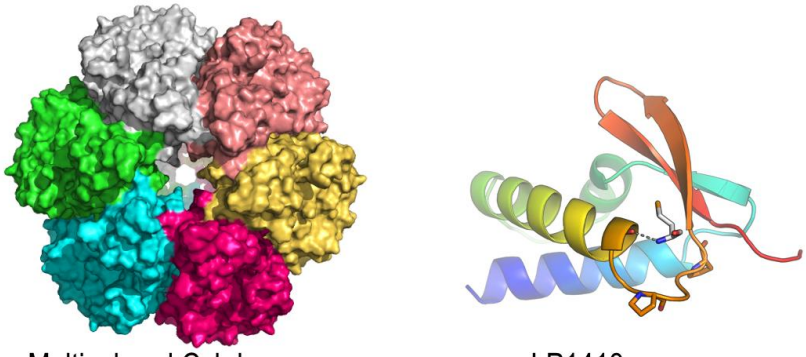
Introduction: Methicillin resistant *Staphylococcus aureus* (MRSA) is a global public health problem. The mobile genetic element responsible for this phenotype is called Staphylococcal Cassette Chromosome *mec* element (SCC*mec*). Although SCC*mec* elements were cataloged as non-replicative mobile elements, in previous work[1] we showed that SCC*mec* elements encode at least three genes whose products are related to DNA replication: an initiator of replication protein with helicase activity, Cch; a single stranded DNA binding protein, LP1413; and a uracil DNA glycosylase inhibitor, SaUGI. Staphylococcal bacteriophages from the *Myoviridae* family have been proposed as good candidates for phage therapy. A subdomain of the phage-encoded polymerase shows predicted structural homology to uracil DNA glycosylase proteins indicating that SaUGI could interfere with phage replication.

Material & Methods: X-ray crystallography to solve the structure of Cch and LP1413 at 2.9 and 2.2 Å respectively. For our phage studies we used two different phages: K and IPLA-RODI.

Results: Cch and LP1413 structures[1,2] are shown in figure1. Artificially expression of SaUGI interfered with the two *Myoviridae* phages tested, presumably by inhibiting phage polymerase.

Conclusion: SCC*mec* conserved genes are related to replication, additionally, SaUGI interferes *in vivo* with *Myoviridae* phages fitness indicating that phage therapy might be a bit harder than expected.

Figure 1 and references



Multicolored Cch hexamer (Cch and LP1413 are not at the same scale)

LP1413 monomer

1 Mir-Sanchis, I. *et al*. (2016). **Staphylococcal SCCmec elements encode an active MCM-like helicase and thus may be replicative**. *Nat. Struct. Mol. Biol*. 23, 891–898.

2 Mir-Sanchis, I. *et al*. (2018). **Crystal structure of a new single-stranded DNA-binding protein encoded by staphylococcal cassette chromosome elements.** *Structure*. In revision.

STATE-OF-THE-ART OF MODELING *IN VIVO* DYNAMICS OF NATURALLY-OCCURRING PHAGES AND *IN VIVO* DYNAMICS OF THERAPEUTIC PHAGES

VOLKOVA, Victoriya[1]; BISWAS, Biswajit[2]; BESSER, Thomas[3]; GRÖHN, Yrjö Tapio[4]

[1] Kansas State University, Manhattan, USA
[2] Naval Medical Research Center, Fort Detrick, USA
[3] Washington State University, Pullman, USA
[4] Cornell University, Ithaca, USA

vv88@vet.k-state.edu

Introduction: Mathematical modeling of naturally occurring and therapeutic phage dynamics has been limited. Modeling natural phage ecology enables evaluating phages' roles in gene distribution in pathogenic bacteria and microbiomes. Modeling phage distribution to infection sites when administered therapeutically and activity against the pathogen is needed for forecasting clinical efficacy. We will discuss the state-of-the-art and our relevant experiences.

Materials and Methods: We developed a model of natural phage infection dynamics in an enteric bacterial species in mammals, and bacterial-gene transduction by the phages (1). We developed a candidate framework for modeling pharmacokinetics of phages administered parenterally in the mammalian body, using ours (2, 3) and others (4, 5) *in vivo* data, coupled with the phage infection dynamics against the pathogen population.

Results, conclusions: Modeling the ecological dynamics of enteric phages suggested that the transfer rate of an antimicrobial-resistance gene in enteric bacteria via transduction is several-fold lower than via plasmid conjugation (1). The model can be adapted to test other hypotheses about natural phage ecology. Development of the framework of PK-PD (pharmacokinetic-pharmacodynamic) modeling of therapeutic phages revealed that phage uptake by the mammalian reticuloendothelial system, phage-specific immune activation, and switch of pathogen susceptibility are major unknowns for forecasting clinical efficacy.

References:

1. *Volkova V. V., Lu Z., Besser T., Grohn Y. T. Modeling the infection dynamics of bacteriophages in enteric Escherichia coli: estimating the contribution of transduction to antimicrobial gene spread. Appl Environ Microbiol 2014 80 (14): 4350-4362*
2. *Merril C. R., Biswas B., Carlton R., Jensen N. C., Creed G. J., Zullo S., Adhya S. Long-circulating bacteriophage as antibacterial agents. PNAS 1996 93 (8): 3188-3192*
3. *Biswas B., Adhya S., Washart P., Paul B., Trostel A. N., Powell B., Carlton R., Merril C. R. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant Enterococcus faecium. Infect Immun 2002 70 (1): 204-210*
4. *Barrow P. A. The use of bacteriophages for treatment and prevention of bacterial disease in animals and animal models of human infection. J Chem Technol Biotechnol 2001 76 (7): 677-682*
5. *Bull J. J., Levin B. R., DeRouin T., Walker N., Bloch C. A. Dynamics of success and failure in phage and antibiotic therapy in experimental infections. BMC Microbiol 2002 2: 35*

BACTERIOPHAGE THERAPY AND URINARY TRACT INFECTIONS

PEREPANOVA, Tamara

N.Lopatkin S.R.Institute of urology and interventional radiology, National medical research center of radiology of the ministry of health of the Russian Federation, Moscow, Russia

Perepanova2003@mail.ru

Introduction The growth of antibiotic-resistant pathogens of urinary infection requires a search for new treatments, with high specificity, and without influence of normal microflora. The possibility of adaptation of bacteriophages to strains of antibiotic-resistant bacteria have increased in recent years, the interest in them.

Material & Methods: In Russia, we hold regular update of phage races from natural sources on phage factory. We sent the strains of microorganisms isolated from patients of the urological hospital to phage factory during the year. Adaptation of the phages to our microorganisms, occurred due to the selection of active phage races from the collection of manufacturers. The collection is formed of highly virulent bacteriophages for many years. Purification of phages is carried out by removal of the lysed bacteria, toxins bacteria. The degree of purification = 98-99%. Drugs do not contain the "temperate" phages capable of transduction or lysogenic conversion.

As a result of the adaptation of the drugs increased the sensitivity of bacteria to phages, especially *E. coli and Proteus mirabilis* with 78.3% and 45,5% to 88% and 85%, respectively.

We studied the dynamics of the excretion of bacteriophages with urine after oral administration of 30 ml of phage investigated in healthy people, patients with homologous and homologous pathogen during the day and in 3-6 days. We proved that after oral administration of bacteriophages entering into the blood and quickly reach the affected organs - the kidneys and urinary tract, lyses bacteria and multiply and are excreted in the urine. In the presence of a bacterial infection related bacteriophages actively proliferate and can be present in the patient's body up to 6-7 days. In healthy people, the phages are released urine during the day.

Therapy with bacteriophages we started in 108 patients after determining the sensitivity to them of uropathogens. We used liquid preparations of bacteriophages (pyocianic, proteaceae, coli-phage, staphylococcal, streptococcal, *Enterobacter a*nd combined pyobacteriophage) topically and orally. The course of treatment was 7-10-14 days.

Results: The efficiency of phage therapy was evaluated by the absence or significant reduction in the number of the pathogen in the urine or in the wound, normalization of body temperature, clinical blood and urine tests, an objective condition of the wound, improvement of the clinical picture of the disease. Bacteriological monitoring is carried out through 3, 10, 20 days of treatment with phages, and 2-3 weeks after completion of therapy.

Clinical and bacteriological effect of treatment of *E. Coli, Proteus spp, Staphylococci* urinary infections made in 86-93%. And even in the treatment of *Pseudomonas aeruginosa* clinical and bacteriological effect was achieved in 81% of cases, *Enterobacter aggl*. in 77% of cases, which is equivalent to antibiotic therapy.

Conclusion: Treatment of urinary tract infections with bacteriophages is an independent effective method of antimicrobial treatment. The adaptability of phages by incorporating phage with proven adsorption properties, with a short latent period, with a high "yield" due to updates and replenish fresh cultures isolated from different natural sources gives them advantages over antibiotics.

EXPERIENCE AND PERSPECTIVES OF PHAGE THERAPY OF CARDOVASCULAR IMPLANT-ASSOTIATED INFECTIONS

RUBALSKII, Evgenii; RUEMKE, Stefan; SALMOUKAS, Christina; KUEHN, Christian; HAVERICH, Axel

Hannover Medical School, Hannover, Germany

Rubalskii.Evgenii@mh-hannover.de

Introduction: Increasing antibiotic resistance is a significant worldwide challenge, especially in medicine. One of the viable alternatives to known antibiotic therapy is the application of lytic bacteriophages. A bacterial infection associated with implanted medical devices is a severe complication for patients after cardiovascular surgery and associated with high mortality. These complications are often hard to treat due to biofilm-mediated antibiotic tolerance.

Patients & Methods: Several patients with severe infections after cardiovascular surgery obtained individualized bacteriophage preparations as *ultima ratio* therapy according to § 37 of the Declaration of Helsinki. Phage therapy applied as an addition to conventional antibiotic therapy.

Results: All patients showed significant reduction of bacteria in infected sites. Development of a phage therapy strategy has been started on the base of these results. The strategy includes both personalized approach as well as universal phage cocktails and the development of new forms for phage application.

Conclusion: Therapy with lytic bacteriophages seems to be efficient and safe. It might be a promising addition for patients who do not respond to conventional antibiotic therapy, especially for patients with infections of implanted medical devices.

CONCEPT OF INDIVIDUALIZED MEDICINE BASED ON PERSONALIZED PHAGE THERAPY FOR INTENSIVE CARE UNIT PATIENTS SUFFERING FROM HEALTHCARE-ASSOCIATED INFECTIONS

ALESHKIN, Andrey[1]; SHKODA, Andrei[2]; BOCHKAREVA, Svetlana[1]; ERSHOVA, Ol'ga[3]; MITROKHIN, Sergei; KISELEVA, Irina[1]; ZUL'KARNEEV, El'dar[1]; RUBAL'SKIY, Evgeniy[1,4]; NOVIKOVA, Lidiya[1]

[1]G.N.Gabrichevsky Moscow Research Institute for Epidemiology and Microbiology, Moscow, Russia
[2]L.A.Vorokhobov Municipal Clinical Hospital No 67, Moscow, Russia
[3]Academician N.N. Burdenko National Medical Research Center for Neurosurgery, Moscow, Russia
[4]Hannover Medical School, Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover, Germany

andreialeshkin@googlemail.com

Effectiveness of serially produced bacteriophages is high in case of intestinal and respiratory infections caused by communityacquired antimicrobial resistant pathogens. However, mass-produced phages don't consider the rapid changes in circulating strains causing healthcare-associated infections (HAIs), the formation of anti-phage immunity, phage pharmacokinetics, etc. We have developed an algorithm (Figure) of personalized phage therapy for intensive care unit (ICU) patients suffering from HAIs which increases the efficiency of phage therapy by 40 per cent. It consists of three consecutive stages:

1. Determination of sensitivity of target bacterium to bacteriophage preparation;
2. Determination by enzyme-linked immunosorbent assay of IgG-antibodies in the patients' sera active against the phages used;
3. Selection of pharmaceutical form and administration route based on the preliminary phage pharmacokinetics study.

Sound and safe update of phage compositions, used against HAI-pathogens, requires the creation of an up-to-date collection of pheno- and genotypically characterized bacteriophages that expect to be included into ready-to-use pharmaceutical forms on the first demand of the hospital.

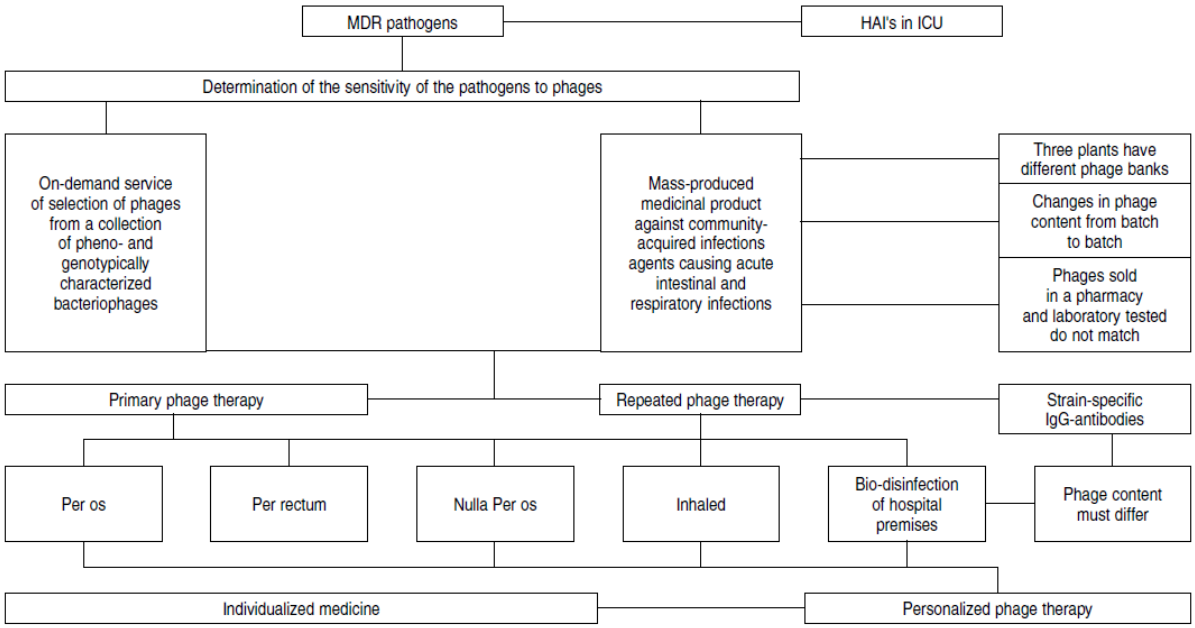


Figure: Algorithm of personalized phage therapy for ICU patients suffering from HAIs.

SAFE AND ACTIVE - SUSTAINED RELEASE OF PHAGES IN GIT

MOGHTADER Farzaneh[1, 2], EGRI Sinan[2, 3] and PISKIN Erhan[1, 2]

[1]Hacettepe University, Institute of Graduate Studies, Nanotechnology and Nanomedicine Division and Chemical Engineering Department, Ankara, Turkey
[2]NanoBMT/Biyomedtek, Cyberpark-Bilkent/KOSGEB-Başkent-Tekmer, Ankara, Turkey
[3]Gaziosmanpaşa University, Bioengineering Department, Tokat, Turkey

farzaneh_moghtader@yahoo.com

The number of antibiotic-resistant bacteria is increasing rapidly at Global level especially in the developing countries, therefore alternative therapeutic approaches to replace antibiotics is becoming one of the most important priorities in public health – Worldwide. Phages are considered as an important alternative to antibiotics. One of the main concerns in phage therapy using oral pharmaceutical formulations (usually emulsion of phages) is the loss of "antibacterial activity" during the passage via gastro-intestinal (GI) tract in which they faced with quite low pH in stomach and destructive enzymes, bile, etc. Therefore, an oral delivery system - "sustained release" - is needed to carry/protect phages from the harsh stomach conditions and release in the intestine safely/actively which is also the main rational of this study. *E.coli* and its specific T4 phages were propagated/purified by rather classical protocols with desired quantities/concentrations. Phages were first encapsulated within alginate beads by following the classical technique – dropping the phage emulsions into a $CaCl_2$ bath for cross-linking of alginate network with calcium cations then coated with two alternative polycations, chitosan and/or branched polyethylene imine (PEI). Here we demonstrate the effects of chitosan and PEI on the stability and sustained release of T4 phage in the simulated body fluid - simulating gastrointestinal track (as described in the "United States Pharmacopeia Convention).

These studies demonstrated that alginate and especially coating with polycations (both chitosan and PEI) improved the stabilities of phages at low pH and allows sustained release kinetics at intestinal conditions. The PEI coating was the most successful one.

PHAGES INTENDED FOR PREVENTING AND TREATING INFECTIONS CAUSED BY *PAENIBACILLUS LARVAE* IN HONEY BEE LARVAE

JOŃCZYK-MATYSIAK Ewa[1], ŚWITAŁA-JELEŃ Kinga[2], HODYRA-STEFANIAK Katarzyna[2], ŁODEJ Norbert[1], NEUBERG Joanna[1], KULA Dominika[1], ORWAT Filip[1], KASZOWSKA Marta[3], BAGIŃSKA Natalia[1], OWCZAREK Barbara[1], POPIELA-PLEBAN Ewa[4], MIGDAŁ Paweł[4], ROMAN Adam[4], WEBER-DĄBROWSKA Beata[1,5], GÓRSKI Andzej[1,5,6]

[1]Bacteriophage Laboratory, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

[2]Pure Biologics Inc, Wrocław, Poland

[3]Laboratory of Microbial Immunochemistry and Vaccines, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

[4]Wrocław University of Environmental and Life Sciences, Faculty of Biology and Animal Science, Department of Environment Hygiene and Animal Welfare, Wrocław, Poland

[5]Phage Therapy Unit, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy (IIET), Polish Academy of Sciences, Wrocław, Poland

[6]Department of Clinical Immunology, Transplantation Institute, Medical University of Warsaw, Warsaw, Poland

ewa.jonczyk@iitd.pan.wroc.pl

Introduction: The vast majority of commercial pollination of crop plants in the world has been done by the honey bee (*Apis mellifera*). The purpose of our studies is to identify a weapon against American Foulbrood – serious health problem of bees – which leads both to the weakening of the vitality of bee colonies and significant economic losses in agriculture and horticulture. The aim of the studies was development of a bacteriophage preparation for the prevention and treatment of American Foulbrood of honeybees larvae.

Material & Methods: For phage isolation biological and environmental samples (e.g. soil, water, wax, bees, honey) obtained both from infected and mature apiaries which have not demonstrated pathological symptoms were used. Phage isolation, lytic spectrum, activity as well as phage amplification were prepared with plate method.

Results: We have obtained a unique collection of phages specific to *Paenibacillus larvae* - the main etiological agent of the American Foulbrood. From the isolated phages we selected and characterized phages (the ultrastructure, morphology, biological properties, storage stability, genome sequencing) to formulate a cocktail which contains the lytic phages with the widest spectra.

Conclusion: We have established conditions to prepare phage preparations that could be used in prophylaxis and treatment bee families infected with American Foulbrood.

MYCOBACTERIOPHAGE BASED PLATFORMS TO DISCOVER DRUG TARGETS FOR *MYCOBACTERIA*

DAS GUPTA, Sujoy, SAMMADAR Sourabh, CALCUTTAWALA Fatema, GHOSH Shreya and SARKAR Apurba

Bose Institute, Kolkata, India

sujoy@jcbose.ac.in

Introduction: Mycobacteriophage D29 infects bacterial species belonging to the genus *Mycobacterium* which includes pathogens such as *M. tuberculosis*, the causative agent of TB. We are interested to know how this phage interacts with its mycobacterial hosts thereby causing their death.

Materials & Methods: *Mycobacterium smegmatis* cells (Msm) were infected with D29, and the temporal changes that happen in terms of viability, optical density, release of superoxide radicals, DNA damage, and cell morphology were monitored. Various phage D29 derived genes were also expressed in Msm to identify those that are host lethal.

Results: Following infection by D29, mycobacterial cells were found to be killed not to a large extent by lysis, but by other mechanisms such as release of superoxide radicals and induction of apoptosis. Screening for possible host lethal phage encoded genes led us to two candidates, one of which encoded a phosphodiesterase and the other a ribonucleotide reductase.

Conclusions:

a) Mycobacteriophage D29 can bring about host lethality through non-lytic mechanisms.

b) Two genes were identified, the translated products of which were found to be host lethal.

c) By understanding the mechanism by which D29 induces mycobacterial cell death we propose to develop novel drugs against TB.

EFFICIENT *IN VIVO* PHAGE THERAPY VIA IMMUNOLOGICAL CLOAKING

NAM, Yoon Sung, YANG, Moon Young, and YU, Jeong Heon

Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea

yoonsung@kaist.ac.kr

Introduction: In vivo anti-bacterial phage therapy is limited by the rapid clearance of phages from systemic circulation. We immunologically cloaked T7 phage to reduce phagocytosis through genetic expression of CD47-derived self-peptide.

Materials & Methods: The T7Select415-1 cloning vector was used to insert the self-peptide-encoding sequence into capsid 10B, generating 'Self-T7'. We performed the phagocytosis inhibition using J774A.1, blood circulation by fluorescence labeling and a plaque-forming assay, biodistribution, and real-time in vivo intravital imaging analysis.

Results: Self-T7 exhibited a higher level of phagocytosis inhibition, and the inserted self-peptide sequence did not affect the bacterial infection rate. Blood plaque assay and intravital mouse imaging demonstrated that Self-T7 exhibited prolonged blood circulation and delayed accumulation in the liver. For *in vivo* phage therapy, mice were i.p. injected with *E. coli*, followed by i.v. and i.p. injections of phages. The group inoculated with only *E. coli* died within 48 h. As for the group treated with the Self-T7, relatively light symptoms appeared 20 h after the *E. coli* inoculation but began to dissipate after 50-60 h. All of the mice in the group were eventually returned to normal conditions.

Conclusion: We demonstrated that the in vivo anti-bacterial activity of lytic T7 phage can be dramatically increased by prolonged blood circulation through immunological cloaking with the self-peptide.

References:

1. *Oldenborg P-A, et al. (2000) Role of CD47 as a marker of self on red blood cells. Science 288:2051-2054.*
2. *Rodriguez PL, et al. (2013) Minimal" Self" peptides that inhibit phagocytic clearance and enhance delivery of nanoparticles. Science 339:971-975.*

BACTERIOPHAGE ΦSA012 HAS A BROAD HOST RANGE AGAINST STAPHYLOCOCCUS AUREUS AND EFFECTIVE LYTIC CAPACITY IN A MOUSE MASTITIS MODEL

IWANO Hidetomo[1]*; FUJIKI Jumpei[1]; TANJI Yasunori[2] and HIGUCHI Hidetoshi[3]

[1]Laboratory of Veterinary Biochemistry, Rakuno Gakuen University, Ebetsu, Japan
[2]Department of Bioengineering, Tokyo Institute of Technology, Yokohama, Japan
[3]Laboratory of Veterinary Hygiene, Rakuno Gakuen University, Ebetsu, Japan

h-iwano@rakuno.ac.jp

Introduction: Bovine mastitis is an inflammation of the mammary gland caused by bacterial infection in dairy cattle. It is the most costly disease in the dairy industry because of the high use of antibiotics. *Staphylococcus aureus* is one of the major causative agents of bovine mastitis and antimicrobial resistance. Therefore, new strategies to control bacterial infection are required in the dairy industry. One potential strategy is bacteriophage (phage) therapy.

Methods & Results: In the present study, we examined the host range of previously isolated S. aureus phages ΦSA012 and ΦSA039 against S. aureus strains isolated from mastitic cows. These phages could kill all S. aureus (93 strains from 40 genotypes) and methicillin-resistant S. aureus (six strains from six genotypes) strains tested. Using a mouse mastitis model, we demonstrated that ΦSA012 reduced proliferation of S. aureus and inflammation in the mammary gland. Furthermore, intravenous or intraperitoneal phage administration reduced proliferation of S. aureus in the mammary glands.

Conclusion: These results suggest that broad host range phages ΦSA012 is potential antibacterial agents for dairy production medicine. Further inspection is necessary about the realistic application of phage therapy for dairy farm.

