24-26 April 2017
Institut Pasteur, France

100th Centennial

1917-2017

Celebration of **Bacteriophage** Research

ABSTRACT BOOK

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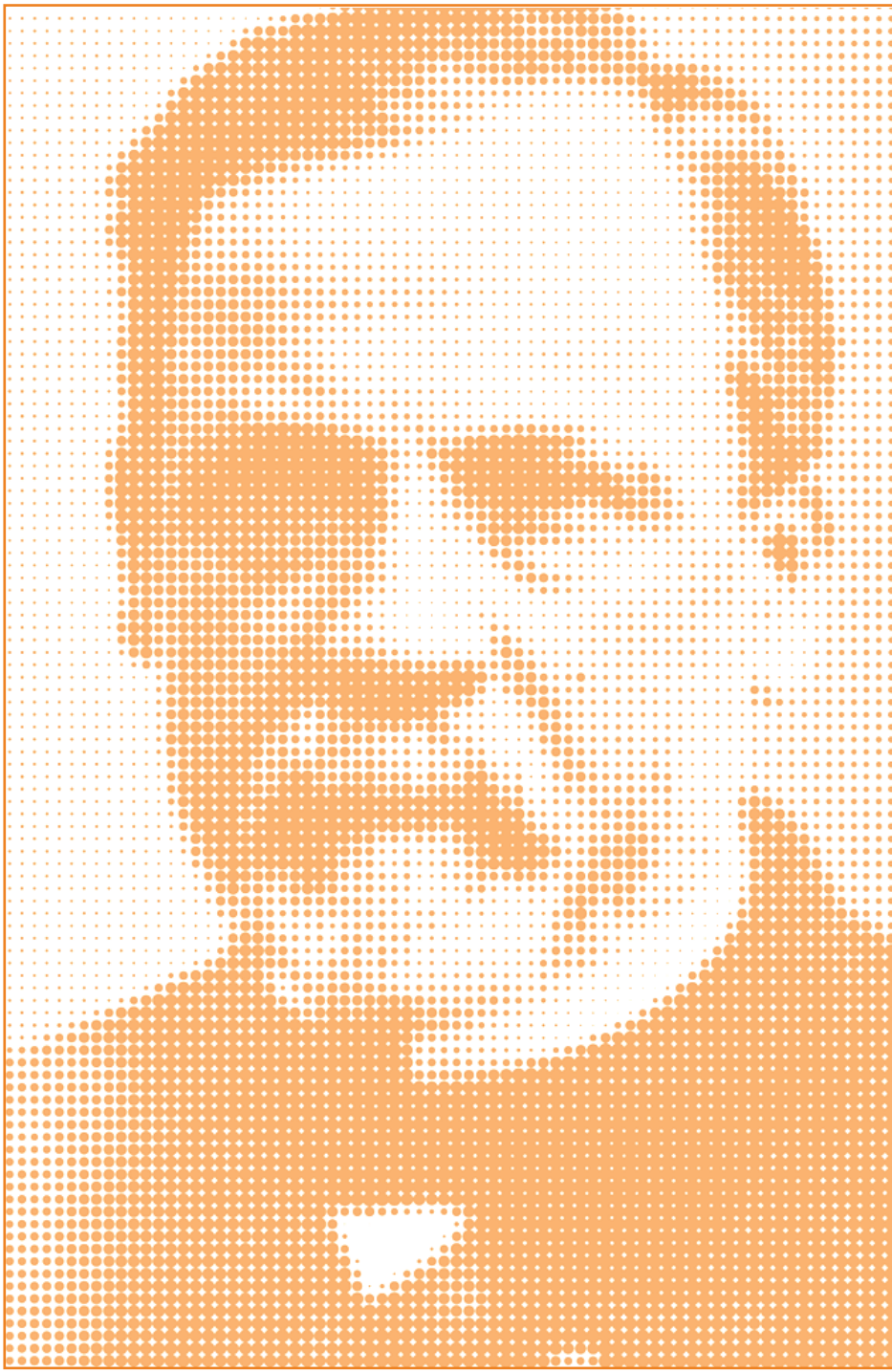


WELCOME ADDRESS

For one century bacteriophages, viruses of bacteria, have served and continue to serve as a basic model for understanding the fundamental principles of life. Indeed, research on bacteriophages is inseparable from the emergence of the field of molecular biology and led to major discoveries, from the identification of DNA as the genetic material and all the way to the discovery of the CRISPR-Cas systems which recently revolutionized many disciplines in life sciences.

The year 2017 marks the 100th anniversary of the initiation of bacteriophage research by Félix d'Herelle at Institut Pasteur. To celebrate this occasion, Institut Pasteur, in collaboration with Eliava Institute, is organizing a special conference in its historic location – the "cradle" of bacteriophages. The event aims at providing a broad overview of the major milestones in the bacteriophage research and will cover recent advances in bacteriophage-host interactions, bacteriophage ecology, the evolutionary heritage of bacteriophages as well as their therapeutic, biotechnological and industrial applications.

This conference is endorsed by the International Society for Viruses of Microbes (www.isvm.org).

SCIENTIFIC COMMITTEE

Laurent DEBARBIEUX	Institut Pasteur, France
Patrick FORTERRE	Institut Pasteur, France
Mart KRUPOVIC	Institut Pasteur, France
Mzia KUTATELADZE	G. Eliava Institute of Bacteriophages, Georgia
David PRANGISHVILI	Institut Pasteur, France

Félix d'Hérelle (1873-1949)

TABLE OF CONTENTS

Summary **Page**

GENERAL INFORMATION

WELCOME DESK - OPENING HOURS

Registration desk opens at 13:30 on April 24th, 2017 in the hall of the "Centre d'Information Scientifique". Please come early to pick-up your badge in order to avoid delays in handing-out badges and to avoid queues.

If your registration is fully covered, you will receive your complete congress kit including your badge, the certificate of attendance and the conference program. Please wear your badge at all time.

If registration was not fully covered, please come directly to the registration desk "on site payment". We accept payment by cash or credit card.

Monday April 24th	Tuesday April 25th	Wednesday April 26th
Welcome desk Cloakroom	***Welcome desk Cloakroom***	***Welcome desk Cloakroom***
13:30 – 19:00	*8:00 – 19:00*	*8:30 – 18:00*

ORAL SESSION

All oral presenters must bring their Power Point presentation on an USB stick to the technical room at least 30 minutes before the session starts. The presentation will be downloaded in the preview system and available from the computer set at the lectern on stage.

GENERAL INFORMATION

POSTER VIEWING

Two poster sessions will be displayed in the exhibition area in the building CIS as following:

Poster session 1: Monday, April 24th
Poster installation 13:30 **Poster with odd numbers**

Poster session 2: Tuesday, April 25th
Poster installation 17:45 **Poster with even numbers**

NB: Poster numbers are in the program book. Check the matching number on the board to display your poster in the right place. Magnets are available at the welcome desk to mount your poster.

LUNCHES & COFFEE BREAKS

Tuesday 25th and Wednesday, April 26th, buffet lunches will be served in the hall of CIS.

During breaks, refreshments and coffee will be available at several buffet points in the hall of CIS.

WIFI

A WiFi connection is available in the auditorium and in the hall of the building.
Use your personal username and password provided in the registration envelope.

SOCIAL PROGRAM

Cocktails

Monday, April 24th: From 17:15 Welcome party - hall of CIS
Wednesday, April 26th: From 17:00 Goodbye party - hall of CIS

Gala dinner (subject to paying registration)

The dinner cruise will be held on the boat "Le Paris" on April 25th 2017 at 20:00.

Boat departure is scheduled at 20:30 and boat return at 23:00.

Make sure you are on time.

Free departure by your own means at the boat.

Participants who have registered to this conference-gala dinner will find their voucher together with their name badge in the registration envelope.

Access:

PARIS SEINE, "Le Paris" boat - Port Debilly

Entrance in front of 26 avenue de New York, Paris 16 district

Metro: Line 9, Iéna station or Alma Marceau (20 minutes from Institut Pasteur)

Bus : n°82 Stop Varsovie; n°72 stop Musée Art Moderne Palais de Tokyo

Public car park: 10 avenue George V, Paris 8 district

SYMPOSIUM

All the Scientific sessions are taking place in the auditorium of the "Centre d'Information Scientifique" (CIS).

CIS Building

Welcome desk - cloakroom

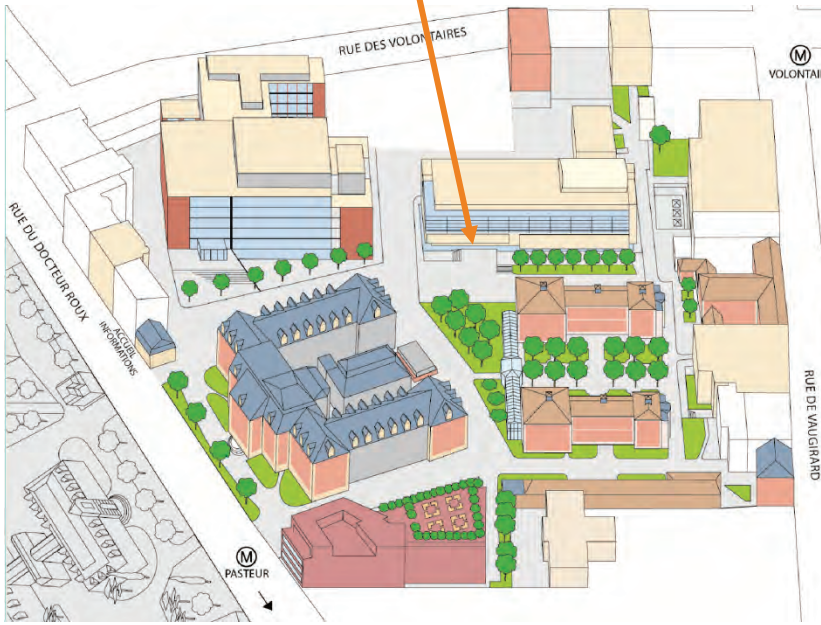
Auditorium (plenary sessions)

Welcome party

Poster viewing

Lunches & coffee breaks

Goodbye Party




INSTITUT PASTEUR

SCIENTIFIC PROGRAM

Welcome addresses by the organizers

CIS Auditorium
24/04/2017 / 15:00-15:15

15:00 **Christian Bréchot, President of Institut Pasteur**

15:05 **Mzia Kutateladze, Director of Eliava Institut**

Keynote Lectures

CIS Auditorium
24/04/2017 / 15:15-17:15

Chair: By the organizers

1 15:15 **The persistent importance but changing significance of the bacteriophage in the last 100 years**
Michel Morange
ENS, Paris, France

2 15:45 **DNA transactions and protein machines: What we have learned, and can still learn, from the T4 family of bacteriophages**
Bruce Alberts
University of California, San Francisco, United States

3 16:30 **Assembly and structure of phage lambda virions**
Roger Hendrix
University of Pittsburgh, United States

Welcome party and Poster Session 1

(odd numbers 1,3,...)

Hall of CIS
24/04/2017 / 17:15-18:30

1 Environmental Virology

CIS Auditorium
25/04/2017 / 09:00-10:30

Chair: David Prangishvili

4 09:00 **The global ocean virome: Re-imagining patterns, processes and paradigms on the high seas**
Matthew Sullivan
The Ohio State University, United States

5 09:30 **Light and depth - main drivers of marine virus diversity and dynamics in the Arctic - Metagenomic analysis of total viral assemblages from surface to the deep**
Ruth-Anne Sandaa
University of Bergen, Norway

6 09:45 **A healthy prophage reservoir associated with the core gut bacterial community**
Pilar Manrique
Montana State University, Bozeman, United States

7 10:00 **Dramatic differences in cyanophage abundances and distribution patterns across environmental gradients in the oceans**
Debbie Lindell
Technion - Israel Institute of Technology, Haifa, Israel

Coffee break and Poster Session 1
(odd numbers 1,3,...)

Hall of CIS
25/04/2017 / 10:30-11:15

2 Evolutionary Legacy of Bacteriophages

CIS Auditorium
25/04/2017 / 11:15-13:00

Chair: Mart Krupovic

8 11:15 **The structure, evolution and function of the major capsid protein of double strand DNA bacteriophage: the unique niche occupied by the HK97 tertiary fold**
John Johnson
The Scripps Research Institute, La Jolla, United States

9 11:45 **Homologous recombination in phages: what's new?**
Marie-Agnès Petit
MICALIS, INRA, Jouy en Josas, France

10 12:00 **Understanding inter-species gene exchange and compatibility in a bacteriophage model**
Franklin Nobrega
Bionanoscience, TU Delft, Delft, The Netherlands

11 12:15 **V-table: The virosphere structured in one comprehensive table**
Victoria Novitska
V-table project, Kyiv, Ukraine

12 12:30 **Ordering the viral universe**
Dennis Bamford
University of Helsinki, Finland

Lunch buffet and Poster Session 1
(odd numbers 1,3,...)

Hall of CIS
25/04/2017 / 13:00-15:30

3 Bacteriophages as Models

CIS Auditorium
25/04/2017 / 15:30-17:45

Chairs: Lucienne Letellier and Patrick Forterre

13 15:30 **Bacteriophage SPP1 (*Subtilis* Phage Pavia–1) Research: From Phage Genetics to Viral NanoMachines at Work**
Paulo Tavares
Institut de Biologie Intégrative de la Cellule (I2BC), Gif-Sur-Yvette, France

14 16:00 **Location of the unique integration site on an *Escherichia coli* chromosome by bacteriophage lambda DNA *in vivo***
Rinat Arbel-Goren
Weizmann Institute of Science, Rehovot, Israel

15 16:15 **Viral Assembly 101: Yet More Lessons For Biology From A Humble RNA Phage**
Peter Stockley
University of Leeds, United Kingdom

16 16:30 **Resistance is futile: Bacteriophage host Range engineering through ACcelerated Evolution (BRACE) and its application to bacterial resistance anticipation.**
Sebastien Lemire
Massachusetts Institute of Technology, Cambridge, United States

17 16:45 **Engineering phages and their hosts for the directed evolution of biomolecules**
Alfonso Jaramillo
University of Warwick, Coventry, United Kingdom

18 17:00 **Assembly and maturation of dsDNA bacteriophage capsids in the light of bacteriophage T5**
Pascale Boulanger
Institute for Integrative Biology of the Cell (I2BC), Gif-Sur-Yvette, France

19 17:15 **Structure and function of bacteriophage T4 tail**
Petr Leiman
UTMB, Galveston, United States

Coffee break and Poster Session 2

(even numbers 2,4,...)

Hall of CIS
25/04/2017 / 17:45-18:45

Gala Dinner on the River Seine

The boat will be open from 20:00 and will leave at 20:30 sharp, make sure you are on time

4.1 Virus - Host Interactions

CIS Auditorium
26/04/2017 / 09:30-10:30

Chairs: Ariane Toussaint and Harald Brüssow

20 09:30 **Deciphering phage-host interactions using CRISPR-Cas systems**
Sylvain Moineau
Université Laval, Québec, Canada

21 10:00 **Some insights from the study of BREX - novel bacteriophage resistance system**
Artem Isaev
Skolkovo Institute of Science and Technology, Moscow, Russia

22 10:15 **Bacteriophages use hypermodified nucleosides to evade host's defence systems**
Witold Kot
Aarhus University, Roskilde, Denmark

Coffee break and Poster Session 2
(even numbers 2,4,...)

Hall of CIS
26/04/2017 / 10:30-11:15

4.2 Virus - Host Interactions

CIS Auditorium
26/04/2017 / 11:15-12:30

Chairs: Ariane Toussaint and Harald Brüssow

23 11:15 **Listeria-phage cooperation during mammalian infection.**
Anat Herskovits
Tel-Aviv University, Israel

24 11:30 **A Eukaryotic-like Serine/Threonine Kinase Protects Staphylococci against Phages**
David Bikard
Institut Pasteur, Paris, France

25 11:45 **Small molecule communication between phages guides lysis-lysogeny decisions**
Rotem Sorek
Weizmann Institute of Science, Rehovot, Israel

26 12:00 **Hostile take-over: Pseudomonas as prey for lytic bacteriophages**
Rob Lavigne
University of Leuven, Belgium

Lunch buffet and Poster Session 2
(even numbers 2,4,...)

Hall of CIS
26/04/2017 / 12:30-15:00

5 Bacteriophage Applications

CIS Auditorium
26/04/2017 / 15:00-16:45

Chair: Laurent Debarbieux

27 15:00 **Immunophage synergy is essential for eradicating pathogens that provoke acute respiratory infections**
Dwayne Roach
Institut Pasteur, Paris, France

28 15:30 **Microfluidic encapsulation of bacteriophage in liposomes**
Salvatore Cinquerrui
Loughborough University, United Kingdom

29 15:45 **Microbiome and stable core virome after human fecal transfer**
Karin Moelling
Max Planck Institute for Molecular Genetics, Berlin, Germany

30 16:00 **Intravenous Application of Phage Therapy to Treat a Terminally Ill Patient Who Was Infected with Multi Drug Resistant *A. baumannii***
Biswajit Biswas
Naval Medical Research Center, Fredrick, United States

31 16:15 **Bacteriophages - wide application for eradication of bacterial pathogens**
Mzia Kutateladze
G. Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia

Poster Prizes

CIS Auditorium
26/04/2017 / 16:45-17:00

Goodbye Party

Hall of CIS
26/04/2017 / 17:00-18:00

ORAL PRESENTATIONS

The persistent importance but changing significance of the bacteriophage in the last 100 years

M. Morange
ENS, Paris, France

The bacteriophage was discovered first by Frederick Twort in 1915, and two years later, independently, by Félix d'Hérelle in Paris. D'Hérelle was obviously the most active proponent of the importance of the bacteriophage. He distinguished two opportunities raised by this discovery: the possibility through bacteriophages of access to the simplest extant forms of life, probably similar to the earliest life forms on Earth; and a way to fight against infectious diseases by the production of bacteriophages adapted to the invading pathogens. D'Hérelle immediately initiated both programmes.

The first rapidly generated the methods adapted to the study of bacteriophages, and of their amplification during bacterial infection. D'Hérelle's efforts were later extended by the American phage group, and bacteriophages played an important role in the rise of molecular biology. The rapid discovery of temperate bacteriophages able to infect bacteria and to remain silent within them for generations – the so-called phenomenon of lysogeny – set in motion work pursued at the Pasteur Institute by Eugène and Elizabeth Wollman, and later by André Lwoff, Elie Wollman and François Jacob, which contributed to the discovery of the mechanisms controlling gene expression.

The second applied programme was also rapidly successful in the hands of Félix d'Hérelle, but it was subject to failures and criticisms. Abandoned after the massive development of antibiotics during the Second World War, it was only recently resurrected as a way of circumventing the increasing resistance to antibiotics of pathogens.

This brilliant legacy should not hide the fact that the bacteriophage was discovered and described in a scientific context that differed greatly from ours. I will explore this scientific context that was so favourable to the development of studies on bacteriophages.

Bacteriophages were extraordinary models for 20th century biology, favouring the progressive description of the living world at the molecular level. They were an obligatory and fruitful point of passage for biological knowledge.

DNA transactions and protein machines: What we have learned, and can still learn, from the T4 family of bacteriophages

B. Alberts
Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, United States

The bacteriophage T4 DNA replication system very efficiently replicates this virus's long double-stranded DNA chromosome, and for 50 years it has served as an effective model for dissecting the detailed mechanisms of DNA replication in all organisms. Because a recombination-initiated form of DNA synthesis predominates during the late stages of T4 bacteriophage infection of *E. coli* cells, the T4 family of bacteriophages is also useful for working out the intimate relationships that are present in all organisms between homologous recombination mechanisms and DNA synthesis events. From such studies, we have learned that protein machines -- complexes of proteins in which ATP hydrolysis drives the ordered movement of component parts in time and space -- form much of the basis for life's chemistry. It has also become clear that a sophisticated chemistry allows a cell to carefully control the assembly on DNA, and thereby the catalytic activities, of each of the many different protein machines that function on its chromosomes. This assembly occurs at specific sites that are recognized by their structure and/or their nucleic acid sequence, and it also responds to specific signals that reflect the state of the cell. Central to these assembly processes are special proteins that serve as "adapters"; the details of adapter protein function have been worked out for some of these proteins, but much more remains to be deciphered by protein biochemists and structural biologists.

As other presentations in this symposium will emphasize, the T4 family of viruses has been enormously successful on Earth despite being able to use only a limited number of proteins to carry out all of its DNA-based processes. For this reason, studies of these bacteriophages can continue to provide critical information for guiding mechanistic studies on the much more complex DNA-based processes in eukaryotes.

Assembly and structure of phage lambda virions

R. Hendrix
University of Pittsburgh, Pittsburgh, United States

Bacteriophage Lambda has played a central role in research on phage lifestyles and on molecular biology more generally since its discovery in 1951 by Ester Lederberg. Lambda research has contributed to our understanding of many different areas of phage biology. Among these I will emphasize lambda's contributions to understanding how virions are assembled from their component protein and DNA parts. This will range from *in vitro* head-tail joining to the role of protein folding chaperones in head assembly to the mechanism of DNA packaging to the role of assembly chaperones in tail assembly.

The global ocean virome: Re-imagining patterns, processes and paradigms on the high seas

M. Sullivan
Department of Microbiology, The Ohio State University, United States

Ocean microbes drive local- and planetary-scale biogeochemical cycling, but do so under constraints imposed by viruses. Viruses can lyse microbes, which is thought to keep organic matter “small” and rapidly recycled (the ‘viral shunt’), but they can also metabolically reprogram their host cells by stealing “auxiliary metabolic genes” genes, or AMGs (e.g., photosynthesis genes for cyanobacterial viruses), from their hosts and using them to make more viruses during infection. Here I will present global-scale analyses that alter both of these paradigms. First, observations from viral metagenomic signatures suggest that viruses ‘sink’ in the oceans, while network and modeling analyses revealed that a handful of viruses, rather than prokaryotes or eukaryotes, best predict carbon flux in the open oceans. These observations call into question the fundamental tenant of the viral shunt, and suggest that ocean viruses may aid sinking of carbon to the deep sea rather than constrain it to rapidly recycle in surface waters. Second, analyses from systematically collected and processed ocean viral metagenomic datasets (the Pacific Ocean Viromes and Global Ocean Viromes datasets) reveal that AMG-mediated viral metabolic reprogramming extends far beyond photosynthesis to also include nearly all of central carbon metabolism, as well as key genes in nitrogen and sulfur cycling. Together these findings suggest that viral roles in ocean biogeochemistry are extensive and provide a new baseline for future work designed to quantitatively evaluate the marine viral shunt.

Light and depth - main drivers of marine virus diversity and dynamics in the Arctic - Metagenomic analysis of total viral assemblages from surface to the deep

J.L. Ray[2], F. Enault[1], T.V. Johanessen[4], G. Bratbak[3], A. Larsen[4], I.H. Steen[4], R.A. Sandaa[3]
[1]Laboratoire Microorganismes: Génome et Environnement, Clermont Université, Université Blaise Pascal, Clermont-Ferrand, Clermont-Ferrand, France [2]Hjort Centre for Marine Ecosystem Dynamics, Uni Research Environment [3]Department of Biology [4]Department of Biology and centre for Geobiology, University of Bergen, Bergen, Norway

High contrast in light and seasonal productivity in Arctic pelagic marine environments bespeaks an environment in which microorganisms must adapt to extremes in light and resource availability. As important regulators of host diversity and nutrient recycling, viruses are critical components of the marine environment and the marine food web. In order to investigate Arctic marine virus assemblages, we conducted research cruises to the northwest of Svalbard across one calendar year, sampling at depths from surface to 1000m. We extracted total nucleic acids (TNA) from 50L of 100,000 pore size (NMWC) tangential flow concentrates (0.45 or 0.2 µm prefiltered) using a QIAsymphony SP automated extraction platform. RNA and ssDNA virus metagenomes were converted to dsDNA using a combination of reverse transcription and second-strand synthesis, respectively. Total libraries, potentially representing all combinations of ss or ds DNA or RNA genomes, were then amplified using a random PCR approach and sequenced using Illumina MiSeq v3 PE300. Single reads from virus metagenomes were quality-trimmed and assembled using Idba_ud and analysed using the MetaVir2 platform. We detected viruses with all different nucleic type. Most abundant was dsDNA viruses belonging to the families *Caudoviridae* and *Phyco- Mega-viridae*. Viral types that have not been previously described for Arctic seawater were also detected, including ssDNA viruses belonging to the families *Microviridae*, *Inoviridae*, and *Circoviridae* (CRESS-DNA viruses) and ssRNA viruses like *Picornaviridae.* The interdependence of viruses on the hosts they infect suggests that virus assemblages in high-latitude marine biomes may vary in step with season- and depth-driven variation in host populations. We did detect dynamic changes in different important viral groups, such as viruses infecting photosynthetic hosts that could be explained by the shift in the host communities due to change in light and seasonal productivity. Our data provide new insight to the role of viruses in a so far nearly undescribed extreme marine habitat and shows that the enormous contrasts in light and depth have significant effects on the microbial community structure, shaping both eukaryotic and prokaryotic host-viral relationships.

A healthy prophage reservoir associated with the core gut bacterial community

P. Manrique[1], J. Munson-Mcgee[1], M. Young[2]
[1]*Microbiology and Immunology* [2]*Plant Sciences and Plant Pathology, Montana State University, Bozeman, United States*

The role of bacteriophages in shaping and influencing the community structure and function of the human gut microbiome is beginning to be investigated. We identified a shared healthy gut phageome (HGP) associated with healthy individuals (1). The HGP is composed of 23 phages found in more than 50% of the individuals (termed core phages) and 132 viruses found in 20-50% of the individuals (termed common phages). Importantly, we showed that there is a significant decrease of HGP phages in inflammatory bowel disease patients (n=102), both in the percentage of individuals that harbor core phages, and in the total number of core phages that each individual carry. We have hypothesized that the HGP phage originate from a large prophage reservoir present in the healthy gut microbial community. In support of this hypothesis, we have determined that 71 HGP phage (45%) are likely prophages present in the healthy gut microbiome. We identified over 500 associations between 80 HGP phages and 105 bacterial species. Our results indicate that HGP phages are more likely associated with the shared bacterial community prevalent in healthy individuals than with non-shared members. Overall, identification of HGP phages lifestyle and hosts supports the hypothesis that the HGP is an activated subset of the total prophage reservoir in the gut and that is associated primarily with the shared bacterial community. Understanding the dynamics between theses phage and their hosts is crucial to determining how phage shape the gut microbiome structure and function, ultimately affecting health and disease.

Dramatic differences in cyanophage abundances and distribution patterns across environmental gradients in the oceans

D. Lindell, I. Maidanik, M. Carlson, S. Kirzner, I. Pekarsky, N. Baran, S. Goldin
Technion - Israel Institute of Technology, Haifa, Israel

Viruses are globally abundant and extremely diverse in their genetic make-up and in the hosts they infect. They influence the abundance, diversity and evolution of their hosts as well as the biogeochemical cycling of matter in the oceans. Yet current methods are inadequate for gaining a quantitative understanding of their impact on these processes. Here, we employ a new culture-independent, solid-phase PCR method, polonies, to gain the first quantitative view of cyanophages at the phage family level. Dramatic differences in abundances and distribution patterns were found for different cyanophage families along a transect traversing large environmental gradients in the North Pacific Ocean. In addition, stark differences in the abundances of different clades of T7-like cyanophages were found over the seasonal cycle in the Red Sea. In both oceanic regions, clade B T7-like cyanophages that encode host-derived photosynthesis genes were considerably more abundant than clade A T7-like cyanophages that lack these photosynthesis genes. Intriguingly, laboratory studies show that the more abundant clade B phages have a longer lytic cycle, are less virulent and produce fewer phages per burst than the clade A phages. These findings highlight the important insights to be gained from investigations of phage ecology in a host-taxon and phage-family specific manner.

The structure, evolution and function of the major capsid protein of double strand DNA bacteriophage: the unique niche occupied by the HK97 tertiary fold

J. Johnson
Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, United States

Double Strand (ds) DNA bacteriophages that have been studied to date have remarkably similar folds of their major capsid proteins. The Hong Kong 97 (HK97) bacteriophage capsid was the first high resolution structure of a dsDNA particle that clearly revealed the fold of the subunit (now known as the HK97 fold). The fold is a mix **a/b** structure (28% **a** helix; 32% **b** strand) organized into two compact, spatially distinct domains that are not sequence contiguous. The axial domain A is near the fivefold and quasi-six fold symmetry axes, and the peripheral domain P, plus extensions (N-arm and E-loop), fill the region between adjacent quasi or icosahedral threefold axes[1]. To date more than 40 different dsDNA bacteriophage or bacterial encapsulins have been shown to have the HK97 tertiary structure, suggesting that capsid proteins of Podovirdae, Myovirdae, Siphovirdae and mammalian infecting Herspesvirdae have a common origin[2]. Since it is estimated that there are ~10^{31} bacteriophage in the biosphere, comprising the largest biomass in nature, this is probably the most abundant protein fold on earth. It is likely that the fold accommodates the acrobatic reorganization of dsDNA bacteriophage and herpes viruses, possibly in a unique manner that occurs during their assembly and maturation. The presentation will discuss the structure-based evolutionary relationships among the major capsid subunits of dsDNA bacteriophages and herpesviruses and demonstrate the novel dynamic properties, both in tertiary structure and quaternary structure, that have elevated the fold to such a lofty position in nature.

1. Wikoff, W., Liljas, L., Duda, R., Tsuruta, H., Hendrix, R., and Johnson, J. 2000. Topologically linked protein rings in the bacteriophage HK97 capsid. *Science* **289:**2129-2133.
2. Suhanovsky, M.M., Teschke, C.M. 2015, Nature's favorite building block: Deciphering folding and capsid assembly of proteins with the HK97-fold. *Virology* **479:** 487–497.

Homologous recombination in phages: what's new?

G. Hutinet[2], M.A. Petit[2], F. Ochsenbein[1], F. Lecointe[2]
[1]IBITECs, CEA, CNRS, Univ. Paris-Sud, Université Paris-Saclay, Institute for Integrative Biology of the Cell, Gif Sur Yvette [2]MICALIS, INRA, Jouy En Josas, France

Bacteriophages are remarkable for the wide diversity of proteins they encode to perform DNA replication and homologous recombination (HR). Indeed, for HR, whereas Bacteria and Eukaryotes all rely on the ubiquitous RecA/Rad51 protein for the key reaction of homology search between DNA strands, phages use four brands of structurally different proteins. One of them is UvsX, homolog to RecA, and quite poorly distributed. Most phages encode rather a Rad52-like protein, an annealase protein also present in Eukaryotes but with rather an auxiliary role in HR. A third family of phage encoded HR proteins encompasses Gp2.5-like proteins that are special kinds of SSB with single strand annealing activity. More recently uncovered, and less well characterized is the fourth type of homology search proteins of phages, Sak4. Sak4 is similar to the archaeal RadB protein, a Rad51 paralog. Here, we report that Sak4 of phage HK620 infecting *Escherichia coli* is an annealase, despite its homology with RecA. A distant homolog of SSB, present almost systematically next to the *sak4* gene

(see figure), stimulates its recombineering activity *in vivo*. As with other phage annealases, recombineering has relaxed fidelity, meaning that Sak4 is active on diverged DNA substrates, up to 12% *in vivo*. *In vitro*, Sak4 binds single-strand DNA and performs single-strand annealing in an ATP-dependent way, pairing up to 20% diverged oligonucleotides. Remarkably, the single-strand annealing activity is stimulated by its cognate SSB. To some extent, and at higher Sak4 concentrations, bacterial SSB proteins of *E. coli* and *Bacillus subtilis* also stimulate annealing. SSB may facilitate annealing both indirectly by removing secondary structures, and directly for the phage SSB, by interacting with Sak4. Sak4 of phage HK620 is the first described annealase using an SSB to enhance its activity.

Understanding inter-species gene exchange and compatibility in a bacteriophage model

F.L. Nobrega[1], Y. Liu[2], N.M. Edner[3], S.J.J. Brouns[1-2]
[1]Bionanoscience, TU Delft, Delft [2]Wageningen UR, Wageningen, The Netherlands [3]Division of Infection and Immunity, University College London, London, United Kingdom

Bacteriophages are highly diverse viral entities that strongly influence the evolution of bacterial communities. While the study of phages has provided significant knowledge in other fields, phages themselves remain relatively obscure entities. Many important questions linger regarding phage modular genome organization, rapid phage evolution, and host-specific interactions. Here we show a tool to study gene function and essentiality, host specificity and evolution. This technique is based on bacteriophage genome shuffling for the creation of chimeric phages. By applying this, from all 3 major families of tailed phages (E. coli phage Myoviridae S2-36s, Siphoviridae S2-55s and Podoviridae S1-55L) we were able to obtain many different recombinant phages with distinct features from the parental phages. Most interestingly, we have sequenced and annotated the genome of one recombinant phage (chimera phiChi3) using Nanopore sequencing, and this revealed rearrangement of genes from the three parental phages. Sixty-five percent of the recombinant phage genes derived from S2-36s, and 29 % and 7 % from phages S2-55s and S1-55l respectively. Although most derived from S2-36s, the recombinant phage surprisingly does not display the same morphology. Instead, it has a Siphoviridae morphology like phage S2-55s. This morphology is mostly derived from the contribution of phage S2-55s to the tail structure of the recombinant phage, while S2-55s provided capsid genes, and S2-36s donated the host recognition structures. However, the host range of the recombinant phage is limited when compared to that of S2-36s, which we discuss to be related to the absence of important receptor binding proteins, as tail fibers with enzymatic activity. This confirm that phages have high levels of flexibility and adaptability to accommodate and re-arrange genetic information from other phages, even from distinct families, posing the hypothesis of exchangeable protein modules complementarity among microbial populations.

V-table: The virosphere structured in one comprehensive table

V. Novitska
V-table project, Kyiv, Ukraine

V-table© is an interactive spherical table of viruses arranged according to their taxonomy, structure and phylogeny simultaneously. The structure of V-table provides an overview on morphology, chemical characteristics and host type of 111 viral families and 26 unassigned genera in 1 comprehensive table. It allows organizing and comparing viruses, understanding the relationships among diverse viruses, as well as facilitates learning virology; it is designed to be expanded.

The structure of V-tableis based on arrangement of multiple parameters, which define unique place in a table on a surface of a sphere for each viral species and family. The place in V-table is defined by specific characteristics of listed parameters.

Each parameter covers the entire surface of the sphere, dividing it into a certain number of parts according to the characteristics of a specific parameter. Each parameter consists of distinct number of characteristics e.g. the parameter II - Type of genetic information - has 7 characteristics representing 7 types of genetic information of viruses according to Baltimore classification. The parameters I – VI have multiple characteristics. The parameters include type of host, type of genetic information, type of capsid symmetry, T-number (if any), capsid size and genome size. These parameters divide the spherical table into layers (rows), vertical slices (columns), sectors and cards and define the unique place in a table for every viral family according to their set of characteristics. Viral species and genera within a particular family usually have identical or similar characteristics of parameters. Thus, card of viral family is a unit of V-table. Along with parameters, which define the place of the specific viral family in V-table, there are supporting parameters VII–X, which usually have only two characteristics.

The functions of V-table are providing structured and easy to overview information about every virus, organizing knowledge about viruses, analyzing and comparing viral species, genera, families and clades according to one or several specific parameters simultaneously. Using V-table helps in perceiving and understanding viral diversity. It is the solution for gathering information about viruses in a structured and informative manner.

Ordering the viral universe

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Viruses are the most abundant living entities in the biosphere outnumbering their host organisms by one to two orders of magnitude. It is conceivable that they cause the highest selective pressure their hosts encounter. As obligate parasites viruses are dependent on their hosts but their origins seem to deviate from that of cellular life.

What are the possible structural principles to build viruses is an open question. However, structural studies on virus capsids and coat protein folds propose that there are only a limited number of ways to construct a virion. This limitation may be based on the limited protein fold space. Consequently, relatedness of viruses is not connected to the type of cells they infect and the same architectural principle of the capsid has been observed in viruses infecting bacteria as well as humans. Using the viral capsid architecture it is possible to group viruses to several structure based lineages that may have existed before the three cellular domains of life (bacteria, archaea and eukarya) were separated. This would mean that viruses are ancient and that early cells were already infected with many different types of viruses proposing that the origin of viruses is polyphyletic opposing to the monophyletic origin of cellular life. To test the hypothesis of limited viral structure space we have pooled information on globally collected environmental viruses infecting archaea and bacteria and compared the obtained information to known viral structures. The latest inclusion of approaches to solve this issue is to extend the comparison to viral protein sequences as their number is increasing rapidly and often only a few compare to anything known before.

Bacteriophage SPP1 (*Subtilis* Phage Pavia–1) Research: From Phage Genetics to Viral NanoMachines at Work

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Bacteriophage SPP1 (*Subtilis* Phage Pavia - 1) is a siphovirus that infects the Gram-positive bacterium *Bacillus subtilis*. The virion was isolated from soil of the botanical garden of Pavia, Italy (Riva et al (1968) J Mol Biol **35**:347). SPP1 became rapidly a model system for phage genetics and molecular biology studies while its generalized transduction activity rendered it a major tool for *B subtilis* genetics. Approximately 60 % of the 40,010 bp SPP1 genome encodes essential DNA replication and viral particle assembly proteins (Alonso et al (1997) Gene **204**:201). The remaining of the genome codes for less characterized functions, mostly dispensable for SPP1 multiplication under laboratory conditions.

Recent research on SPP1 combined knowledge of the repertoire of phage effectors, and in numerous cases of their atomic structures, with in vitro reconstitution assays and genetic approaches to uncover the molecular mechanisms of viral DNA replication and of viral particles assembly. This interdisciplinary approach will be illustrated by studies on the molecular mechanisms how (**i**) SPP1 determines the amount of DNA that is tightly packed inside the viral capsid (headful packaging mechanism) and (**ii**) how the release of phage DNA from the capsid is promoted when SPP1 interacts with its cellular receptor YueB. The genome gatekeeper present at the phage capsid-to-tail interface is shown to play a central role in both processes.

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

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Viral infection and the subsequent integration of a viral genome into a host chromosome are fundamental in the generation of genetic diversity in prokaryotes. After entering an *E. coli* cell, the ~5x10^4 bp-long bacteriophage λ DNA must locate a unique site among ~5 million possible sites on the bacterial genome, with high efficiency and within physiological times, to integrate and establish lysogeny. What are the mechanisms that allow it to do this efficiently in physiologically relevant time scales? To address this question, we followed the targeting process in individual live *E. coli* cells in real-time, by fluorescently labeling both the phage DNA after entry into the host, and a chromosomal sequence near the integration site. To our surprise, we found that λ DNA does not carry out an active search, even though it is much smaller than the bacterial chromosome. Instead, it remains confined near its entry point into the cell following infection, preferentially at the poles, where it undergoes limited diffusion. The encounter between the 15 bp-long target sequence on the chromosome and the recombination site on the viral genome is facilitated by the directed motion of bacterial DNA generated during chromosome replication and segregation. Moving the native bacterial integration site to different locations on the genome and measuring the integration frequency in these strains reveals that the frequencies of the native site and a site symmetric to it relative to the origin are similar, whereas both are significantly higher than when the integration site is moved near the terminus, consistent with the replication-driven mechanism of target location that we propose. This mechanism adds to the list of profligate use of host functions by λ, brought about by coevolution of host−phage processes.

Viral Assembly 101: Yet More Lessons For Biology From A Humble RNA Phage

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Since its discovery in the 1960s bacteriophage MS2 has proved to be a robust model for investigation of a plethora of molecular mechanisms occurring throughout biology. These include the first complete genetic sequence for an organism, discovery of translational repression, regulation of gene expression by RNA higher order conformation, providing a major paradigm for understanding the sequence-specific recognition of RNA by proteins and, mostly lately, providing a system in which the regulation of virion assembly can be studied in fine detail. Recently, it became the first virus to have its asymmetric structure determined at high resolution by cryo-electron microscopy, revealing the unique conformation of the encapsidated genome and how the single copy Maturation Protein (MP) imposes asymmetry on an otherwise icosahedrally symmetry surface lattice of coat protein subunits. I will describe our studies on the assembly of this phage and how it lead to a remarkable new insight into the mechanism of virion assembly for many ssRNA, including major human pathogens. This will include a discussion on how the asymmetric structure differs from previous ideas based around the traditional icosahedral view of virus architecture.

Resistance is futile: Bacteriophage host Range engineering through ACcelerated Evolution (BRACE) and its application to bacterial resistance anticipation

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Bacterial antibiotic resistance development is a growing and serious public health threat but few solutions have been put forth. One promising option lies in adapting the traditional practice of phage therapy, whereby natural viral predators of bacteria are used to eradicate pathogens. However, naturally sourced bacteriophages often have a restricted host range evolved from their vital need to establish a form of ecological equilibrium with their host. Therefore, proper therapeutic coverage of a pathogen is achieved through empirically updated cocktails of different phages which collectively target all the relevant bacterial strains which presents a significant regulatory burden.

We have developed several complementary phage genome engineering approaches to modify determinants of host range in bacteriophages and probe the design principles behind host recognition.

We have shown that it is possible to drastically change the host range of members of the T7-family of phages by swapping out various parts of the phage tail machinery for those of related phages targeting different bacteria but this strategy requires the possession of a phage with the desired host range to clone host determinants from and may therefore be ill-suited to track fast evolving bacteria.