References:

1. *Synnott, A.J.; Kuang, Y.; Kurimoto, M.; Yamamichi, K.; Iwano, H.; Tanji, Y. Isolation from sewage influent and characterization of novel Staphylococcus aureus bacteriophages with wide host ranges and potent lytic capabilities. Appl. Environ. Microbiol. 2009, 75, 4483–4490.*
2. *Iwano H., Inoue Y., Takasago T., Kobayashi H., Furusawa T., Taniguchi K., Fujiki J., Yokota H., Usui M., Tanji Y., Hagiwara K., Higuchi H. and Tamura Y.Bacteriophage ΦSA012 Has a Broad Host Range against Staphylococcus aureus and Effective Lytic Capacity in a Mouse Mastitis Model. Biology (Basel). 2018;7(1). pii: E8. doi: 10.3390/biology7010008.*
3. *Iwano H., Inoue Y., Takasago T., Kobayashi H., Furusawa T., Taniguchi K., Fujiki J., Yokota H., Usui M., Tanji Y., Hagiwara K., Higuchi H. and Tamura Y. Characterization of the Lytic Capability of a LysK-Like Endolysin, Lys-phiSA012, Derived from a Polyvalent Staphylococcus aureus Bacteriophage Pharmaceuticals (Basel). 2018;11(1). pii: E25. doi: 10.3390/ph11010025.*

COMPARISON OF EFFECTIVENESS OF EXPERIMENTAL PHAGE COCKTAIL, SINGLE PHAGE AND COMMONLY USED ANTIBIOTICS IN ERADICATION *OF SALMONELLA ENTERICA* SEROTYPES FOUND IN POULTRY

KOSZNIK-KWAŚNICKA Katarzyna[1], GRABOWSKI Łukasz[2]; SPIRIDON Andreea Georgiana[3]
BOROWSKA Karolina[2]; JURCZAK-KUREK Agata[4]; WĘGRZYN Alicja[1]

[1]Laboratory of Molecular Biology, Institute Biochemistry and Biophisics, Polish Academy of Sciences, Gdańsk, Poland
[2]Department of Molecular Biology, University of Gdańsk, Gdańsk, Poland
[3]Department of Molecular Genetics, Alexandru Ioan Cuza University, Romania
[4]Department of Molecular Evolution, University of Gdańsk, Gdańsk, Poland

katarzyna.kwasnikca@biol.ug.edu.pl

Introduction: In recent years we observe a rapid development of bacterial resistance against antibiotics. One of the reasons of this phenomenon is the fact that antibiotics are often uses as feed additives for prevention of disease within a herd or for treatment of an outbreak. Use of bacteriophages is seen as one of alternatives to avoid further spread of antibiotic resistance. However, there are certain concerns regarding use of phages in farm animals and in food industry. Lower effectiveness and development of phage resistance being some of those objections.

Material & Methods: Phages infecting various *Salmonella enterica* serotypes were characterized (host range, lysis profile, phage burst) and three of them were combined into a cocktail. We have then tested the effectiveness of cocktail, single phage and commonly used antibiotics against eight *Salmonella enterica* serotypes found in poultry. We have also tested the bacteria for resistance development.

Results: We have observed that effectiveness of a single phage and phage cocktail depended on used *S. enterica* serotype, while antibiotics tended to be equally effective against all tested *S. enterica* serotypes.

Conclusion: Phages and phage cocktails can be effectively used in fighting contamination caused by *Salmonella* rods, however their range of effectiveness is more narrow than antibiotics.

ACENITOBACTER PROPHAGE MINING FOR PRODUCTION OF SPECIFIC ENDOLYSINS

ABDULAMIR, Ahmed Sahib; NSAIF, Hayder

Microbiology Dept. College of Medicine, Alnahrain University, Baghdad, Iraq

ahmed.sahib@colmed-alnahrain.edu.iq

Introduction: *Acenitobacter baumannii* infection is a wide-spread multi-drug resistant infection in wounds, burns and post-op sector in Iraq; therefore *A, baumanii* was dubbed Iraqibacter [1]. This study exploited the ability of *A. baumanii* to harbor 3-5 prophages for induction and extracting native phage endolysins. Induced prophage- endolysins mining protocols were attempted and optimized.

Material & Methods: 23 mid-exponential *A. baumannii* broth cultures were treated with 2 g/ml mitomycin-C for 16h to induce prophages [2]. The induced prophage suspension was detected using phage amplification assays. Molecular weight chromatography G-100 Sephadex and Western blot were used to extract and purify phage endolysins.

Results: 18/23 of bacterial isolates were positive for phage induction, later, only 11 phages showed consistent lytic inhibition zones. We succeeded in forming specific endolysins from induced prophages in 5 days.

Conclusion: Induction of prophages in *A. baumanii* can be an endless and quick source for therapeutic phage endolysins.

References:

1. *Abedon ST, Kuhl SJ, Blasdel BG, et al.2011. Phage treatment of human infections. Bacteriophage. 1: 66–85.*
2. *Lood R, Winer BY, Pelzek AJ, et al. 2015. Novel phage lysin capable of killing the multidrugresistant Gram-negative bacterium Acinetobacter baumannii in a mouse bacteremia model. Antimicrob Agents Chemother 59:1983–1991.*

USE OF A BIOLOG™ SYSTEM FOR MONITORING AND OVERCOMING PHAGE AND ANTIBIOTIC RESISTANCE DURING THE TREATMENT OF MDR INFECTIONS IN HUMANS

BISWAS Biswajit[1], SCHOOLEY Robert[3], HENRY Matthew[1, 2], QUINONES Javier[1, 2], BISHOP-LILLY Kimberly A[1] and HAMILTON Theron[1]

[1]Biological Defense Research Directorate, Naval Medical Research Center, Fort Detrick, USA
[2]The Geneva Foundation, Tacoma, USA
[3]University of California, San Diego, USA

biswajit.biswas.civ@mail.mil

Introduction: Global surveillance indicates that multi drug resistant (MDR) bacteria are emerging at an alarming rate. In this regard a properly formulated phage cocktail would be highly effective for overcoming antimicrobial resistance in any class of bacteria. We developed a rapid Biolog™ based process [1] to select combinations of phages for generating personalized phage cocktails, which can overcome the emergence of phage resistant bacteria in vivo [2, 3]. The details of these processes will be discussed within the context of recent therapies directed at MDR infections in human patients.

Material & Methods: (i) Vast numbers of phages were isolated from environmental samples using diverse bacterial isolates to generate a working library of phages (ii) Clinical isolates were screened against the library using our Biolog™ based process to monitor phage-host interaction. (iii) Selected phages were purified by ultra-filtration [4] for intravenous injection.

Results: Intravenous injection of our personalized phage cocktails didn't produce adverse effects in any patient and effectively steriled the blood of any circulating bacteria.

Conclusion: Our modified Biolog™ process is an ideal platform for studying phage-bacteria interactions. This system provides an incomparable high-throughput capability to study bacterial growth in presence of single or multiple phages in a continuous and automated fashion.

References:

1. *Henry, M., et al., Development of a high throughput assay for indirectly measuring phage growth using the OmniLog(TM) system. Bacteriophage, 2012. 2(3): p. 159-167.*
2. *Schooley, R.T., et al., Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails To Treat a Patient with a Disseminated Resistant Acinetobacter baumannii Infection. Antimicrob Agents Chemother, 2017. 61(10).*
3. *Regeimbal, J.M., et al., Personalized therapeutic cocktail of wild environmental phages rescues mice from A. baumannii wound infections. Antimicrob Agents Chemother, 2016.*
4. *Biswas, B., et al., Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant Enterococcus faecium. Infect Immun, 2002. 70(1): p. 204-10.*

A PHAGE-DISPLAY-GENERATED PEPTIDE THAT TRANSPORTS BIOLOGICS AND PHAGES THROUGH THE MUCOSA

KENNGOTT, Elisabeth[1]; PERNTHANER, Anton[2]; HAMANN, Alf[1]

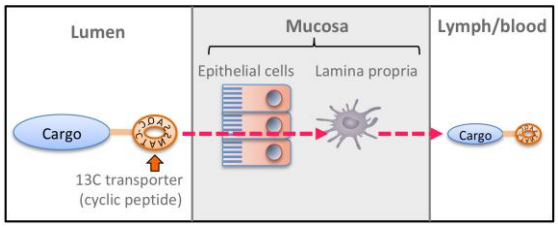
[1]Deutsches Rheuma-Forschungszentrum, Berlin, Germany
2Hopkirk Research Institute, AgResearch Ltd., Grasslands Research Institute, Palmerston North, New Zealand

hamann@drfz.de

Introduction: Large molecules such as proteins as well as hydrophilic compounds are usually not taken up upon application via the oral route, unless specific transport receptors are targeted. To overcome this hurdle for therapeutic application we developed transporter peptides that carry cargos through the mucosa.

Material & Methods: Peptides were isolated from a phage display library by in vitro and in vivo selection for binding to and transcytosis through the gut mucosa of sheep. These transporter peptides were then fused with antigenic peptides during synthesis or coupled to streptavidin via biotin. Uptake after oral, nasal or rectal application was measured by fluorescence microscopy, radioactive labeling or functional readout (local immune activation).

Results: Phages displaying the selected transporter peptides were found to cross the gut wall and to reach lymph and blood circulation. The same was found for antigenic peptides or reporter proteins (streptavidin) fused to the transporter peptide 13C. Fluorescent conjugates were first detectable in a subset of mucosal epithelial cells (most likely goblet cells) and later in dendritic cells of the underlying lymphoid tissue as well as in the blood circulation. Transgenic T cells could be stimulated by mucosal delivery of conjugates of the transporter peptide with antigenic peptide in all lymphoid organs.



Conclusion: Although only a fraction of the material is taken up, the inclusion or the transporter peptide in biologicals as well as in living phages might be a way to target these agents to and through the mucosa, allowing oral or nasal application of the therapeutic agents.

References:

1. *Kenngott EE, Pfeil J, Hoffmann U, Lauer U, Kuhl AA, Rigby A, Pernthaner A, Hamann A. 2017. Facilitated Peptide Transport via the Mucosal Epithelium: Impact on Tolerance Induction. Front Immunol 8: 216/1-10*
2. *Kenngott EE, Cole S, Hein WR, Hoffmann U, Lauer U, Maass D, Moore L, Pfeil J, Rosanowski S, Shoemaker CB, Umair S, Volkmer R, Hamann A, Pernthaner A. 2016. Identification of Targeting Peptides for Mucosal Delivery in Sheep and Mice. Mol Pharm 13: 202-10*

BIOLOGICAL REPROSPECTING OF FDA APPROVED DRUGS FOR IDENTIFICATION OF POTENT QS INHIBITOR AND ANTIBIOFILM AGENT; TARGETING *PSEUDOMONAS AERUGINOSA* LASR THROUGH ENHANCED MOLECULAR DOCKING AND DYNAMICS STUDIES

VERMA Nidhi, KANTIWAL Usha, PANDEY Janmejay, GOYAL Pankaj

Department of Biotechnology, School of Life Sciences, Central University of Rajasthan, Rajasthan, India

nv.nidhi3@gmail.com

Introduction: *Pseudomonas aeruginosa* is an opportunistic human pathogen that uses quorum sensing signaling cascade to regulate virulence genes and biofilm development. Therefore, quorum sensing system is regarded as potential target for biofilm control. Previously reported triphenyl compounds are structurally distinct from QS triggering homoserine lactone and interact specifically with LasR receptor; they may be used for screening of novel QS and biofilm inhibitors. The broad objective of the present study is to carry out bio-reprospecting of FDA approved drugs to identify potent antibiofilm agents.

Material & Methods: The present study was carried out to explore triphenyl compound TP-5 analogues as potential LasR inhibitors. FDA approved Drugs from ZINC database were used for e-Pharmacophore based virtual screening, molecular docking and dynamics and ADMET analysis.

Results: Among 1000 Phase screened FDA-approved Drugs, top 10 drug like molecules showed the extra precision Glide score from -13.395 to -12.88 and mmGBSA binding energy from -117.92 to -103.785 Kcal/mol. These results were corroborated by molecular simulations.

Conclusion: The findings indicate that the newly identified ligands were more stable in the active site of LasR protein compared to TP-5 and therefore they may serve for development of anti-biofilm therapeutics against *P. aeruginosa*.

Targeting Phage & Antibiotic Resistance

Abstracts for Day 2

May 17, 2018

ANTIMICROBIAL DISCOVERY FROM EXTREME HALOPHILES

GILMORE, Brendan F.

School of Pharmacy, Queen's University Belfast, Belfast, UK

b.gilmore@qub.ac.uk

Introduction: To date, the vast majority of clinically useful, naturally-derived antimicrobials have been discovered and isolated from terrestrial microorganisms. The application of the 'Wkasman platform' for discovery of antibiotic compounds from soil bacteria has yielded numerous important classes of antibiotics, but problems with emerging resistance and mining the same sources of terrestrial microbes has led to stagnation in antibiotic discovery. One relatively untapped source of novel chemical diversity may be organisms derived from extreme environments. Extreme halophiles (which require high salt concentrations for survival) are increasingly being explored for their biotechnological potential [1], and the aim of this study was to screen halophilic microorganisms from Kilroot salt mine, Northern Ireland, a Triassic era subterranean halite deposit for potentially novel antimicrobial activities.

Results: Screening a large culturable library of halophilic bacteria and archaea has demonstrated that over 50% of halophiles exhibit some antimicrobial activity. In addition, we have observed novel quorum sensing cross-talk between bacteria and archaea, and the production of potent archaeal quorum sensing inhibitors which when used in combination with conventional antibiotics increase biofilm sensitivity to antimicrobial challenge.

Conclusion: These data suggest that extremely halophilic bacteria and archaea are potential, yet largely unexplored, sources for novel antibiotic and anti-virulence compounds.

Reference:

A. Oren, "Industrial and environmental applications of halophilic microorganisms" Environmental Technology ***31*** *(8-9) (2010) 825-834.*

MICROARRAY PATCHES FOR PREVENTION AND TREATMENT OF INFECTIOUS DISEASES AND THEIR POTENTIAL FOR REDUCING ANTIBIOTIC RESISTANCE

RODGERS, Aoife

Queen's university Belfast, United Kingdom

arodgers11@qub.ac.uk

The human gut microbiome is a key source of antibiotic-resistance genes. Oral delivery of antibiotics contributes to development of resistance. Intravenous administration of renally-excreted antibiotics can minimise development of resistant bacteria in the gut and avoid disruption of the gut microbiome, preventing dysbiosis-associated health probolems.

However, it is impractical to admit patients to hospital for intravenous treatment every time they need an antibiotic. Novel transdermal delivery systems can deliver antibiotics directly to the systemic circulation, bypassing the gut.

We are currently developing such systems, with a view to extending the lifetime of existing antibiotics.

REINCARNATION OF A STAPHYLOCOCCAL PATHOGENICITY ISLAND AS AN ANTIBACTERIAL DRONE

NOVICK, Richard P., ROSS, Hope F. and RAM, Geeta

School of Medicine, New York University, New York, USA

Richard.Novick@med.nyu.edu

Staphylococcus aureus, long considered a dangerous, antibiotic resistant pathogen, has recently become more virulent, more contagious and more resistant, especially to β-lactams (MRSA) and glycopeptides (VRSA). Today, it causes a wide variety of life-threatening infections, many of which cannot be treated effectively with conventional antibiotics. Consequently, there is an urgent need for new ways to treat these infections, which annually cause some 18,000 deaths in the US.
We have developed a novel non-antibiotic method for treating staphylococcal infections. This method is based on the naturally-occurring, highly mobile staphylococcal pathogenicity islands (SaPIs). The SaPIs are ~15 kb genetic elements that are stably inserted in the staph chromosome but can be induced by "helper" phages to excise and replicate.

The replicated SaPI DNA is packaged in infectious phage-like particles which are released from the bacterial cell upon phage-induced lysis, resulting in high frequency SaPI transfer. The SaPIs carry and disseminate genes encoding superantigen toxins and other virulence factors. Instead of working on the prevention of SaPI spread, we hit upon the idea of exploiting SaPI spread by converting these agents of disease into agents of therapy – antibacterial drones (ABDs). To create the ABDs,we have re-engineered the SaPIs, deleting their natural cargo (toxin genes), increasing their packaging capacity from 15 to >40 kb, and inserting antibacterial modules. We have also modified the helper phage so that ABD particles are produced in the absence of functional phage. The ABD particles are administered to an infected animal (or plant), where they attach to the infecting bacteria, insert their DNA, express their antibacterial cargo genes and thus abrogate the infection.

As proof of principle, we have begun by incorporating into ABDs either CRISPR/cas9 or CRISPR/dcas9 modules with spacers targeting a chromosomal gene or the promoter region of a global virulence regulator (*agr*), respectively. Preliminary studies have shown that The CRISPR/cas9-containing ABD kills *S. aureus* in vitro by DNA cleavage, blocks the development of a subcutaneous *S. aureus* abscess, and rescues mice given a lethal dose of *S. aureus* intraperitoneally. The CRISPR/dcas9-containing ABD blocks the expression of staphylococcal virulence in vitro and blocks the formation of a subcutaneous abscess in vivo.

As infectious particles, ABDs are akin to therapeutic phages. However, they have the following major advantages:
–**Obviating resistance:** ABDs may contain 2 or more unrelated modules targeting the same process, so that resistance to one would be covered by the other(s).
–**Versatility**: Therapeutic phages kill bacteria, period, and their genomes cannot be greatly expanded. ABDs can target virtually any bacterial function and can target several simultaneously.
–**Broader host range**: Phages depend on the metabolic and biosynthetic systems of the host bacterium and are therefore subject to a wide variety of resistance mutations; ABDs express their cargo genes immediately following entry and cannot be blocked by mutation.
–**Penetration:** ABDs may express secreted lysins such as lysostaphin that can penetrate biofilms and other sites of bacterial sequestration that phages may not be able to reach.

Our plans for the future include two major initiatives: Adding new antibacterial modules to the basic system, and expanding the system to other bacterial pathogens.

MICROBIOTS & MEDICINE REVOLUTION: THE STRATEGIC ROLE OF PHAGE

EDEAS, Marvin

Cochin Institute-INSERM 1016, University Paris Descartes, Cochin Hospital, Paris, France

marvin.edeas@inserm.fr

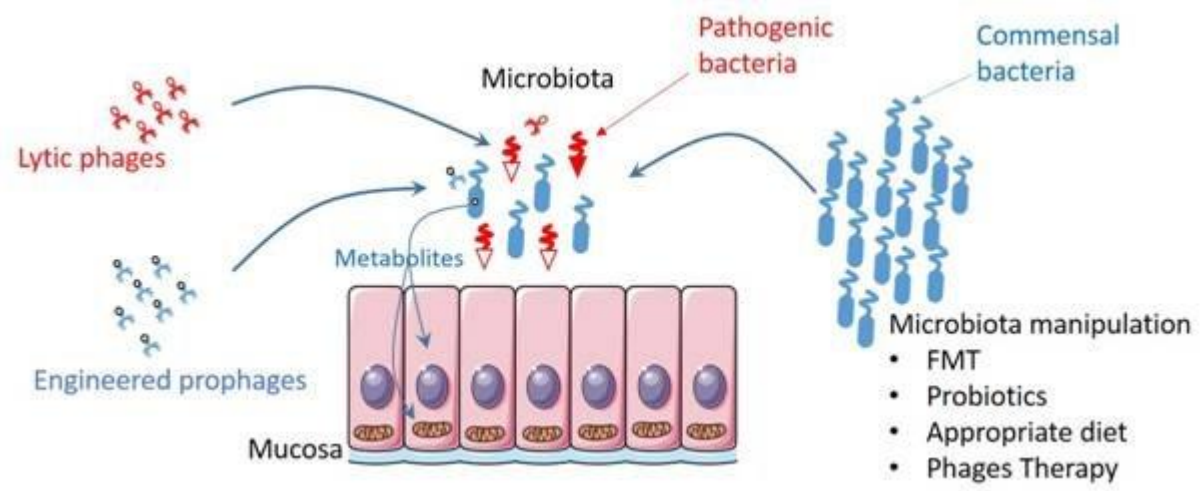
Phage therapy has been extensively used in Eastern Europe to reduce pathogenic bacteria and arise as a new method to modulate microbiota quantity and quality.

Unbalanced microbiota displayed low bacterial diversity and potentially increase proportion of pathogenic bacteria that favor mucosal inflammation.

The most strategic question is how to modulate and manipulate Gut and skin microbiota?

Manipulation of microbiota by Fecal Microbiota Transplantation (FMT); probiotics or specific diet are currently in use. Alternatively, lytic phage can be used to selectively reduce pathogenic bacteria. In addition, prophages that carry biosynthesis genes of metabolites that positively regulates mucosal inflammation can be engineered to genetically modified commensal bacteria.

New development of genetically modified phages may be an efficient tool to increase production of bacterial metabolites and subsequently decrease systemic low-grade chronic inflammation associated with chronic diseases.



Phage Therapy to modulate microbiota quality and quantity

Reference:

Saint-Georges-Chaumet, Y. and Edeas, M. Microbiota–mitochondria inter-talk: consequence for microbiota–host interaction. Pathogens and Disease, 2016, 74(1)

INHIBITION OF SHIKIMATE KINASE FROM *M. TUBERCULOSIS* AND *H. PYLORI* FOR ANTIBIOTIC DISCOVERY

GONZÁLEZ-BELLO Concepción, PRADO Verónica*, LENCE Emilio and MANEIRO María

Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares and Departamento de Química Orgánica, Universidade de Santiago de Compostela, Jenaro de la Fuente s/n, Santiago de Compostela, Spain

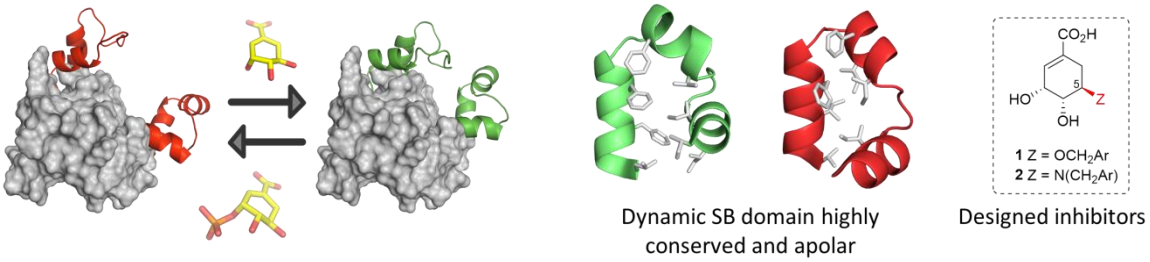
concepcion.gonzalez.bello@usc.es

Introduction: The possible development of new antibiotics by the selective and effective inhibition of an essential enzyme in important pathogenic bacteria (*M. tuberculosis* and *H. pylori*) that does not have any counterpart in human cells, shikimate kinase (SK, *aroK* gene) has been explored.

Material & Methods: The binding requirements of the natural substrates in the Michaelis Complex model and the essential enzyme motion for catalysis and product release was studied by Molecular Dynamics (MD) simulation studies. Diverse shikimic acid derivatives 1-2 were synthesized, the K_i of the compounds were measured with the isolated enzymes and the agar dilution method was used to determine their *in vitro* anti-*Helicobacter pylori* activity.

Results: Potent reversible competitive inhibitors of the SK enzyme that stabilize the open conformation for product release and capture the catalytic arginine far from the ATP binding site were developed. The 3-nitrobenzyl and 5-benzothiophenyl derivatives proved to be the most potent inhibitors. An ester prodrug was the most efficient derivative in achieving good *in vitro* activity against *H. pylori* (4 µg/mL).

Conclusion: A good scaffold for the design of reversible competitive inhibitors of the SK enzyme has been identified by targeting the dynamic apolar pocket surrounds the natural substrate.