BRACE uses the structure of the phage's host determinant as a guide to target evolution and generate genetic diversity in the tail fiber's domains believed to participate most in host recognition thus minimizing potential damage to the overall structure of the protein. BRACE also permits sampling a much wider sequence space than is possible with traditional natural evolution and selection schemes. Because of the similarity of our process with antibody specificity engineering, we termed the phage variants created through BRACE phagebodies. We illustrate the power of BRACE by screening phagebody libraries for members targeting T3-resistant mutants of its host, *E. coli*. Among those mutants, some had a vastly broadened host range and eradicated bacteria up to ~700x better than wild-type T3 while preventing resistance appearance during extensive co-culturing with *E. coli* suggesting their genetic make-up allowed them to "anticipate" the evolutionary pathways of their host and eliminate most if not all mutants that are resistant to the wild-type ancestral phage. Analysis of the mutations introduced into those phagebodies is revealing informative trends on receptor recognition mechanisms that are being used to improve phagebody designs.

We anticipate knowledge gained from such studies will impact both fundamental research on phage-host interactions and help design simplified therapeutic phage applications.

Engineering phages and their hosts for the directed evolution of biomolecules

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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. 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. The implementation of negative selections allowed the engineering of specificity. 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*. We also show how to evolve riboswitches using cycles of positive and negative selections. Our methodology for directed evolution can be implemented in many phage systems to evolve proteins, nucleic acids and phage tropism determinants.

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Assembly and maturation of dsDNA bacteriophage capsids in the light of bacteriophage T5

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Capsids of dsDNA bacteriophages are extraordinarily robust macromolecular assemblies capable of withstanding the strong internal pressure generated by the packaged DNA. Their assembly is a regulated stepwise process, exhibiting conserved features in all tailed bacteriophages. An empty icosahedral procapsid is initially assembled and then packaged with DNA. This procapsid is built from several hundred copies of one or two major capsid proteins arranged as pentamers at the vertices and hexamers on the faces of the icosahedron. A dodecameric portal protein occupies a unique vertex and forms a gate through which DNA is translocated by the terminase, a powerful molecular motor. The procapsid undergoes expansion during packaging, leading to an increase in the internal volume that allows for accommodation of the full-length genome and accessory proteins often decorate the surface of the DNA-filled capsid.

Assembly of the large bacteriophage T5 capsid (T=13) has been investigated by combining genetic, biochemical and structural approaches (cryo-electron microscopy, small angle X-ray scattering and NMR). While following the paradigm of the smaller HK97 capsid (T=7), T5 capsid assembly exhibits distinct features. Initial assembly of T5 procapsid requires a maturation protease and is regulated by the portal complex [1,2]. Expansion of this procapsid proceeds through a highly cooperative structural rearrangement of the 775 capsid protein subunits, which yields the highly stable mature capsid, with no need for reinforcement by inter-subunit crosslinking or additional cementing proteins [3]. The decoration protein pb10 anchors onto the surface of the expanded capsid, at the center of each of the 120 hexamers formed by the capsid protein. By solving the NMR solution structure of pb10 and investigating its capsid binding properties by SPR, we provided new insights into the quasi-irreversible and highly cooperative mechanism of capsid decoration [4]. All together, these features make T5 a very attractive system for investigating the maturation of dsDNA bacteriophage capsids.

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Structure and function of bacteriophage T4 tail

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The tail of bacteriophage T4 is an archetypical member of a diverse class of complex multicomponent organelles called Contractile Injection Systems (CISs). Besides phage tails, CISs contain the bacterial Type VI Secretion System (T6SS), Antifeeding Prophages of *Serratia*, *Photorhabdus* Virulence Cassette, Metamorphosis-Associated Contractile arrays of *Pseudoalteromonas luteoviolacea*, R-type pyocins of *Pseudomonas* and similar complexes of *Clostridium*. These systems have a common architecture and functional mechanism that involves a contractile sheath wrapped around a central rigid tube, which carries a spike-shaped protein at its tip. The initial extended conformation is a high-energy metastable state of the assembly. Upon interaction with a target cell, the sheath contracts and propels the tube through the cell envelope. The event of sheath contraction is coupled to delivery of toxins in T6SS and constitutes a prerequisite for subsequent protein and DNA translocation in phages. The sheath contraction triggering process is controlled by a multicomponent baseplate at the end of the tail. Using cryo-electron microscopy, X-ray crystallography and modeling, we have determined the atomic structure of the 6 MDa baseplate of bacteriophage T4 in two states – in its pre- and post-host cell attachment conformations. This information allowed us to describe – in atomic detail – how the baseplate undergoes its massive structural rearrangement that results in deployment of the short tail fibers and release of the central membrane-piercing spike complex while simultaneously initiating sheath contraction. We identified a critical disulfide bond that is essential for connecting the tail fiber network to the rest of the baseplate. Finally, we have established a minimal composition of the baseplate in all contractile injection systems.

Deciphering phage-host interactions using CRISPR-Cas systems

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Clustered regularly interspaced short palindromic repeats (CRISPR) and their associated *cas* genes protect microbial cells against infection by foreign nucleic acids, including phage genomes. Exploiting this system has resulted in the development of the much-publicized CRISPR-Cas9 technology for precise genome manipulation of various organisms. The absence of genetic tools to efficiently edit the genome of virulent phages partly explains why many viral proteins have no known function. Recently, we used CRISPR-Cas9 to mutate uncharacterized phage proteins and study their roles in vivo. This seminar will recall the biology of CRISPR-Cas systems and the use of CRISPR-Cas9 to edit viral genomes

Some insights from the study of BREX - novel bacteriophage resistance system

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BREX is a novel phage defense system widespread in bacterial and archaeal genomes. In this work we have studied the BREX gene cluster from *Escherichia coli* HS strain that consists of 6 genes, including methyltransferase, alkaline-phosphatase and Lon-like protease. When BREX was expressed from the low-copy number plasmid (pBTB) in a sensitive strain BW25113, it conferred resistance to a spectrum of phages with different life strategies and genome organization, such as T7, λ, ΦX174, but not to RNA-containing phages. In deletion experiments it was shown that all genes, except *brxA*, are essential for the protection and *brxL* deletion leads to decreased cell viability.

Adsorption of phage particles to a cell is not affected by the BREX system. Therefore we decided to see whether accumulation of phage DNA changed in BREX+ cells. In control cells, transfected with an empty vector, accumulation of phage DNA and degradation of host DNA became visible 15 min after addition of T7 phage. In BREX+ cells degradation of host DNA was significantly delayed and T7 DNA accumulation was not observed following 3 hour incubation with the phage. This indicates that T7 life cycle progression was inhibited or that phage DNA was degraded. These results were also supported by T7 transcription analysis. Transcription efficiency was measured by quantitative PCR and the overall level of T7 transcripts in BREX+ cells was 2 orders of magnitude lower, but no timing shifts in the expression of different gene classes was observed.

Besides protection from phages, BREX also inhibits horizontal gene transfer. Efficiency of transformation, transduction and conjugation to the BREX+ cells was inhibited to more than one order of magnitude. Deletion of methyltransferase gene - *brxX* or mutation in the catalytic center of this protein (Y519A) leads to restoration of the efficiency. From HPLC analysis of nucleosides we may conclude that BREX+ cells contain increased amounts of N6-methyl-adenine, suggesting that modification status of DNA is important for self-nonself discrimination. It was proved in λ lysogenisation assay, where λ induced from prophage state in BREX+ cells were able to overcome BREX protection, probably due to the presence of modifications.

Bacteriophages use hypermodified nucleosides to evade host's defence systems

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Since the very beginning of life, primitive cells were forced to face selfish genetic elements like viruses or plasmids. Bacteria, continually exposed to infections, developed several phage resistance mechanisms e.g. restriction-modification and CRISPR-Cas systems. On the other hand, bacteriophages developed several strategies to evade these defence mechanisms. Ultimately, this led to the oldest and still running arms race - microorganisms vs. their molecular parasites.

We here describe a remarkable new strategy used by the recently isolated *Escherichia coli* phage CAjan belonging to *Seuratvirus* genus. CAjan, as do the rest of the phages in this genus and the closely related genus *nonagvirus*, contains a set of genes with a high degree of similarity to queuosine biosynthesis genes. Queuosine is a hypermodified nucleoside, which is present in some classes of RNA in bacteria and eukaryotes. Phages belonging to these two genera show a remarkable resistance to restriction endonucleases. This fact, combined with the discovery of hypermodified bases in the DNA from these phages by LC-MS/MS [1], strongly suggests that hypermodified bases are utilized to evade host's defence systems by bacteriophages. In order to investigate this mechanism in detail we have used several methods including transcriptomics, direct plaque sequencing, restriction endonuclease analysis and CRISPR-Cas genome editing. Through generation of specific mutants, we were able to introduce a restriction sensitive phenotype in the CAjan bacteriophage providing new insight on use of alternative bases by bacteriophages.

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Listeria-phage cooperation during mammalian infection

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Unlike lytic phages, temperate phages maintain a long-term association with their bacterial host through lysogeny. In this context mutual beneficial interactions can evolve that support efficient co-reproduction. We recently identified a new type of bacteria-phage interaction in which a prophage integrated within a critical bacterial gene serves as an active regulatory switch to regulate the gene expression via genome excision, a phenomenon we termed active lysogeny.

Regulation of the *comK* gene in the human pathogen *Listeria monocytogenes* is mediated via phage genome excision and re-integration. ComK itself is the activator of the competence system, which was shown to promote *L. monocytogenes* infection of mammalian cells. Interestingly, prophage excision is specifically induced during *L. monocytogenes* intracellular growth, primarily within the mammalian cells phagosomes, allowing ComK expression. Yet, unlike classic prophage induction, progeny virions are not produced and bacterial lysis is avoided, turning the prophage into a genetic switch that regulates host behavior. To further investigate this phage-host interaction we performed a transcriptional analysis of the phage in its three life states: lytic, lysogenic and active lysogenic; using genome-wide RNA-seq and Nano-string analyses. Notably, a unique transcriptional profile was revealed for each state, and critical regulators were identified. This is the first study to demonstrate a unique transcriptional behavior of a prophage, which is a result of bacteria-phage co-adaptation.

A Eukaryotic-like Serine/Threonine Kinase Protects Staphylococci against Phages

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Organisms from all domains of life are infected by viruses. In eukaryotes, serine/threonine kinases play a central role in antiviral response. Bacteria, however, are not commonly known to use protein phosphorylation as part of their defense against phages. Here we identify Stk2, a staphylococcal serine/threonine kinase that provides efficient immunity against bacteriophages by inducing abortive infection. A phage protein of unknown function activates the Stk2 kinase. This leads to the Stk2-dependent phosphorylation of several proteins involved in translation, global transcription control, cell-cycle control, stress response, DNA topology, DNA repair, and central metabolism. Bacterial host cells die as a consequence of Stk2 activation, thereby preventing propagation of the phage to the rest of the bacterial population. Our work shows that mechanisms of viral defense that rely on protein phosphorylation constitute a conserved antiviral strategy across multiple domains of life.

Small molecule communication between phages guides lysis-lysogeny decisions

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Temperate viruses can integrate into the genome of their host cells, a process called lysogeny. In every infection, such viruses need to decide between the lytic and the lysogenic cycles, i.e., whether to replicate and lyse their host or to lysogenize and keep the host viable. Here we show that viruses (phages) of the spBeta group use a small-molecule communication system to coordinate lysis-lysogeny decisions. During infection of its *Bacillus* host cell, the phage produces a 6aa communication peptide that is released to the medium. In subsequent infections, progeny phages measure the concentration of this peptide and lysogenize if the concentration is sufficiently high. We found that different phages within the phage group encode different versions of the communication peptide, demonstrating a phage-specific peptide communication code for lysogeny decisions. We termed this communication system the "*arbitrium*" system, and further show that it is encoded by 3 phage genes: *aimP*, producing the peptide, *aimR*, the intracellular peptide receptor, and *aimX*, a negative regulator of lysogeny. The *arbitrium* system enables an offspring phage to communicate with its predecessors, i.e., to estimate the amount of recent prior infections and hence decide whether to employ the lytic or lysogenic cycle.

Hostile take-over: Pseudomonas as prey for lytic bacteriophages

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Pseudomonas-infecting phages mirror widespread and diverse nature of their hosts. Therefore, Pseudomonas spp. and their phages are an ideal system to study the molecular interactions that are involved in the struggle for cell control. Understanding these interactions not only yields novel insights into phage biology, but also advances the development of phage therapy, phage-derived antimicrobial strategies and innovative biotechnological tools. Our lab studies the phage-encoded mechanisms which allow the evasion of host defense mechanisms and induce the subversion of the host metabolism.

Protein interaction studies on a diverse set of Pseudomonas phages allowed us to identify phage-proteins which impact transcription & RNA stability, replication & cell division and the modulation of the host metabolism. This lecture will offer specific examples of these mechanisms and attempts to embed them in a systems biology view of the phage infection.

Immunophage synergy is essential for eradicating pathogens that provoke acute respiratory infections

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Pseudomonas aeruginosa is an important cause of life-threatening nosocomial pneumonia and prone to multidrug resistance. Especially at risk are patients with weakened immune systems and chronic respiratory disorders. Considering the scarcity of new antibiotics, the use of bacteriophage phages) as an alternative therapy has re-emerged. While several recent animal studies have demonstrated the therapeutic potential of phages for treating pseudomonal lung infection in healthy immunocompetent animals, it remains unknown whether phages are effective in the setting of immunodeficiency or have untoward immunostimulatory effects during inflammation (e.g. cystic fibrosis). Here, we provide a systematic investigation of host innate immune status on the efficacy of phage curative and prophylactic therapies for pneumonia caused by *Pseudomonas aeruginosa*. We show that inhaled phage therapy ability to cure acute pneumonia, as well as phage prophylaxis to prevent infection, was innate and adaptive lymphocyte-independent. In contrast, both phage therapy and prophylaxis efficacies were dependent on host neutrophil contribution. By integrating *in vivo* and mathematical modelling, we demonstrate that phage killing and host neutrophils work in concert to reduce phage-sensitive and phage-resistant bacterial burden in the lungs. Importantly, host neutrophils were needed to prevent phage-resistant bacterial outgrowth. This 'immunophage synergy' contrasts with the predominant view that phage therapy efficacy relies largely on the bacterial permissiveness to phage killing. Nonetheless, we show that phage therapy can still be efficacious in hosts with weakened innate immunity that manifest emerging phage resistance. Concerning untoward immune effects from respiratory treatment, high dose of phages did not promote obvious cytokine production in the lung tissues. This study supports that phage therapy is a safe and efficient respiratory antibacterial treatment in the context of specific immunodeficiencies.

Microfluidic encapsulation of bacteriophage in liposomes

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Increasing antibiotic resistance in pathogenic microorganisms has led to renewed interest in bacteriophage therapy both in humans as well as in animals. It is generally recognised that phage do not diffuse across eukaryotic cell membranes and therefore would 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 for the treatment of important infections e.g. Mycobacterium tuberculosis or infections caused by other intracellular bacteria e.g. S. aureus or E. coli. There are relatively few published studies looking at encapsulation of bacteriophage in liposomes [2–6]. Recent animal studies in chickens [3] have shown that liposome encapsulated Salmonella phage were retained and released in the caecum for a significantly longer duration in comparison with free phage and also resulted in reduction in intestinal colonisation for longer in infected animals. Liposome encapsulated K. pneumonia phage (delivered via intraperitoneal injection) were shown to remain in systemic circulation in mice for longer compared with free phage [4]. Liposome nanoparticle access to infection sites deep in intestinal mucosa may be achieved thereby improving phage therapy efficacy by targeting bacterial refugees residing in these difficult to access niches ([7], [8]). 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. Bacteriophage K (lytic, S. aureus, myovirus) was used in the study. We report results for encapsulation of phage in sub-micron liposomes (shown using TEM and confocal microscopy) and their subsequent release (phage titre measured using plaque assay). The effects of lipid and solvent composition, temperature, phage titre and micro-channel setup were investigated on phage encapsulation, phage viability and storage stability.

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Microbiome and stable core virome after human fecal transfer

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We recently 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). 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. 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). Moreover, 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). This was unexpected since enteric viromes are normally highly variable, assumed to influence the bacterial host community and change with environmental conditions. 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). Our findings that are based on sequence information and protein domain analysis seem to suggest that stable phage properties correlate with successful FMT better than the changing bacterial communities. We speculate that we here preferentially detected a stable core virome, which dominated over a variable flexible virome that may have been too heterogeneous for experimental detection, or was underrepresented in the databases. The virome is possibly the determining factor in the composition of the gut microbiome and stool transfer. It will be interesting to analyze whether the enteric virome allows for predicting the clinical outcome of FMT for rCDI and other diseases such as inflammatory bowel disease or obesity.

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Intravenous Application of Phage Therapy to Treat a Terminally Ill Patient Who Was Infected with Multi Drug Resistant *A. baumannii*

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Current global surveillance indicates that multi drug resistant (MDR) bacteria are emerging at an alarming rate. There is also a significant concern about the potential of generating highly virulent microorganisms using genetic engineering and synthetic biology. With the growing problem of rapidly occurring and spreading antimicrobial resistance, alternative treatment must be sought and developed. Phages or bacterial viruses are the most abundant biomolecules on the surface of the earth and known to be bacterial killers. Furthermore without any partiality phage will kill both MDR and non-MDR bacterial pathogens. In this regard a properly formulated phage cocktail would be highly effective for overcoming antimicrobial resistance (AMR) of any bacteria. Currently we are developing phage therapy to treat MDR bacterial infections in human. In this project we invented a rapid process to select combination of broad spectrum phages which can overcome the emergence of phage resistance bacteria and ultimately enhance the therapeutic efficacy of the phages. Recently we received a request from University of California, San Diego (UCSD) to provide cocktail phages which are enabled to kill an MDR A. baumannii clinical isolate called the TP strain. We immediately engaged to evaluate the killing efficacy of 98 A. baumannii phages from our library on the TP strain using our proprietary liquid assay system. Our rapid assay results indicated that the phage library contained 10 very virulent phages which very effectively killed the TP strain. After further analysis using our proprietary system we selected 4 phages to prepare cocktail which can overcome the emergence of phage resistance when used of TP strain. Selected phages were amplified on TP strain and purified using cesium chloride density gradient technique to prepare phage cocktail. The cocktail was sent to UCSD where physician used the phage cocktail to treat a terminally ill patient who was infected with MDR A. baumannii. This phage therapy was done as an FDA-approved, off-use intravenous infusion to treat patient's systemic infection. Within 48 hours of intravenous administration of phage cocktail the patient came out of his coma and within 7 days patient's temperature and white blood cell count became normal and his creatinine level improved significantly. Additionally phage therapy also re-sensitizes the TP strain to several antibiotics to which it was previously acquired resistant. The detail of this phage therapy study will be presented here.

Bacteriophages - wide application for eradication of bacterial pathogens

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G. Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia that was established in 1923 by Georgian microbiologist, Prof. Giorgi Eliava and French-Canadian Prof. Felix D'Herelle still remains as one of the major centers in bacteriophage research and elaboration of appropriate methodologies for biological preparations for human and animal protection. Historically, the Eliava Institute was performing investigation in several directions, but phage research and applications were the main focus of its activity. Phage preparations elaborated and produced by the Eliava Institute have been successfully used in the entire Soviet Union and other Socialist countries for treatment and prophylaxis of various infectious diseases for decades. In addition to the therapeutic preparations, the Eliava Institute produced standard (typical) and indicator bacteriophages for diagnostics of different infections.

Today, the Eliava Institute continues its activity in selection and detailed studies of phages that are active against various bacterial pathogens, including multi-drug resistant bacteria. The strains (human or animal isolates) are obtained from different geographical zones; phages (commercially available and from the Institute's collection) are being tested against the strains, and active phages are selected for further characterization.

Several projects are dedicated to phage application for animal protection, e.g. against bacterial pathogens in aquaculture, against bovine mastitis, enteropathogenic *E.coli* in calves. Prove of concept of successful application of phages is confirmed in plants, as well (bacterial infection in rice, cotton, potato, grapes and others).

Main application of the Eliava phages is directed for treatment and prophylaxis of human bacterial diseases. Phages are successfully used to treat acute, as well chronic infections caused by antibiotic-resistant bacterial strains. At Eliava, phages are mainly used for treatment of urologic problems, gynecological diseases, gastrointestinal problems, skin and soft tissue diseases, respiratory system diseases, and secondary infections in cystic fibrosis patients.

The next step of our activity is commercialization of phage products according to International standards. The Institute plans to continue and extend its activity for elaboration of phage preparations and to explore the most appropriate organizational development for broader production, application and marketing of its biological preparations.

POSTER SESSION

Poster session 1: Monday, April 24th
Poster installation 13:30 / Poster with odd numbers

Poster session 2: Tuesday, April 25th
Poster installation 17:45 / Poster with even numbers

Topic	Page

Dynamics of unculturable microbes and their viruses in a natural environment

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In the last several decades a range of models have been proposed to describe the dynamics of interacting bacterial and phage populations in natural environments. These models have their basis in the classic Lotka - Volterra concept of dynamic changes of predator and prey numbers. In accordance to Kill-the-Winner, the most well-known model of population dynamics of phage-host interactions, an increase in abundance of a bacterial host causes a subsequent increase in numbers of a corresponding phage, which in turn decimates the host population. A range of experimental systems showed dynamics that are consistent with the Kill-the-Winner model. Recently, a new extension to the Kill-the-Winner model has been proposed, the so-called Piggyback-the-Winner model. It suggests that an increase of host population density can cause integration of their temperate phage predators into the host genome, leading to decreased phage/bacterial ratio. Findings from another recent study showed that the temperate/lytic switch may be regulated by phage-phage communication via short peptides. Beside these models, other mechanisms may be at play and contribute to host-pathogen dynamics, and the true spectrum of possible interactions between phages and bacteria in natural environments remains unknown.

To assess the range of possible host-pathogen interactions in natural microbiomes, we analysed a temperate freshwater lake with a clear seasonal dynamic. We generated time series metagenomic samples and identified peaks of individual bacterial and viral populations throughout the year. Out of 8950 of newly assembled viruses, 225 could be linked to their hosts using matching CRISPR spacers. One-third of these phage-host pairs (31.5%) displayed dynamic changes in accordance with the Kill-the-Winner model, where the peak in viral abundance coincides with, or directly follows the peak of its host. Analysis of the rest of the pairs allowed us identify another frequent pattern (16.5% of pairs), where the peak of viral abundance preceded the peak of corresponding host. We propose that this unexpected dynamic pattern can be explained by the acquisition of bacterial resistance to the phage followed by outgrowth of the immunised bacteria. Moreover, we may expect that similar dynamics can be the result of Piggyback-the-Winner interactions, if phages with increasing host densities enter the lysogenic cycle. Together, we present the first community-scale quantification of the different possible phage-host interaction dynamics in a natural environment.

A Snapshot of Bacteriophage Diversity in Gasworks-contaminated Groundwater

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Groundwater contamination and environmental pollution is a serious issue today and has been since the advent of industrialization. Microorganisms, particularly bacteria, can metabolize and clean-up events of contamination through a process commonly described as bioremediation. Bacteriophages play important roles in the ecology, diversity and adaptation of bacteria but, despite their relevance, the study of bacteriophages in groundwater and bioremediation has been widely overlooked to date: thus far, no study exists on the influence of bacteriophages in groundwater microbial degrader community activities and, moreover, no comprehensive study has been published yet on the taxonomic and functional diversity of viruses in contaminated environments.

Here we present the analysis of the first two metaviromic datasets of an experiment that aims to follow the diversity and evolution of microbes and viruses in contaminated (C1) and non-contaminated (NC1) groundwater. To do this, microbial and viral DNA fractions were isolated from an old gasworks site in Northern Ireland and sequenced using Illumina HiSeq 4000 Technology.

Assembly of C1 and NC1 viral contigs was performed with metaSPAdes yielding 169,658 and 311,306 contigs with a maximum size of 90,312 and 196,114 bp from respective samples. Annotation of features was performed with Prokka, finding 132,686 CDS for C1 and 322,970 CDS for NC1. Using DIAMOND, we found that only 11% and 9% protein BLAST hits were found for C1 and NC1 in the RefSeq viral database and out of all BLAST hits, 91% matched previously known taxa in both samples. The taxonomic and functional analysis was performed using MEGAN6 and we found that while C1 and NC1 are populated by different phages, most of the phages found are part of the Myoviridae, Podoviridae and Siphoviridae families. Functional annotation was performed using GO, COG and SEED subsystems.

Ongoing work aims to evaluate relations between bacterial and viral communities. In particular, we want to investigate the possibility of the phage impact on bioremediation and the usefulness of phages as biomarkers.

Two *Bacillus licheniformis* bacteriophages isolated from the brackish water lake Lisi, Georgia

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Bacilli are endospore-forming, Gram-positive bacteria ubiquitously distributed in the environment and playing an important role in the biogeochemical cycling. In spite of ubiquity, little is known about diversity and distribution of bacilli and their bacteriophages in the natural aquatic environments in different parts of the world.

Here, we describe two *B. licheniformis* phages isolated from the brackish water lake Lisi, Georgia.

Our studies showed that *Bacillus spp.* are frequently cultured from different water environments in Georgia.

After series of isolation using enrichment technique, the phage mixture infecting environmental strains of *B. licheniformis*, LW 2.5 and LW 1.10 was obtained. Following single plaque purification, two phage clones were selected and named BLCH 1.2 and BLCH 2.1. Transmission electron microscopy showed that both phages belong to the Myoviridae family. The phages had a broad-host range, being active against all *B. licheniformis* strains (23 strains) and 45 % of all *Bacillus spp.* (in total 187 strains), isolated from the Black Sea and the lake Lisi water and sediment environments. We assumed that high lytic activity of the phages could promote genetic exchange between the hosts. The phage BLCH 1.2 was tested for the ability to transduce the erythromycin resistance gene among the selected bacilli isolates. In our experiments transduction of the erythromycin resistance via phage BLCH 1.2 wasn't revealed.

The whole genome sequencing showed that genome size of both phages was 148kb, with 39% GC content and showing high identity (97%) to Bacillus phage SIO phi**.** Gene prediction identified 225 and 224 open reading frames (ORF) in BLCH 1.2 and BLCH2.1 phages, respectively. The genes encoding proteins for DNA replication, DNA biosynthesis, morphogenesis, as well as genes responsible for the lysogeny were identified. Interestingly, two genes, encoding thioredoxin and the host intergartion factor appear to be acquired from the bacterial host.

Based on obtained data we can conclude that the isolated phages can shape bacterial populations through lysis as well as through lysogeny and possibly can be involved in transduction of host derived genes among bacilli populations.

Characterization of a *Siphoviridae* phage infecting *Citrobacter freundii*

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Citrobacter freundii is a member of the Gram-negative *Enterobacteriaceae* and is an opportunistic pathogen associated with several infectious diseases including urinary tract and wound infections as well as neonatal meningitis. This pathogen is increasingly resistant to antibiotics, which emphasizes the need of alternative therapeutics and preventive approaches. In this study, we explored phages as antimicrobial agents against this pathogen. From a sewage sample in Tunisia, we isolated and characterized a novel virulent phage, named SH8, using the strain *Citrobacter freundii* CF3 as a host bacterium. Genomic analyses revealed that phage SH8 (49,339 bp, 42% G+C, 77 ORFs) belongs to the *Siphoviridae* family and the *T1virus* genus with a high degree of nucleotide sequence identity to the virulent phages Stevie (86%) and Tls (83%) infecting respectively *Citrobacter freundii* and *Escherichia coli*. Genes coding for an integrase were not identified in SH8 genome suggesting its obligatory virulent lifestyle thus reducing the risk of horizontal gene transfer. SH8 genome was also scrutinized for gene coding for known virulence factors and none were identified. Among 30 enterobacterial strains tested including strains of *Citrobacter*, *E. coli*, *Salmonella*, *Shigella*, *Yersinia* and *Cronobacter*, phage SH8 was able to lyse only one other strain of *Citrobacter freundii* (CF4). Moreover, phage SH8 was found to have a short latent time of 17 min and a burst size of 150 ± 13 PFU per infected cell. This phage was sensitive to acidic conditions under pH 5. Mass spectrometry analysis of purified phage particles revealed twelve structural proteins, including the portal, capsid, tail and tail fibers. Because the emergence of bacteriophage insensitive mutants (BIMs) is a concern for the therapeutic use of phages, we explored the frequency of their occurrence in presence of phage SH8 either alone or in combination with other *Citrobacter* phages belonging to the *Myoviridae* (SH9) and the *Podoviridae* (SH3 and SH4) families. Our results indicate that the use at high MOI of a cocktail of phages belonging to different phage families reduce the appearance of BIMs. Taken altogether, the newly isolated lytic phage SH8 can be added to our arsenal of phages to combat *C. freundii*.

Genetic and functional diversity of ubiquitous DNA viruses in selected Chinese agricultural soils

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Viral community structures in complex agricultural soils are largely unknown. Tailed bacteriophages, spherical and filamentous particles were identified by the morphological analysis. Based on the metagenomic analysis, single-stranded DNA viruses represented the largest viral component in most of the soil habitats, while the Jiangxi-maize soil contained predominantly double-stranded DNA viruses belonging to the *Caudovirales* order. The majority of functional genes belonged to the subsystem “phages, prophages, transposable elements, and plasmids”.

Non-metric multidimensional analysis of viral community showed that the environment medium type was the most important driving factor for the viral community structure. For the major viral groups detected in all samples (*Microviridae* and *Caudovirales*), these clades gathered viruses from different sites and similar genetic composition, indicating that viral diversity was high on a local point but relatively limited on a global scale. This is a novel and systematic report of viral diversity in Chinese agricultural soils, and the abundance, taxonomic, and functional diversity of viruses that were observed in different types of soils will aid future soil virome studies and enhance our understanding of the ecological functions of soil viruses.

New Phi29 related isolates letting small things grow bigger

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Phi29 like viruses have been known for decades and represent one of the best described viruses. Special features of its type strain Phi29 are the protein caped linear genome, the protein priming mechanism during replication initiation and the fastest known DNA-polymerase described so far. Viruses of this subfamily infect species of the genus Bacillus and reveal Podoviridae morphology with virion dimensions of approximately 50 nm width and 85 nm height.

The genome size ranks between 18 and 21 kb and codes for 24 to 35 protein coding genes. Here, we present results on a newly isolated virus vB_BthP-Goe4 of Bacillus thuringiensis. Morphological investigation via transmission electron microscopy revealed key characteristics of phi29viruses but with a prolonged head resulting in larger virion particles of approximately 50 nm width and 120 nm height. Genome sequencing and analysis resulted in a genome of approximately 26 kb, harboring 40 protein encoding genes. Sequence comparison confirmed its relation to the phi29virus subfamily and disclosed genomes of further related strains. A holistic analysis of all potential members of this subfamily indicated the formation of a new clade around vB_BthP-Goe4 and its relation to the established members of the Phi29virus subfamily.

Stumbling across the same phage: Comparative genomics of broadly distributed bacteriophages reveal an omnipresent temperate phage for *V. anguillarum*

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Nineteen *V. anguillarum*-specific temperate bacteriophages isolated across Europe and Chile from aquaculture and environmental sites were genome sequenced and analyzed for host range, morphology and life cycle characteristics. The phages were classified as Siphoviridae with genome sizes between 46,006 and 54,201 bp. All 19 phages showed high genetic similarity, and 13 of phages were genetically identical. Apart from sporadically distributed SNPs, genetic diversifications were located in 3 variable regions (VR1, VR2 and VR3) in 6 of the phages genomes. Identification of specific genes, such as N6-adenine methyltransferase and lambda like repressor, as well as the presence of a $tRNA^{Arg}$, suggested a both mutualistic and parasitic interaction between phages and hosts. During short term phage exposure experiments, 28 % of a *V. anguillarum* host population was lysogenized by the temperate phages, and a genomic analysis of a collection of 31 virulent *V. anguillarum* showed that the isolated phages were present as prophages in >50 % of the strains covering large geographical distances. Further, phage sequences were widely distributed among CRISPR-Cas arrays of publicly available sequenced *Vibrio*s. The observed distribution of these specific temperate Vibriophages and prophages across large geographical scales may be explained by efficient dispersal of phages and bacteria in the marine environment combined with a mutualistic interaction between temperate phages and their hosts which selects for co-existence rather than arms race dynamics.

Keywords: bacteriophages, temperate, *Vibrio anguillarum*, Siphovirus, genetic similarity, omnipresent, lysogenic conversion, repressor, superinfection exclusion, N6-adenine methyltransferase, counter-defense.

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Characteristics, completed genome sequences and phylogenetic tree of *Salmonella* Chi-like bacteriophages isolated from Thailand

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Salmonella enterica serovar Typhimurium (*S*. Typhimurium) is a worldwide major cause of food-borne disease. Bacteriophage (phage), virus of bacteria, is commonly found closely associated with the bacteria in the environment and has impact on diversity and composition of their bacterial host. In this study, 421 chicken droppings were randomly collected from Thailand using *S*. Typhimurium as a host strain. Amplification of 108 phages isolated from this collection with capsid protein E primers, common among phages in the genus Chi, revealed that 46 % of phages gave positive reactions with the PCR suggested that they are Chi virus and this genus is predominant in Thailand, at least in our collection. Three of the PCR positive phages including φSTm-101, φSTm-118 and φSTm-374 which show different plaque morphologies, host range infection and one-step phage growth curve data were selected for whole genome sequencing. Genome sequence analysis indicated that *Salmonella* phages φSTm-101, φSTm-118 and φSTm-374 have genome size around 59 Kb and shared 92.9%, 92.8% and 91.9% identity to φSPN19 (a member in the Chi genus), respectively confirming the PCR result that these phages are Chi virus. Furthermore, electron micrographs of these phages correlated with the previous data that Chi virus are members of *Siphoviridae* family. Phylogenetic tree, constructed from sequences of the isolated phages and the available genome sequences in database, found that φSTm-374 is outgroup of the phylogenic tree while φSTm-101 and φSTm-118 are in the same root.

Exploration of cyanophage-mediated horizontal gene transfer in *Prochlorococcus*

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With an estimated population of 10^{27} cells globally, *Prochlorococcus* is the most abundant photosynthetic organism on Earth. This marine cyanobacterium is responsible for producing an estimated 4 gigatons of fixed carbon each year, which is approximately the same net primary productivity as global croplands. Cyanophage that infect *Prochlorococcus* represent a notable fraction of the total viral population in some parts of the ocean. *Prochlorococcus* strains possess hyperviariable genomic islands that have been linked to ecological niche dimensions. Several lines of evidence suggest that phage-mediated horizontal gene transfer plays an important role in moving flexible genes in genomic islands.

Our objective is to understand the mechanisms underlying cyanophage horizontal gene transfer and their impact on the evolution and diversification of *Prochlorococcus* genomes.

We have set up an experimental procedure to quantify the presence of host genes within phage particles. Our results provide a first glance into the amount of *Prochlorococcus* DNA travelling inside phage. As an example, we were able to detect a 35 fold increase in packaging frequency for transposon DNA present inside genomic islands. By observing *in vivo* the packaging of host DNA in different phage families, and in different conditions, we are trying to identify the major drivers of phage horizontal gene transfer in the environment.

A novel type of icosahedral viruses infecting hyperthermophilic archaea

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Encapsidation of genetic material into icosahedral particles is one of the most common structural solutions employed by viruses infecting hosts in all three domains of life. Here, we describe a new virus of hyperthermophilic archaea, *Sulfolobus* icosahedral virus 1 (SIV1), which condenses its circular double-stranded DNA genome in a manner not previously observed for other known icosahedral viruses. The genome complexed with virion proteins is wound up sinusoidally into a spherical coil which is surrounded by an envelope and further encased by an outer icosahedral capsid apparently composed of the 20 kDa virion protein. Lipids selectively acquired from the pool of host lipids are integral constituents of the virion. None of the major virion proteins of SIV1 show similarity to structural proteins of known viruses. However, minor structural proteins, which are predicted to mediate host recognition, are shared with other hyperthermophilic archaeal viruses infecting members of the order Sulfolobales. The SIV1 genome consists of 20,222 bp and encodes 45 open reading frames, only one fifth of which could be functionally annotated. The SIV1 infection does not cause lysis of the host cells. However, the mechanism of how a non-enveloped virus penetrates through the cellular membrane without breaking it remains unclear.

Mutation of *Acidianus* filamentous virus 1 under laboratory conditions

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DNA viruses infecting Crenarchaeaota represent a unique, however integral part of the virosphere (Prangishvili, 2013). They reveal astounding diversity of unique virion morphotypes, including spindle-shaped (families *Fuselloviridae* and *Bicaudaviridae*), bottle-shaped (*Ampullaviridae*), droplet-shaped (*Guttaviridae*), coil-shaped (*Spiraviridae*) particles. Unique for the Crenachaeota are also filamentous viruses with double-stranded DNA genomes (families *Lipothrixviridae*, *Rudiviridae*, *Clavaviridae* and *Tristromaviridae*). Also the genomes of crenarchaeal viruses are exceptional with more than 90% of genes without homologues in extant databases.

Members of the family of *Lipothrixviridae* are enveloped, lipid-containing filamentous viruses with linear dsDNA genomes. *Acidianus* filamentous virus 1 (AFV1) is the most extensively studied member of this family (Bettstetter *et al*., 2003). The termini of the virion are decorated with claw-like structures which clasp around pili-like appendages present on the host cell surface. AFV1 infects two species in the genus *Acidianus*, *A. hospitalis* and *A. infernus*. AFV1 is not lytic and upon infection establishes a stable carrier state. The 20.869-bp genome of AFV1 carries 40 open reading frames and terminal sequences, resembling telomeres of eukaryotic chromosomes.

As a results of continuous propagation of AFV1 in the laboratory for more than a decade, the virus AFV1 has mutated. The mutant is about 10% shorter than the native virion and carries a genome of 18.173 bp. The deletion of 2.696 bp has occurred in the left part of the genome and affected 8 ORFs. Among the deleted genes were those encoding for the experimentally characterized nuclease AFV1_ORF157 (Goulet *et al*., 2010) and Zinc-finger DNA-binding protein (Guillière *et al*., 2013) as well as CopG family transcriptional regulator (AFV1_ORF59b) conserved in crenarchaeal viruses. Additional cultivation of the mutant resulted in further virus adaptation which was accompanied by introduction of a stop codon in the truncated ORF1, presumably due to toxicity of the corresponding read-through gene product. These results provide new insights into the genome evolution of crenarchaeal viruses.

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First bacteriophages detection in food matrices in Abidjan, West Africa

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Phages are widely apply to screen, to detect and to eliminate bacterial in different applications. In Africa, the emergence of antibiotic resistance strains and the fecal contamination in the aquatic environment require to explore the phage therapy. In Abidjan, the microbiology lacks in food chain represents a risk in the country for several food products. However, fishes and chicken are the most consumed products in West Africa. The aim of this study is to identify phages in fishes and chicken samples and to test their virulence in multidrug resistant strains.

Fresh fishes and chicken were collected from fish market in Abidjan. The intestinal organs were first diluted in buffer and pre-filtered through 0.45µm. 0.1 ml of samples were inoculated in LB media supplemented with 3 ml of filtrates. The enrichment of bacteria and phages was taken in LB media at 37°C, 24h and virulence tests were performed by plate method.

A Total of 15 phages were isolated in this study. All isolated phages have virulence against *E. coli B*. The virulence has showed against for antibiotic multidrug resistant strains *Pseudomonas aeruginosa, Salmonella sp, Enterobacter aerogenes.* No virulence was detected against *Klebsiella pneumoniae* and *Enterobacter aerogenes*. This study reports the first isolation of 15 novel phages in West Africa against multidrug resistant's strains and can be apply as a new approach for eliminating of pathogens.

Keywords: Fishes, chicken, Multidrug resistant'strains, phages, biocontrol, West Africa

Structuration of the viral community at the mouth of the Charente river estuary

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The mouth of the Charente estuary (Atlantic coast, France) was monthly monitored from April 2002 to July 2009. Globally dynamics of viruses and heterotrophic prokaryotes were synchronized in summer and decoupled in spring and autumn (May and November) when viruses peaked and prokaryotes dropped concomitantly to an increase in Chl *a*. Multivariate analysis (including variance partitioning for the 2008-2009 survey) discriminated as principal explicative variables of the viral community structure, the frequencies of lysogens and infected prokaryotic cells, the viral and bacterial productions, and the viral and prokaryotic abundances. 34.5% of the variance of the viral diversity pattern were explained by a biological set of explanatory variables (abundance of microbial autotrophs and heterotrophs and physiological parameters of prokaryotes and viruses) and 34.7% by the structure of the prokaryotic assemblage. Lysogeny was weak (up to 4%) and no lysogen was inducible in December-January and April-May; however the frequency of infected cells remained moderate all along the year (but increased up to 15% in April-May and October-November). Changes in the diversity patterns of the viral community should thus occur in April and December as confirmed by the two fingerprint analysis and metagenomic analysis.