References:

1. *González-Bello C et al. J. Am. Chem. Soc. 2013, 135, 12366.*
2. *González-Bello C et al. J. Med. Chem. 2016, 59, 5471.*
3. *González-Bello C et al. Chem. Eur. J. 2016, 22, 17988.*

GUT MICROBIOME AND VIROME AFTER HUMAN FECAL TRANSFER

MOELLING, Karin[1,2] and BROCKER, Felix[1,2,3]

[1]Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland
[2]Max Planck Institute for molecular Genetics, Berlin, Germany
[3]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, USA

moelling@molgen.mpg.de

Introduction: We described the 4.5-year time course of the enteric bacterial microbiota and virome of a patient cured from recurrent *Clostridium difficile* infection (rCDI) by fecal microbiota transplantation (FMT).

Materials & Methods: We analyzed bacterial and viral compositions in the intestine of the recovered rCDI patient and the stool donor using 16S rRNA gene and metagenomic sequencing approaches.

Results: The virome contained dsDNA viruses, mainly *Caudovirales* phages. Unexpectedly, sequences related to giant algae-infecting *Chlorella* viruses were also identified. Our findings indicated that intestinal viruses can be implicated in the establishment of gut microbiota, as phages and their host bacteria were frequently co-detected (1,2). We found the patient's phage population to exhibit highly donor-similar characteristics following FMT, which remained stable for the whole period tested (up to 7 months). In contrast to the virome, the bacterial microbiota varied indeed for more than seven months with ongoing dysbiosis before it reached donor similarity 4.5 years post-FMT (3,4).

Conclusion: Our findings are based on sequence information and protein domain analysis and suggest that stable phage properties correlate with successful FMT better than the changing bacterial communities.

References:

1. *Digestion 2013;88: 243-251.*
2. *Cold Spring Harb Mol Case Stud 2016; 2:a000448.*
3. *Gut Microbes 2016;Dec 9:1-7.*
4. *Bacteriophage 2016;6:e1251380.*

GENOME EDITING OF VIRULENT STAPHYLOCOCCAL PHAGES USING CRISPR-CAS10

HATOUM-ASLAN, Asma; BARI, S. M. Nayeemul; WALKER, Forrest C.

University of Alabama, Tuscaloosa, USA

ahatoum@ua.edu

Introduction: Virulent staphylococcal phages are potent antimicrobials that show significant promise as alternatives to conventional antibiotics. However, since over half their genes have unknown functions (*1*), these phages carry inherent risk to cause unexpected downstream side-effects. Further, their swift and destructive reproductive cycles make them intractable by current genetic engineering techniques. Here, we present strategies to genetically engineer virulent staphylococcal phages using CRISPR-Cas10, an elaborate prokaryotic immune system that uses small RNAs and a multi-subunit protein complex to detect and destroy foreign nucleic acids (*2, 3*).

Materials & Methods: A two-step process was used to harness the native CRISPR-Cas10 system in *Staphylococcus epidermidis* as a counter-selection tool to edit virulent staphylococcal phages (*4*).

Results: We show that CRISPR-Cas10 elicits robust immunity against staphylococcal phages belonging to both virulent families, *Podoviridae* and *Myoviridae*. This immunity facilitates the recovery of recombinant phages that have acquired desired mutations from a donor DNA construct. Variations of this approach can be used to facilitate the editing of toxic phage genes and access phages that infect CRISPR-less staphylococci (*4*).

Conclusion: CRISPR-Cas10 offers a powerful genetic tool that can be used to study unknown phage genes and design genetically defined phage-based antimicrobials that combat specific *Staphylococcus* species.

References:

1. *Deghorain, M. and Van Melderen, L. (2012) The staphylococci phages family: An overview. Viruses, 4, 3316–3335.*
2. *Marraffini, L.A. and Sontheimer, E.J. (2008) CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Science, 322(5909), 1843-1845.*
3. *Hatoum-Aslan, A., Samai, P., Maniv, I., Jiang, W. and Marraffini, L.A. (2013) A ruler protein in a complex for antiviral defense determines the length of small interfering CRISPR RNAs. J. Biol. Chem., 288, 27888–27897.*
4. *Bari, S.M.N., Walker, F.C., Cater, K., Aslan, B., and Hatoum-Aslan, A. (2017) Strategies for editing virulent staphylococcal phages using CRISPR-Cas10. ACS Synth. Biol., 6(12), 2316-2325.*

CRISPR-CAS9 PROMOTES THE RE-SENSITIZATION OF *ENTEROBACTERIACEAE* CLINICAL STRAINS TO B-LACTAMS

TAGLIAFERRI, Thaysa; GUIMARÃES, Natália; PEREIRA, Marcella; VILELA, Liza; FARIAS, Luiz; MENDES, Tiago; SANTOS, Simone; HORZ, Hans-Peter

RWTH Aachen University Hospital, Aachen, Germany
Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
Universidade Federal de Viçosa, Viçosa, Brazil

tltferri@gmail.com

Introduction: Considering the uncontrolled emergence and spreading of antimicrobial resistance, the aim of this study was to reverse β-lactam resistance using the CRISPR-Cas9 technology.

Material & Methods: The gRNA (guide RNA) designed to target the bla_{TEM} gene was synthesized and inserted into the CRISPR-Cas9 vector. The functionality of the Cas9 was proven by RT-qPCR and the phenotype reversal was assessed by growth curves with ampicillin, initially in *E. coli* BL21. The modified bla_{TEM} gene was sequenced for verification. The CRISPR-Cas9 was also applied to clinical *E. coli, K. pneumoniae* and *E. cloacae* strains and its efficiency was assessed by antibiograms, plasmid sequencing and whole genome sequencing (WGS).

Results: The expression of the Cas9 enzyme could be demonstrated in all control experiments. Growth curves demonstrated resistance reversal in the *E. coli* model. Sequence analysis of the edited gene showed a gRNA-mediated frameshift mutation. While clinical *E. coli* was entirely re-sensitized to 5 antimicrobials, a resistance reduction was also achieved with *K. pneumoniae* and *E. cloacae* for six antimicrobials. WGS revealed the presence of additional resistance genes with similar broad spectrum as bla_{TEM} in *K. pneumoniae.*

Conclusion: The CRISPR-Cas9 system has the potential to reverse antibiotic resistance in multidrug resistant Gram-negative bacteria.

Reference:
O'NEIL, J. Tackling drug-resistant infections globally: final report and recommendations. Review of Antimiciobial Resistance, 2016.

THERAPEUTIC APPLICATION OF PHAGE OMKO1 IN TWO CASES OF ANTIMICROBIAL RESISTANT *PSEUDOMONAS AERUGINOSA*

CHAN, Benjamin K.[1], KORTRIGHT, Kaitlyn E.[1], APPELL, Casey[2], KANU, Adaobi[2], TURNER, Paul E.[1]

[1]Yale University, New Haven, CT USA
[2]Texas Tech University Medical Center, Lubbock, TX USA

benjaminchanphd@gmail.com

Introduction: The increasing prevalence of antimicrobial resistant infections coupled with the lack of viable alternatives has presented the opportunity to re-examine phage therapy as a potential means by which these infections could be managed. We recently described phage OMKO1, a phage observed to force an evolutionary trade-off resulting in re-sensitization to chemical antimicrobials. Here, we present the clinical course and therapeutic application of phage OMKO1 in two cases of antimicrobial resistant *Pseudomonas aeruginosa* infection. These cases suggest that clinical application of this phage can be highly effective at either eradication or antibiotic re-sensitization of *P. aeruginosa* and merit further examination.

Materials & Methods: Phage OMKO1 was applied with ceftazidime at the distal portion of a draining aorto-cutaneous fistula associated with a contaminated Dacron prosthesis. The second application consisted of nebulization for 10 days in a cystic fibrosis associated PDR-*P. aeruginosa* infection.

Results: Complete resolution of infection was observed in the contaminated indwelling prosthesis after 6 weeks without recurrence for two years. Complete re-sensitization to antimicrobials was observed in sputum culture following phage nebulization in the second case.

Conclusion: Therapeutic use of phage OMKO1 can result in eradication or re-sensitization to antimicrobials in divergent cases of *P. aeruginosa* infection.

MYCOBACTERIAL TUBERCULOSIS NADD, A PROMISE FOR TARGETING LATENT AND DRUG-RESISTANT TUBERCULOSIS

SORCI, Leonardo[1],*; RODIONOVA, Irina[2]; SERGIENKO, Eduard[2]; RHODE, Kyle[3]; CATANZARO, Antonino[4]; KOROTKOV, Konstantin[5]; OSTERMAN, Andrei[2]

[1]Polytechnic University of Marche, Ancona, Italy
[2]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, United States
[3]University of Central Florida, Orlando, FL, United States
[4]University of California San Diego, La Jolla, CA, United States
[5]University of Kentucky, Lexington, KY, United States

l.sorci@staff.univpm.it

Introduction: conventional treatments to combat Tuberculosis (TB) epidemic are falling short, thus encouraging the search for novel antitubercular drugs acting on unexplored molecular targets.

Material & Methods: MICs of the compounds were determined using bioluminescent strains of *M. abscessus* (Mab) and *M. tuberculosis* (Mtb) in solid white 384-well microtiter plates. Intracellular NAD level was assessed by commercial kit (MAK03, Sigma).

Results: we screened 1,500 mycobactericidal compounds against *Mycobacterium tuberculosis* NaMN adenylyltransferase (*Mtb*_NadD), a key enzyme in the biogenesis of NAD cofactor that was recently validated as a new drug target(1-2) for dormant and active tuberculosis(3-4). We found three chemotypes that efficiently inhibit *Mtb*_NadD at low micromolar range *in vitro*. SAR and cheminformatics studies of commercially available analogs point to a series of substituted benzimidazoles with bactericidal activity on *M. tuberculosis* (including MDR-Mtb), *M. smegmatis* (in replicative and dormant states), and *M. abscessus*. The on-target activity was supported by a rapid decline in NAD(H) levels in *M. smegmatis*. A co-crystal structure of *Mtb*_NadD with N2-8 inhibitor reveals that the compound is anchored to a hydrophobic pocket adjacent to the active site, paving the way for the development of a new generation of selective *Mtb*_NadD bioactive inhibitors.

Conclusion: these results strongly suggest that pharmacological inhibition of *Mtb*_NadD is an effective strategy to combat dormant and resistant Mtb strains.

References:

1. *Sorci, L., et al., J Bacteriol 2013, 195 (4), 726-32.*
2. *Sorci, L., et al., Chem Biol 2009, 16 (8), 849-61.*
3. *Rodionova, I. A., et al., MBio 2014, 5 (1), e00747-13.*
4. *Rodionova, I. A., et al., J Biol Chem 2015, 290 (12), 7693-706.*

USING PHAGE TO SELECT FOR EVOLUTION OF REDUCED VIRULENCE IN PATHOGENIC BACTERIA

TURNER, Paul E.[1,4]; CHAN, Benjamin K.[1]; KORTRIGHT, Kaitlyn E.[1]; SISTROM, M.[2];
WERTZ, John E.[1]; LAWRENZ, M.[3]; WARAWA, J.[3]; NARAYAN, D.[4]

[1]Yale University, New Haven, USA
[2]University of California, Merced, USA
[3]University of Louisville School of Medicine, Louisville, USA
[4]Yale School of Medicine, New Haven, USA

paul.turner@yale.edu

Introduction: Increasing prevalence of multi-drug-resistant (MDR) bacterial pathogens necessitates novel antibacterial strategies. Ideally, new approaches would select for reduced pathogenesis in target bacteria, because evolution of therapy resistance is inevitable(1-2).

Material & Methods: We isolated lytic bacteriophage OMKO1 (family Myoviridae) of *Pseudomonas aeruginosa* that utilizes outer membrane porin M (OprM) of multidrug efflux systems MexAB and MexXY in cell binding. We conducted *in vitro* and mouse studies with phage OMKO1 to test whether adjunctive therapy (phage plus antibiotics) is superior to traditional antibiotics, when targeting MDR *P. aeruginosa*.

Results: *In vitro* studies show that phage selection produces an evolutionary trade-off in MDR *P. aeruginosa*: evolution of phage resistance changes the efflux pump mechanism, causing increased drug sensitivity across antibiotic classes(1). Additional experiments show phage-antibiotic synergy in reducing bacterial biofilm densities on artificial substrates such as Dacron(3). Similarly, safety/efficacy experiments in a mouse model of acute respiratory disease show phage-antibiotic synergy that improves treatment of pneumonia caused by *P. aeruginosa*.

Conclusion: Phages such as OMKO1 represent a new approach where viruses select for MDR bacteria to become increasingly sensitive to traditional antibiotics, thereby extending the lifetime of current antibiotics and potentially reducing incidence of antibiotic resistant infections.

References:

1. *Chan, B.K., M. Sistrom, J.E. Wertz, K.E. Kortright, D. Narayan, and P.E. Turner. 2016. Phage selection restores antibiotic sensitivity in MDR* Pseudomonas aeruginosa. *Scientific Reports 6:26717.*
2. *Chan, B.K., K. Brown, K.E. Kortright, S. Mao, and P.E. Turner. 2016. Extending the lifetime of antibiotics: how can phage therapy help? Future Microbiology 11(9):1105-1107.*
3. *Chan, B.K., P.E. Turner, S. Kim, H.R. Mojibian, J.A. Elefteriades, and D. Narayan. 2018. Case Study: Phage treatment of an aortic graft infected with* Pseudomonas aeruginosa. *Evolution, Medicine and Public Health 2018(1):60-66.*

PHAGE-BASED ANTIMICROBIALS: NOVEL APPROACHES FOR MANAGING DRUG-RESISTANT BACTERIA

SULAKVELIDZE Alexander

Intralytix, Inc., Baltimore, USA

asulakvelidze@intralytix.com

Bacteriophages are bacterial viruses that are arguably the oldest (ca. 3-billion-years-old) and most ubiquitous organisms (ca. 10^{30}-10^{31} virions) on Earth. Lytic phages have remarkable *bactericidal* potency against their specific bacterial host strains. In contrast to broad-spectrum antibiotics, phages usually only lyse strains or subgroups of strains of their targeted bacterial species, which makes targeted bacterial killing possible. Importantly, the mechanisms by which phages kill bacteria, and the mechanisms of bacterial resistance to phages, are different from those for antibiotics. Therefore, phages potentially can help to reduce the emergence of antibiotic resistance in two major ways:
(1) using phages in agricultural applications; e.g., to reduce contamination of foods with specific foodborne bacteria (the approach commonly called “phage biocontrol”), and
(2) prophylactic or therapeutic applications of bacteriophages for managing infectious diseases of humans and domesticated, commercially important animals (the approach commonly called “phage therapy”).

The presentation will give the audience a current perspective about the history of bacteriophage therapy research, key differences between antibiotic and phage therapies and how one approach can complement another, and the crucial regulatory and human safety issues concerning the use of bacteriophages in various applications ranging from food safety to dietary supplements / probiotics to therapeutic applications.

NOVEL PHAGE BASED THERAPEUTICS TO ADDRESS ANTIBIOTIC RESISTANCE

ZAK, Naomi

BiomX, Israel

naomiz@biomx.com

BiomX is mission focused on developing novel therapeutics to alleviate human disease, stemming from the imbalance of the microbiome. Microbiome is a fast-developing new field at the interface of nutrition and pharmaceuticals, driven by the realization that microbials residing mainly in our gut, stomach and skin play a meaningful role in causing/alleviating disease.

We utilize a 3 tier platforms offering an end-to-end solution for the rapid analysis of complex microbiomes, identifying natural or designing synthetic phage and/or adding bacteria which undergo development into microbiome modulated drugs. Our platform applies expertise of our scientific founders, Prof. Rotem Sorek, phage biology expert, Dr. Eran Elinav, world leader microbiome research and Prof. Timothy K. Lu of MIT, phage synthetic modulation expert.

Our pipeline is diverse – addressing Acne, IBD, gastric and colorectal cancer and cancer immune therapy drugs. Our most advanced program for acne and IBD are at the pre-clinical development stages and are comprised of phage cocktails that eradicate specific bacteria shows to be resistant to antibiotics. For example, BX002 is a naturally occurring phage cocktail aimed at eradicating several proprietary bacteria targets associated with the onset of IBD. The associated IBD bacteria are resistant to antibiotics and necessitate revision of current approaches. BX002 offers a novel means to eradicate these bacteria and provides a unique therapeutic approach to the disease.

BiomX recently completed a series A financing round of $24 million, led by OrbiMed, Johnson & Johnson Innovation – JJDC, Inc. and Takeda Ventures, Inc. with participation from Seventure Partners, MiraeAsset, SBI Japan-Israel Innovation Fund and other European investors. Proceeds are used to drive the product pipeline to clinic and improve our technological platform.

ENGINEERING BACTERIOPHAGE RECOGNITION BASEPLATES IN STAPHYLOCOCCAL PHAGES

RADDING, Jeffrey; WARNER, John; FERULLO, Daniel

EnBiotix, Inc., Cambridge, MA, USA

jeff.radding@enbiotix.com

Introduction: Staphylococcus aureus (*Sau*) isolates from prosthetic joint infections (PJIs) were infected by *Picovirinae* phages. Comparison of phage sequences and range of infectivity in *Sau* isolates among related phages implicated ORF14 as a determinant of phage infectivity. We engineered GRCS phage using Cas9 technology to replace ORF14 with homologous genes from other phages and tested for host range specificity[1,2,3].

Material & Methods: Phage infectivity was tested against 33 Sau clinical isolates from human PJIs by efficiency of plating. *S. pyogenes* CAS9 with GRCS-targeting crRNAs was cloned into pCN33. Sau RN4220 harboring Cas9 was infected by GRCS to induce DNA cleavage. Subsequent phage genome editing by allelic exchange was achieved by provision of a second plasmid with flanking DNA homology.

Results: A) Phage immunity was induced by targeting dual sites in the phage genome, B) allelic exchange was demonstrated using major capsid protein genes from different phages, C) allelic exchange of ORF14 homologues, resulted in altered phage infectivity for *Sau* isolates.

Conclusion: Cas9 engineering of small phage genomes enables the alteration of phage properties including bacterial recognition. This approach will allow for rapid elaboration and modification of natural phages to more efficient and effective therapeutics.

References:

1. *Bikard, D., et al., Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. Nat Biotechnol, 2014. 32(11): p. 1146-50.*
2. *Bari, S.M.N., et al., Strategies for Editing Virulent Staphylococcal Phages Using CRISPR-Cas10. ACS Synth Biol, 2017.*
3. *Lemay, M.L., D.M. Tremblay, and S. Moineau, Genome Engineering of Virulent Lactococcal Phages Using CRISPR-Cas9. ACS Synth Biol, 2017. 6(7): p. 1351-1358.*

BRIDGING A GAP IN PHAGE THERAPY: TOWARDS FAST AND EFFICIENT PRODUCTION OF HIGHLY PURIFIED PHAGES FOR VARIOUS APPLICATIONS

SMREKAR, Frenk; KUZNIK, Beti

JAFRAL, Ljubljana, Slovenia

frenk.smrekar@jafral.com

Phage product is usually multivalent product, meaning that more phages compose final product. This provides manufacturing challenges as each phage usually requires its own development work, including host pairing, growth optimization, downstream optimization and formulation studies. This can discourage companies or research groups to manufacture phages and consequently provide phage-based products for end users.

JAFRAL implemented phage manufacturing platform that can link early stage research to process focused development and manufacturing, thereby facilitating progression towards phage applications. Extensive know-how and careful selection of analytical methods enable fast development of multicomponent highly purified phage products that can be adjusted for various phage applications, such as in veterinary, cosmetics, diagnostics, food industry or as human therapeutics.

Each product is first produced at 1-5L scale by applying scalable, industriable, GMP-compliant materials, which is then followed by 10L scale and finally 50-100L large scale production. Our products are highly concentrated (>10e11PFU/mL) and highly purified (low levels of endotoxins, proteins and DNA). By frequently implemented analytical methods along the process, we optimize the process, which consequently enables to include higher number of component phages in multivalent product. This results in increasing efficacy of phage product, at more affordable costs.

JAFRAL is a contract manufacturing organization (CMO) and contract research organization (CRO) specialized in phage production. JAFRAL provides a valuable resource that is helping companies and research groups develop and produce their applied phage products at desired quantity and quality.

References:

1. *Cornelissen A. 2010. Single-step concentration and purification of bacterial viruses using CIM monolithic columns MSS 2010, Portoroz.*
2. *Smrekar F. 2008. Purification and concentration of bacteriophage T4 using monolithic chromatographic supports. J Chrom B*
3. *Smrekar F. 2011. Optimization of lytic phage manufacturing in bioreactor using monolithic supports. J Sep Science*
4. *Smrekar F. 2010. Characterization of methacrylate monoliths for bacteriophage purification. J Chrom A*

ADDRESSING CHALLENGES FOR THE CLINICAL DEVELOPMENT OF PHAGE PRODUCTS

MORALES, Sandra

AmpliPhi Biosciences, Sydney, Australia

spm@ampliphibio.com

A century of reported successful treatment of recalcitrant bacterial infections supports the general belief that bacteriophage therapy can be safe and efficacious. However, the shortage of randomised, blinded, placebo-controlled clinical trials creates healthy skepticism about overall efficacy and whether phage therapy can be implemented in widespread clinical practice. Running such trials involves a number of scientific and practical challenges.

In addition to regulatory and logistical requirements for thorough phage characterisation and consistent manufacturing to scale, the design of trials themselves can be difficult. Classical challenges for investigating the clinical efficacy of an investigational product include endpoint and comparator selection as well selection of inclusion and exclusion criteria that can affect enrollment rates. Novel challenges for phage therapy include the development and validation of new assays for outcome assessments and the identification of optimal dosing regimens without the benefit of the PK/PD methods typically used to bridge animal and human antibiotic dosing. AmpliPhi Biosciences has run Phase I studies following Good Clinical Practice guidelines and manufactured phage products under current Good Manufacturing Practices (cGMP) at AmpliPhi's fully owned GMP-certified manufacturing facility.

We will discuss the unique scientific and CMC (Chemistry, Manufacturing, and Controls) considerations for the advancement of clinical phage therapy.

DETECTION OF BACTERIA IN AIR BY USING AN AIR SAMPLER CARRYING PHAGES AND GOLD NANOPARTICLES BY RAMAN PROBE

MOGHTADER Farzaneh[1,2], HABERAL Orhan Erdem[2,3] and PISKIN Erhan[2,4]

[1]Hacettepe University, Institute of Graduate School of Science and Engineering,
Nanotechnology and Nanomedicine Division, Beytepe, Ankara, Turkey
[2]NanoBMT/Biyomedtek, Cyberpark-Bilkent/KOSGEB- Baskent Univesrsity -Tekmer, Ankara, Turkey
[3]Baskent University, Biomedical Engineering Department, Baglica, Ankara, Turkey
[4]Hacettepe University, Chemical Engineering Department

erhanpiskin@icloud.com

Introduction: A specially designed air-sampler was used to collect the bacteria suspended in the air in a working air-conditioned chamber. It was possible to change/control the air-flow through rate and therefore the amount of air recirculated through the air sampler.

Material & Methods: The positively charged nano-fibrous PCL-chitosan matrices were electrospun from the respective polymers and used as the bacterial collecting platforms in the air sampler. Gold nanoparticles were deposited onto these platforms (filters) by ultrasound-triggered reduction chemistry from gold salt solutions. A Raman probe was mounted onto the air-sampler which was used for monitoring phage-target bacteria interactions on the filter. The target bacteria - *E.coli* and its specific T4 phage were propagated/purified by rather classical protocols with desired quantities/concentrations. *S.aureus* was used as the negative controlled (i.e. non-target). T4 phages were immobilized physically onto the electrospun matrice surfaces. The air flow rate was optimized to catch the bacteria in healthy forms. The air in the air chamber was contaminated with the target or non-target bacteria with the desired (different) quantities/concentrations.

Results: Airborne bacteria were trapped onto the filters were detected by Raman probe in real-time. It was possible to enhance significantly the bacterial spectral peaks by using the plasmonic gold nanoparticles. The rapid-time dependent changes as a result of bacterial invasion by phages - the steep increase in their concentration on the filters clearly monitored by the Raman probe.

Conclusion: These studies demonstrated that bacterial contaminations in air could be detected/monitored real-time by using this novelistic air-sampler.