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The first known virus isolates from Antarctic sea ice

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Sea ice is one of the largest habitats on Earth covering up to 7% of Earth surface, most of which is located in the coldest place on Earth, Antarctica. However, sea ice is full of life. Cold adapted microbes live inside liquid brine channels and pockets, which are formed during the freezing process when salts and nutrients from the sea water concentrate between ice crystals. The sea-ice microbial community consists of protists, bacteria, archaea and their viruses. However, the role of viruses in the sea ice is extremely understudied. We participated an austral winter expedition to Antarctica, to sample the sea ice for virus isolation. The abundance of virus-like particles (VLP) was measured by flow cytometry from 10 ice cores sampled from the Weddell Sea. Numbers were in range of 10^5–10^6 ml^{-1} in bulk ice and the virus-to-bacteria ratio (VBR; more precisely VLP to prokaryotic cell ratio) was 0.7–13.4 suggesting that the microbial community is active. We also isolated viruses from the sea ice samples and describe the first cultivable viruses from Antarctic sea ice. These unique bacteriophages PANV1, PANV2, OANV1 and OANV2 grow on bacterial hosts which originate from the same sea ice samples and belonged to the common sea-ice bacterial genera *Paraglaciecola* and *Octadecabacter*. The bacteriophages were purified and characterized by their morphology and structural components, and their host range and the effect of temperature to the infection were determined. Most of the phages were very specific in their host recognition, but OANV1 was able to infect bacterial strains from two different classes, Alphaproteobacteria (*Octadecabacter*) and Gammaproteobacteria (*Paraglaciecola*). All bacteriophages were cold-active. They could produce plaques only at temperatures below the maximum growth temperatures of the host. In all, bacteriophages may be abundant and controlling bacterial community composition and diversity even in the Antarctic winter sea ice.

Detection and quantification of P-SSP7 prophage in *Prochlorococcus* MED4 genome

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The marine cyanobacteria *Prochlorococcus* is the smallest and most abundant photosynthetic organism on Earth paying important and various ecological roles of ocean ecosystems. They are adapted to living in low- nutrient oceanic regions and are physiologically and genetically diverse. Strain to strain variability is often clustered in genomic islands, the hypervariable regions of the chromosome, which contain signatures of phage and mobile element activity, including phage- like integrase genes.

Although lysogeny has been extensively studied in heterotrophic bacteria, and in some cyanobacterial systems, there is no direct evidence of this phenomenon in *Prochlorococcus*. The only sign of phage integration seen among the hundreds of Prochlorococcus genomes available (including single-cell genomes), is a single contig containing a hybrid fragment of a phage genome (similar to the podovirus P-SSP7) fused to a host genome in a wild single cell.

The podovirus P-SSP7 contains an integrase gene upstream of a 42- bp exact match to its host strain *Prochlorococcus* MED4, representing a putative integration site. Despite these converging clues, however, only lytic infection has been reported in the past. In a series of recent experiments, however, we have been able to show, for the first time, the integration of cyanophage P-SSP7 as a prophage during infection of *Prochlorococcus* MED4. This lysogeny, which is happening in a small fraction of the phage population for a short period of time, doesn't seem to fit the canonical lysogenic process. Further description and quantification of prophage formation in this abundant cyanobacterium will help us better understand selection pressures shaping phage – host dynamics in marine ecosystems.

Phenotypic Comparison of *Salmonella* Phage Isolated from Open versus Close Animal Production Systems

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Introduction: The genus *Salmonella* represent a worldwide distributed pathogen that can be transmitted to humans mainly as foodborne or by contact with infected animals. *Salmonella* diversity is represented by 2,600 serovars, which show geographic and animal host distribution. Bacteriophages are abundant and widely distributed; through constant host lysis, phage play important roles driving host diversity. The ability of phages to lyse different hosts can be tested, this allowing to evaluate the affinity of phages in a given environment to certain *Salmonella* serovars, through of Host range test.

Purpose: The purpose of this study was to compare the host range of *Salmonella* phages obtained from open and closed animal systems in Chile

Methods: We isolated *Salmonella* and phages; for phage isolation we used as host the predominant *Salmonella* serovar in Chile (Enteritidis, Typhimurium, Infantis, and Heidelberg).

Our study systems included i) backyard poultry and pigs (n=35), ii) industrial pigs (n=36), iii) backyard cattle (n=47), iv) industrial cattle (n=160), v) wild bird in peri – urban locations (n=49), vi) wild bird – wetland (n=271), and vii) backyard – South American camelids (n=49). The host range was characterized with a panel of 23 *Salmonella* serovars and analyzed through hierarchical clustering. Narrow host range was defined for phages lysing 1–4 hosts, and wide host range for phages lysing 5–23 hosts.

Results: *Salmonella* was found in all systems, except for industrial cattle. Phages were isolated in all systems in a proportion of three times more often than *Salmonella* (93 *Salmonella* versus 322 phages). Phage host range differ by system, the widest host range was found in backyard cattle, wild bird – wetland, backyard poultry, all open systems with low human intervention. While in close systems, phages lysed *Salmonella* serovar commonly associated with human cases; contrastingly the open systems lysed serovars not frequently isolated in Chile.

Significance: This is the first study that conduct a wide comparison of *Salmonella* and its phages in different animal systems. Differences described here could indicate the potential application of using phages as prediction tools to investigate *Salmonella* diversity.

A new siphovirus infecting *Nodularia* sp. AV2

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We have isolated a new siphovirus that infects filamentous nitrogen fixing and toxin producing cyanobacterium *Nodularia* sp. AV2. The virus designated as vB_NpeS-2AV2 was shown to infect several strains of *Nodularia* sp. originating from the Baltic Sea. In addition to the host range, we have characterized the virus in terms of its genome sequence, structural proteins and infection cycle.

Detection of bacteriophages as indicator of fecal contamination in fresh produce in the Philippines

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Worldwide, increasing popularity of fresh and fresh-cut produce has been observed together with the consumer perception that these products are healthy, tasty, convenient and fresh. The fruit and vegetable industry has experienced solid growth over the past ten years as illustrated by increasing consumption of these produce. Preference for these products has however brought consumers greater risks of contracting microbiological foodborne illnesses and even outbreaks (i.e. severe diarrhea and other gastrointestinal diseases). Fresh produce have become one of the vehicles of transmission of various opportunistic pathogens (e.g. enteric microorganisms) which are typically linked to fecal contamination from different farm animals. A comprehensive survey of the presence of somatic coliphage in fresh produce from different open air markets in the Philippines was done by double agar layer assay. Various vegetables that were typically eaten raw (carrot, bell pepper, lettuce, mung bean sprout, and tomato) were screened for possible contamination. Out of the 132 vegetable samples collected and 21 (15.91%) coliphage isolates were obtained. Somatic colipage Phi-X-174 was used as positive control to ensure presence of the phages. This correlates to the observed 12 (9.09%) *Escherichia coli* isolates detected. This study shows that bacteriophages can be used as indicators of fecal contamination and may be used in detection instead of pathogenic viruses. The enteric microbial loads in the vegetable samples can pose great risk among the health of human consumers. Hence, monitoring on the handling of different vegetable produce in open air markets in the Philippines is highly important.

Inferring virus-microbe infection networks from time-series data

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Microbes are found in high abundances in the environment and in human-associated microbiomes, often exceeding one million per milliliter. Viruses of microbes are estimated to turn over 10 to 40 percent of microbes daily and, consequently, are important in shaping microbial communities. Yet, the interactions among microbes and viruses are difficult to pin down in situ. Deducing which pairs interact in complex virus-microbe communities, i.e., the "inference problem", remains an open question. Contrary to widespread use, we find that correlation-based approaches are poor indiactors of interactions when such interactions are not already known in advance. Instead, we demonstrate the value of model-based inference by discretizing a nonlinear mechanistic model to infer virus-microbe interactions from time-series (Jover, Romberg & Weitz, Roy. Soc. Open., 2016). We extend this approach to infer virus-microbe interactions even when microbes interact with each other heterogeneously. We find that, unlike the correlation-based approach, the model-based inference is robust to variation in network structure and life history traits. We discuss ways to implement model-based inference using sequence-based measurements in the lab and field.

Deciphering the regulation of *Listeria monocytogenes* 10403S monocin

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Listeria monocytogenes is a gram-positive, food-borne, facultative intracellular pathogen. This specie harbors a conserved phage-derived genomic region that was shown to encode for a phage tail-like protein complex functioning as a bacteriocin, which is released to the media upon DNA stress and capable of efficiently killing competing related bacteria. This region was named monocin or lma region and was also shown to express proteins inducing an immune response in mammalian hosts upon bacterial infection. While the function of this region was deciphered, the regulatory elements governing the expression of the monocin have not been investigated. In this study, we have constructed deletion mutants and over expression strains of different genes of the locus and characterized the phenotypes using functional lysis assays and quantitative reverse transcription PCR analysis under different conditions. We found this region to actively cause lysis upon DNA stress in a holin-endolysin dependent manner, furthermore, we have characterized the main regulators of the monocin.

Evolution of bacteriophage transduction rates

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Horizontal gene transfer appears to be common in nature, where it underlies the spread of factors that affect human health, such as antibiotic resistance genes and pathogenicity islands. However, the evolutionary and ecological mechanisms underlying gene transfer in natural populations have been understudied. One of the major modes of bacterial horizontal gene transfer involves the packaging of bacterial DNA into phage capsids, which deliver the DNA to another cell where it can recombine into the chromosome. This process of generalized transduction has long been used to transfer genes in laboratory experiments. To investigate the role of the environment in the evolution of transduction rate, we used agent-based evolutionary models of bacteria, generalized transducing phage, and antibiotic selection pressure to investigate. We verified that increasing the intensity of antibiotic treatment results in the evolution of antibiotic-resistant bacteria. We found that transduction rates were higher in spatially structured populations. However, this observation is sensitive to bacteria-phage community stability, as unstructured environments more quickly lead to phage extinctions, in turn leading to greater bacterial densities. We also found that the mean transduction rate of the phage population declined over time in all environments, but that it declined the most rapidly in environments with less spatial structure. For future work, we plan to test our model using laboratory populations of *Salmonella* and bacteriophage P22. We envision that an understanding of how transduction rates evolve will aid in the prediction of pathogen evolution, helping to inform policy and best practices or long-term effectiveness of antimicrobial drugs and therapeutic phages.

Microbiota-Driven Evolution of Bacteriophage Host Spectra

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While the therapeutic potential of virulent bacteriophages (phages) in killing gut pathogens has already been demonstrated in experimental settings as well as in humans, nothing is known about their long-term coevolution in the complex gastrointestinal environment.

We setup a model of *Escherichia coli* gut colonisation in mice where the commensal strain MG1655 and the Adherent-Invasive *E. coli* (AIEC) strain LF82 persist over several weeks. We then selected LF82_P10, a Felix01-like phage, unable to infect or adsorb to strain MG1655 but active against different AIEC strains including LF82. The phage was fed to co-colonised mice or added to liquid co-cultures of these strains, and viral populations were monitored over time. Interestingly, LF82_P10 phages were isolated from mice faecal samples having acquired the ability to infect strain MG1655.

Also, the same *in vivo*-adapted phages significantly broadened or decreased their host-range when tested against a collection of more than one hundred Enterobacteria. On the other end, *in vitro*-evolving LF82_P10 never adapted to infect strain MG1655.

The genomic basis of such drastic variations were identified in a panel of mutations within the tail fiber genes which were not detected in populations evolving *in vitro*. We also found that the ecological explanation behind such positive mutation selection in the gut relies on the pressure exerted by the coevolving microbiota. Indeed, LF82_P10 adaptation to strain MG1655 occurred via multiple steps involving at least one intermediate *E. coli* host strain that we isolated from the murine intestine. *In vitro* reconstitution of a possible adaptation path was obtained when LF82_P10 was mixed with the three *E. coli* strains.

Our work shows that bacteriophage-bacterial co-evolution in complex microbial environments, like the gut microbiota, has a strong impact on viral genetic diversity and infectivity.

The virocell concept

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Historically, viruses were assimilated to the ultrafiltrable infectious agents responsible for the transmission of several contagious diseases. When observed by electron microscopy, these agents turned out to be viral particles, triggering the assimilation of viruses to their virions. This led to several important consequences on virus definition and consideration. For instance, viruses were defined as containing only one type of nucleic acid, although DNA viruses contain both DNA and (messenger) RNA. The number of viral particles in the environment is systematically assimilated to the number of viruses, as if the number of fish eggs in the ocean was used to count the number of fishes! More dramatically, viruses were not considered to be living organisms but inert molecular machine deprived of metabolic functions. As a consequence, the possibility for viruses to produce their own gene was dramatically underestimated, leading to the current idea that viruses are mainly pickpockets of cellular genes. However, as recently suggested by some philosophers, viruses are not simply virions but processes (a definition valuable for all organisms), the virion being a transient materialization of this process (Forterre, 2016). Another materialization is the infected cell that I suggested calling a virocell (Forterre, 2013). The virocell concept reminds us that viruses are fundamentally cellular organisms and focus our attention on the active (living?) phase of the viral life cycle, when the viral genome is actively expressed and replicated, remodelling the cellular metabolism (Rosenwasser et al., 2016), creating eventually new genes. Many eukaryotic viruses produce viral factories in the cytoplasm of the infected cell that can be assimilate to nuclei of virocells. Recently, Pogliano and co-workers discovered that some bacteriophages produce viral nuclei in the infected bacterium, as well as mitotic spindle-like structure (4). The discovery of these “synkaryotic virocells” supports the controversial idea that the eukaryotic nucleus could have evolved from the nucleus of a proto-eukaryotic virocell (5).

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Whole genome sequences & analysis of a novel group of bacteriophages infecting the dairy bacterium *Streptococcus thermophilus*

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Streptococcus thermophilus is a globally employed dairy bacterium used mainly in the production of cheese, yoghurt and related products. The Generally Regarded As Safe (GRAS) status of this species and favourable acidification and texturisation properties have ensured its widespread use in the international dairy industry. Despite a relatively recent general improvement in sanitary standards, increased technical knowledge and genetic information, contamination of dairy production lines by bacteriophages is a persistent and costly problem.

A total of thirteen genomes of phages infecting *S. thermophilus* have been published to date with a large degree of conservation observed within the defined groupings. Whole genome sequencing of these phages has enabled their genome-wide nucleotide-level comparison and elucidation of their putative mechanisms of evolution in dairy fermentations. Traditionally, the classification of phages of *S. thermophilus* has been based on 1) morphology, i.e. as Siphoviridae, 2) a variable genomic region thought to be (at least in part) responsible for host determination (VR2 region) or 3) the mode of DNA packaging and major structural protein content (cos-type or pac-type). More recently, a morphologically distinct and genetically divergent *S. thermophilus* phage (named 5093) containing neither cos- or pac-defining structural elements (nor a confirmed receptor binding protein-encoding gene) was described, necessitating the creation of a third group ('5093-type'). The genomic content of phage 5093 highlights the genetic plasticity in, and potential for diverse lineages of *S. thermophilus* phages.

Here, we present the complete genome sequences of four novel phages capable of infecting *S. thermophilus* BMD2, an industrial dairy starter strain. These phages appear to have undergone considerable genetic exchange with an unknown *L. lactis* phage of the P335 species. Comparative genomic analysis was performed on all four phages with archetypal phages infecting *S. thermophilus* and *L. lactis*. The phages were also characterized by microscopic analysis and adsorption analysis on industrial strains of both *S. thermophilus* and *L. lactis*. The proposed structural proteins of one representative phage of this group were also confirmed by mass spectrometry.

The Role of Transduction in the Adaptation of Microbial Communities

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The evolutionary dynamics of microbial communities are strongly driven by horizontal gene transfer. Adaptive traits such as antibiotic resistance genes can be hastily disseminated through lateral transmission, both within and between microbial populations. Transduction by bacteriophages is one of the vehicles through which these adaptive traits might be horizontally transferred. It is particularly challenging to understand the role of phage transduction in bacterial adaptation, since phage also act as predators of bacteria. Therefore, understanding in which conditions phage might enhance the dissemination of adaptive traits in bacteria is of tremendous importance. Given the increasingly important role of phage therapy, it is crucial to acquire knowledge regarding the ability of phage to act as drivers of adaptation, particularly towards pathogenic traits.

We are currently using a multidisciplinary approach to better understand the conditions in which transduction of adaptive traits, such as antibiotic resistance genes, can occur and impact the development of resistance in bacterial communities. We develop theoretical expectations for these events, and analyse genomes in search of their evidence. Combining theoretical modelling of bacterial-phage dynamics with comparative genomics in a large number of genomes, we propose a novel and comprehensive framework to understand the dual role of phage in impacting microbial communities.

Structure of the bacteriophage T5 tail tube

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The vast majority (96%) of bacteriophages possess a tail that allows host cell recognition, cell wall perforation and safe viral DNA channelling from the capsid to the cytoplasm of the bacterium. 60% of all tailed phages, the *Siphoviridae*, bear a long flexible tail, which tube is formed of the tail tube protein (TTP) that polymerises in stacks of hexamers around the tape measure protein (TMP). At the distal end of the tail, the tail tip complex harbours the receptor binding proteins (RBP). For these phages, little is known on the mechanism that triggers DNA ejection after binding to the host. We report the crystal structure at 2.2 Å resolution of pb6, an unusual trimeric TTP, of siphophage T5. Structure analysis however confirms the homology of pb6 with all TTPs and related tube proteins of bacterial puncturing devices (type VI secretion system and R-pyocin). We fit this structure into the cryo-electron microscopy map of the tail tube determined at 6 Å resolution. Comparing the structure of the tail tube before and after interaction with the host receptor, we show that unlike previously proposed, the host binding information is not propagated to the capsid by the tail tube, as the two structures, at that resolution, are identical.

Convergent evolution toward an improved growth rate and a reduced resistance range in *Prochlorococcus* strains resistant to phage

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Prochlorococcus is an abundant marine cyanobacterium that grows rapidly in the environment and contributes significantly to global primary production. This cyanobacterium coexists with many cyanophages in the oceans, likely aided by resistance to numerous co-occurring phages. Spontaneous resistance occurs frequently in *Prochlorococcus* and is often accompanied by a pleiotropic fitness cost manifested as either a reduced growth rate or enhanced infection by other phages. Here, we assessed the fate of a number of phage-resistant *Prochlorococcus* strains, focusing on those with a high fitness cost. We found that phage-resistant strains continued evolving toward an improved growth rate and a narrower resistance range, resulting in lineages with phenotypes intermediate between those of ancestral susceptible wild-type and initial resistant substrains. Changes in growth rate and resistance range often occurred in independent events, leading to a decoupling of the selection pressures acting on these phenotypes. These changes were largely the result of additional, compensatory mutations in noncore genes located in genomic islands, although genetic reversions were also observed. Additionally, a mutator strain was identified. The similarity of the evolutionary pathway followed by multiple independent resistant cultures and clones suggests they undergo a predictable evolutionary pathway. This process serves to increase both genetic diversity and infection permutations in *Prochlorococcus* populations, further augmenting the complexity of the interaction network between *Prochlorococcus* and its phages in nature. Last, our findings provide an explanation for the apparent paradox of a multitude of resistant *Prochlorococcus* cells in nature that are growing close to their maximal intrinsic growth rates.

Cell-surface receptor recognition proteins of lipopolysaccharide-specific bacteriophages: Infection initiators and valuable diagnosis tools for pathogens

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Bacteriophages have to identify, bind and subsequently infect their hosts in a biochemically diverse environment. For this they use highly specific tail fiber or spike proteins that recognize bacterial cell wall structures. In lipopolysaccharide (LPS) specific phages tailspike proteins (TSP) bind and enzymatically cleave long O-antigen polysaccharide stretches in extended interaction grooves.[1] In the temperate podovirus P22, outer membrane fixation via its TSP is a prerequisite for successful infection.[2] Moreover, homologous TSP genes are often found exchanged between phages infecting Gram-negative bacteria to extend their host range.[3] We have analyzed the O-polysaccharide binding interfaces of different TSP with surface plasmon resonance, isothermal titration calorimetry and X-ray crystallography to define the origins of receptor specificity and affinity. We have designed TSP high affinity mutants that can be used for the sensitive detection and the monitoring of O-antigen phase variations in *Salmonella*.[4] With this we show that engineered TSP are a valuable tool to study the fundamentals of phage particle O-antigen binding in host cell adsorption and infection and for the use in phage-based antimicrobial strategies.

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Novel use for cationic peptides as anti-bacteriophage agent. Its relevance on Escherichia coli O157:H7 Shiga Toxin-Encoding Bacteriophages

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Shiga toxin (Stx) is the main virulence factor in Escherichia coli O157:H7 Shiga Toxin-Encoding Bacteriophages. Cationic antimicrobial peptides shown effects on multirresistant bacteria. Previously we reported inactivation of bacteriophage encoding Stx after chitosan treatment, which is a linear polysaccharide polymer with cationic properties.

With the aim to evaluate if cationic properties are responsible for bacteriophage inactivation, we tested four cationic peptides as anti-bacteriophage agent. Zeta potential were analyzed to evaluate bacteriophage charges and peptide-bacteriophage interaction. We observed bacteriophage inactivation after incubation with cationic peptides, suggesting that inactivation of bacteriophage charges are relevant for infection. Those peptides could be used to avoid infection of intestinal bacteria, consequently avoiding bacteriophage replication and stx expression.