References:

1. *Moghtader F, Eğri S, Piskin E. Phages in modified alginate beads. Artificial cells, Nanomedicine and Biotechnology, 45.2 (2017): 357-363.*
2. *Moghtader F, Congur G, Erdem A, Zareie H, Piskin E, Impedimetric detection of pathogenic bacteria with bacteriophages using gold nanorod deposited graphite electrodes. RSC Advances 6.100 (2016): 97832-97839.*

SALMONELLA RISSEN ϕ1: A MOLECULAR SWITCH

PAPAIANNI, Marina; FULGIONE, Andrea; CONTALDI, Felice; IANNACCONE, Marco; CAPPARELLI, Rosanna

Department of agricultural, University of Naples Federico II, Naples, Italy

marina.papaianni@unina.it

Introduction: Bacteria develop resistance against phages by losing the phage receptor (Labrie et al., 2010; Capparelli et al; 2010) or reducing its binding specificity or by a temporary change of phage receptor specificity (Drexler et al., 1989). Here we describe the phage resistance mechanism adopted by S. Rissen which acts through a molecular switch.

Material & Methods: Phage was isolated from the *S.* Rissen strain (R^W) and used to select the phage resistant strain R^Rϕ1+. We evaluate both the differences in bacterial morphology and the genetic variations between the two strains by biochemical and comparative genomic analyses.

Results: Biochemical analyses showed that the presence of the phage influences biofilm production (fig 1), phage resistance and the switch of the O-antigen from smooth to rough (fig 2), Genomic analysis showed that the sensitive and resistant strains differ by 10 genes. Only the *phosphomannomutase_1* and 2 genes, involved in mannose synthesis pathway, showed different expression levels (fig. 3). The SNP of the two genes are located near HTs known to regulate phase variation (Scott et al., 2007). We used *S* rissen to see whether a character under strong selection pressure- such as phage resistance is repeatable. In four independent experiments, phage resistance was acquired by the same molecular mechanism.

Conclusion: *S.* rissen uses the same and evolutionary flexible tool (phase variation) to control several characters: biofilm production, phage resistance, and O-antigen structure.

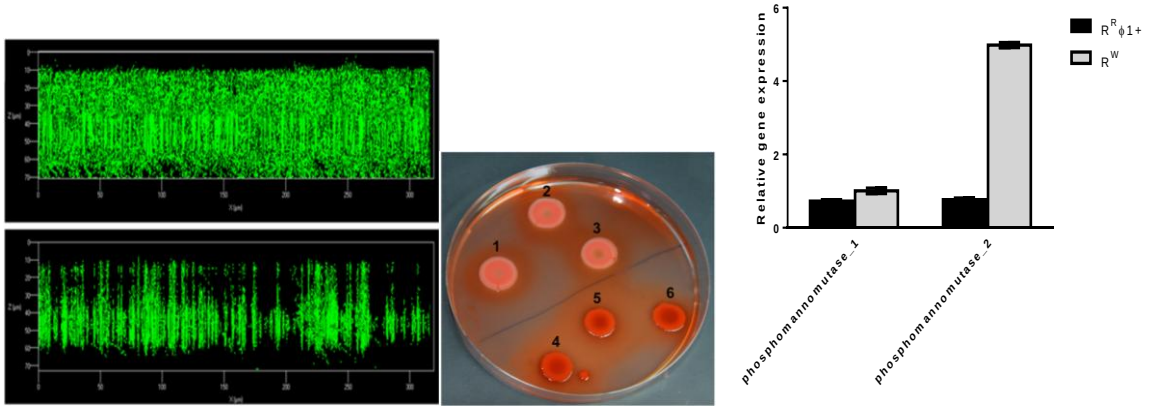


Fig. 1: *Confocal Laser Scanning Microscopy (CLSM). CLSM biofilm analysis of S. Rissen R^Rϕ1+, and S. Rissen R^W Green fluorescence (SYTO9) indicates viable cells and red fluorescence (PI) dead cells.*

Fig. 2: *R^W and R^Rϕ1+ cells phenotypes. R^W (1,2,3) displays the pdar phenotype while R^Rϕ1+ (4,5,6) that rdar,*

Fig. 3: *Real time PCR. The resistant strain (R^Rϕ1+) displays significantly reduced expression levels of both genes compared to the sensitive strain (R^w).*

References:

1. *Labrie, S.J., Samson, J.E., Moineau, S., (2010). Bacteriophage resistance mechanisms. Nat. Rev. Microbiol. 8(5): 317-327.*
2. *Capparelli R., Nocerino N., Iannaccone M., Ercolini D., Parlato M., Medaglia C., Iannelli D. (2010). Bacteriophage therapy of Salmonella enterica: a fresh appraisal of bacteriophage therapy. J Infect Dis.;201(1):52-61*
3. *Drexler, K., Riede, I., Montag, D., Eschbach, M.L., Henning, U. (1989). Receptor specificity of the Escherichia coli T-even type phage Ox2. Mutational alterations in host range mutants. J. Mol. Biol. 207(4): 797-803.*
4. *Scott, A.E., Timms, A.R., Connerton, P.L., Loc, C.C., Adzfa, R.K., Connerton, I.F. (2007). Genome dynamics of Campylobacter jejuni in response to bacteriophage predation. PLoS Pathog. 3(8):e119.*

A PHASE 1 CLINICAL TRIAL TO EVALUATE THE SAFETY, TOLERABILITY AND PRELIMINARY EFFICACY OF BACTERIOPHAGES IN PATIENTS WITH *STAPHYLOCOCCUS AUREUS* CHRONIC RHINOSINUSITIS

VREUGDE, Sarah[1] on behalf of
Ooi, Mian Li,[1]; DRILLING, Amanda J [1]; MORALES, Sandra[3]; MORAITIS, Sophia[1]; MACIAS-VALLE Luis[1,2]; PSALTIS, Alkis J[1]; WORMALD, Peter-John[1]

[1]The University of Adelaide, Adelaide, Australia
[2]Universidad La Salle, Mexico City, Mexico
[3]AmpliPhi Australia, Brookvale, New South Wales, Australia

sarah.vreugde@adelaide.edu.au

Background: *S. aureus* infections are associated with recalcitrant chronic rhinosinusitis (rCRS). The emergence of multidrug resistant *S. aureus* stresses the need for the development of new antimicrobial therapies. Phage therapy has gained significant interest recently, however its clinical application is hindered by a lack of safety and efficacy data.

Methods: rCRS patients presenting with signs and symptoms of sinus infection with positive *S. aureus* cultures sensitive to the bacteriophage-based treatment AB-SA01 were included in the study. Three patient cohorts (n=3 patients/ cohort) were serially dosed with twice daily sinus irrigations of AB-SA01 at a concentration of (1) $3x10^8$ PFU for 7 days; (2) $3x10^8$ PFU for 14 days; (3) $3x10^9$ PFU for 14 days. Safety observations included vital signs, physical examinations and clinical laboratory tests. Preliminary efficacy was assessed comparing pre- and post-treatment microbiology results, endoscopic Lund Kennedy Scores (LKS) and symptom scores.

Results: Topical AB-SA01 treatment was safe and well tolerated with no dose-limiting side effects in any of the 3 cohorts. Preliminary efficacy results support decreased bacterial load and improvements in endoscopic findings and symptom scores.

Conclusion: Sinus irrigation with AB-SA01 is safe and well tolerated up to $3x10^9$ PFU for 14 days with promising preliminary efficacy results.

ISOLATION OF ENVIRONMENTAL CAMPYLOBACTER PHAGES AND THEIR APPLICATION FOR MEAT DECONTAMINATION AND PHAGE-THERAPY IN POULTRY

APREA, Giuseppe[1]; D'ANGELANTONIO, Daniela[1]; CONNERTON, Pippa[2]; NERI, Diana[1]; DI SERAFINO, Gabriella[1]; BONI, Arianna[1]; SCATTOLINI, Silvia[1]; CONNERTON, Ian[2]; CALISTRI, Paolo[1]; MIGLIORATI, Giacomo[1]; POMILIO, Francesco[1]; DI GIANNATALE, Elisabetta[1]

[1] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy
[2] The University of Nottingham, Sutton Bonington- Leicestershire, UK

g.aprea@izs.it

Introduction: *Campylobacter jejuni* is a zoonotic agent involved in human gastrointestinal infections and neuropathies (Guillain-Barré syndrome). With a number of 246,307 cases in EU in 2016, it is the most commonly reported pathogen in humans (1) and poultry is recognized as the principal reservoir. Phages have the potential to reduce the contamination loads on poultry meat and in live broilers before slaughtering, thus reducing the number of *Campylobacter* entering the human food chain. This work shows the results deriving from the isolation of new phages from the environment, their *in vitro* activity and ability to reduce bacterial counts in chicken meat and in broilers before slaughtering.

Material & Methods: Nine *Campylobacter jejuni* strains have been used for phage isolation in New Zealand Casamino Yeast media. One-hundread and three environmental samples were analysed for phage isolation (2). Lytic spectrum was assessed and phage genomes were analysed by Pulsed-filed-gel-electrophoresis and sequencing. Fourty-five pieces of poultry meat were assessed for phage activity in *Campylobacter* decontamination, with samples stored at three different conditions. For the *in vivo* experiment, 2 different phages were orally administered (MOI 0.1 and 1) to 75 broilers experimentally infected with *C. jejuni* (10^8 ufc/gr of cecal content).

Results: Thirty-six lytic phages were isolated and two (Φ7 and Φ16; Fig. 1 and 2) were selected for decontamination and phage-therapy experiments. PFGE revealed a similar size for all phages of about 140 Kb. Phage application in meat decontamination led to a successful *Campylobacter* load decrease in all and three storage conditions (average 2 Log reduction). The *in vivo* experiment (phage-therapy) resulted in *Campylobacter* counts falling between 1 (moi 0.1) and 2 (moi 1) $\log_{10}$ cfu/g of cecal content compared to untreated control.

Conclusions: The results provide evidence of the efficacy of phage to treat *Campylobacter* contaminated meat and live poultry and are in line with those reported in literature (3). Our findings, according to EFSA opinion (4), could lead to a 90% reduction of the risk for the human consumers to get campylobacteriosis. Since veterinary use of antibiotics becomes more and more controversial due to increased antibiotic resistance, phage-therapy may become more generally accepted, giving an important contribution in enhancing food safety standards.

References:

1. *EFSA and ECDC. 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA J. 15 (12), 5077*
2. *Sørensen M.C.H. et al. 2011. Bacteriophage F336 recognizes the capsular phosphoramidate modification of Campylobacter jejuni NCTC11168. J. Bacter. 193 (23), 6742-6749*
3. *Carvalho C.M. et al. 2010. The in vivo efficacy of two administration routes of a phage cocktail to reduce numbers of Campylobacter coli and Campylobacter jejuni in chickens. BMC Microbiol. 1, 232*
4. *EFSA. 2011. Scientific Opinion on Campylobacter in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA J. 9, 2105-2145*

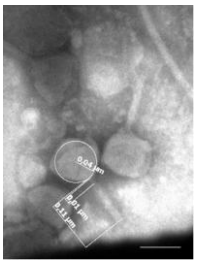


Fig. 1 Phage 7 under transmission electron microscope observation (50,000x).

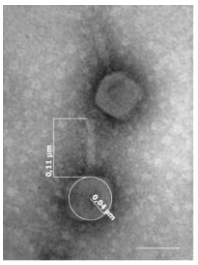


Fig. 2 Phage 16 under transmission electron microscope observation (50,000x).

ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES ACTIVE AGAINST AVIAN PATHOGENIC *E. COLI*

JAKOČIŪNĖ[1], Džiuginta; BAIG[2], Abiyad; NIELSEN[1], Søren Saxmose; TROTEREAU[3], Angelina SCHOULER[3], Catherine; WAGEMANS[4], Jeroen; CHRISTENSEN[1], Jens Peter; ATTERBURY[2], Robert BARROW[2], Paul; LAVIGNE[4], Rob; MOODLEY[1], Arshnee

[1]University of Copenhagen, Frederiksberg C, Denmark
[2]University of Nottingham, Sutton Bonington, United Kingdom
[3]INRA, Nouzilly, France
[4]KU Leuven, Leuven, Belgium

djak@sund.ku.dk

Introduction: Colibacillosis is a common bacterial disease in egg laying hens leading to major economic losses[1]. We tested 100 bacteriophages for their ability to kill dominant APEC serotypes (n=12, in total 21 strains) and the strain used in Poulvac®, a commercial modified-live *E.coli* vaccine.

Material & Methods: Phage host range was determined using a Bioscreen. OD_{600} values were plotted as a function of time. Growth inhibition of APEC was defined by a cutoff of $<OD_{600} = 0.5$ at around four hours and plotted in a heatmap. All phages were sequenced and compared to published genomes using BLAST.

Results: Twenty-five phages were selected for the Bioscreen assay based on their genetic uniqueness. They belonged to *Siphoviridae* (n=3) and *Myoviridae* (n=22) and their genomes ranged from 50-174 Kb. Individual phages were able to inhibit the growth of between 2-12 APEC strains belonging to 10 serotypes in total. Only two phages were active against the Poulvac® *E.coli*.

Conclusion: We have phages active against dominant APEC serotypes and with little activity against the vaccine *E.coli*. These are promising candidates for an alternate mean of eliminating pathogenic *E.coli* in birds, particularly in view of increasing antibiotic resistance and restrictions on antibiotic use in agriculture.

Reference:
Kabir SML, Lutful SM. Avian Colibacillosis and Salmonellosis: A Closer Look at Epidemiology, Pathogenesis, Diagnosis, Control and Public Health Concerns. Int J Environ Res Public Health. 2010; 7(1):89-114.

EFFECT OF DILUTION RATE ON CONTINUOUS PRODUCTION OF PHAGES USING TWO CHEMOSTATS IN SERIES

MANCUSO, Francesco; BENYAHIA, Brahim; MALIK, Danish Javed

Loughborough University, UK

F.mancuso@lboro.ac.uk

Introduction: Development of cost effective scalable cGMP production platforms for high titres of purified bacteriophages is needed to address demand for future phage therapies. Lab scale production processes using flasks followed by purification with CsCl density gradients is slow, not scalable and not cost effective[1].
The aim of this research was to demonstrate a robust scalable process for continuous production of bacteriophages using two bioreactors operated as chemostats, resulting in control over phage production rates. The process allowed investigation of key process parameters including dilution rate and host bacteria concentration on phage production as well as the effect of chemostat operation on aspects of downstream processing.

Material & Methods: *E. coli* (ATCC 11303) and the bacteriophage T3 (ATCC 11303-B3) were grown in synthetic medium[2]. Intracellular proteins and DNA in the lysate and following purification were assessed using size exclusion gel chromatography.

Results: Using synthetic medium continuous production of phage could be achieved with control over phage production by manipulating the dilution rate. The process was modeled using simple 1st order kinetics. The synthetic medium resulted in with lower level of impurities in the lysate, leading to a faster overall purification.

Conclusion: Biochemical engineering approaches for phage production can significantly improve productivity, quality and cost effectiveness of phage production[3]. Controlling important parameters such as dilution rates, bacterial growth rates and MOI affect yield and impact on downstream purification unit operations.

References:

1. *Gill, J. J.. & Hyman, P. Phage Choice, Isolation and Preparation for Phage Therapy. Curr. Pharm. Biotechnol. 11, 2014 (2010).*
2. *Li, Z., Kessler, W., Heuvel, J. van den & Rinas, U. Simple defined autoinduction medium for recombinant protein production in E. coli T7 expression system. 1–40 (2010).*
3. *Krysiak-baltyn, K. & Martin, G. J. O. Computational Modelling of Large Scale Phage Production Using a Two-Stage Batch Process. 1–14 (2018)*

METAGENOME ANALYSIS OF A RUSSIAN AND GEORGIAN COCKTAILS AND A PLACEBO-CONTROLLED SAFETY TRIAL OF A SINGLE PHAGE VERSUS PHAGE COCKTAIL IN HEALTHY *STAPHYLOCOCUS AUREUS* CARRIERS

MCCALLIN, Shawna; SARKER, Shafiq A; SULTANA, Shamima; OECHSLIN, Frank
MOREILLON, Philippe; BRUSSOW, Harald

University of Lausanne, Lausanne, Switzerland
KU Leuven, Laboratory of Gene Technology, Department of Biosystems, Leuven, Belgium
International Centre for Diarrhoeal Diseases Research, Dhaka, Bangladesh

Shawna.mccallin@gmail.com

Introduction: Bacteriophage therapy is a commonly used treatment for *Staphylococcus aureus* infections in countries of the former Soviet Union. The few data available on which phages are used to target specific bacteria in Eastern phage cocktails prompted an investigation into commercially-available products, which include *S. aureus* as part of their therapeutic range.

Material & Methods: Metagenomics was used to investigate the composition of the cocktails, and the safety of broad-spectrum cocktails was tested by comparing the effects of nasal and oral exposure to Eliava Pyophage, a monospecies counterpart, or placebo in healthy human carriers of *S. aureus*.

Results: Comparison of eight metagenomes revealed different phages in highly-variable proportions for most bacteria, while closely-related Myoviruses were, conversely, a unique and unanimous component against *S. aureus*, except for the inclusion of a secondary Podovirus in one Russian product. Several probable prophage sequences were detected, but without genetic safety risks in terms of virulence factors or antibiotics resistance genes. No safety concerns were associated with phage application during phase I studies.

Conclusions: The lack of *in silico* safety risks and adverse effects in any of the treatment groups supports the clinical safety of *S. aureus* phages administered as a single phage or as part of a complex, broad-spectrum cocktail.

LISTERIA MONOCYTOGENES' INFECTIVE PROPHAGE THAT PROMOTES VIRULENCE IS CONTROLLED BY AN ANCIENT CRYPTIC PROPHAGE, AN EVIDENCE FOR THE CO-OPTATION OF PHAGE REMNANT REGULATORY GENES

ARGOV, Tal, RAN-SAPIR, Shai, PASECHNECK, Anna, BOROVOK, Ilya, SIGAL, Nadejda and HERSKOVITS, Anat A.*

Department of Molecular Microbiology and Biotechnology, Faculty of Life Sciences, Tel Aviv University, Tel- Aviv, Israel

anathe@post.tau.ac.il

Most bacterial pathogens are lysogens, namely carry DNA of infective and cryptic prophages within their genome, yet the impact of this phenomenon on their behavior during mammalian infection is not known. Several years ago, we uncovered a novel example of a pathogen-prophage interaction, in which a prophage promotes the virulence of its host, the intracellular pathogen *Listeria monocytogenes* (*Lm*), via a cooperative behavior.

We identified an infective prophage that stably inhabits the *Lm* chromosome, serving as an intervening DNA element that regulates bacterial gene expression (via genome excision), some of which are important for virulence. In a search for determinants that mediate this phage behavior, we identified a critical metalloprotease encoded by a cryptic prophage element (cPE). We found that this metalloprotease functions as the main anti-repressor of both prophages, φ10403S and cPE, simultaneously cleaving each cI-like repressor, thereby co-triggering their induction. Overall, these findings reveal that φ10403S, though fully infective, is a non-autonomous prophage completely dependent on an ancient phage remnant regulatory factor.

Our results provide an intriguing molecular insight into the intricate interactions between bacteria and their inhabiting prophages, demonstrating the co-optation of phage remnant regulatory genes for the control of newly incoming phages.

POTENTIAL USE OF PHAGES AS SANITIZING AGENTS TO REDUCE HOSPITAL PATHOGENS ON HARD SURFACES

CASELLI, Elisabetta[1]; D'ACCOLTI, Maria[1]; SOFFRITTI, Irene[1]; BISI, Matteo[1]; KUTATELADZE, Mzia[2]; MAZZACANE, Sante[1]

[1]University of Ferrara, Ferrara, Italy

[2]G. Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia

elisabetta.caselli@unife.it

Introduction: Hospital-acquired infections (HAI) can be transmitted by pathogens persistently contaminating hospital surfaces,[1] often multidrug-resistant (MDR), and not efficiently controlled by conventional sanitation protocols, which indeed contribute to selection of drug-resistant strains.[2] Due to the selective killing of specific bacteria, bacteriophages have been repeatedly suggested as decontaminating agents.[3,4] This work was aimed to assess phage usability as sanitizing agents in routine hospital sanitation.

Materials & Methods: Phage activity was assessed *in vitro* and *in situ,* in aqueous buffer or probiotic eco-sustainable detergents,[5] on glass, plastic or ceramic surfaces artificially contaminated by *S. aureus*, *E. coli* and *P. aeruginosa*. Both ATCC strains and wild-type MDR hospital isolates were used, at a density consistent with what detected on hospital surfaces.

Results: Phage application significantly reduced (up to 90%) all tested bacteria on all treated surfaces. Notably, phages suspended in probiotic detergents not only retained their full activity, but resulted even more effective especially at later times.

Conclusions: Results suggest that phages might be successfully included in probiotic detergents currently used for hospital sanitation, potentially resulting in innovative products highly effective in the safe elimination of MDR nosocomial pathogens from the hospital environment.

Targeting Phage & Antibiotic Resistance

Abstracts for poster presentation by alphabetic order

HUMORAL RESPONSE TO STAPHYLOCOCCAL MONOVALENT PHAGES DURING PHAGE THERAPY

ŁUSIAK-SZELACHOWSKA Marzanna[1], WEBER-DĄBROWSKA Beata[1,2], MIĘDZYBRODZKI Ryszard[1,2,3], FORTUNA Wojciech[1,2,4], ROGÓŻ Paweł[2,5], JOŃCZYK-MATYSIAK Ewa[1], BAGIŃSKA Natalia[1], HARHALA Marek[1], LETKIEWICZ Sławomir[2,6], WOJCIECHOWSKA Renata[1], GÓRSKI Andrzej[1,2,3]

[1]Bacteriophage Laboratory, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy (IIET), Polish Academy of Sciences, Wrocław, Poland
[2]Phage Therapy Unit, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy (IIET), Polish Academy of Sciences, Wrocław, Poland
[3]Department of Clinical Immunology, Transplantation Institute, Medical University of Warsaw, Warsaw, Poland
[4]Department of Neurosurgery, Wrocław Medical University, Wrocław, Poland
[5]Clinical Department of Traumatology and Hand Surgery, Wrocław Medical University, Wrocław, Poland
[6]Medical Sciences Institute, Katowice School of Economics, Katowice, Poland

Natalia.Baginska@iitd.pan.wroc.pl

Introduction: Phage therapy (PT) may induce antiphage antibodies. Recent studies indicate that the level of phage neutralization depends on the route of phage administration, type of disease and type of phage preparation. Approximately 50% of patients subjected to PT are immunodeficient. The aim was to investigate the antiphage activity of sera (AAS) in three types of diseases treated with staphylococcal monovalent phages.

Material & Methods: 15 patients with three types of diseases were treated at the Phage Therapy Unit in Wrocław. Patients with bone infections, soft tissue infections or upper respiratory tract infections used locally or locally and orally applied staphylococcal monovalent phages 676/Z, fi 200 or 676/T. AAS was estimated during a 30-minute reaction of phage with diluted serum at 37°C using a plate neutralization test.

Results: The same phage was neutralized with low (mean 48% of patients of all groups) or high (mean 52% of patients of all groups) levels by sera in the same group.

Conclusion: Identical phages can elicit different antibody responses in patients treated with PT which probably depends on immune status of patients. There is a strong positive correlation between the duration of PT and antibodies neutralizing phages as measured by the coefficient K.

ENDOLYSIN LYSF1, NATURAL DELETION MUTANT OF LYSK WITH A BROAD RANGE OF LYTIC ACTIVITY

BENESIK, Martin; JANDA, Lubomir; PANTUCEK, Roman; DOSKAR, Jiri; FUGLIK, Vitezslav; MOSA, Marek

Masaryk University, Brno, Czech Republic
Charles University, Prague, Czech Republic
MB Pharma, Prague, Czech Republic

martin.benesik@mail.muni.cz

Introduction: Bacteriophage endolysins are useful tool for phage therapy. In this work we focused on LysF1, natural deletion mutant endolysin of well described LysK, lacking whole Ami domain.

Material & Methods: We used zymogram to confirm lytic activity of CHAP domain and LysF1. We determined lytic spectrum of phages compared to endolysin on 43 staphylococcal species using plate lysis assay and turbidity reduction assay.

Results: In this work, LysF1 and both its domains were prepared as recombinant proteins and their function was analyzed. LysF1 had an antimicrobial effect on 31 *Staphylococcus* species of the 43 tested. SH3b domain positively influenced antimicrobial activity of LysF1, since the lytic activity of the truncated variant containing the CHAP domain alone was decreased.

Conclusion: The new naturally raised deletion mutant endolysin LysF1 is smaller than LysK, has a broad lytic spectrum and therefore is an appropriate enzyme for practical use. The Endolysin LysF1 can be used for its high specific activity as an enzybiotic against most frequent *Staphylococcus* pathogenic species, such as *S. aureus, S. epidermidis*, but also against other significant pathogens such as *S intermedius, S. lugdunensis, S. haemolyticus, S. saprophiticus,* and *S. warneri*.

Reference:
Benesik et al. Virus Genes 2018; 54:130-139.

ISOLATION OF PHAGES TARGETING DIFFERENT *ESCHERICHIA COLI* PATHOTYPES

BOLOCAN, Andrei; CLOONEY, Adam; SHKOPOROV, Andrey; ALMEIDA BETTIO, Pedro; DRAPER, Lorraine; ROSS, Paul; HILL, Colin

APC Microbiome Ireland, University College Cork, Cork, Ireland

andrei.bolocan@ucc.ie

Introduction: The threat of an antibiotic resistance crisis has revived interest in diverse biological approaches against infectious diseases, including the use of phages against bacterial infections. The aim of this study was to isolate phages from human faeces to target multiple *E. coli* pathotypes.

Material & Methods: Seventeen *E. coli* strains, including representatives of the *E. coli* pathotypes *(*uropathogenic eneterotoxigenic, enteropathogenic, adherent invasive, enterotoxigenic and enteroaggregative), were used in the screening study. Phages were isolated from faeces using two enrichment steps, whereby the faecal material was mixed with *E. coli* strains in LB broth supplemented with CaCl2 and MgSO4, and incubated while shaking for 24 h at 37°C. Samples were analyzed for the presence of the phages using standard spot and double-layer plaque assay techniques.

Results: Of 50 plaques tested, we found at least four distinct phage types on the bases of RAPD-PCR and DNA enzymatic restriction profile analysis. Genome sequencing revealed the virulent nature of two of the phages. The four phages demonstrated broad host range specificity in a spot test, especially against UPEC and AIEC.

Conclusion: The two lytic phages can be further investigated for their therapeutic potential for some of the diseases in which these pathotypes are implicated as causative agents.

NANOENCAPSULATION OF BACTERIOPHAGES IN UNILAMELLAR LIPOSOMES PREPARED USING MICROFLUIDIC MICROMIXING

CINQUERRUI, Salvatore; Vladisavljevic, Goran T.; MALIK, Danish J.

Chemical Engineering Department, Loughborough University, UK

S.Cinquerrui@lboro.ac.uk

Introduction: Increasing antibiotic resistance in pathogenic microorganisms has led to renewed interest in bacteriophage therapy. It is generally recognised that phage do not diffuse across eukaryotic cell membranes and therefore be unable to infect intracellular pathogens e.g. mycobacteria, (1). A 'Trojan Horse' approach utilising liposome encapsulated phage may however permit phage access to intracellular pathogen. There are relatively few published studies looking at encapsulation of bacteriophage in liposomes (2–4).

The aim of the present study was to evaluate the use of a novel microfluidic based technique for encapsulation of bacteriophage. Previous studies have in nearly all cases used a thin-film hydration method for liposome preparation which does not afford precise control over the resulting liposome size and phage encapsulation.

Material & Methods: bacteriophage K ATCC 1985-B1 and E. coli bacteriophage ATCC® 11303-B3 were encapsulated. Liposomes were produced using an in-house fabricated microfluidic device and the method of direct co-flow alcohol injection.

Results: Issues such as phage aggregation into larger clusters and phage interaction with lipid bilayers was observed which made phage nanoencapsulation challenging.

Conclusions: This study presents insights into factors affecting phage nanoencapsulation in liposomes formed during spontaneous self-assemble of vesicles during lipid-alcohol-water micromixing in a microfluidic channel.

References:

1. Nieth A, et al., Springer Sci Rev. 2015;3:1–11.

2. Nieth A, et al., Expert Opin Drug Deliv. 2015;12(9):1411–24.

3. Colom J et al., Appl Environ Microbiol. 2015;81(14):4841–9.

4. Singla S, et al., J Infect Dis. 2015;212(2):325–34.

DIAGNOSIS OF *STAPHYLOCOCCUS* SPP. PROSTHETIC JOINT INFECTIONS WITH BACTERIOPHAGE K

CÖR, Andrej; ŠUSTER, Katja

Valdoltra Orthopedic Hospital, Ankaran, Slovenia

andrej.coer@ob-valdoltra.si

Introduction: Prosthetic joint infection (PJI) is a serious complication of arthroplasty and staphylococci are the major pathogens involved. At this point, there is still need for a diagnostic method for accurate diagnosis of PJI. Our aim was to develop alternatives to conventional microbiological diagnostic procedures, based on specific detection of live Staphylococci in sonicate fluid of PJI, with the use of bacteriophage K.

Material & Methods: 104 sonicate fluid obtained after revision surgery, because of the prosthesis loosening, were analysed. Quantitative RT-PCR with primers specific for bacteriophage K DNA to monitor its amplification in the presence of staphylococci and bioluminescence detection after intracellular ATP release by bacteriophage K mediated lysis of present staphylococci were used.

Results: The bioluminescence method took 3h with a limit of detection (LOD) in the bacterial concentration range of 10^3 CFU/mL and with 62.5% sensitivity. The method with qPCR took 4h and had a LOD of 10^2 CFU/mL and 81.25% sensitivity. Sensitivity of both methods improved to 98.12% with overnight incubation before testing. Specificity of both methods was 100%.

Conclusion: The developed models for detection of staphylococci within sonicate fluid of PJI using bacteriophage K are rapid, sensitive and specific and allow the detection of staphylococci.

IN VIVO EFFICACY OF A COMBINATION OF THERAPEUTIC BACTERIOPHAGES IN A MOUSE MODEL OF *S. AUREUS* DIABETIC FOOT ULCER (DFU) INFECTION

ALBAC Sandrine[1], Hayez Davy[1], MEDINA Mathieu[2], LABROUSSE Delphine[1], ANZALA Nelson[1], BONNOT Dorian[1], LAURENT Frédéric[3], DUBLANCHET Alain[4], CHAVANET Pascal[1-5], FEVRE Cindy[2], GABARD Jérôme[2], CROISIER Delphine[1]

[1]Vivexia, Dijon, France

[2]Pherecydes Pharma, Romainville, France

[3]Hospices Civils de Lyon, University of Lyon, France

[4]Ancien de laboratoire de microbiologie, Centre Hospitalier Intercommunal Villeneuve-Saint-George, France

[5]Infectious Diseases Department, Centre Hospitalier Universitaire de Dijon, France

delphine.croisier@vivexia.fr

Introduction: DFUs are the most common cause of hospitalization among diabetic patients and *Staphylococcus aureus* is the most prevalent pathogen implicated. Where all antibiotic treatment fails, bacteriophages represent an alternative for the treatment of such infections. We evaluated the efficacy of a combination of three lytic bacteriophages, in comparison to linezolid, in a mouse model of *S. aureus* DFU infection.

Material & Methods: An acute hindpaw infection was established in streptozotocin induced diabetic BALB/c mice. After infection, mice received an intraperitoneal administration of linezolid or a local administration of bacteriophages at 1 Multiplicity of Infection (MOI) or 10MOI. Control mice were infected but untreated. Mice were sacrificed every day during five days and the residual bacterial load in the hindpaw was enumerated.

Results: A single injection of 1MOI or 10MOI of bacteriophages showed a significant bacterial reduction on day 1 and its efficacy was comparable to linezolid. The strongest bacterial reduction was observed on day 3 using the highest bacteriophage 10MOI dose (- 2.4 Log).

Conclusion: This work highlights the efficacy of bacteriophages therapy for *S. aureus* DFU infections that are one of the leading causes of amputation and morbi-mortality among the diabetic population.

SYNTHESIS OF ANTIBACTERIAL PROTEINS AGAINST GRAM-NEGATIVE BACTERIA IN THE GREEN ALGA *CHLAMYDOMONAS REINHARDTII*

DA COSTA RAMOS Juliana, PURTON Saul

University College London, London, UK

juliana.ramos.14@ucl.ac.uk

Introduction: Widespread antibiotic resistance among pathogenic bacteria increases the need for new antibacterial drugs. Endolysins, bacteriophage enzymes that lyse the bacterial cell wall, have potential as novel antibiotics. Microalgae can be an alternative recombinant protein production platform due to established techniques for foreign gene expression, inexpensive cultivation, and ease of large-scale production. Here, bacteriophage endolysins were produced in the chloroplast of the microalga *Chlamydomonas reinhardtii*.

Material and Methods: The chloroplast was transformed with the gene of interest by the glass bead method (Kindle et al., 1991). After SDS-PAGE, a quantitative western blot analysis was performed using the Odyssey® Infrared Imaging system to confirm protein accumulation. Enzymatic assays were done by measuring the optical density of the bacteria with a crude extract of microalgae containing endolysin using the ELx 808 microplate reader at 30ºC.

Results and Conclusion: A synthetic gene encoding an endolysin "JR1" against the Gram-negative pathogen *Acinetobacter baumannii*, and two Artilysins (fusion between endolysin and an antimicrobial peptide) against both *A. baumannii* and *Pseudomonas aeruginosa* were designed and succesfully integrated into the chloroplast genome. Protein accumulation in the alga was confirmed. Antibacterial assays with *C. reinhardtii* extract containing JR1 showed a significant reduction in bacteria colony formation, and the activity of the Artilysins is currently being assessed.

References

Lai, M.-J., Lin, N.-T., Hu, A., Soo, P.-C., Chen, L.-K., Chen, L.-H., & Chang, K.-C. (2011). Antibacterial activity of Acinetobacter baumannii phage ϕAB2 endolysin (LysAB2) against both gram-positive and gram-negative bacteria. Applied Microbiology and Biotechnology, 90(2), 529–39.

Stoffels, L., Taunt, H. N., Charalambous, B., & Purton, S. (2016). Synthesis of bacteriophage lytic proteins against Streptococcus pneumoniae in the chloroplast of Chlamydomonas reinhardtii. International Journal of Laboratory Hematology, 38(1), 42–49.

Briers, Y., Walmagh, M., Van Puyenbroeck, V., Cornelissen, A., Cenens, W., Aertsen, A., … Lavigne, R. (2014). Engineered Endolysin-Based "Artilysins" To Combat Multidrug- Resistant Gram-Negative Pathogens. mBio, 5(4), e01379-14-e01379-14. http://doi.org/10.1128/mBio.01379-14

STRUCTURAL CHARACTERIZATION OF THE FMTA PROTEIN, MODULATOR OF THE WALL TEICHOIC ACIDS OF *STAPHYLOCOCCUS AUREUS*

DASANTILA Golemi-Kotra[1]; VIKRAM Dalal[2]; PRAVINDRA Kumar[2],

[1]York University, Toronto, Canada,
[2]Indian Institute of Technology, Roorkee, India

dgkotra@yorku.ca

Introduction: FmtA is a D-amino esterase that removes the D-Ala group of wall teichoic acid (WTA) of *Staphylococcus aureus.* Wall teichoic acids are polymers involved in many physiological aspects of *S. aureus*, among them the recognition and infection by *S. aureus* specific phages. FmtA is similar to a penicillin-binding protein but it is devoid of such function. This protein is involved in biofilm formation and its knockout re-sensitizes MRSA to β-lactams. We have undertaken structural biology studies to reveal the structural elements that enablethe D-amino esterase activity of FmtA.

Material & Methods: X-Ray crystallography was used to determine the crystal structure of FmtA.

Results: 3D structure of FmtA resembles largely that of DD-carboxypeptidase enzymes and Class C β-Lactamases. However, Local structural changes to the active site of the enzyme are unique to FmtA and hold thekey to the substrate specificity of FmtA.

Conclusion: FmtA offers an excellent target to understand the structural elements that drive substrate specificity among penicillin recognition proteins and their diversity in function. The crystal structure provides insights into targeting FmtA with the purpose of increasing *S. aureus* sensitivity to β-lactam antibiotics and inhibiting biofilm formation.

CHARACTERIZATION OF A NOVEL THREE-COMPONENT TOXIN-ANTITOXIN MODULE FROM THE PATHOGENIC BACTERIUM *ESCHERICHIA COLI* O157:H7

DE BRUYN, Pieter; VANDERVELDE, Alexandra; VAN MELDEREN, Laurence, LORIS, Remy

Vrije Universiteit Brussel, Brussel, Belgium

Pieter.de.bruyn@vub.be

Introduction: The *paaR2-paaA2-parE2* operon is a unique three-component toxin-antitoxin module present in pathogenic *Escherichia coli* O157:H7 and a possible target for the development of new antibacterial drugs (Hallez et al., 2010; Sterckx et al., 2016). Aside from the toxin (ParE2) and antitoxin (PaaA2), the operon encodes an additional regulator (PaaR2) that is involved in the regulation of the transcription of the operon. The objective of this work is to understand how PaaR2 autoregulates the transcription of the operon and if this regulation is possibly linked to the onset of persistence.

Material & Methods: Via a combination of structural biology, biochemistry and biophysical experiments, a mechanistic model for the autoregulation of the *paaR2-paaA2-parE2* operon is constructed.

Results: PaaR2 forms an octameric complex that represses transcription of the *paaR2-paaA2-parE2* operon and of a neighboring operon via recognizing four imperfect palindromic repeats with a consensus sequence GTTTAGTG. The repressor of that neighboring operon also represses transcription of both operons.

Conclusion: PaaR2 and an adjacent repressor have been found to regulate transcription of the *paaR2-paaA2-parE2* operon and of a neighboring operon in a mechanism that resembles the CI-Cro repression mechanism from bacteriophage λ.

References:

1. *Hallez, R., Geeraerts, D., Sterckx, Y., Mine, N., Loris, R., & Van Melderen, L. (2010). New toxins homologous to ParE belonging to three-component toxin-antitoxin systems in Escherichia coli O157:H7. Molecular Microbiology, 76(3), 719–32.*
2. *Sterckx, Y. G.-J., Jové, T., Shkumatov, A. V., Garcia-Pino, A., Geerts, L., De Kerpel, M., Lah, J., De Greve, H., Van Melderen, L., Loris, R. (2016). A unique hetero-hexadecameric architecture displayed by the E. coli O157 PaaA2-ParE2 antitoxin-toxin complex. Journal of Molecular Biology, 428(8):1589-603.*

USE OF BIOSYNTHETIC NANOPARTICLES TO FIGHT RESISTANT BACTERIA

DE DONATO, Marcos[1], ESPAÑA-SANCHEZ, Beatriz[2]; OZA, Goldie[2]; ROJAS-AVELIZAPA, Norma[3], RODULFO, Hectorina[1].

[1]Tecnologico de Monterrey, Escuela de Ingenieria y Ciencias, Queretaro, Mexico.
[2]Centro de Investigacion y Desarrollo Tecnologico en Electroquimica, Queretaro, Mexico.
[3]Centro de Investigacion en Ciencia Aplicada y Tecnologia Avanzada, Instituto Politecnico Nacional, Queretaro, Mexico.

mdedonate@itesm.mx

In past two decades, there has been an increase in the emergence and wider dissemination of multi-drug resistant (MDR) bacteria to several types of antibiotics, with some strains showing extreme resistance (to all but one or two antibiotics) and even panresistance (resistant to all known antibiotics) in a disturbing regularity. The high frequencies of resistant strains are reported in bacteria that cause common health-care associated infections, as well as community-acquired infections (e.g. urinary tract infection, pneumonia) worldwide.

Recently, the use of metallic nanoparticles (NPs) has gained scientific/industrial attention due to the high bactericidal properties, associated with their morphological characteristics and their interaction with pathogens, and have been considered as the new generation of antimicrobials. The capability of biological systems such as microbes, fungi, algae and plants by green chemistry methods, to sequester metal ions and meticulously define the dimensions via fetter like capping proteins, is intriguing giving it a monodispersed size and forming stable NPs. Our group have used, *Nocardia farcinica* (bacteria) and *Fusarium oxysporum* (fungus) to synthesize metallic spheroid NPs of uniform size (15-20 and 1-6 nm in diameter, respectively), by extracellular enzymes. We have described, for the first time, the use of an industrial sulfur waste to achieve extracellular biosynthesis of CdS quantum dots using biomass of *F. oxysporum*, which showed improved characteristics over other physico-chemically produced CdS nanoparticles. Biosynthesized quantum dots were circular with diameter of 6.1±2.1 nm and had a wurtzite crystalline structure. These biosynthetic nanoparticles were hydrophilic and biocompatible, due to their free carboxylic groups, being able to react with macromolecules, making them suitable for use against bacterial cells with low probability of interfering with the cell functions in the host. Unlike commercial antibiotics, NPs have antimicrobial activities through combinations of mechanisms such as disruption of cellular morphology, inactivation of vital cellular enzymes and proteins, DNA condensation, loss of DNA replication, depletion of ATP. Other modus operandi includes, protein denaturation, inhibition of ribosome interaction, accumulation at lethal concentration in cell, generation of reactive oxygen species (ROS), oxidative stress, and modulation of cellular signaling, which make them ideal for targeting a broad range of microorganisms. In this sense, our group have used Ag and Cu based NPs and demonstrated antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aureus, although this effect is dependent of the concentration.

We have shown that NPs adhere to bacterial cells, which produces morphological changes in the structure of the bacteria, which was associated with damaged proteins of the outer cell membrane.

In the future, research should focus on the understanding of the mode of action of both metallic NPs and functionalized NPs as antimicrobial therapy as well as the study of their innocuity to higher eukaryotic cells.

DIFFUSION OF STAPHYLOPHAGES ACROSS BACTERIAL BIOFILMS

GONZÁLEZ, Silvia; FERNÁNDEZ, Lucía; GUTIÉRREZ, Diana; RODRÍGUEZ, Ana; GARCÍA, Pilar

Instituto de Productos Lácteos de Asturias (IPLA-CSIC, Villaviciosa, Asturias, Spain

lucia.fernandez@ipla.csic.es

Introduction: Bacteriophages are considered promising biofilm control agents. However, the development of successful antibiofilm strategies requires in-depth understanding of the parameters that govern phage-host interactions within attached microbial communities. Here, we evaluate the diffusion and propagation in biofilms of two phages, vB_SauM_philPLA-RODI and vB_SepM_philPLA-C1C, with potential for the treatment of staphylococcal infections (1, 2).

Material & Methods: Strains from different species exhibiting varying degrees of susceptibility to the phages and biofilm-forming ability were selected for this study. Single-species or mixed-species biofilms were allowed to form for 24 hours on polycarbonate membranes located inside the inserts of Transwell plates. These biofilms were subsequently treated with a phage suspension for 24 h and the phage titer in the biofilm and the flow-through was determined (3) and compared to the initial phage concentration.

Results: Our results showed that diffusion across biofilms depend on the strain(s) forming the biofilm. Some of the factors involved were the amount of attached biomass, susceptibility of the strain, initial phage titer, phage entrapment in the extracellular matrix, and phage inactivation.

Conclusion: This technique is useful to study phage-bacteria interactions in biofilms, an information that will be valuable for the development of phage-based antimicrobial products.

References:

1. *Gutiérrez D, Vandenheuvel D, Martínez B, Rodríguez A, Lavigne R, García P. 2015. Two phages, philPLA-RODI and philPLA-C1C, lyse mono- and dual-species staphylococcal biofilms. Appl Environ Microbiol 81:3336-3348.*
2. *González S, Fernández L, Campelo AB, Gutiérrez D, Martínez B, Rodríguez A, García P. 2017. The behavior of Staphylococcus aureus dual-species biofilms treated with bacteriophage philPLA-RODI depends on the accompanying microorganism. Appl Environ Microbiol 8:e02821-16.*
3. *Gutiérrez D, Martínez B, Rodríguez A, García P. 2010. Isolation and characterization of bacteriophages infecting Staphylococcus epidermidis. Curr Microbiol 61:601-608.*

THE MINOR FLAGELLIN (FLAB) OF *CAMPYLOBACTER JEJUNI* CONFERS DEFENSIVE PROPERTIES AGAINST BACTERIOPHAGE INFECTION

FINK, Michael[1]; LIS, Lukas[1]; CONNERTON, Ian F.[2]

[1]PTC Phage Technology Center GmbH, Bönen, Germany
[2]University of Nottingham, Sutton Bonington Campus, Loughborough, United Kingdom

Michael.Fink@finktec.com

Introduction: *Campylobacter jejuni* is a major food born pathogen with approx. 246,000 reported cases in the EU in 2016*. This microorganism has been target of numerous biocontrol studies, hence the focus of this study laid on the identification of surface structures and virulence factors that influence bacteriophage infection, and could affect biocontrol. Screening of bacteriophages on a monogenetic mutant library of *C. jejuni* NCTC12662 PT14 revealed an effect of FlaB on bacteriophage infection.

Material and Methods: Spot test/plaque assay, liquid growth culture, motility assay, one-step growth curve, adsorption assay.

Results: Deactivation of the *flaB* gene generated increased susceptibility of PT14 to infection by two types of bacteriophages in liquid media and clearer lysis in spot test assays. This was accompanied by an increase in liberated bacteriophage progeny after 24 hours in liquid culture. Further, the bacteriophage adsorption constant increased 2-fold for the *flaB* mutant strain and phage growth parameters showed a 2-fold increase in burst-size, relative to wild-type. Disruption of *flaB* resulted in no major motility reduction.

Conclusion: Multiple stages of bacteriophage infection were affected by the absence of functional FlaB. This indicates a role different from motility, which may be part of a novel phage defense mechanism.

References:

** EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA Journal 2017;15(12):5077, 228 pp.*

COAGULASE-NEGATIVE STAPHYLOCOCCI AS A SOURCE OF ANTIMICROBIAL RESISTANCE

FISAROVA, Lenka; DOSKAR Jiri; PANTUCEK, Roman; BOTKA, Tibor; MOSA, Marek

Masaryk University, Brno, Czech Republic
MB Pharma, Praha, Czech Republic

lena.fisarova@seznam.cz

Introduction: Coagulase-negative staphylococci (CoNS) cause various diseases in both humans and animals. Furthermore, they are considered to be important carriers of resistance determinants located mainly on mobile genetic elements, which can spread to different strains by horizontal transfer.

Material & Methods: In this study we analysed antimicrobial resistance and plasmid content in 62 CoNS strains of human and veterinary origin. We detected resistance by disc diffusion method, isolated plasmid DNA using isolation kits, cleaved it by restriction endonucleases, detected genes by PCR and cured plasmids by SDS.

Results: We detected resistance to all classes of antibiotics in most strains. The majority of strains harboured 1-6 resistance plasmids. Sequence analysis proved that some strains belonging to different species of CoNS and *S. aureus* carry identical plasmids, which indicates that these plasmids are transferred between them.

Conclusion: CoNS are important carriers of antimicrobial resistance genes and serve as their source for other, even more pathogenic bacteria. Therefore, it is essential to know on which MGE these genes are localized and how they spread.

Reference:
Schwarz S., Shen J., Wendlandt S. et al. (2015) Plasmid-Mediated Antimicrobial Resistance in Staphylococci and Other Firmicutes, In PLASMIDS: Biology and impact in biotechnology and discovery, ASM Press, Washington, DC.

THERAPEUTIC EFFECTS OF BACTERIOPHAGE ΦEF24C-P2 ON *ENTEROCOCCUS FAECALIS* ENDOPHTHALMITIS IN MICE

FUKUDA, Ken; KISHIMOTO, Tatsuma; ISHIDA, Waka; SUZUKI, Takashi; UCHIYAMA, Jumpei; MATSUZAKI, Shigenobu; DAIBATA, Masanori; FUKUSHIMA, Atsuki

Kochi Medical School, Kochi, Japan
Toho University, Tokyo, Japan
Azabu University, Kanagawa, Japan

k.fukuda@kochi-u.ac.jp

Introduction: To develop novel topical phage therapy for infectious eye diseases, we investigated the therapeutic effects of bacteriophage ΦEF24C-P2 on *Enterococcus faecalis* endophthalmitis in mice.