A virulence-associated filamentous bacteriophage increases the host-cell colonisation by *Neisseria meningitidis*

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The mechanism by which *Neisseria meningitidis*, commensal bacterium commonly carried asymptomatically in the human nasopharynx, becomes invasive is not well understood. Comparison of the genome of strains revealed the presence of an 8kb island in strains belonging to invasive clonal complexes. Epidemiological investigations confirmed that the presence of this island is associated with the ability of bacteria to be invasive (Bille *et al.,* 2005; Bille *et al*., 2008). This island was designated MDA for Meningococcal Disease Associated. Highly conserved among meningococcal isolates, its genomic organisation is similar to that of a filamentous phage such as CTXPhi of *Vibrio cholerae*. Subsequent molecular investigations showed that the MDA island has indeed all the characteristics of a filamentous prophage which can enter into a productive cycle, is secreted using the type IV pilus (tfp) secretin PilQ and infects new bacteria using the tfp like receptors (Meyer *et al.*, 2016).

The analysis of the genome content of the prophage did not reveal any obvious virulence factor. The use of isogenic derivatives deleted in the prophage did not show any role in phenotypes associated with the bloodstream phase of meningococcemia (growth in serum, interaction with endothelial cells). On the other hand, when interacting with epithelial cells, phage production increased as microcolonies grew on the apical surface of the monolayer, thus leading to a thicker biomass than in deleted isogenic variants. The analysis of the bacteria covering the epithelial cells revealed that meningococci are bound to the host cells by layers of heavily piliated bacteria. At the opposite, in the upper layers, bacteria were surrounded by phage particles and surprisingly not by tfp that are the only identified attribute allowing aggregation of encapsulated meningococci. Connection between bacteriophage filaments bound to different bacterial cells seemed increasing bacteria-bacteria interactions. We hypothesized that inside biofilm, phage production in place of tfp maintain bacteria-bacteria interactions favouring bacterial colonization onto a monolayer of epithelial cells, thus suggesting that the gain of invasiveness of strains producing this phage rely on its ability to increase colonization at the port-of-entry.

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Structure of bacteriophage T5 tail tube: revisiting the trigger for DNA ejection

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The vast majority (96%) of bacteriophages possess a tail that allows host cell recognition, cell wall perforation and safe viral DNA channelling from the capsid to the cytoplasm of the bacterium. 60% of all tailed phages, the *Siphoviridae*, bear a long flexible tail, which tube is formed of the tail tube protein (TTP) that polymerises in stacks of hexamers around the tape measure protein (TMP). At the distal end of the tail, the tail tip complex harbours the receptor binding proteins (RBP). For these phages, little is known on the mechanism that triggers DNA ejection after binding to the host. We report the crystal structure at 2.2 Å resolution of pb6, an unusual trimeric TTP, of siphophage T5. Structure analysis however confirms the homology of pb6 with all TTPs and related tube proteins of bacterial puncturing devices (type VI secretion system and R-pyocin). We fit this structure into the cryo-electron microscopy map of the tail tube determined at 6 Å resolution. Comparing the structure of the tail tube before and after interaction with the host receptor, we show that unlike previously proposed, the host binding information is not propagated to the capsid by the tail tube, as the two structures, at that resolution, are identical.

Swiss Army knife-like equipment of cell surface recognizing proteins enables myovirus Det7 to infect a broad host range

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Bacteriophages recognize the host cell wall by specific tailspike proteins (TSP) or fibres. The receptors targeted are highly diverse and range from bacterial pili or flagella to cell wall components like outer membrane proteins or parts of the lipopolysaccharide (LPS). These reversible interactions often precede irreversible adsorption to secondary, often protein receptors and have been shown to be non-essential in many phages. However, in model podovirus P22 the fixation to the LPS O-antigen and its degradation by P22TSP are sufficient to elicit *in vitro* DNA ejection.[1] This highly O-antigen specific process is slow compared to the *in vivo* genome internalization rate and can be accelerated in presence of OmpA.[2] In order to further analyze the molecular trigger for *in vitro* particle opening we have analyzed *Viunalikevirus* Det7. The phage has a contractile tail and its genome encodes for four types of TSP and one tail fiber,[3] and we show that this enables Det7 to infect a broad host range. Two different TSP from Det7 have been isolated and structurally characterized so far. Det7TSP, a close homolog to P22TSP is used to recognize the same *Salmonella* Typhimurium host strain as podovirus P22.[4] In this work we describe a second TSP from Det7, DettilonTSP. It recognizes *Salmonella* Anatum LPS and is a homolog to a TSP from podovirus epsilon15. We have measured time-resolved *in vitro* DNA release from Det7 in the presence of different LPS serotypes and have analyzed the role of Det7TSP and DettilonTSP. We found the size of LPS-aggregates in solution to be crucial for successful DNA-ejection *in vitro*, supporting the hypothesis that a membrane contact is sufficient to trigger particle opening.

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How does T4-like environmental phage ΦCBH8 activate ToxIN$_{Pa}$-mediated "altruistic suicide" in bacterial host?

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Abortive infection (Abi) is an anti-phage mechanism in which a virally-infected bacterium initiates its own premature death and reduces the production of viral progeny. This protects clonal siblings in the bacterial population and is considered a form of "altruistic suicide". ToxIN$_{Pa}$, a Type III toxin-antitoxin, is found to initiate Abi. ToxIN$_{Pa}$ consists of endoribonuclease toxin and RNA antitoxin. The latter inhibits the toxin by forming a heterohexameric complex with the toxin until specific phage infection "activates" the toxin.

To investigate how phages "activate" ToxIN$_{Pa}$, we introduced ToxIN$_{Pa}$ into *Serratia* sp. ATCC 39006. Using *Serratia* as host, we isolated a T4-like phage, ΦCBH8, from a treated sewage outflow point into the river Cam in Cambridge. ΦCBH8 is susceptible to ToxIN$_{Pa}$-mediated abortive infection and produced spontaneous mutants that escape ToxIN$_{Pa}$ by multiple routes. Whole-genome sequencing of wild type ΦCBH8 and escape mutants has shown that escapes can arise by mutating the *asiA* gene, or by deleting a large region of its DNA with sizes ranging from 6.5kb to 10kb. Predicted open reading frames within these large regions have no known function, but analysis of their individual expression in *Serratia* has shown interesting phenotypes. More in-depth investigation of these escape mutants may help uncover the nature of the phage product(s) responsible for the activation of ToxIN$_{Pa}$.

To which extent an ancestral infection mechanism is conserved during the evolution of two bacteriophage genera?

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While the evolution of tailed bacteriophages has increasingly been better understood through comparisons of their DNA sequences, the functional consequences of this evolution on phage infectious strategies have remained unresolved. In this study, we comprehensively compared the transcriptional strategies of two related myoviruses, PAK_P3 and PAK_P4, infecting the same *Pseudomonas aeruginosa* host strain. Outside of the conservation of their structural clusters, their highly syntenic genomes display only limited DNA similarity. Despite this apparent divergence, we found that both viruses follow a similar infection scheme, relying on a temporal regulation of their gene expression, likely involving the use of antisense transcripts, as well as a rapid degradation of 90% of the host non ribosomal mRNA, as previously reported for PAK_P3. However, the kinetics of the mRNA degradation is remarkably faster during PAK_P4 infection. Moreover, we found that each virus has evolved specific adaptations, as exemplified by the distinct patterns of their core genes expression as well as the specific manipulation of the expression of iron-related host genes by PAK_P4. This study enhances our understanding of the evolutionary process of virulent phages, which relies on adjusting globally conserved ancestral infection mechanisms.

Host-phage interactions between novel jumbo phage PA5oct and *Pseudomonas aeruginosa*

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The PA5oct, a novel jumbo myovirus, has a giant linear A+T-rich double-stranded DNA genome (287,182 bp), making it the third largest sequenced *Pseudomonas* phage genome. Based on DNA & RNA sequencing, 462 genes could be confirmed, of which only a small number could be functionally assigned, further supported by structural mass spec analysis. The genetic relationships between PA5oct and other jumbo bacteriophages and other *Caudovirales* viruses, a protein-sharing network was constructed.

The PA5oct, *Escherichia* phages 121Q/PBECO4, *Klebsiella* phage vB_KleM-RaK2, *Klebsiella* phage K64-1, and *Cronobacter* phage vB_CsaM_GAP32 presented closer relationships based on shared conserved core genes. Furthermore, PA5oct, PBECO4, GAP32, T5, FelixO1 appears to be distantly diverged members of *Tevenvirinae*.

To examine phage transcription across the temporal phases of infection cycle at single-nucleotide resolution as well as the phage- host interactions, RNA-seq was performed based on three selected infection time points (5, 15 and 25 min) . Reads originating from the phage and the host at each stage of infection were mapped to the phage and host genomes respectively, revealing that PA5oct progressively dominates host transcription. Indeed, PA5oct transcripts represent 21% of total non-rRNA transcripts within 5 minutes and eventually proceed to 69% then 92% by middle and late infection, respectively. The PAO1 host-mediated transcriptional response to PA5oct infection is similar to 14-1, PEV2, YuA, and LUZ19 infection response. Interestingly, PA5oct progressively dominates the mRNA and sRNA environment of the cell with strong transcription, as well as the expression of an RNase-H-like protein putatively involved in degrading host transcripts. This shift from host transcripts to phage transcripts de-enriches all host transcripts relative to the total in the cell.

Comparative metabolomics of different clades of Pseudomonas phages

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Since bacteriophages do not possess their own metabolism, they have to exploit their host cells' metabolism to fuel viral replication. As nature's own engineers, phages have evolved to modify, adapt and control this metabolism through billions of years of co-evolution[1]. Indeed, many viral genomes encode acquired host-derived genes with metabolic functions, which are hypothesized to overcome specific metabolic bottlenecks, so-called 'auxiliary metabolic genes' (AMGs)[2]. If the phage-encoded set of AMGs impacts the metabolic response to phage infection remained poorly studied. To investigate this, we studied the metabolism of the ubiquitous, gram-negative soil bacterium, *Pseudomonas aeruginosa*, under attack of six different lytic dsDNA phages using a "state of the art" metabolomics technique[3].

A total of 6006 ions were measured, of which 375 unique ions were retained after filtering, corresponding to 810 *P. aeruginosa* metabolites. In comparison to the metabolic steady state of the control, we identified phage-dependent metabolic alterations upon infection, ranging from depletion to an active modulation of the host metabolism[3]. Furthermore, the predicted AMGs alone fail to explain the metabolic diversity, confirming an important role for unpredicted, "non-enzymatic" AMGs (termed "metabolic modulators") in host metabolism take-over.

To identify such metabolic modulators, we fused five central carbon metabolism enzymes to a C-terminal strepII-tag using an in-house strategy[4]. Next, *in vivo* pull downs were performed during phage infection with four distinct phages (LUZ19, PEV2, phiKZ and YuA). Preliminary results led to the co-purification of six phage proteins of unknown function. Future work will focus on the characterization of these potential metabolic modulators.

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CRISPR as a tool to study phage-bacteria interactions in the gut

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The human gut microbiota has been the subject of extensive research, particularly in the past decade. Such interest is due to the accumulating evidence that it is playing a crucial role in human health. A large and diverse population of bacteriophages, viruses infecting bacteria, also colonizes the gastrointestinal tract. However, very little is known about them and how they impact the bacterial composition, via their specific interactions. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) loci, together with their *cas* genes, are found in nearly half of the bacterial genomes that have been sequenced so far. CRISPR-Cas systems have been shown to provide acquired immunity against foreign DNA elements, such as bacteriophages. The mechanism behind such adaptive defence machinery is through the incorporation of short DNA sequences, called spacers, in the CRISPR locus. These spacers are derived from foreign elements such as phage genomes. The addition of new spacers is mostly polarized at the same end of the CRISPR array, just like adding a new page to a book. It has been suggested that this molecular bookkeeping may serve as a chronological archive or memory of past phage-bacteria interactions.

To investigate the interactions between bacteriophages and bacteria in the human gut environment, we analysed the CRISPR loci and the spacers diversity among bacterial isolates isolated from faecal samples. Those isolates originated from the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) 2010 cohort. We began by characterizing the CRISPR loci of several *Escherichia coli* isolates. Primers were redesigned following the alignment of conserved sequences and so far, PCR amplifications were performed on 1769 *E. coli* isolates. We detected high spacers diversity within isolates and an average of 10 spacers were found per isolate. Furthermore, only 3.3% of the spacers analysed so far (157 out of 4720 distinct spacers) are homologous to a known phage sequence available in public databases. These included the temperate phages RCS47, SJ46, P7 and P1. It is tempting to speculate that a vast number of unknown coliphages remain to be discovered in the human gut. The analyses of CRISPR loci of other bacterial species are also underway.

Dynamic biofilm architecture confers individual and collective mechanisms of phage protection

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Phages are the most abundant replicating entities on Earth, and biofilm formation is the most common mode of bacterial life. Biofilm-phage interactions are thus fundamental to microbial life and pathogenesis. However, the mechanisms and spatiotemporal dynamics of interactions between phages and biofilm-dwelling bacteria are largely unknown. By using *Escherichia coli* biofilms exposed to T7 phages, we discovered that polymers of a specific secreted protein (CsgA) protect individual cells and groups of cells against phage attack *via* two separate, novel mechanisms. First, collective protection results from phage transport inhibition and can be fully recapitulated in synthetic biofilms composed of *in vitro* assembled CsgA amyloid fibers and micro-beads that mimic inert bacterial cells. Second, CsgA fibers protect cells individually by coating them and preventing phage attachment. These insights into biofilm-phage interactions have broad-ranging implications for the design of antibacterial therapy based on phages, and the evolutionary dynamics of phages with their bacterial hosts.

Characterization of *Pseudomonas aeruginosa* phage-resistant clones obtained by controlled phage infection

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Phage therapy is a promising strategy to combat bacterial infections, but it has also some drawbacks. Besides difficulties in the standardization of phage preparations and regulatory issues related to the use of phage products in humans, a major biological problem is the onset of phage resistance upon phage treatment. The emergence of the phage-resistant mutants has been recognized in phage therapy application. One of the simplest, effective, and widely accepted practice of counteracting the effects of phage resistance is the use of phage cocktails containing several phages with a similar host spectrum, but different attachment mechanism.

Most microbial surface structures recognized by phages are also important bacterial virulence factors that strongly stimulate the immune system of infected humans. Therefore, changes in the expression level or the structure modification of these receptors reduce bacterial virulence. In this study, we examined the diversity of *Pseudomonas aeruginosa* clones obtained as a result of controlled infection by single bacteriophages and cocktails composed of two or three bacteriophages. Three well-characterized bacteriophages of the *Caudovirales* order were selected for these experiments: phiKZ (fimbriae-dependent, giant *Myoviridae*) LUZ7 (fimbriae-dependent, *Podoviridae*) and KTN6 (LPS-dependent, *Myoviridae*). Isolated bacterial mutants were tested for twitching, swarming and swimming motility, the intensity of pyoverdine, pyocyanin and biofilm production, the LPS patterns and the sensitivity to antibiotics, phages, complement system and phagocytosis.

Our results indicate that the selection pressure caused by phage infection leads to modification or even loss of important virulence factors (i.e. type IV fimbriae or LPS O-antigen). It was confirmed that the virulence of isolated phage-resistant clones was reduced. In most cases, observed changes remain stable, what suggests their mutational character. Nevertheless, bacterial resistance based on the loss or modification of the particular receptor did not always result in cross-resistance to other phages recognizing the same receptor.

Host metabolic reprogramming of *P. aeruginosa* by Phage-based Quorum Sensing modulation

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One of the main challenges in phage biology is the functional elucidation of the *in silico* annotated open reading frames that lack any similarity to known genes, the so-called viral 'dark matter'. A large portion of this consists of phage genes expressed early in infection, that are hypothesized to play a major role in the host metabolism take-over to ensure efficient production of new phage particles. Indeed, the vast majority of known phage proteins that inhibit, activate or functionally redirect host proteins are early proteins. To understand the biological function of these phage proteins, identification of the bacterial target protein can give a first clue[1]. By focusing on growth inhibitory phage proteins, functional genomics research will yield new insights into phage biology and provide a basis for innovative phage-derived antibacterials[2].

In this research, we identified a hypothetical *P. aeruginosa* protein, predicted to be involved in leucine biosynthesis and regulated by quorum sensing[3], that partially alleviated the toxic effect of an early expressed protein of the *P. aeruginosa*-infecting podovirus LUZ19 (a phiKMV isolate). Multiple protein-protein interaction assays confirmed that both proteins interact directly and located the interaction site at the catalytic domain of the host protein. Furthermore, both proteins showed acetyl transferase activity potentially modulating the supply of essential components or energy for phage replication[4]. Metabolomics indeed revealed alterations in various pathways, including the PQS quorum sensing system, in response to expression of this toxic phage protein.

To our knowledge, this is the first report of a phage protein modulating the PQS levels in the host, resulting in toxicity and metabolic reprogramming. Recently the PQS system was found to regulate CRISPR mediated immunity[5], thus we hypothesize that this phage protein may be part of a novel phage mechanism to evade CRISPR resistance, which will be further investigated.

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Phage bacteria evolutionary dynamics and how it affects phage therapy

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The rise of bacterial variants in the presence of lytic phage has been one of the basic grounds for evolution studies. However there are incongruent results among different studies investigating the effect of acquiring phage resistance on bacterial fitness and virulence. We employed an *in vitro* burn wound model to study the development of phage resistance and associated variations in virulence and biofilm formation for the resulting phage-resistant PA phenotypes. Experimental evolution was used to generate *Pseudomonas aeruginosa* variants under selective pressure from different homogeneous and one heterogeneous phage environment. The phages were chosen to target different receptors to decrease the chance of cross-resistance. The variants were categorized into seven colony morphotypes. Studying phenotypic traits of the variants revealed significant changes in various fitness and virulence determinants such as growth, motilities, biofilm formation, resistance to oxidative stress and production of siderophores and chromophores compared to the control. We further showed that mRNA transcription for genes associated with certain phenotypic traits had checked the genome for SNP's. The phenotype of the three classes of variants studied also showed significant differences from each other, suggesting the cost of resistance for this system to be context dependent. Variants resistant to a bacteriophage mixture were observed to suffer a greater change in phenotypic traits. Furthermore, the appearance of melanogenic variants and the increase in pyocyanin and pyoverdin production for some variants is believed to affect the virulence of the population. The choice of therapeutic phage (or phage cocktail) allowed for the control of evolution of resistant phenotypes (*i.e.,* selective pressure). Choosing a mixture of phages that directly or indirectly use known PA virulence factors as receptors led to direct killing, or selected for inactivation of the receptors, causing loss of those virulence determinants and impeding the mutants' ability to invade cultured skin cell lines (keratinocytes).

This work is currently being followed up by invivo experiments on various models and also using microfabricated microenvironments for a more mechanistic investigation. The knowledge gained from this study will fundamentally contribute to our understanding of the evolutionary dynamics of bacteria under phage selective pressure, which is crucial for the efficient utilization of bacteriophages for phage therapy.

A widespread family of polymorphic toxins carried by temperate phages

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Phages strongly impact bacterial population dynamics. Polymorphic toxins secreted by bacteria to kill or modulate the growth of bacterial competitors are another critical parameter in the dynamics of bacterial communities.

We have shown that one super-family of poorly studied phage-specific proteins containing a domain named MuF is present in a very large fraction of lysogenic and lytic phages. Indeed, by analyzing the proteomes of 1 845 bacteriophages and 2 464 bacterial genomes, we found that 35% of the sequenced tailed phages and 30% of the prophages harbored a *muf* gene.

We found that MuF proteins exhibit two main architectures: i) short proteins with solely a MuF domain and ii) proteins where MuF domain is associated with a C-terminal toxin domain. Most of these toxin domains are predicted to have ribonuclease or metallopeptidase activities. Interestingly, MuF toxins were exclusively encoded within temperate phages (and their prophages) mostly infecting Firmicutes.

Hence, this polymorphic toxin super-family is likely to play an important role in ecosystems such as the human microbiota. We propose that MuF toxins could be delivered by phages into host bacteria and either influence the lysogeny decision or serve as bacterial weapons by inhibiting the growth of targeted bacteria.

Clinical and livestock-associated *S. aureus* strains have distinct phage profiles

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Infections caused by antibiotic-resistant bacteria are major problems in medicine. WHO regards antibiotic resistance of *Staphylococcus aureus* as one of the most important concerns for public health. Phage therapy has recently been revisited as one of the potent alternative treatments for *S. aureus* infections.

Approximately 30% of the human population is colonized with *S. aureus* and from the epidemiological point of view, these bacteria represent the community-acquired (CA) *S. aureus*. Traditionally, the hospital-acquired (HA) *S. aureus* strains have been considered as the most important health risk, but recently, livestock-associated (LA) *S. aureus* strains have become more common, especially among farmers and veterinarians.

We have recently isolated three *S. aureus* –specific phages; one (fRu-Sau02) from commercial Microgen phage therapy product, and two (fPf-Sau02 and fPf-Sau04) from pig stool samples collected from two Finnish pig farms. We assessed the host ranges of these phages by analysing their infection profile in four different types of *Staphylococcus* strains: 35 clinical methicillin-sensitive *S. aureus* (MSSA), 15 clinical methicillin-resistant *S. aureus (*MRSA), 73 MRSA strains isolated from pigs (LA-MRSA), and 30 clinical coagulase-negative *Staphylococcus* (CoNS), representing altogether 6 different species.