Material & Methods: Endophthalmitis was induced in mice by injection of 1×10^4 *E. faecalis* bacteria into the vitreous body. BacteriophageΦEF24C-P2 was then injected into the vitreous 6 h after bacterial injection. Eyes were examined 1 day after infection to grade disease severity according to an established scale. The number of viable bacteria in the eye was determined, and infiltrated inflammatory cells in the eye were quantitated by assay of myeloperoxidase activity.

Results: Injection of the eye with *E. faecalis* induced severe endophthalmitis, with the ocular fundus being invisible due to fibrin precipitation or hemorrhage in the anterior chamber at 24 h. Single-dose administration of phageΦEF24C-P2 into the vitreous resulted in significant attenuation of disease severity and preserved the structural integrity of the retina. ΦEF24C-P2 treatment also suppressed the number of viable bacteria and neutrophil infiltration in the infected eye.

Conclusion: Administration of phage into the vitreous body is a potential novel adjunct or alternative option for the treatment of bacterial endophthalmitis.

NANOENCAPSULATION OF PHAGE LYTIC PROTEINS IN pH-SENSITIVE LIPOSOMES

PORTILLA, Silvia; FERNÁNDEZ, Lucía; GUTIÉRREZ, Diana; RODRÍGUEZ, Ana; and GARCÍA, Pilar

Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Villaviciosa, Asturias, Spain

pgarcia@ipla.csic.es

Introduction: Endolysins represent an interesting alternative to conventional antibacterials. However, their implementation in the food industry requires formulations that ensure their stability upon its use. Nanoencapsulation in liposomes is an innovative technology to deliver proteins. Indeed, liposomes and nanoliposomes have already been employed to encapsulate flavoring and nutritive agents (1). In this study, we have tested the efficacy of liposome encapsulation of a staphylococcal endolysin.

Material & Methods: Encapsulation was performed with a commercial preparation of proliposomes, Prolipo-pH sensitive (PNS-pH), able to release their content at pH < 5.5. For the entrapment efficiency (EE) determination, vesicles were mixed with 1 mL of PBS 0.1 M, 135 mM NaCl, pH 5, and the activity was measured by the turbidity assay.

Results: Liposomes containing the endolysin showed a size distribution of 450 nm as measured by dynamic light scattering (DLS) and an EE of 47.5%. Encapsulated endolysin showed lytic activity against *Staphylococcus aureus* cultures in TSB at pH 5. Time-kill experiments demonstrated a reduction in viable bacteria of 2 log units after 30 min of incubation.

Conclusion: Encapsulation of endolysins in liposomes would be a useful technique for the controlled delivery of antimicrobials in food.

Reference:

Dias MI, Ferreira IC, Barreiro MF. 2015. Microencapsulation of bioactives for food applications. Food Funct. 6(4):1035-52.

OPTIMIZATION OF PHAGE PROPAGATION AND PURIFICATION PROCESSES

ANUSZKIEWICZ, Paula[1]; KARCZEWSKA-GOLEC, Joanna[2]; GOLEC, Piotr[2]

[1]University of Gdansk, Gdansk, Poland
[2]University of Warsaw, Warsaw, Poland

pgolec@biol.uw.edu.pl

Introduction: Bacteriophages are widely used in medical, industrial and scientific settings. Regardless of phage application purpose, rapid and efficient production and purification methods are essential in phage preparation. In this work, we present optimization of phage propagation and purification.

Material & Methods: We carried out optimization of propagation of PRA33, WIS42 and DG67 phages infecting *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Staphylococcus pseudintermedius*, respectively. The propagation stage was performed in bacterial flask cultures at various OD and MOI values during the infection step. After propagation, we carried out optimization of phage preparations` purification with the use of ACTA purifier system and CIM® monolithic columns (QA1 and DEAE). In the purification process, phage preparations in LB medium or after a former PEG concentration were tested.

Results: Three different bacteriophages were propagated with various phage/bacteria ratios. Under optimal conditions, the final phage concentration was at least 10^{10} pfu/ml. The best results were obtained for CIM® QA1 monolithic column for which the resulting phage recovery rates were up to 100%.

Conclusion: We identified propagation and purification conditions which enabled us to perform both steps in one day. Phages prepared with our rapid protocol are suitable for medical or industrial applications.

RELEASE OF BACTERIOPHAGE K FROM POLY(LACTIC ACID)-POLY(ETHYLENE GLYCOL) MATRICES FOR THE TREATMENT OF WOUND INFECTIONS

GWYNNE, Lauren[1]; DREW, Kate[1]; MAILLARD, Jean-Yves[2]; JENKINS, Toby[1]

[1]University of Bath, Bath, United Kingdom

[2]Cardiff University, Cardiff, United Kingdom

L.Gwynne@bath.ac.uk

Introduction: Current treatments to eliminate wound infections are becoming less effective due to antibiotic resistance. This study outlines the development of a novel system where bacteriophage are released from a matrix consisting of poly(lactic acid) (PLA) and poly(ethylene glycol) (PEG). Triggered release can be achieved by placing a shell of PLA around this matrix. Bacteria secrete proteases, which are able to break down this shell, thus selectively releasing bacteriophage, and subsequently eliminating pathogenic bacteria at the wound site.

Material & Methods: PLA-PEG films were fabricated by the solvent-casting method, and bacteriophage-loaded PLA-PEG were investigated for loading efficiency, *in vitro* release, and antibacterial efficacy against *Staphylococcus aureus.*

Results: The release profile of bacteriophage-loaded films showed a burst release (96.28 ± 2.15% within 40min), followed by a consistent phage titre for 24h. This could be attributed to the large pore size (72.26 ± 46.31µm) and the high degradation rate of the matrix. Bacteriophage-loaded porous films were also capable of reducing *S. aureus* cell count (c.5 x 10^5 CFU/ml; MOI 1:1) within 6h, with complete elimination by 24h.

Conclusion: Bacteriophage-loaded PLA-PEG films were successfully prepared, and shown to inhibit *S. aureus* growth. Work is ongoing to utilize proteases as a trigger for the release of bacteriophage from this matrix.

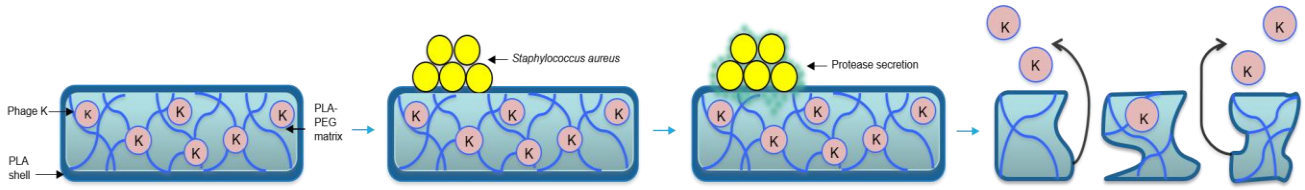


Figure 1: Protease-responsive wound dressing concept

HOST RANGES OF PHISA012 AND PHISA039 AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* PREVALENT STRAINS

HANAWA, Tomoko[1]; MIYANAGA, Kazuhiro[2]; ARAKI, Koji[1]; OHNISHI, Hiroaki[1];
KAMIYA Shigeru[1]; TANJI, Yasunori[2]; MATSUDA Takeaki[1]

[1]Kyorin University, Tokyo, Japan
[2]Tokyo Institute of Technology, Tokyo, Japan

thanawa@ks.kyorin-u.ac.jp

Introduction: Application of relevant phages to target bacteria is essential for successful phage therapy. In the present study, clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) were characterized for genotypes, and their lytic susceptibility to *S. aureus* phages, phiSA012 and phiSA039[1, 2] was analyzed to test the possibility of the therapeutic utility.

Material & Methods: One-hundred and five MRSA clinical isolates were randomly selected from the Kyorin University Hospital in 2015-2016, and Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was performed with phage-open reading frame typing kit (Kanto Chemical Co., Inc., Tokyo)[3,4]. Lytic activity of the phages was evaluated by spot test and efficacy of plating assay.

Results: Forty-eight healthcare associated (HA)-MRSA and 55 community associated (CA)-MRS type clones classified by SCC*mec* type[5] were used in this study. In total, 75.7% of MRSA strains were lysed by either or both of phiSA012 and phiSA039 phages which displayed similar host ranges. HA-MRSA clones were more susceptible than CA-MRSA clones (89.6% vs 63.6%, P=0.002) to these phages.

Conclusion: phiSA012 and phiSA039 showed wide host range for HA-MRSA, and could be promising therapeutic agents for its infection. However, isolation of novel phages may be required to combat CA-MRSA infection.

References:

1. *Synnott AJ, Kuang Y, Kurimoto M, Yamamichi K, Iwano H, Tanji Y. Appl Environ Microbiol. 2009 75:4483-90.*
2. *Iwano H, Inoue Takasago et al., Biology (Basel). 2018 7. pii: E8.*
3. *Suzuki M, Tawada Y, Kato M, et al., J Appl Microbiol. 2006 101:938-47.*
4. *Maeda, T., Saga, T., Miyazaki, T. et al. J Infect Chemother 2012 18: 906.*
5. *Deurenberg RH, Vink C, Kalenic S, Clin Microbiol Infect. 200 13:222-35.*

CHARACTERISTICS OF HEALTHY AND ACNE HUMAN SKIN COLONIZATION BY BACTERIOPHAGES OF *PROPIONIBACTERIUM ACNES* AND *STAPHYLOCOCCUS EPIDERMIDIS* AND THEIR HOSTS

JANEŽ, Nika[1]; ŠTOKELJ, Maja[1]; TUŠAR, Jasmina[1]; NEMEC, Kristina[1]; ACCETTO, Tomaž[2]; ŠPEHAR, Slivija[3]; JANEŽIČ, Sandra[4]; BALKOVEC, Valerija[5]; PETERKA, Matjaž[1]

[1]Centre of Excellence for Biosensors, Instrumentation and Process Control, Ajdovščina, Slovenia
[2]University of Ljubljana, Biotechnical Faculty, Animal sciences department, Domžale, Slovenia
[3]Hemptouch d.o.o, Novo mesto, Slovenia
[4]National Laboratory for Health, Environment and Food (NLZOH), Maribor, Slovenia
[5]General Hospital Novo mesto, Department of Dermatovenerology, Novo mesto, Slovenia

nikolaja.janez@cobik.si

Introduction: *Propionibacterium* spp. and *Staphylococcus* spp. were identified as the predominant and stable inhabitants of healthy human skin. They are considered to be commensal microorganisms though they are associated with development of acne and clinically relevant infections.

We aim to determine *Propionibacterium acnes* and *Staphylococcus epidermidis* co-colonization characteristics of human skin potentially playing a role in health or disease of the human skin. Therefore we investigated prevalence of *P. acnes* and *S. epidermidis* bacteria and their bacteriophages in healthy (6) and acne (13) volunteer skin samples. All healthy skin samples carried *P. acnes* and *S. epidermidis* along with *P. acnes* specific bacteriophages and one sample carried as well *S. epidermis* bacteriophage. On a strain level *P. acnes* isolates were identified as type I and majority of *S. epidermidis* isolates belonged to ST73. The prevalence of *P. acnes* was much lower (38%) on acne skin and the recovered staphylococci were identified as *S. epidermidis* in only 69%. From acne skin samples only *P. acnes* specific bacteriophages were recovered. The newly isolated bacteriophages were all similar to each other in terms of morphology and were able to lyse majority of hereto isolated *P. acnes* strains regardless to the isolation source. In contrary, the newly isolated *S. epidermidis* bacteriophage was able to lyse mainly ST73 strains. It seems that also volunteer's age and gender affect co-colonization. The most potent among *P. acnes* bacteriophages was sequenced and analyzed.

Our small scale study results suggest that *P. acnes*, *S. epidermidis* and their bacteriophages are able to co-inhabit healthy human skin, but on acne skin this balance seems to be altered. The bacteriophages were examined more in detail to evaluate their possible ecological and therapeutic potential.

DIRECTED EVOLUTION OF BIOMOLECULES USING ENGINEERED PHAGES

JARAMILLO, Alfonso[1,2,3]

[1]Warwick Integrative Synthetic Biology Centre & School of Life Sciences, University of Warwick, UK
[2]CNRS-UMR8030 iSSB, Université Paris-Saclay and Évry and CEA, DRF IG, Genoscope, Évry, France
[3]Institute for Integrative Systems Biology (I2SysBio), University of Valencia-CSIC, Paterna, Spain

Alfonso.Jaramillo@synth-bio.org

Introduction: *In vivo* directed evolution techniques allow engineering protein and nucleic acids with targeted functions inside living cells. The efficiency of such techniques is determined by the evolution speed and sampling size inside the organism. Viruses with fast replicative cycles and able to support high mutagenesis rates allow implementing a faster evolution, where the host cell is re-engineered according to the desired selection. Phages are specially suited due to their small size, fast replication and the ease of engineering of their genomes and their hosts.

Material & Methods: We have developed directed evolution systems based on filamentous (M13) and lytic (T7) phages. We have engineered their genomes and hosts by removing from the phages genes required for their replication to later complement them within the host.

Results: We demonstrate the usefulness of our system by engineering the largest known set of orthogonal transcription factors able to activate and/or repress cognate or combinatorial promoters in *E. coli*. The implementation of negative selections allowed the engineering of specificity. We also show how to evolve riboswitches using cycles of positive and negative selections.

Conclusion: Our methodology for directed evolution can be implemented in many phage systems to evolve proteins, nucleic acids and phage tropism determinants.

References:

1. *Broedel AK, Isalan M, Jaramillo A. Engineering of biomolecules by bacteriophage directed evolution. Curr Opin Biotechnol. 2017 Nov 22; 51:32–8.*
2. *Broedel AK, Jaramillo A, Isalan M. Intracellular directed evolution of proteins from combinatorial libraries based on conditional phage replication. Nat Protoc. 2017 Sep; 12(9):1830–43.*
3. *Jaramillo A. Synthetic biology: Engineered stable ecosystems. Nat Microbiol. 2017 Jul 25;2(8):17119.*
4. *Broedel AK, Jaramillo A, Isalan M. Engineering orthogonal dual transcription factors for multi-input synthetic promoters. Nat Comms. 2016 Dec 16; 7:13858.*
5. *Sagona AP, Grigonyte AM, MacDonald PR, Jaramillo A. Genetically modified bacteriophages. Integr Biol. 2016 Apr 18; 8(4):465–74.*

EVALUATION OF ADDING BACTERIOPHAGES TO RENDERED ANIMAL MEALS AS FEED ADDITIVE TO CONTROL *SALMONELLA* INFECTION IN ANIMAL

JIANG, Xiuping; KINLEY, Brandon

Clemson University, Clemson, USA

xiuping@clemson.edu

Introduction: The presence of *Salmonella* spp. has been documented in rendered animal by-products as well as in environmental swab samples from the rendering facilities. Rendered animal meals, as a major ingredient of animal feeds, if contaminated with foodborne pathogen *Salmonella*, can serve as a vehicle for pathogenic bacteria to enter the food chain. Therefore, the objective of this study was to determine if a bacteriophage cocktail could be used as a feed additive to control *Salmonella* infection in animal.

Material & Methods: A five-strain cocktail of bacteriophages specific for five *Salmonella* serotypes (Enteritidis, Typhimurium, Mbandaka, Johannesburg, and Idikan) was optimized, lyophilized and mixed in 3 types of rendered animal meals (poultry, blood, and feather). During storage at 30^{o}C for 4 wk, the rendered animal meals were rehydrated at room temperature for 24 h, and *Salmonella* was enumerated. To conduct animal trial, female Balb/c mice (4-6 wk, n=10) were given a dough diet (Bio-Serv) supplemented with the bacteriophage cocktail at a multiplicity of infection (MOI) of 100, and then orally administered with *S. Enteritidis* H4717 (10^6 CFU/mouse) using gavage needles. *Salmonella* enumeration and histological analysis of mice were performed during a 4-wk trial.

Results: Bacteriophage stability testing indicated that the titer of lyophilized bacteriophages in rendered animal meals decreased by 1.5 log PFU/g at 30°C over 4 wk. Although the lyophilized bacteriophages did not reduce *Salmonella* levels in the animal meals during storage, *Salmonella* levels after animal meal rehydration for 24 h were reduced from 0.8~1.5 and 1.0~2.0 log CFU/g more in those samples treated with lyophilized bacteriophages when using a MOI of 10 and 100, respectively, than with those not treated. Balb/c mice receiving feed supplemented with the bacteriophage cocktail showed no signs of infections (shedding in feces or histological analysis), while mice not receiving the bacteriophages shed *Salmonella* in the feces for a period of 2.5 wk and histological examination revealed signs of inflammation in the liver.

Conclusion: These results indicate that the bacteriophage cocktail can be used as an effective feed additive for *Salmonella* infection control in animals.

References:

1. *Crump, J.A., P.M. Griffin, and F.J. Angulo. 2002. Bacterial contamination of animal feed and its relationship to human foodborne illness. Clin. Infect. Dis. 35, 859-865.*
2. *Kinley, B., P. Dawson, J. Rieck, and X. Jiang. 2010. Analysis of* Salmonella *and enterococci isolated from rendered animal by products. Can. J. Microbiol. 56:65-73.*
3. *Knox, W.A., N.C. Galbraith, M.J. Lewis, G.C. Hickie, and H.H. Johnston. 1963. A milkborne outbreak of food poisoning due to* Salmonella *Heidelberg. J. Hyg. 61, 175-185.*

CHARACTERIZATION OF THE LYTIC CAPABILITY OF LYS-PHISA012 DERIVED FROM POLYVALENT STAPYHLOCOCCUS AUREUS BACTERIOPHAGE

JUMPEI, Fujiki*; TOMOHIRO, Nakamura; HIDETOMO, Iwano

Rakuno Gakuen University, Ebetsu, Japan

j-fujiki@rakuno.ac.jp

Introduction: Alternative strategies against Antibiotic-resistant bacteria (ARB) are required urgently as ARB have spread widely and rapidly. Bacteriophages and their endolysins have received significant attention as novel approaches against ARB. We previously reported the isolation of a bacteriophage, phiSA012, its wide host range and its effective lytic capability towards *Staphylococcus aureus* strains [1, 2].

Material & Methods: We purified an endolysin, Lys-phiSA012, which harbors dual lytic domains (CHAP and Amidase) and a SH3b cell wall binding domain. Turbidity reduction assays and minimum inhibitory concentration (MIC) assays were performed to investigate the lytic capability of Lys-phiSA012.

Results: Lys-phiSA012 exhibits high lytic activity towards staphylococcal strains including MRSA. Deletion analysis revealed that only mutants possessing the CHAP and SH3b domains could lyse *S. aureus*, indicating that the CHAP and SH3b domain are required to the lytic activity. The lytic activity was enhanced by the presence of at least 1 mM Ca^{2+} and 100 μM Zn^{2+} in a turbidity reduction assay. Furthermore, a MIC assay showed that the addition of Lys-phiSA012 decreased the MIC of oxacillin.

Conclusion: Our results suggest that endolysins are a promising alternative approach and may contribute to the proper use of antibiotics, leading to the reduction of ARB.

References:

1. *Synnott, A.J.; Kuang, Y.; Kurimoto, M.; Yamamichi, K.; Iwano, H.; Tanji, Y. Isolation from sewage influent and characterization of novel staphylococcus aureus bacteriophages with wide host ranges and potent lytic capabilities. Appl Environ Microbiol 2009, 75, 4483-4490.*
2. *Iwano, H.; Inoue, Y.; Takasago, T.; Kobayashi, H.; Furusawa, T.; Taniguchi, K.; Fujiki, J.; Yokota, H.; Usui, M.; Tanji, Y., et al. Bacteriophage phisa012 has a broad host range against staphylococcus aureus and effective lytic capacity in a mouse mastitis model. Biology (Basel) 2018, 7.*

THERAPEUTIC EFFECTS OF INTRAVITREOUS BACTERIOPHAGE ON VANCOMYCIN RESISTANT ENTEROCOCCAL ENDOPHTHALMITIS IN MICE

KISHIMOTO, Tatsuma; ISHIDA, Waka; SUZUKI, Takashi; UCHIYAMA, Jumpei; MATSUZAKI, Shigenobu; DAIBATA, Masanori; FUKUDA, Ken; FUKUSHIMA, Atsuki

Kochi Medical School, Kochi, Japan
Toho University, Tokyo, Japan
Azabu University, Kanagawa, Japan

jm-kishimoto@kochi-u.ac.jp

Introduction: Endophthalmitis can cause irreversible vision loss. We examined the effects of intravitreous bacteriophage on vancomycin-resistant (VR) enterococcal endophthalmitis in mice.

Material & Methods: Endophthalmitis was induced by injection of 1×10^4 VR *Enterococcus faecalis* bacteria into the vitreous. Bacteriophage ΦEF24C-P2 was injected intravitreously 6 h later. The clinical score was evaluated 24 h after bacterial injection, and eyeballs were isolated for enumeration of viable bacteria, quantitation of inflammatory cell infiltration by assay of myeloperoxidase (MPO) activity, and pathological examination.

Results: The bacteria could grow in the presence of vancomycin (2 mg/ml) in vitro. The fundus of mice was invisible at 24 h after bacterial injection as a result of fibrin precipitation or bleeding in the anterior chamber or of vitreous opacity. The number of viable bacteria increased to ~1×10^7 CFU and MPO activity was elevated in vehicle-treated eyes. The clinical score, number of viable bacteria, and MPO activity were significantly decreased by injection of ΦEF24C-P2. Pathological examination revealed inflammatory cell infiltration and retinal detachment in vehicle-treated eyes, whereas cell infiltration was attenuated and retinal structure maintained in phage-treated eyes.

Conclusion: Intravitreous bacteriophage injection is effective for treatment of VR enterococcal endophthalmitis.

PHAGE SELECTION AGAINST VIRULENCE FACTORS INVOLVED IN INTRACELLULAR SPREAD OF *SHIGELLA FLEXNERI*

KORTRIGHT, Kaitlyn E.[1]; SLOAN, Dylan[2]; MODY, Akshay[3]; CHAN, Benjamin K.[1]; TURNER, Paul E.[1]

[1]Yale University, New Haven, USA
[2]Hopkins High School, New Haven, USA
[3]Peddie High School, Hightstown, USA

kaitlyn.kortright@yale.edu

Introduction: *Shigella flexneri* has a large repertoire of plasmid and chromosomally encoded virulence factors[1]. Some of these virulence factors are surface expressed, leaving the bacterium vulnerable to infection from phages able to use these virulence factors as receptor binding sites. In particular, outer membrane proteins OmpA and OmpC of contribute to intracellular spread of *S. flexneri*[2,3].

Material & Methods: From environmental water samples, we isolated 62 new phages able to infect *S. flexneri*. Lytic spectrum, phage growth, and absorption assays were used on knock-out and complementation strains to identify phage that utilized OmpA or OmpC as receptors. Spontaneous phage-resistant mutants were generated.

Results: Of the 62 isolated phages, we identified 12 that require OmpA and/or OmpC to infect *S. flexneri*. We further demonstrate that spontaneous phage resistant mutants are phenotypically similar to engineered Δ*ompA* and Δ*ompC* strains.

Conclusion: Using knock-out strains of *S. flexneri*, we were able to identify 12 bacteriophages able to utilize virulence factors as receptor binding sites. Genomic data of phage resistant mutants may reveal that the sites undergoing selection are in the operons of *ompA* and/or *ompC*. We are currently determining the *in vitro* impact of these phage on virulence in a tissue culture model of infection.