Clinical and LA *S. aureus* strains were shown to have clearly distinct phage profiles: Phage fRu-Sau02 infected 96% of tested clinical strains (both MSSA and MRSA) but only 47% of LA-MRSA strains. Only two out of 30 tested CoNS strains were sensitive to fRu-Sau02. The phages isolated from pig stools did not infect any of the clinical *S. aureus* or coagulase-negative *Staphylococcus* strains studied. According to the preliminary tests with 73 LA-MRSA strains, the sensitivities were 99% and 89% for fPf-Sau02 and fPf-Sau04, respectively. The study clearly illustrates the need for isolating *S. aureus* phages also from different livestock environments, as therapeutic phages used to treat infections caused by CA- or HA-strains may not be effective against LA-MRSA.

Optimized phage-robust cultures

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Lactic acid bacteria are widely used as starter cultures for the manufacture of cheese and other fermented milk products.

However, during the fermentation process the strains can become susceptible to bacteriophage attack, resulting in delay or even failure of the fermentation process and thereby causing significant economic loss.

As a starter culture supplier, DSM takes the phage robustness aspect of its cultures very seriously and takes care to supply its customers with cultures of the highest phage robustness standard. The basis for the development of these phage robust cultures consist of high throughput characterization of phage-host interactions and rational design of blends based on these data.

In addition, the market performance of our cultures is monitored by extensive whey testing and phage analysis. By using state-of-the-art software analysis this data is visualized to spot trends in phage developments, performance of specific culture product ranges and benchmarking of specific dairy factories against the overall industry. This allows us to be ahead of the game and being able to take corrective action early on.

In this poster, several examples of these methods will be shared.

Translocation of viral DNA across bacterial membranes

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The first challenge for a replicating bacteriophage is to transport its DNA into a host cell. While T4 phage uses the contraction of its tail to penetrate the bacterial cell wall, T7 with its stubby tail injects 3 different internal head proteins (gp14, 15, 16) into the periplasm that then assemble to a DNA-translocating device. For their translocation into the periplasm the 3 proteins have to pass through the phage head portal and to do so, they have to at least partially unfold. The subsequent assembly in the periplasm allows the transport of the phage DNA across the host outer membrane, the periplasm and the inner membrane.

We have purified and studied the 3 proteins and found that they can refold after thermal unfolding. Gp14 forms small cylindrical rings, gp15 binds to DNA and gp16 to liposomes. Gp15 and gp16 form a 2:1 complex that appears as spiral rings of various sizes. All these structures of the proteins show drastic differences from their appearance inside the phage head. We are now attempting to assemble a gp14-15-16 complex in vitro, also in the presence of liposomes.

Another fascinating DNA-translocating system is the extrusion of M13 progeny phage. M13 assembles in the membrane and the single-stranded DNA is coated by proteins while it is extruded through the inner membrane of E. coli. It is assumed that two proteins gp1 and gp11 are involved in this process. We have isolated a deca histidine-tagged version of gp1 which co-eluted gp11 and OmpF. Now we are attempting to resolve its structural details. The sequence of gp1 shows two motifs of putative “Walker boxes” that could play a role for M13 assembly. We tested various mutations in the two motifs and can conclude from the results that both Walker boxes in gp1 are essential for a functional phage reproduction.

Viral factories organization in time and space during phage infection

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During the co-evolution of viruses and cells, viruses exploited numerous ways to hijack cell machineries for their proficient multiplication and dissemination. SPP1 is a well-characterized model tailed bacteriophage that infects the Gram-positive bacterium *Bacillus subtilis*. More than 300 copies of the viral genome are synthesized in the first 25 minutes after initiation of SPP1 infection. Phage DNA was shown to be localized in defined viral DNA replication *foci* in the cytoplasm. This process requires the fast recruitment of most of the bacterial replisome proteins orchestrated by the SPP1 helicase gp40 that is known to bind to the DnaG primase and to DnaX, a subunit of DNA polymerase III (Wang et al., 2008; Martínez-Jiménez et al., 2002). Their accumulation in phage genome replication factories few minutes after infection suggests that the host replisome machinery is massively redirected and dedicated for optimal SPP1 DNA replication.

Moreover, for the first time in prokaryotic cells, our studies showed that assembled viral particles are stored in defined *foci* at positions distinct from the genome replication factory. These virion warehouses are found mostly side by side from the viral DNA replication factory. This spatial and temporal distribution in the bacterial cytoplasm highlights a sequential program of molecular interactions leading to an extensive re-organization of the cell to achieve formation of about 150 infective particles within 25 minutes of infection. Structuration of viral factories appears as a very efficient strategy for SPP1 to exploit the bacterial resources for its own profit.

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Enterobacterial O-antigens as a non-specific shield limiting phage infection

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The susceptibility of the bacteria to bacteriophages is largely determined by the availability of appropriate receptors on the host cell surface. Many enterobacterial phages exploit conserved outer membrane proteins as their receptors. This results in such phages having wide host ranges with respect to a diverse panel of *E. coli* strains. It is generally understood that the O-antigen can to some extent hinder the interactions of phages with protein receptors thus even if the cell has appropriate protein receptors they are partially blocked by the O-antigen. However, in contrast many other viruses, particularly the podoviruses, are strictly dependent on particular O-polysaccharides (OPS) as the receptors. To address this conundrum as to how the O-antigen both hinders and helps phage binding, we have investigated a range of *E. coli* phages. We demonstrate that in wild *E. coli* isolates O-antigens are highly effective non-specific shield that protects the cell from phage infection completely unless the virus acquires specific proteins for recognition and in some cases enzymatic degradation of the OPS. Moreover we found that the alterations of the O-unit including the lateral modification and the alterations in OPS chain length distribution can significantly influence the efficacy of the non-specific cell protection. We have used comparative genomics and experimental approaches to demonstrate that the adaptation for use of the hosts that produce different O-antigens and we demonstrate that this is one of the driving forces of microevolutionary adaptation of the coliphage adsorption machinery.

Our findings are particularly significant in the context of phage therapy as which requires broad host range phages that target *E. coli*. It would clearly be beneficial to apply a rational strategy to a collection of phages by including collections of viruses that can effectively infect different O-serotypes and use them as the sources of the receptor binding proteins genes for artificial host range management. We also speculate that the inhibitors of the key enzymes of the OPS synthesis pathways may be used to enlarge the *in vivo* host ranges of the phages used for therapy.

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Which are the host factors influencing the replication of virulent bacteriophages in the gastrointestinal tract?

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Virulent bacteriophages have been frequently proposed nowadays as an alternative to target bacterial pathogens resistant to multiple antibiotics. Often, some of these pathogens are asymptomatically carried in the gastrointestinal tract (GIT) of mammals. However, despite several attempts using different animal models, the use of virulent bacteriophages to efficiently reduce carriage levels have often been disappointing achieving at best a moderate reduction. We believe the lack of knowledge on the factors that influence the replication of bacteriophage in the GIT is impeding the development of strategies to improve overall bacteriophage efficacy.

To address this question we characterized nine bacteriophages isolated using three different strains of *Escherichia coli*: the enteroggregative strain 55989, the adherent invasive strain LF82 and the uropathogenic strain AL505. Each of these *E. coli* strains was used to colonize the GIT of mice. Using homogenized GIT samples from these mice, we assessed the *ex-vivo* replication of these bacteriophages in different sections of the GIT. We found that some bacteriophages replicate efficiently regardless of the GIT sections while others display a differential efficacy.

Therefore, these data support our hypothesis that the local GIT environment is influencing the infectivity of bacteriophages, raising questions regarding the identification of the actors involved and how, in the future, we could design ways to improve overall efficacy of phage therapy treatments.

Natural diversity of CRIPSR spacers of *Thermus* bacteria, its variation between distant hot springs, and its role in a record of history of phage-bacteria interactions

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Prokaryotes have developed a broad range of mechanisms against invasive genetic elements such as viruses and plasmids. One of defence mechanisms -- CRISPR-Cas system -- provides adaptive immunity in bacteria and archaea. Short sequences (spacers) acquired from the genomes of viruses or plasmids are incorporated into the repetitive locus (CRISPR array). The CRISPR array is transcribed and processed into CRISPR RNAs (crRNAs). The complex of crRNA and Cas proteins perform RNA-interference based degradation of invader DNA (or RNA). The CRISPR array contains historical knowledge of past encounters. The metagenomic analysis of CRISPR spacers can reveal the difference in viral populations and virus-host interactions on sites.

We investigate the diversity of CRISPR spacers of uncultured communities of *Thermus* strains from hot springs in Italy (m. Etna and m. Vesuvius) and Chile (Termas del flaco and el Tatio). Thousands of spacers were revealed in each site and compared to each other and to *Thermus* CRISPR database. Most (58% to 72%) spacers in samples were unique, with shared spacers in samples from distant location not exceeding several percent of the total. The intersection with known Thermus spacer set from database was 7%.

This observation suggests rapid local evolution of CRISPR arrays in *Thermus* communities. 8% of spacers displayed significant similarities to *Thermus* phages, plasmids and chromosomal DNAs of different *Thermus* strains. Four new *Thermus* phages were isolated from m. Vesuvius and el Tatio sites. The percentage of spacers with matches to *Thermus* phages isolated from the same location was significantly higher than to phages from distant locations. The result clearly indicates that *Thermus* bacteria tend to acquire spacers locally. Interestingly, we found that different CRISPR-Cas systems appear to be specialized and “prefer” to attack different types of phages.

The Bacteriphage Like Infection Cycle of the Large Paramecium Bursaria Chlorella Virus-1

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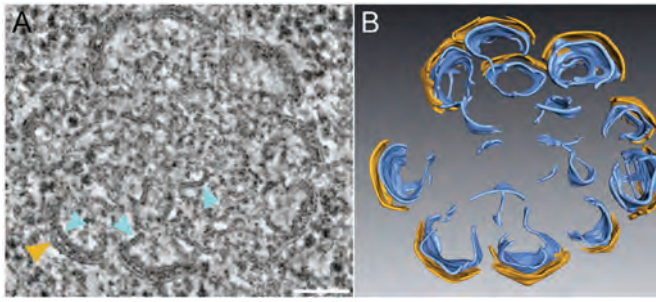
Paramecium Bursaria Chlorella Virus-1 (PBCV-1) is the prototype member of the *Phycodnaviridae* family and shares common features with other members of the Nucleo Cytoplasmic Large DNA Viruses (NCLDV) clade. The virus harbors ~331 Kbp of dsDNA genome enclosed by one internal membrane. With the exception of Mimivirus and Vaccinia Virus, all DNA viruses replicate their genomes in the host nucleus. By combining advanced high-resolution fluorescence and electron microscopy techniques we have shown that, in contrast to other members of the NCLDV, PBCV-1 infection is initiated with a unique mechanism reminiscent that of bacteriophages. Using Scanning Transmission Electron Microscopy (STEM) tomography we showed that soon after adsorption to the cell wall, PBCV-1 generates a hole in the cell wall through which a narrow membranous tunnel is formed by the fusion of the plasma and viral membranes, and is used for viral genome delivery into the cytoplasm.

Our studies revealed that soon after being ejected into the host cytoplasm, viral genomes are transported to the nucleus. The infection proceeds with the generation of elaborate "viral organelles", termed viral factories that are generated in the cytoplasm of the host. The viral factories generated by PBCV-1 are profoundly different from those produced by other group members of the NCLDVs, Vaccinia, ASFV and Mimivirus. We used several techniques that provide 3D information with nanometer resolution, namely, STEM tomography and Focused Ion Beam Scanning Electron Microscopy (FIB-SEM), in order to investigate the 3D organization and function of the factories produced by PBCV-1. These assays were corroborated by florescence microscopy studies. Our studies revealed the distinct rosette-like architecture of the factories, with viral genomes at the outer periphery, host cisternae at the inner periphery, and single bilayer membranes used as capsid templates in the central region. Similar to other group members of the NCLDVs we demonstrated that cisternae derived mainly from ER are the origin of the viral internal membranes. Overall, our results point towards the notion that the eukaryote-infecting PBCV-1 infection initiates and proceeds through a bacteriophage-like infection.



A. A 10 nm-thick tomographic slice from a STEM tomogram showing a rosette-like cytoplasmic factory of PBCV-1 in a 2h post infection *Chlorella* cell. Light blue arrowheads point to open membrane sheets that accumulate in the center of PBCV-1 factories, and yellow arrowheads indicate capsids generated at the outer sites of the membrane bilayers. **B.** Surface rendering of the tomogram showing top view of a PBCV-1 factory. Open membrane sheets and capsids are depicted in light blue and yellow, respectively. Scale bar: 100 nm.

Ribosomal Proteins in Viruses

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Interactions between viruses and cells have played a major role in the evolution of life. In particular, viruses have a high impact on the composition of microbial communities due to their abundance and profound significance as agents of microbial mortality. Numerous studies have previous shown that viruses and their hosts are engaged in bilateral horizontal gene transfer which have significant consequences for the mode of virus-host interaction. Viruses infecting marine cyanobacteria present one of the most illustrious examples. These viruses are known to carry their own genes encoding some of the key components of photosynthesis. It has been also shown that these host-derived genes are translated and the produced proteins boost the energy metabolism in the infected cell, thereby increasing viral fitness. More recently, metagenomic studies have also contributed to expanding the repertoire of such metabolic host-derived genes found in viruses. Among the most unexpected discoveries was the presence of a high number of ribosomal proteins-encoding genes within bona fide viromes. Ribosomal proteins interact with the rRNA to form the ribosome, the molecular machinery for protein biosynthesis, and are the hallmark of cellular life forms. Here we explored the presence of ribosomal proteins within publicly available viral genomes. Fifteen viral genomes (14 bacteriophages and one eukaryotic virus) encoding ribosomal protein homologues were identified. This was surprising because even giant viruses of protists, such as mimiviruses and pandoraviruses, lack such genes in their genomes. Five different ribosomal proteins were found to be encoded by viruses, although the functional importance of these acquisitions is still speculative. Our results break the long-standing dogma that viruses do not encode ribosomal proteins and show that genes from virtually all functional categories are subject to horizontal transfer.

Molecular Characterization of The Activity and Requirements of A Novel and Promiscuous Bacteriophage Integrase

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Stx bacteriophages are responsible for the dissemination to and production of Shiga toxin genes (stx) in the Enterohaemorrhagic *E. coli* (EHEC). These toxigenic bacteriophage hosts can cause severe, life-threatening illness, and Shiga toxin (Stx) is responsible for the severe nature of EHEC infection. At the point of infection, the injected phage DNA can direct its integration into the bacterial chromosome becoming a prophage; the host cell is then known as a lysogen. Unusually, our model Stx phage, Φ24B, can integrate into at least four distinct sites within the *E. coli* genome that shared no easily identifiable recognition sequence pattern. The identification of what are actually required for phage and bacterial DNAs recombination has been tested using both an *in vitro* and *in situ* recombination assays. These assays enable easy manipulation of bacterial attachment site (attB) and phage attachment site (attP) sequences. The aim of our study is to fully characterize the requirements of this promiscuous integrase, carried by the Stx phage Φ24B (IntΦ24B), to drive integration. So far, a number of successful assays have enabled us to identify the minimal necessary flanking sequences for all of four *attB* sites identified (21 bp and 49 bp from the right and lift the cross over region) and *attP* site (200 bp each side). Furthermore, we identified that Φ24B integrase does not need Integration Host Factor (IHF) to drive integration. Finally, as this integrase can integrate the phage genome inside more than four different bacterial attachment sites *attBs*, four of these *attBs* were identified, sequenced and cloned in different compatible plasmids to be transformed to one cell, and the frecuency of each recombenation was tested by means of qPCR.

Contrasting evolutionary fortunes for prophages in an epidemic *Salmonella* lineage

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Genomic analyses have established that pathogenic lineages of *Salmonella* carry characteristic prophage repertoires. However, much of the biology and ecology of temperate phages in epidemic *Salmonella* strains has remained enigmatic.

We have used a combination of genetic and experimental approaches to characterise the prophages associated with African epidemic *S.* Typhimurium ST313 lineages.

S. Typhimurium ST313 is closely related to gastroenteritis associated *Salmonella,* but is associated with bloodstream infection in sub-Saharan Africa. The representative ST313 strain, D23580, contains 5 full-length prophages: BTP1, Gifsy-2, ST64B, Gifsy-1 and BTP5. A genetic strategy was used to remove each prophage from the chromosome of D23580, allowing the functional viability and phenotypic contribution of each prophage to be determined.

Novel P22-like BTP1 and P2-like BTP5 prophages were convergently acquired during the evolution of ST313 lineages 1 and 2, and both prophages are functional. Stationary-phase cultures of strain D23580 produced a high titer of BTP1 virus particles (~10^9 *per* ml), the greatest spontaneously-induced phage titer yet reported for a bacterial prophage and indicating the phage-mediated lysis of around 0.2% of the lysogenic population.

Contrastingly, evidence is presented that the archetypical *S.* Typhimurium prophages Gifsy-2, Gifsy-1 and ST64B, were functionally inactivated by mutations during the evolution of ST313. The single nucleotide mutation responsible for Gifsy-1 phage inactivation is identified, and it is shown that this nucleotide is conserved in all ST313 isolates.

The data are consistent with an important biological role for acquisition, conservation and degradation of resident prophages during the evolution of African *S.* Typhimurium ST313.

Interspecies plasmid transduction mediated by a *Staphylococcus sciuri* phage

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Coagulase-negative and novobiocin-resistant *Staphylococcus sciuri* is a bacterial pathogen associated with animal diseases including dog dermatitis, exudative epidermitis in pigs, and with a number of nosocomial diseases of humans, namely endocarditis, pelvic inflammation, and wound infections. It was speculated that the *S. sciuri* species group is a potential reservoir of virulence and antimicrobial resistance genes.

Homologues of the *mecA* gene coding for PBP2a-like protein responsible for methicillin-resistance in staphylococci, carried on the staphylococcal cassette chromosome mec (SCC*mec*), are often found in bacteria from the *S. sciuri* group. The acquisition of the SCC*mec* element by methicillin-susceptible *S. aureus* was crucial for the bacterium to become a successful pathogen. It is hypothesized that *S. aureus* acquired this element directly from coagulase-negative staphylococci by transduction. Transduction by temperate bacteriophages is considered to be a major path for the horizontal gene transfer of virulence and resistance determinants in staphylococci.

Here we presented two intraspecies and one interspecies transduction of a 5,667-bp-long tetracycline and aminoglycoside pSSC723 resistance plasmid by novel *S. sciuri* siphophage phi879. Plasmid pSSC723 was transduced from a donor *S. sciuri* strain to two *S. sciuri* strains and *S. aureus* strain RN4220 with a frequency about 10^{-11}. Real-time PCR proved the ability of phage phi879 to package plasmid pSSC723 and *mecA* gene from its bacterial host into virions. The packaging frequency of pSSC723 was 5.7×10^{-3} and that of *mecA* gene as part of SCC*mec* was 3×10^{-5} as determined by qPCR analysis. The ability of phage to package and transmit mobile elements and also to adsorb onto the cells of other staphylococcal species indicates a possible interspecies horizontal transfer of mobile genetic elements by *S. sciuri* siphophages.

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The morphology, genome and receptor of the lytic leptophages LE3 and LE4

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Leptospirosis is a zoonotic disease caused by infection with gram-negative bacteria of the genus *Leptospira*. This genus includes 22 species, including saprophytes and pathogens, that have the ability to persist in freshwater. We have previously isolated three bacteriophages of *Leptospira* (leptophages) called LE1, LE3 and LE4. All of them are specific to the saprophyte *Leptospira biflexa* and they belong to the *Myoviridae* family. The genome of the lysogenic phage LE1 was previously sequenced and its replication origin was used to design a replicative vector. The lytic phages LE3 and LE4 are characterized in this study to further evaluate the diversity of leptophages.

LE3 and LE4 have a similar morphology and their genomes are very closely related. The LE4 genome contains 48 kb and 81 open reading frames (ORFs). Putative functions could be assigned to only 6 gene products (7% of gene content) based on sequence homology and amino acid motif searches. Interestingly, similar prophage-like regions were found in the genomes of *Leptospira* pathogenic strains.

Additionally, we selected phage-resistant *L. biflexa* mutants to identify the phage receptor. Whole genome sequencing of a resistant strain, called RLE4, allowed the identification of a single nucleotide polymorphism in a gene of the O-antigen locus that introduced a premature stop codon.

In conclusion, our analyses suggest that LE3 and LE4 are unrelated to other known bacteriophages. Further studies will be required to identify the function of the vast majority of these “novel” genes. The genomes of LE3 and LE4 will be further used for the detection of similar prophages in leptospiral genomes and their roles in the evolution of pathogens.