References:

1. *Daskaleros PA and Payne SM. 1985. Cloning the gene for Congo red binding in Shigella flexneri. Infection and Immunity.*
2. *Bernardini ML, Sanna MG, Fontaine A and Sansonetti PJ. 1993. OmpC is involved in invasion of epithelial cells by Shigella flexneri. Infection and Immunity.*
3. *Ambrosi C, Pompili M, Scribano D, Zagaglia C, Ripa S, and Nicoletti M. 2012. Outer Membrane Protein A (OmpA): A New Player in Shigella flexneri Protrusion Formation and Inter-Cellular Spreading. PLOS.*

THE STUDIES ON THE LYTIC SPECTRA OF BACTERIOPHAGES SPECIFIC TO ESBL-PRODUCING *KLEBSIELLA PNEUMONIAE* STRAINS

KULA Dominika[1]; JOŃCZYK-MATYSIAK Ewa[1]; ŁODEJ Norbert[1]; NEUBERG Joanna[1]; OWCZAREK Barbara[1]; ORWAT Filip[1]; ŁUSIAK-SZELACHOWSKA Marzanna[1], WEBER-DĄBROWSKA Beata[1,2]; GÓRSKI Andrzej[1,2,3]

[1]Bacteriophage Laboratory, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland

[2]Phage Therapy Unit, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy (IIET), Polish Academy of Sciences, Wrocław, Poland

[3]Department of Clinical Immunology, Transplantation Institute, Medical University of Warsaw, Warsaw, Poland

dominika.kula@iitd.pan.wroc.pl

Introduction: In 2017, the World Health Organization (WHO) published a list of bacteria that pose the greatest threat to human health. *Klebsiella* is mentioned among those pathogens. *Klebsiella* strains are opportunistic pathogen and also belonging to ESKAPE group.

Material & Methods: The lytic activity of 95 *Klebsiella* phages were examined both on the ESBL-positive strains which were isolated from the patients (n=45) and host strains that are used for phage amplification (n=52).

Results: It was observed that 35% of the strains were resistant to the tested phages. The bacteriophages tested on ESBL-positive *Klebsiella pneumoniae* strains were characterized by a narrow lytic spectrum (2,2 to 31%). The titers of phages amplified on the antibiotic-resistant strains were lower than those obtained with strains used for standard phage amplification. Searching for new therapeutic phages in environmental samples (n=251) has not yielded positive results.

Conclusion: The results of the study demonstrate that antibiotic-resistant *Klebsiella* strains are only weakly sensitive to our phages. β-lactam resistant bacterial strains could not be used as host strains for phage amplification. There is an urgent need for the isolation of new therapeutic phages against *Klebsiella pneumoniae*, in particular against ESBL-positive bacteria.

BACTERIOPHAGE-ANTIBIOTIC COMBINATIONS FOR DISRUPTION OF GRAM-POSITIVE BIOFILMS ON LEFT VENTRICULAR ASSIST DEVICE DRIVELINES

LEUCK, Anne-Marie[1], NILSON, Jennifer[2], AHMAD, Adeel[1], and DUNNY, Gary M.[2]

[1] Division of Infectious Diseases and International Medicine, Department of Medicine

[2] Department of Microbiology, University of Minnesota, Minneapolis, Minnesota

Background: Left ventricular assist devices (LVADs) have revolutionized the treatment of heart failure, but LVAD driveline infections affect up to 30% of patients per year. Most driveline infections are due to Gram-positive pathogens. We have previously shown that common antibiotics have little effect on biofilms grown on LVAD drivelines *in vitro*. Here, we investigate the effects of lytic bacteriophages (phages) alone and in combination with antibiotics.

Methods: *Enterococcus faecalis* or *Staphylocccus aureus* biofilms were grown on sections of Dacron-coated silicone LVAD drivelines (enterococci) or silicone disks (staphylococci) for 24 hours in liquid growth medium. Antibiotics and lytic phage were added for an additional 24 hours. Planktonic and biofilm bacteria were quantified.

Results: For *E. faecalis*, a combination of ampicillin and phage eradicated the planktonic cells and was more effective than either phage or ampicillin alone ($p < 0.05$). For biofilm cells, combination treatment was more effective than either phage or ampicillin alone ($p < 0.05$). *S. aureus* shows similar promise with multiple antibiotics.

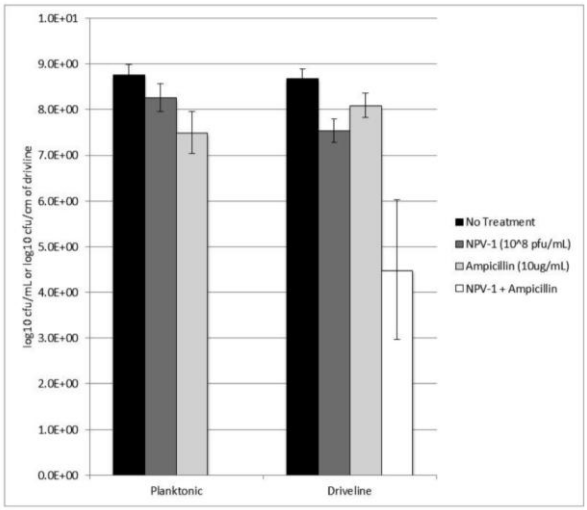


Figure 1: Effect of NPV-1 bacteriophage and ampicillin on *Enterococcus faecalis* grown on LVAD driveline material.

Conclusion: Phage therapy may prove to be an important option for LVAD driveline infections and will be especially important for the many patients in whom antibiotics alone have failed and device removal is not an option.

ERADICATION OF MULTIDRUG-RESISTANT *STAPHYLOCOCCUS AUREUS* BIOFILMS USING TWO NEWLY ISOLATED PHAGES

ŁUBOWSKA, Natalia[1]; GRYGORCEWICZ, Bartłomiej[2]; PIECHOWICZ, Lidia[1]

[1]Department of Medical Microbiology, Faculty of Medicine, Medical University of Gdansk, Gdansk, Poland
[2]Department of Immunology, Microbiology and Physiological Chemistry, Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology, Szczecin, Poland

nlubowska@gumed.edu.pl

Introduction: *Staphylococcus aureus* is one of the most common nosocomial pathogens. An important virulence factor of *S. aureus* is its ability to form biofilm. The biofilm forming *S. aureus* strains are responsible for causing a number of diseases. They are a serious challenge for today's medicine. An alternative approach for the treatment of *S. aureus* infections is bacteriophage therapy. This study assessed the potential of using two newly isolated lytic phages to eradicate multidrug-resistant *S. aureus* biofilms.

Material & Methods: The study examined two newly isolated phages on three multidrug-resistant clinical isolates of *S. aureus*. Biofilms formed on microtiter plates were treated with phage lysates in different concentrations. The removal of established biofilms was investigated by crystal violet staining, MTT assay, scanning electron microscopy (SEM) and colony forming unit enumeration.

Results: Both phages tested resulted in a significant reduction of biomass (61-93%) and a decrease in the metabolic activity (50-94%) of biofilm produced by selected isolates. SEM analysis revealed the reduction of the number of cells in all biofilms treated with phages. The amount of the extracellular matrix in some of them was higher than in untreated biofilms.

Conclusion: This study indicates that isolated phages can effectively remove *S. aureus* biofilms.

References:

1. *Alves D.R., Gaudion A., Bean J.E., Perez Esteban P., Arnot T.C., Harper D.R., Kot W., Hansen L.H., Enright M.C., Jenkins A.T.A.: Combined use of bacteriophage K and a novel bacteriophage to reduce Staphylococcus aureus biofilm formation. Appl. Environ. Microbiol., 2014; 80: 6694–6703;*
2. *Fong S.A., Drilling A., Morales S., Cornet M.E., Woodworth B.A., Fokkens W.J., Psaltis A.J., Vreugde S., Wormald P.-J.: Activity of Bacteriophages in Removing Biofilms of Pseudomonas aeruginosa Isolates from Chronic Rhinosinusitis Patients. Front. Cell. Infect. Microbiol., 2017; 7: 1–11;*

IDENTIFICATION OF PROPHAGES TYPES IN COAGULASE-NEGATIVE *STAPHYLOCOCCUS PETRASII* STRAINS

MAŠLAŇOVÁ, Ivana; KOVAŘOVIC, Vojtěch; VRBOVSKÁ, Veronika; PANTŮČEK, Roman; and DOŠKAŘ, Jiří

Masaryk University, Faculty of Science, Department of Experimental Biology, Brno, Czech Republic

iva.maslanova@gmail.com

Introduction: The recently described coagulase-negative and novobiocin-susceptible species *Staphylococcus petrasii* is considered to be an opportunistic pathogen, recovered mostly from young children and older patients. Four subspecies have been described: *S. petrasii* subsp. *petrasii, S. petrasii* subsp. *croceilyticus*, *S. petrasii* subsp. *jettensis* and *S. petrasii* subsp. *pragensis*. In coagulase-negative staphylococci many mobile genetic elements including bacteriophages have been discovered and these can cause virulence, antimicrobial resistance, adaptation to the environment and evading the immunity system of the host.

Material & Methods: Whole genome sequencing allowed target phage-borne genes for prophage typing. The integrase and amidase prophage typing by multiplex PCR assay was designed.

Results: Prophages in bacterial genomes can carry genes beneficial for the bacterium and after induction, they transfer genes in the bacterial population. Only a little information is known about bacteriophages and prophages in *S. petrasii* species. New prophages classified to 8 types with a unique combination of integrase and amidase genes have been identified in set of 66 *S. petrasii* strains.

Conclusion: In this study, we developed prophage typing assay and demonstrated prophages types' diversity in *S. petrasii* with potential role in spreading resistance genes among staphylococci.

DISSEMINATION OF GENES CODING FOR ANTIBIOTIC-RESISTANCE IN CANINE *STAPHYLOCOCCUS PSEUDINTERMEDIUS*

MERONI, Gabriele; MARTINO, Piera Anna

Università degli Studi di Milano, Milan, Italy
Department of Veterinary Medicine

gabriele.meroni@unimi.it

Introduction: *Staphylococcus pseudintermedius* (SP) is an opportunistic pathogen often associated with otitis externa and pyoderma in dogs (1,2). The aim of this study is to focus on the distribution of genes coding for antibiotic resistance in SP isolates.

Material & Methods: This study analyses 116 SP strains. Twenty-two different antibiotic molecules were used in the Kirby-Bauer disk diffusion test; the antibiotics were divided in three groups (G_1, G_2 and G_3). All the strains were exposed to G_1 (*OX_5, AMC_{30}, CL_{30}, CVN_{30}*, *EFT_{30}, DA_{10}, DO_{30}, ENR_5, MAR_5*). The presence of *mecA*, *blaZ*, *tetM*, *tetK* and *aacA-aphD* genes *was assessed using M-PCR.*

Results: 42 strains were Multi-Drug resistant (MDR). All these strains were exposed to G_2 (AK_{30}, *CN_{30}*, *N_{30}*, *TOB_{10}, $Prado_5$, RD_{30}, CRO_{30}, $Linco_{10}$*) and G_3 (*AML_{10}, CAR_{100}, AZM_{15} E_{30}, K_{30}*). The 27% were methicillin-resistant. An high rate of resistance to cephalosporins was found. Only the MDR strains were genetically studied by PCR. Among methicillin-resistant strains (MRSP), 83% were *mecA*-positive and 95% were *blaZ*-positive. The percentage of *tetK*, *tetM* and *aacA-aphD* positive strains was respectively 31%, 45% and 81%.

Conclusion: All the MRSP isolates exhibited a simultaneous resistance to three or more antibiotics, indicating that they are very dangerous MDR strains.

References:

1. *Kang MH, Chae MJ, Yoon JW, Kim SG, Lee SY, Yoo JH, et al. Antibiotic resistance and molecular characterization of ophthalmic Staphylococcus pseudintermedius isolates from dogs. J Vet Sci. 2014;15(3):409–15.*
2. *Osland AM, Vestby LK, Fanuelsen H, Slettemeås JS, Sunde M. Clonal diversity and biofilm-forming ability of methicillin-resistant Staphylococcus pseudintermedius. J Antimicrob Chemother. 2012;67(4):841–8.*

EFFECT OF URIDINE ON THE NF-KB SIGNALING PATHWAY AND THE EXPRESSION OF HSP72 IN SPLEEN LYMPHOCYTES FROM INFLAMMATION-BEARING MICE

MIRONOVA, Galina[1]; KHRENOV, Maxim[2]; TALANOV, Eugeniy[1]; GLUSHKOVA, Olga[2]; BELOSLUDTSEVA, Natalia[1]; PARFENYUK, Svetlana[2]; NOVOSELOVA, Tatyana[2]; LUNIN, Sergey[2]; NOVOSELOVA, Elena G.[2]; LEMASTERS, John[1,3]

[1]Institute of Theoretical and Experimental Biophysics RAS, Pushchino, Moscow Region, Russia
[2]Institute of Cell Biophysics RAS, Pushchino, Moscow Region, Russia
[3]Medical University of South Carolina, Charleston, USA

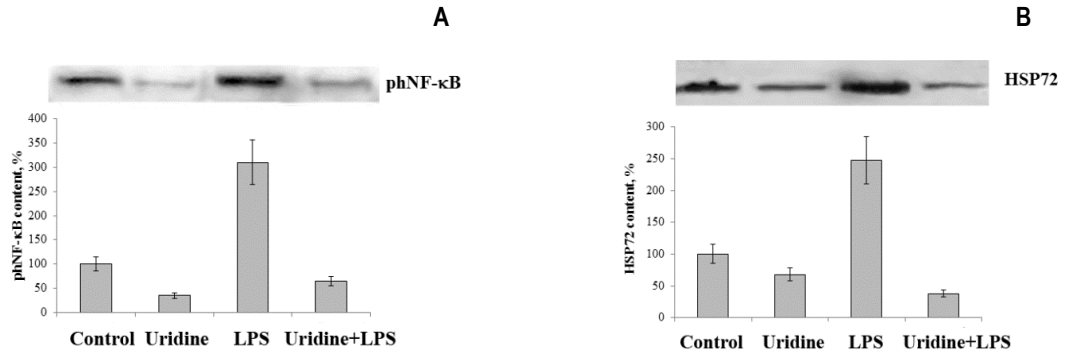
mironova40@mail.ru

Introduction: The study was designed to study the effect of uridine on plasma cytokine's profile, expression of heat shock protein Hsp72 and activity of the NF-κB signaling cascade in spleen lymphocytes after treatment of male Balb/c mice to *E.coli* lipopolysaccharide (LPS).

Material & Methods: Mice were treated with uridine (30 mg/kg body weight, i.p.) or vehicle followed 1 h after LPS (2.5 mg/kg, i.p.) injected. ELISA Development Kits were used for mouse cytokines measuring, and *Western blot analysis* was used to determine the signaling and stress protein.

Results: Endotoxin increased the cytokine's level in plasma by 2 times, expression of heat shock protein Hsp72 by 2.4 times and also activated NF-κB signaling in splenic lymphocytes. Prior treatment with uridine prevented all of these effects. Inhibitory analysis showed that the mechanism of uridine action is associated with the forming of the UDP - activator mitochondrial K_{ATP} channel [1], and the UTP - activator of the synthesis of the glycogen. MitoK_{ATP} inhibitors, 5-hydroxydecanoate and glybenclamide, as well as inhibitor of glycogen synthesis, - galactosamine, prevent the effects of uridine.

Conclusion: The mechanism of uridine effects is associated with the activation of glycogen synthesis and the opening of the mitochondrial KATP channel, which, in turn, increases the energy potential of the cell and reduces oxidative stress [2]. The work was supported by the Russian Science Foundation (No. 16-15-00157).



Effect of uridine on phospho-NF-κB (A) and Hsp72 (B) production by spleen lymphocytes from LPS-injected mice

References:

1. *G.D. Mironova et al., J Biol. Chem. 279(31) (2004) 4127–4135.*
2. *I.B. Krylova et al.,, Exp. Gerontol. 41(7) (2006) 697-70311.*

ISOLATION OF BACTERIOPHAGES FROM WASTEWATER AND THEIR LYTIC ACTIVITY IN UROPATHOGENIC *ESCHERICHIA COLI* STRAINS

GONZÁLEZ-VILLALOBOS Edgar[1, 2]; RIBAS-APARICIO Rosa María[1]; BELMONT-MONROY Laura[1, 2]; SAN MIGUEL-GARCÍA Axel[1, 2]; APARICIO-OZORES Gerardo[1]; ESLAVA-CAMPOS Carlos Alberto[2]; HERNÁNDEZ-CHIÑAS Ulises[2]; CHÁVEZ-BERROCAL Ma. Elena[2]; MOLINA-LÓPEZ José[2].

[1]Escuela Nacional de Ciencias Biológicas, IPN, Ciudad de México, México
[2]UNAM – Hospital Infantil de México "Federico Gómez", Ciudad de México, México

joseml@unam.mx

Introduction: Uropathogenic *Escherichia coli* (UPEC) is the main etiologic agent of urinary tract infections (UTIs). The selection of multiresistant UPEC strains has been resulted in medical complications and inefficient treatments, which has made necessary to search for new therapeutic alternatives. Phage therapy is an option based in their bactericidal activity.

Material & Methods: Searching for phages in residual water samples was carried out by agar double layer, only lytic phages were selected, each was propagated and purified by polyethylene glycol precipitation, their lytic activity was evaluated in UPEC strains (50 from acute and 50 from recurrent UTIs).

Results: Three phages were selected (φEc1, φEc3, and φEc4). They show a lytic activity in 30%, 18% and 13% for φEc1, φEc3, and φEc4 respectively in UPEC from acute, otherwise, in strains from recurrent UTIs we found the following percentages of 14%, 6% and 6% for φEc1, φEc3, and φEc4 respectively. φEc3 and φEc4 infected the same strains in both groups suggesting they use same receptors for recognition.

Conclusion: UPEC lytic phages were isolated from wastewater, the susceptibility of the strains from acute and recurrent UTIs was different, φEc1 proved to be more effective in UPEC from acute UTI´s than recurrent.

References:

1. *Lehman SM, y Donlan RM. (2015). Bacteriophage-mediated control of a two-species biofilm formed by microorganisms causing catheter-associated urinary tract infections in an in vitro urinary catheter model. Antimicrob. Agents Chemother. 59:1127-1137*
2. *Wittebole X, De Roock S, Opal SM. (2013). A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. Virulence, 5:226–235.*
3. *Clokie MR, Millard AD, Letarov AV, Heaphy S. (2011). Phages in nature. Bacteriophage, 1(1), 31–45.*

ISOLATION AND CHARACTERIZATION OF NOVEL BACTERIOPHAGES ACTIVE AGAINST *STREPTOCOCCUS UBERIS*

JAKOČIŪNĖ, Džiuginta[1]; MOODLEY, Arshnee[1]

[1]University of Copenhagen, Frederiksberg C, Denmark

asm@sund.ku.dk

Introduction: Bovine mastitis is one of the most common infections in dairy cows worldwide with *Streptococcus uberis* being the causative agent of a large proportion of these infections. There are no studies on the use of phages for treatment or prevention of *S. uberis*-mastitis. Our aim was to isolate and characterize phages against *S. uberis*.

Material & Methods: Phages were isolated from the environment ofdairy farms with chronic *S. uberis* infections in Denmark. Samples were enriched using BHI containing $CaCl_2$and a mix of 20 clinical *S. uberis* strains isolated from the same farms, which were then spotted on individual bacterial strains. Purified phages were sequenced and compared to published genomes using BLAST. Lytic host range was determined by the spot method on the 20 *S. uberis* strains.

Results: Sixteen phages were isolated from seven environmental samples. 14/16 phages were closely related (99% similarity). The remaining two phages were similar to each other (92%) but distinct from the other 14 phages. Based on BLAST, phages appear to be unique with only partial similarity to a streptococcal prophage. Phages exhibited a narrow host range, only infecting one *S. uberis* strains.

Conclusion: We describe novel phages active against mastitis causing *S. uberis*.

PHAGE THERAPY AGAINST INTRACELLULAR BACTERIAL PATHOGENS: DEVELOPING NEW LIPOSOMAL DELIVERY STARTEGIES

MUELLER, Peter F.; ROEMER, Winfried

University of Freiburg, Freiburg im Breisgau, Germany

peter.fritz.mueller@bioss.uni-freiburg.de

Intracellular bacteria are a perpetual threat of the humankind. The ability to hide from the immune system and the development of antibiotic resistances turn those pathogens into a severe danger. *Mycobacterium tuberculosis* causes one of the most critical infectious diseases with 6.3 million newly reported cases in 2016, almost 10% of those antibiotics resistant [1]. Therefore, most promising hope in fighting this major health problem is the treatment with bacteriophages [2-3].

Studies from our laboratory have successfully shown the liposomal encapsulation and intracellular delivery of bacteriophage [4]. The most efficient and gentle techniques for phage-filled vesicle formation are lipid film swelling on top of a polyvinyl alcohol support [5] and by generation of an inverted emulsion [6]. With our expertise in the production of liposomes we aim to create constructs affording the encapsulation and intracellular delivery of bacteriophage. By using various lipid compositions we will be able to address different cellular locations, such as the cytosol by fusogenic liposomes or endosomal compartments by directed phagocytosis.

Using those liposome based delivery strategies, we aim to establish a versatile therapeutic tool to target different intracellular bacteria, despite the presence of antibiotic resistances.

References:

1. *Global tuberculosis report 2017 (2017). Geneva: World Health Organization. Licence: CC BY-NCSA 3.0 IGO; ISBN 978-92-4-156551-6*
2. *Abedon, S. et al. (2011). Phage treatment of human infections. Bacteriophage 1(2), 66–8.*
3. *Sulakvelidze, A. et al. (2001). Bacteriophage Therapy. Antimicrobial Agents and Chemotherapy 45(3), 649–659.*
4. *Nieth, A. et al. (2015). A first step toward liposome-mediated intracellular bacteriophage therapy. Expert Opinion on Drug Delivery 12(9), 1411-1424.*
5. *Weinberger, A. et al. (2013). Gel-Assisted Formation of Giant Unilamellar Vesicles. Biophysical Journal 105(1), 154–164.*
6. *Pautot, S. et al. (2003). Production of Unilamellar Vesicles Using an Inverted Emulsion. Langmuir 19(7), 2870–2879.*

UNDERLYING MECHANISM OF PHAGE-ANTIBIOTIC SYNERGY (PAS)

MYUNG, Heejoon; HWANG, Yoonjung;

Hankuk University of Foreign Studies, Yong-In, Korea

hjmyung@hufs.ac.kr

Introduction: When phages infect bacteria cultured in the presence of sub-lethal doses of antibiotics, the sizes of the phage plaques are significantly increased. This phenomenon is known as phage-antibiotic synergy (PAS). In this study, the observation of PAS was extended to a wide variety of bacteria-phage pairs using different classes of antibiotics.

Results: PAS was shown in both Gram-positive and Gram-negative bacteria. Cells stressed with β-lactam antibiotics filamented or swelled extensively, resulting in increased phage production. PAS was also sometimes observed in the presence of other classes of antibiotics with or without bacterial filamentation. The addition of antibiotics induced *recA* expression in various bacteria, but a *recA*-deletion mutant strain of *E. coli* also showed filamentation and PAS in the presence of quinolone antibiotics. The phage adsorption efficiency did not change in the presence of the antibiotics when the cell surfaces were enlarged as they filamented. Although an increased availability of phage components were observed in these cells, it had little effect in the actual number of phages produced. Instead, the prolonged assembly period due to delayed lysis was the main reason for PAS. The increase in the cell surface area far exceeded the increase in phage holin production in the filamented host cells, leading to a relatively limited availability of intracellular holins for aggregating and forming holes in the host membrane. Reactive oxygen species (ROS) stress also led to an increased production of phages, while heat stress only showed a limited increase in phage production.

Conclusion: We have elucidated the underlying mechanism of PAS.

METHOD FOR EFFECTIVE *CAMPYLOBACTER* PHAGE ISOLATION FROM POULTRY SOURCES

NAFARRATE, Ibai; LASAGABASTER, Amaia; AMÁRITA, Felix; MARTINEZ DE MARAÑON, Iñigo

AZTI, Food Research Division, Derio (Bizkaia), Spain

inafarrate@azti.es

Introduction: Campylophages have been isolated with variable success rates from 0 to 100%, which could be due to different isolation methods or kind of samples. This study aimed to compare the efficiency of three methods on campylophage recovery from poultry sources.

Material & Methods: Chicken skin and feces samples were inoculated with phage AZT501 at 10^4 PFU/g and processed by a stabilization method and two enrichment methods in BHI and Bolton broth, respectively. Phage recovery was assessed by the spot test.

Results: No differences among tested methods were found for chicken feces, observing phage recovery rates from 63 to 80 %. Similar efficiencies of 65 and 69 % were obtained in skin samples treated by the first two methods. Nevertheless, higher efficiency of 160 % was achieved in samples enriched with Bolton, which could be consequence of a great phage replication. Differences on recovery rates in skin and feces samples could be due to a matrix effect.

Conclusion: Bolton broth enrichment could be considered the most efficient methodology to recover campylophages from chicken skin. Further research is needed to study the effect of the matrix, the phage titer and the kind of phage on the recovery efficiency.

COMPARATIVE ANALYSIS OF NEW *BACILLUS ANTHRACIS* PHAGES AND THEIR LYTIC ENZYMES

NAKONIECZNA, Aleksandra[1]; ŁOBOCKA, Małgorzata[2]; MIZAK, Lidia[1]; GRYKO, Romuald[1]

[1] Military Institute of Hygiene and Epidemiology, Puławy, Poland
[2] Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Warsaw, Poland

a.nakonieczna1@gmail.com

Introduction: Antibiotic therapy in anthrax infections takes a long time and can save lives only when administered immediately after the exposure. Decontamination of places infected naturally as well as intentionally after using a biowarfare (*B. anthracis* spores) is difficult without using strong chemicals. Phages or their lytic enzymes could be applied standalone or as a support to the regular treatment or as natural decontaminants. Therefore, we tried to find new phages and lysins killing *B. anthracis* cells.

Material & Methods: A couple of new phages were identified and sequenced. Sequences of their full genomes and of their endolysins were compared and analyzed.

Results: Genomic sequences of the tested phages appear to be in 70-90 % identical to each other. Their lysins are of the same length and show only a few amino acid sequence differences. However, their predicted secondary structures may be slightly different which possibly implies variations in specificities. Bacteriolytic activities of new lysins will be tested in further experiments with pure protein preparations.

Conclusion: *B. anthracis* infecting phages isolated from different sites show noticeable differences but their lysins are highly similar. Whether slight differences between them result in specificity differences will be tested.

PHAGE THERAPY FOR VIBRIOSIS

RAMASAMY Palaniappan

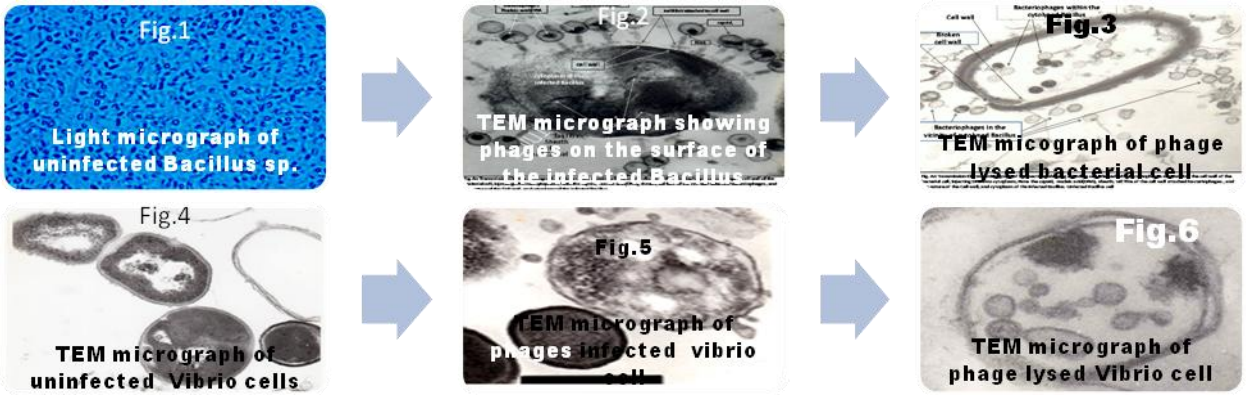
University, R&D Wing, BIHER-Bharath University, Chromepet, India

researchsbmch@gmail.com

Introduction: The phage therapy is one of the most important control strategies envisaged for the management of bacterial diseases in the aquatic environment (1-3). There are no other effective alternative approaches for the natural control of Vibriosis while phage therapy remains the best method which has not yet been exploited. In the present study, the occurrences, morphology, infectivity, lytic activities and therapeutic potentials of the phages of *Vibrios and Bacillus* were demonstrated.

Materials and methods: Agar bioassay method and one-step growth experiments of the lytic phages infected *Vibrio* spp *and Bacillus spp.*, *in vitro* experiments were carried out to determine the efficacy of phage therapy on the host bacterial population (*1-3).*

Results: *In vitro* experiments, efficacy of phage therapy on the host bacterial population, Agar bioassay method and one-step growth experiments of the phage infected *Vibrio* spp *and Bacillus spp.* validated their lytic activities, killing effects of the host bacterial cells, growth inhibitory effects, burst sizes, and latent periods. The study demonstrated the occurrences of plagues of lytic phages of *Vibrio* sp and *Bacillus* spp and their control effects of vibriosis and thus established the application and efficacy of the phages of *Vibrio/ Bacillus* against the host bacterial pathogens (Figs.1-6).



Conclusion: The study demonstrated the occurrences of lytic phages, their morphology, infectivity, lytic activities and therapeutic potentials of the phages of *Vibrios and Bacillus* and their usefulness in phage therapy.

References:

1. *Srinivasan, P., and P.Ramasamy, Morphological characterization and biocontrol effects of Vibrio vulnificus phages against Vibriosis in the shrimp aquaculture environment Microbial Pathogenesis* 111:472-480. doi: 10.1016/j.micpath.2017.09.024. Epub 2017 Sep 13.*
2. *Srinivasan, P., Ramasamy, P. Brennan, G.P. and Hanna, R.E.B.Inhibitory effects of bacteriophages against shrimp pathogenic Vibrio spp. of the Indian aquaculture environment. Asian Journal of Animal and Veterinary Advances*, 2007.2(4): 166-183*
3. *Vaseeharan, B. and Ramasamy, P., Control of Pathogenic Vibrio Spp. Bacillus Ubtilis BT23, a possible probiotic treatment for black tiger shrimp penaeus monodon. Letters in Applied Microbiology*,2003; 1-5.*

STRATEGIES TO MINIMISE ANTIBIOTIC RESISTANT BACTERIA IN FARM ANIMALS

RANDALL, Luke; HORTON, Robert; LEMMA, Fabrizio; MARTELLI, Francesca; DAVIES, Robert

Animal and Plant Health Agency (Weybridge), New Haw, Addlestone, UK

Luke.Randall@apha.gsi.gov.uk

Introduction: Strategies and policies to reduce antibiotic resistance (AMR) in farm animals are considered based on recent studies performed at the Animal and Plant Health Agency (APHA), UK.

Materials & Methods: Recent studies performed by the APHA have investigated the presence of Extended Spectrum β-lactamase (ESBL) resistant *E. coli* in meats,[1] farm animals and in waste milk that is fed to calves;[2] fluoroquinolone resistance in *E. coli* in pigs and poultry and *mcr-1* plasmid-mediated colistin resistant *E. coli* in pigs.[3] Based on these and other APHA studies, we comment on strategies and policies that may reduce AMR bacteria in farm animals and food.

Results: Cessation or reduction in use of relevant antimicrobials in animals was followed by a reduction in ESBL *E. coli* in chickens and a reduction of *mcr-1 E. coli* (below the detection limit) in pigs previously positive. Cessation of feeding waste milk containing cephalosporin residues to calves is likely to reduce the prevalence of ESBL bacteria in those calves. Dose optimisation[4] and/or combined antimicrobial therapy[5] may help reduce emergence of fluoroquinolone resistant bacteria in animals.

Conclusion: A multifaceted approach to reducing AMR in food animals is likely to have a greater impact.

References:

1. *Randall LP, Lodge MP, Elviss NC et al. Evaluation of meat, fruit and vegetables from retail stores in five United Kingdom regions as sources of extended-spectrum beta-lactamase (ESBL)-producing and carbapenem-resistant Escherichia coli. International Journal of Food Microbiology 2017; 241: 283-90.*
2. *Randall L, Heinrich K, Horton R et al. Detection of antibiotic residues and association of cefquinome residues with the occurrence of Extended-Spectrum beta-Lactamase (ESBL)-producing bacteria in waste milk samples from dairy farms in England and Wales in 2011. Res Vet Sci 2014; 96: 15-24.*
3. *Anjum MF, Duggett NA, AbuOun M et al. Colistin resistance in Salmonella and Escherichia coli isolates from a pig farm in Great Britain. J Antimicrob Chemother 2016; 71: 2306-13.*
4. *Randall LP, Cooles SW, Coldham NC et al. Modification of enrofloxacin treatment regimens for poultry experimentally infected with Salmonella enterica serovar Typhimurium DT104 to minimize selection of resistance. Antimicrob Agents Chemother 2006; 50: 4030-7.*
5. *Randall L, Ridley A, Lemma F et al. In vitro investigations into the use of antimicrobials in combination to maintain efficacy of fluoroquinolones in poultry. Res Vet Sci 2016; 108: 47-53.*

CHARACTERIZATION OF LYTIC BACTERIOPHAGES AGAINST BIOFILM-FORMING MULTI DRUG RESISTANCE PSEUDOMONAS AERUGINOSA

SARSHAD Koderi Valappil[1], ZOLTÁN Deim[1], GABRIELLA Terhes[2], EDIT Urbán[2], GYÖRGY Schneider[3]
TAMÁS Kovács[4], GÁBOR Rákhely[1]

[1]Department of Biotechnology, University of Szeged, Hungary
[2]Institute of Clinical Microbiology, University of Szeged, Hungary
[3]Department of Medical Microbiology and Immunology, University of Pécs, Hungary
[4]Department of Biotechnology, Nanophagetherapy Center, Enviroinvest Corp. Pécs, Hungary

Way2sarshad@gmail.com

Introduction: Phage therapy is a promising treatment for multi-drug resistant (MDR) bacteria. The single infection burst out to make an exponential pattern, showing a faster killing effect than other therapeutic agents. P.aeruginosa, opportunistic MDR pathogen is poised to become a common disease problem. Humans readily encounter P. aeruginosa, which thrives in environments, varying from estuaries to hospitals and household sink drains.

Materials & Method: The serotypes of clinical strains were determined. The morphology of phages was analyzed by electron microscopy, the genomes were studied by NGS Technologies. The effectivity of phage cocktails on recalcitrant biofilm architecture was examined by confocal laser scanning microscope.

Results: More than twenty *P. aeruginosa* strains belonging various serotypes and 13 lytic phages were isolated and characterized. The biofilm-forming ability of the strains greatly varied. The serotype specificity of the phages was determined using cultures and bacterial biofilms. A defined cocktail composed of 5 phages with distinct serotype specificity was effective against any biofilm-former strains.

Conclusion: Increasing prevalence of multi-drug-resistant bacterial infections has necessitated novel antibacterial strategies. This approach, using phage cocktail as targeted antibacterial, could extend and reduce the incidence of extreme common adaption of Biofilm Infections.

LYTIC BACTERIOPHAGES FOR CONTROL OF MULTIPLE DRUGS RESISTANT SALMONELLA ISOLATES FROM POULTRY AND HUMAN STOOL SAMPLES

SATTAR Sadia; ULLAH Inam; SHAMSI Bushra; KHALID Kehkashan; BOSTAN Nazish; AKBER Haji

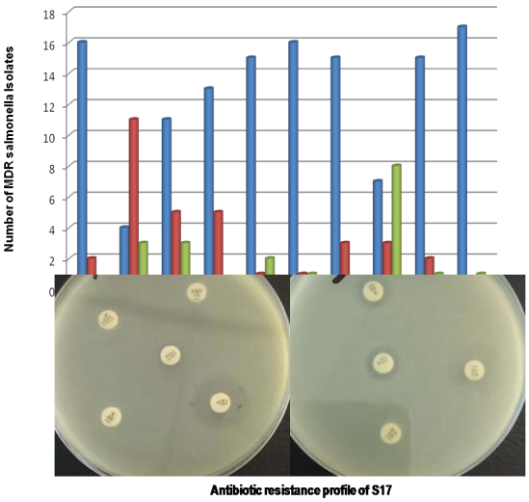
Comsats Institute of Information Technology, Islamabad, Pakistan

Sadia.sattar@comsats.edu.pk

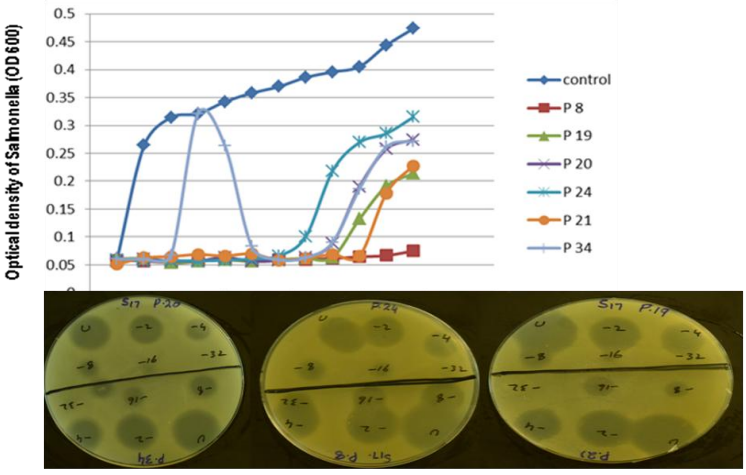
Introduction: Salmonella is endemic food borne pathogen which causes acute enteric infections in humans and poultry due to contaminated food. Poultry is the main reservoir of salmonella. Use of sub therapeutic doses of antibiotics as growth promoters in poultry is mainly responsible for emergence of multiple drug resistant salmonella strains. This study was designed with the aim of finding extent of resistance in salmonella strains circulating in retail poultry in Punjab province, Pakistan. In total 18 multiple drug resistant salmonella isolates were characterized in this study (n=30). Majority isolates were resistant to more than six antibiotics. We also isolated and characterized 6 bacteriophage isolates which had promising results for the reduction of drug resistant isolates.

Material & Methods: Sample collection: Poultry and human stool samples were collected from different regions of Punjab province Pakistan. Samples were processed for the presence of salmonella and bacteriophages. Antibiotic resistance profile of salmonella strain: was carried out by disc diffusion method. Bacteriophages assays a) determination of Phage titer: was carried out by spot assay, plating assay and dilution method previously described by (Doria et al., 2013). Bacterial growth reduction assay: was carried out according to previously mentioned protocol by in order to evaluate the lytic potential of phages. Infection of bacteria with the phages was monitored for 24 hours. The bacterial reduction caused by phages was compared with control. Determination of thermal and PH stability of phages: Thermal and PH stability of phages were evaluated by incubating phage suspensions at various Temperatures (37, 50, and 70 ° C) and PH (2, 5, 7, 9,11, respectively) at 25°C for 1 hours. Assay were carried out according to previously mentioned protocol by (Carey-Smith, Billington, Cornelius, Hudson, & Heinemann, 2006). Bacteriophage genome extraction and analysis: Genome extraction were performed by PCI (phenol-chloroform-isoamylalcohol) method previously reported by. Gel electrophoresis were performed for observing the extracted genome.

Results:



Antibiotic resistance profile of S17



Phage growth on S17 a highly resistant salmonella isolate.

Conclusion: All six bacteriophage isolates characterized had promising potential to control multiple drug resistant salmonella isolates

References:

1. *Carey-Smith, G. V., Billington, C., Cornelius, A. J., Hudson, J. A., & Heinemann, J. A. (2006). Isolation and characterization of bacteriophages infecting Salmonella spp. FEMS Microbiology Letters, 258(2), 182–186.*
2. *Doria, F., Napoli, C., Costantini, A., Berta, G., Saiz, J. C., & Garcia-Moruno, E. (2013). Development of a new method for detection and identification of Oenococcus oeni bacteriophages based on endolysin gene sequence and randomly amplified polymorphic DNA. Applied and Environmental Microbiology, 79(16), 4799–4805.*
3. *Matsuzaki, S., Rashel, M., Uchiyama, J., Sakurai, S., Ujihara, T., Kuroda, M., Imai, S. (2005). Bacteriophage therapy: A revitalized therapy against bacterial infectious diseases. Journal of Infection and Chemotherapy, 11(5), 211–219.*

PURIFICATION, SEPARATION AND DETECTION OF *KAYVIRUS* USING ELECTROPHORETIC TECHNIQUES

HORKÁ, Marie[1]; ŠTVERÁKOVÁ, Dana[2,5]; ŠALPLACHTA, Jiří[1]; ŠLAIS, Karel[1]; ŠIBOROVÁ, Marta[3]; RŮŽIČKA, Filip[4]; PANTŮČEK, Roman[2]

[1]Institute of Analytical Chemistry of the CAS, Brno, Czech Republic
[2]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
[3]Central European Institute of Technology, Masaryk University, Brno, Czech Republic
[4]Department of Microbiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic
[5]MB Pharma, Praha, Czech Republic

dana.stverakova@gmail.com

Introduction: The *Myoviridae* bacteriophage K1/420 belonging to genus *Kayvirus* was extensively studied as an antibacterial agent to combat infections caused by antibiotic-resistant *Staphylococcus aureus* strains. Development of clinical applications of phages in the western world is facing regulatory and technical hurdles and suitable diagnostic techniques to control the quality of the phage are being discussed.

Material & Methods: Here, we present electrophoretic and mass spectrometric methods for the separation, pre-concentration and characterization of staphylococcal phage K1/420.

Results: The conditions for the simultaneous separation and detection of phage K1/420 and *S. aureus* by capillary zone electrophoresis and by capillary isoelectric focusing were found, and the phage isoelectric point was determined to be 3.6. The phage was successfully purified and pre-concentrated by preparative isoelectric focusing (IEF). After IEF the harvested phage had a decreased ability to infect *S. aureus*. However, it was suitable for phage separation, detection and identification by capillary electrophoretic methods, MALDI-TOF mass spectrometry and electron microscopy.

Conclusion: Due to the decrease in infection ability of the phage, IEF is only suitable for analytical purposes. Very good quantitative responses were achieved in subsequent capillary electrophoretic methods. MALDI-TOF MS and electron microscopy can be used for diagnostics of phages after IEF.

TAILORING PHAGE PREPARATIONS FOR CONTROL OF LEADING BACTERIAL PATHOGENS IN ACUTE CARE HOPSITALS

TEDIASHVILI Marina[1]; T. KOKASHVILI[1]; N.JANELIDZE[1]; T SULADZE[1]; E. DIDEBULIDZE[1]; S. TSERTVADZE[2]; G.KASHIBADZE[3]; N. ROSTIASHVILI[4]; E.VALADE [5]

[1] George Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia [2] M. Iashvili Children's Central Hospital, Tbilisi, Georgia [3]Center of Thermal Injuries, Tbilisi, Georgia [4] Gori Military Hospital, Gori, Georgia [5] Institut de Recherche Biomédicale des Armées (IRBA), Bretigny Sur Orge, France

m.tediashvili@pha.ge

Introduction: The efficient control of health care associated infections largely depends on susceptibility of prevalent agents to various antimicrobials. Application of targeted bacteriophage mixtures can aid in elimination of hospital infections.

Materials & Methods: From 406 clinical samples collected from patients and hospital environment at 3 Georgian clinics 515 bacterial isolates were obtained and identified by biochemical profiling and by PCR. The susceptibility to phages and antibiotics was determined by standard methodology.

Results: Gram $^{+}$ bacteria prevailed leaded by *S.aureus* and coagulase - negative staphylococci. Among Gram $^{-}$ bacteria *P. aeruginosa* was the most frequently isolated, followed by *Klebsiella spp.*, other Enterobacteriaceae, *Acinetobacter baumannii,* etc. Higher bacterial diversity was revealed at children's hospital. Several pathogens persisted at burn wound center and ICU of multiprofile hospital. The multidrug resistant profiles of leading bacterial isolates, especially *P. aeruginosa,* was shown. The susceptibility to Eliava commercial phage preparations and individual phages was varying by pathogen with highest susceptibility for *S. aureus* and *P. aeruginosa,* considerably less for *Acinetobacter spp.*, *Klebsiella spp*. and *E.coli*.

Conclusion: No correlation was observed between phage and antibiotic resistance of clinical isolates. The experimental phage mixtures enriched with newly isolated phages have been tailored for studied hospitals.

HALOPHILES A NOVEL SOURCE OF ANTIMICROBIAL NATURAL PRODUCTS

THOMPSON Thomas P.; GILMORE Brendan F.

Queen's University Belfast, Belfast, Northern Ireland, UK

tthompson08@qub.ac.uk

Introduction: This study aims to establish the potential of extremely halophilic microorganisms from Kilroot Salt Mine, Northern Ireland, as a novel source of natural product chemistry with the objective to eventually isolate and structurally elucidate original anti-infective compounds from this unique microbiome.

Material & Methods: Different isolation techniques were utilised to obtain a collection of halophilic bacteria and archaea. Following 7 days of growth, organic extracts were screened for antimicrobial activity against the ESKAPE pathogens, and for their ability to produce quorum sensing inhibitory compounds.

Results: Initial screening suggests that many of the strains displayed antimicrobial activity against numerous ESKAPE pathogens. Furthermore, the presence of a putative quorum sensing inhibitory compound was observed. A type strain, *Haloferax volcanii,* was also investigated for its bioactivity; this crude extract had MICs and MBCs of 8mg/ml against *Staphylococcus aureus*, MRSA and, importantly, *Klebsiella pneumoniae*. Additionally, the MBEC assay revealed anti-biofilm properties against MRSA. Initial characterisation of this crude extract suggests the presence of several active compounds.

Conclusion: Bio-assay guided fractionation of crude extracts is on-going. Halophiles remain a promising reservoir possessing broad antimicrobial activity, and there is no doubt that exploitation of extreme environments have an important role to play in AMR.

CHARACTERIZATION OF TWO LYTIC PHAGES ACTIVE AGAINST CARBAPENEM-RESISTANT *ACINETOBACTER BAUMANNII*

VUKOTIC, Goran[1,2] OBRADOVIC, Mina[2]; NOVOVIC, Katarina[2]; STANISAVLJEVIC, Nemanja[2]; MIRKOVIC, Nemanja[2]; JOVCIC Branko[1,2]; FIRA Djordje[1]; NEVE, Horst[3]; MCAULIFFE, Olivia[4]; KOJIC, Milan[2]

[1]Faculty of Biology, University of Belgrade, Belgrade, Serbia
[2]Institue of Molecular Genetics and Genetic Engineering, University Of Belgrade, Belgrade, Serbia
[3]Department of Microbiology and Biotechnology, Max Rubner-Institut, Kiel, Germany
[4]Department of Food Biosciences, Teagasc Food Research Centre, Moorepark, Fermoy, Ireland

vukoticg@bio.bg.ac.rs

Introduction: Carbapenem-resistant *Acinetobacter baumannii* is a leading cause of healthcare-associated infections worldwide and presents a serious therapeutic challenge. It is characterized by various intrinsic and acquired mechanisms of antibiotic resistance.

Material & Methods: Two lytic phages active against *A. baumannii* 6077/12, NOVI and ISTD, were isolated from Belgrade wastewaters and screened against 40 bacterial strains from our laboratory collection. Phages were purified using CsCl gradient ultracentrifugation and analyzed by SDS-PAGE and TEM. In addition to standard spot tests, phage activities towards grown bacterial lawns were assessed. Antibiofilm effect of phage NOVI was analyzed by viable cell count of *A. baumannii* 6077/12 in biofilm before and after 16h incubation with different phages-to-bacteria ratios.

Results: Both phages showed good host range, where phage NOVI was active on 18%, while ISTD was active on 53% of strains. Translucent and growing halos were observed around the plaques in all sensitive strains. Halos tested positive for presence of active phages. In addition, ISTD was capable of inducing halos even on lawns of overnight grown bacteria. Phage NOVI reduced biofilm viable cell count by one order of magnitude.

Conclusion: Phages NOVI and ISTD are potentially polyvalent and applicable in combat against multi-drug resistant *A. baumannii* infections.

5th World Congress on Targeting Infectious Diseases 2018 - Index of Speakers