Inferring rates of phage acquisition from host phylogenies

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In the past decade, the number of bacteriophage genome sequences available through public repositories has increased dramatically, and even greater increases are expected with the advent of more public metagenomes as well as more accurate means of recovering prophage genomes from within bacterial hosts. While a great deal of effort has been placed on how to classify these novel phages within the wider context of viral diversity and taxonomy, new methods are needed for exploring the ecology and evolution of these viruses. An open question with novel prophage genomes is often how active the phage is in the environment and how recently it was acquired by its host. Here, we borrow tools for the phylogenetic estimation of rates of trait evolution to make predictions about the relative rates of acquisition of diverse prophages found in bacteria. We apply this technique to phages from *Gardnerella vaginalis*, *Pseudomonas aeruginosa*, and *Lactococcus lactis* and demonstrate a wide range of rates of acquisition, with most prophages appearing to spread randomly and rapidly through a bacterial taxon. We also highlight a minority of phages that appear to have been transmitted largely through vertical transmission. This work provides a new lens for studying the ecological and evolutionary histories of prophages.

Transcriptional regulation of membrane transporters confers phage-resistant phenotype in *Vibrio alginolyticus*

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Phage therapy interest has been revived during the last decade in an attempt to tackle antibiotic resistant bacteria, but its application is hampered by the development of phage-resistant bacterial strains. Although bacterial intracellular molecular mechanisms of resistance development against phage infections have been well characterized over the years, the knowledge about defensive mechanisms that include alterations in membrane receptors remains inadequate. Bacteria can develop resistance against phages not only through costly constitutive mutations, but also by altering, shutting down or diminishing the expression of specific receptors. Transcriptional changes of membrane and transmembrane transporters of the Gram negative fish pathogen *Vibrio alginolyticus* were monitored on phage-resistant strains against lytic *Vibrio* phages belonging to "schizoT4like" and "phiKZlike" clades of *Myoviridae* family and a lytic bacteriophage belonging to *Siphoviridae* family. Phage-resistant strains of *V. alginolyticus* revealed also phenotypic differences on growth rate, motility and metabolic activity compared to the wild type strain. We correlated these differences with changes in the transcription levels of sugar, amino acid and other transporters. More specifically, the targeted transporters were downregulated between 3 to 12 times, whereas some transcript levels were almost totally depleted. These results suggest that phage resistant bacteria are able to diminish the transcription levels of the membrane receptors that are used by the phages as a defensive response, implying that they are able to reprogram their metabolism in order to avoid infection. Resistant strains are, however, subjected to a metabolic cost, which could potentially result in altered phenotypic properties and differentiate virulence. The present work promotes the binary model lytic phages: *Vibrio* sp. for studying phage-host interactions, strengthens the concept for developing phage cocktails against resistant to antibiotics bacteria using bacteriophages able to infect cells adsorbed by different receptors and underlines the importance of characterizing phages' receptors specificity.

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Broad host-range T5-like *Yersinia enterocolitica* phage phiR2-01

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Here we report the complete genome sequence and morphological characterization of the T5-like siphovirus vB_YenP_phiR2-01 (in short phiR2-01) infecting *Yersinia enterocolitica*, a zoonotic food-borne pathogen causing yersiniosis in humans and animals. Bacteriophage phiR2-01 encodes for 152 open reading frames (ORFs) and 19 tRNA-molecules on its 122,696 bp-long double-stranded DNA genome including 9,901 bp terminal repeats. A total of 115 of the ORFs are similar to genes of the well-characterized bacteriophage T5, including all genes involved in phage morphogenesis. The genome of phiR2-01 is mostly syntenic to that of T5, and the major differences between the genomes reside in areas with genes encoding for hypothetical phiR2-01 proteins. Other phages similar to phiR2-01 are the *Salmonella enterica* serovar Typhimurium phage Stitch, the *Escherichia* phage EPS7, the *Salmonella* phage Shivani, the *Escherichia* phage vB_EcoS_FFH1, the Enterobacteria phage DT57C and DT571/2, the *Escherichia* phage AKFV33, and the *Salmonella* phage SPC35. The host range of the phage is broad among *Y. enterocolitica* and it infects strains of both pathogenic and non-pathogenic serotypes. It does not infect strains of *Y. pseudotuberculosis, Y. nurmii, Y. pekkanenii, Y. mollaretii, Y. frederiksenii, Y. intermedia, Y. ruckeri, Y. bercovieri, Y. kristenseni* (except one strain), and *Y. aleksiciae.* The phage also failed to infect *Escherichia coli* and *Shigella* strains. Isolation of phage-resistant mutants from a transposon-insertion library revealed that the phage receptor is the BtuB protein.

Bacteriophage-mediated antibiotics resistance gene transfer in human gut microbiota

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Broad antibiotics usage in medicine and industry has led to increases in antibiotics resistance in microbial communities. Antibiotics resistance genes can emerge *de novo* as a result of mutation or spread through the process of horizontal gene transfer (HGT), which is carried out by mobile genetic elements such as plasmids, transposones and bacteriophages.

The aim of our study was to search for evidence for potential bacteriophage-mediated antibiotics resistance (AR) gene transfer in human gut microbiota.

This was performed using three *in silico* approaches: mapping metagenomic VLP reads on antibiotics resistance genes database sequences, analyzing *Enterococcus* isolates through coding sequences homology search and analysing AR genes and prophages colocalization in human gut metagenomes.

In order to find AR genes in virus-like particles' sequences, 257 viral metagenomes obtained from various microbial environments were downloaded and analyzed. Metaviral reads were mapped on antibiotics resistance genes sequences from CARD database. As a result, we detected 75 AR genes of several types in 21 of the 257 viromes analyzed.

Prophages carrying AR-genes were annotated in genomic assemblies of 20 *Enterococcus* strains isolated from human gut microbiota using PHASTER and Resistance Gene Identifier tools. Using this approach, a prophage carrying efrAB efflux pump genes was identified in 3 of the 20 analyzed strains, and a prophage carrying membrane mprF protein homolog was identified in one of the strains.

In order to find metagenomic variants of known resistance genes and known phage genes we have annotated the integrated human gut microbiota genes catalog (IGC) using specific Hidden Markov Models (HMMs). Metagenomic assemblies from which IGC genes were extracted were used for predicting phage-like regions and antibiotics resistance genes inside that regions. As a result, we have found 862 phage-like regions containing 1 or more antibiotics resistance genes.

The obtained results can be considered as evidence for phage-mediated antibiotics resistance genes transfer, though, a more detailed prophage structure analysis is required for better understanding of how antibiotics resistance is spread via horizontal gene transfer.

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Predicted transposable prophages in *Spirochaetes* reveal a new genome organization in the "*Saltoviridae*" family

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Transposable phages and prophages (the proposed “Saltoviridae” family) have already been detected in a range of $Gram^-$ and $Gram^+$ bacteria.

Escherichia coli phage Mu and *Pseudomonas aeruginosa* phage B3 stand as the paradigms for two genetic organizations of the “Saltoviridae” genomes, with the immunity repressor vs. late positive regulator at the left end, and respectively two vs. three transposition proteins, which are distant enough to belong to distinct protein families (Uniprot, ACLAME etc.) and contain different pfam domains.

Sequence similarity search with the most conserved "Saltoviridae" proteins (GemA, the late transcriptional regulator Mor, the portal protein and the transposition proteins) identified 6 putative prophages in Spirochaetes genomic scaffolds. All 6 harbor B3-type transposition proteins but are otherwise more related to Mu and have a new genomic organization that differs from both Mu and B3.

This further expands the host range of transposable phages and further illustrates their tendency to exchange functional modules.

New insights into A1, a multitasking pre-early protein of bacteriophage T5

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Immediately after delivery of their genome into the host, lytic phages defeat bacterial defences and hijack host cell machineries to establish a favourable environment for their multiplication. The early-expressed genes governing host takeover are highly diverse from one phage to another and most of them have no assigned function. They thus represent a pool of novel genes whose products potentially subvert or disrupt bacterial cell vital functions for phage profit. So far, however, their characterization is still at an early stage.

Bacteriophage T5 uses a unique two-step mechanism to deliver its 121 kb DNA into its host *Escherichia coli*[1]. At the onset of infection only 8 % of the genome enters the cell before the transfer temporarily stops. During the pause, the genes encoded by this DNA portion are expressed and their products lead to the host chromosome degradation, shut-off of the host gene expression and inactivation of the host defence systems (restriction/methylation, DNA repair)[2]. After a few minutes, T5 DNA transfer resumes allowing expression of the T5 middle and late genes and further phage productive growth. This original mechanism of DNA delivery facilitates the identification and functional characterization of the early genes responsible for host takeover, as they are clustered on the genome.

Two early proteins of T5, A1 and A2, are required for resuming the DNA transfer and additionally A1 is essential for host DNA degradation. We have demonstrated that purified A1 has an exo- and endo-nuclease activity *in vitro*. Moreover, ectopic expression of A1 in *E. coli* is sufficient to observe host DNA degradation by fluorescence microscopy. Taken together, our results indicate that A1 might be the so far elusive phage factor responsible for the massive host DNA digestion observed after the first-step transfer. Using bioinformatics tools, site-directed mutagenesis and complementation assays, we have identified some amino acids that are essential for infection and nuclease activity. Our findings raise the intriguing question of how A1 nuclease activity is coupled to the completion of phage DNA transfer.

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New insights into replication and structure of bacteriophage Bam35

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Bacteriophages belonging to *Tectiviridae* family can be divided into two distinct groups, lytic phages that infect Gram-negative bacteria and temperate phages whose hosts are Gram- positive bacteria. However, despite these differences, both groups share important structural and genomic features. Thus, the best characterized member of this family, PRD1, which infects several Gram-negative enterobacteria containing conjugative plasmids, is morphologically indistinguishable from the Bam35 bacteriophage whose host is *Bacillus thuringiensis*. Although the sequence identity at the nucleotide level is low, the genome of both phages is a linear double-stranded DNA with terminal inverted repeats. We recently determined that the Bam35 DNA polymerase (B35DNAP) displays highly processive polymerization coupled to strand displacement. Interestingly, although this enzyme is a highly faithful replicase, it is able to carry out the translesion synthesis of abasic sites[1]. The B35DNAP initiates the genome replication using a terminal protein (TP, coded by gene 4) as a primer and the third base at the 3´ends of the DNA as a template. Thus, the B35DNAP catalyses the formation of a phosphoester bond between a thymidine and the OH group of tyrosine 194 of the TP, that remains covalently linked to the 5´ ends of the genome (TP-DNA). Subsequently, the genetic information of the first two bases can be recovered by a novel single-nucleotide jumping back mechanism[2].

In the TP-DNA of Bam35, 32 open reading frames (ORFs) have been predicted, although only 17 encode proteins with a proposed or established function. With the aim of elucidating the function of some unknown ORFs, we carried out a comprehensive yeast two-hybrid analysis among all the putative proteins codified by the Bam35 genome. The map of protein interactions obtained comprises 25 proteins, of which 12 have an unknown function. The results allow us to propose that the P17 protein is the minor capsid protein of Bam35 and P24 is the penton protein, while P20 may be the stabilizing protein of the spike. These structural roles are supported by protein structure predicted through iterative threading. Moreover, the P26 inner membrane hydrolytic protein could have an additional structural role.

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Insights into the Determination of the Templating Nucleotide at the Initiation of Phi29 DNA Replication

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Bacteriophage Phi29 from *Bacillus subtilis* starts replication of its terminal protein (TP)-DNA by a protein-priming mechanism. To start replication, the DNA polymerase forms a heterodimer with a free TP that recognizes the replication origins, placed at both ends of the linear chromosome, and initiates replication using as primer the OH-group of Ser232 of the TP. The initiation of Phi29 TP-DNA replication mainly occurs opposite the second nucleotide at the 3' end of the template. Previous assays showed that the TP priming domain is the main structural determinant that dictates the internal 3' nucleotide used as template during initiation. At the end of the priming domain is placed the priming loop that contains the priming residue, Ser232. The alignment of the corresponding priming loop of the TPs from different viruses that initiate the replication of their genomes with a TP, shows the presence of aromatic residues close to the initiating (priming) residue. Interestingly, there seems to be a correlation between the distance of the aromatic residue to the priming amino acid and the initiation position.

We have analysed the role of the aromatic residue Phe230 of the Phi29 TP priming loop, proximal to the priming Ser232. The results showed that mutations in the aromatic residue Phe230 changed the specificity for the templating nucleotide, as initiation occurred mainly opposite the third 3' T instead of the second one. This aromatic residue could act as a structural barrier that limits the internalization of the 3' end of the template strand, conditioning the placement at the polymerization active site of the correct templating nucleotide. It could be interacting with the 3' terminal base and this contact would block further internalization, allowing the second position to be placed at the polymerization active site.

Characterization of the regulatory mechanism of a deep-sea filamentous phage SW1 and the interaction with its bacterial host *Shewanella piezotolerans* WP3

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Virus production in the deep-sea environment has been found to be high, and viruses have been suggested to play significant roles in the overall functioning of this ecosystem. Nevertheless, little is known about these viruses, including the mechanisms that control their production, which makes them one of the least understood biological entities on Earth. Previously, we isolated the filamentous phage SW1, whose virus production and gene transcription were found to be active at low temperatures, from a deep-sea bacterium, *Shewanella piezotolerans* WP3. In this study, the operon structure of phage SW1 is presented, which shows two operons with exceptionally long 5′ and 3′ untranslated regions (UTRs). In addition, the 5′UTR was confirmed to significantly influence the RNA stability of the SW1 transcripts. Our study revealed novel regulation of the operon and led us to propose a unique regulatory mechanism for Inoviruses. This type of RNA-based regulation may represent a mechanism for significant viral production in the cold deep biosphere. Moreover, the deep-sea phage and host interactions under in situ environmental conditions (20 MPa and 4°C) were investigated. Phage SW1 was shown to be active under in situ environmental conditions by transmission electron microscopy and qPCR. Further comparative analysis showed that SW1 had a significant influence on the growth and transcriptome of its host. The transcription of genes responsible for basic cellular activities, including the transcriptional/translational apparatus, arginine synthesis, purine metabolism and the flagellar motor, were down-regulated by the phage. Our results present the first characterization of a phage–host interaction under high-pressure and low temperature conditions, which indicated that the phage adjusted the energy utilization strategy of the host for improved survival in deep-sea environments.

Nanopore sequencing of bacteriophage genomes

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The idea of using nanopores to sequence DNA can be traced back to the late 1980s. However, it took more than two decades for the advances in the development of nanopore sensing to finally materialize through the release of the benchtop MinION sequencer by Oxford Nanopore Technology. The first version of the product showed limited yield and accuracy, but continuous improvements in the engineering of nanopore proteins and chemistry have significantly raised both metrics. We investigated the application of such technology to bacteriophage genome sequencing and developed a computational analysis pipeline for the assembly of the reads. In this presentation, we will report our results, emphasizing both the strengths and limitations of the technology, and present some ideas of future analysis that can be done with the MinION sequencer.

Experimental evolution reveals stronger selective constraints at higher temperature for an RNA coliphage MS2

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Experimental evolution in laboratory populations facilitates the detailed investigation of adaptation in real time. RNA viruses provide several advantages for studying evolution in action due to their large populations, short generation times, small genomes and high mutation rates. In this study, we used MS2, an RNA bacteriophage with a tremendous burst size and a tiny genome. We characterized the phage's response to a higher than usual temperature, comparing the identity and fitness effects of mutations at 41°C, vs. the optimal growth temperature of 37°C, using high-fidelity deep sequencing.

Our experiment began with a nearly homogenous population derived from a single clone, which was serially passaged and sequenced at high and low temperatures. We first inferred the phage mutation rate based on the change in frequency of neutral mutations. Our results revealed a mutation rate one order of magnitude lower than what has been previously reported. We next set out to infer the fitness of all mutations detected, based on the change in their frequency over time. While multiple deleterious mutations appeared and then became extinct throughout the course of the experiment, our data also revealed several interesting adaptive mutations that occurred in coding regions at 37°C and/or 41°C, both synonymous and non-synonymous. The proportion of nonsynonymous and synonymous fixation rates has been used to measure the level of selective pressure on protein coding genes. Our results show that, over time, the proportion of non-synonymous mutations drops, indicating that the vast majority of mutations are deleterious and under purifying selection. Interestingly, the slope at 41°C was found to be substantially steeper compared to 37°C, suggesting that non-synonymous mutations tend to be more harmful at the high temperature, i.e., purifying selection is stronger in MS2 at 41°C. This trend was confirmed by constructing a distribution of fitness effects, which showed a distribution far more enriched with deleterious mutations at 41°C. To conclude, our study shows that MS2 mutation and consequent evolution are more constrained at the higher temperature, suggesting that not only the MS2 consensus sequence but also MS2 mutants are optimzed for growth at 37°C.

Characterisation of four toxic proteins encoded by the *Bacillus subtilis* phage SPO1

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The subversion of the bacterial host during a bacteriophage (phage) infection is a multifaceted process involving a variety of elegant mechanisms. This often involves small phage-encoded proteins that sabotage or repurpose host biosynthetic processes, leading to more efficient phage replication and development. The genome of the *Bacillus subtilis* phage SPO1 contains 26 genes in a segment referred to as the host-takeover module (HTM).

Many of the HTM genes are uncharacterised and their mode of action may potentially reveal mechanisms of host-takeover by phages. Here, we present the characterisation of four HTM genes, which attenuate bacterial growth when overexpressed in *B. subtilis* in the absence of SPO1 infection. The results are discussed in the context of the bacterial targets of these HTM genes and their potential biological role in the acquisition of B. subtilis by SPO1.

Viral DNA packaging initiation: recognition and cleavage of the bacteriophage SPP1 *pac* site

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Specific recognition of the viral genome is an essential step in the assembly of most viruses, including tailed bacteriophages. In numerous phages including SPP1, genome packaging is initiated by recognition and cleavage of a specific sequence *pac*, by the small (TerS) and large (TerL) terminase subunits. This cleavage generates a DNA free end in the viral DNA concatemer that is the starting point for the unidirectional packaging of DNA into the procapsid. It was previously shown that the SPP1 *pac* region has two sequences where TerS binds (*pac*R and *pac*L) flanking the segment where TerL cleaves the SPP1 DNA (*pac*C). However, the *pac* specific sequences required to achieve this endonucleolytic cut were not established. Their characterization is essential to understand the underlying mechanism.

In this study we used a plasmid minimal system encoding SPP1 *pac*, TerS and TerL that mimics specific *pac* recognition and its auto-regulated cleavage in *Bacillus subtilis*, the SPP1 host. We show that the *pac*R sequence localized within 35 bp downstream of the *pac* cut can be extensively degenerated, and that only disruption of a 5 bp polyadenine tract impairs *pac* cleavage. This result together with deletion analysis of *pac*L shows that the specific DNA sequences required for targeting the terminase for *pac* cleavage are considerably shorter than the large region bound by TerS. Furthermore, extensive degeneration of the 6 bp target sequence within *pac*C where *pac* cleavage occurs, reveals that TerL maintains, remarkably, its precise position of cleavage. Studies with SPP1-related phages show conservation of the cut position, irrespectively of sequence variation in *pac*C, in *pac*R or changes in *pac*L-*pac*C distance. Mechanistically our data is compatible with a model in which TerS interactions with part of the *pac*L sequence and a poly-A tract in *pac*R are sufficient to orient very accurately the TerL nuclease to a defined *pac*C position. They also demonstrate that the resulting precise cut at *pac*C is independent of the targeted DNA sequence.

The near-atomic resolution cryo electron microscopy structure of the lactococcal siphophage 1358 virion mature full capsid

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Lactococcus lactis, a Gram(+) lactic acid-producing bacterium used for the manufacture of several fermented dairy products, is subject to infection by diverse virulent tailed phages, leading to industrial fermentation failures. Despite the availability of several antiphage measures, new phages keep emerging in this ecosystem. This constant viral risk has led to a sustained interest in the study of their biology, diversity, and evolution.

Lactococcal phages now constitute a wide ensemble of at least 10 distinct genotypes within the Caudovirales order, many of them belonging to the Siphoviridae family. Lactococcal siphophage 1358, currently the only known member of its group, displays a noticeably high genomic similarity to some Listeria phages as well as a host range limited to a few L. lactis strains. These genomic and functional characteristics stimulated our interest in this phage.

Here, we report the 4.5 Angstroms resolution cryo electron microscopy (cryo-EM) structure of the 1358 virion mature full capsid. Recent advances in cryo-EM have enabled structure determination of macromolecular complexes at near-atomic resolution. However, structure determination and atomic modelling still remains a challenging task susceptible to model bias and overfitting especially while using *de novo* methods. This study demonstrates a practical approach to obtain a rigorously validated atomic resolution cryo-EM structure allowing to complement the previously published complete structural picture of a unique lactococcal phage at lower resolution and have deeper insight into structural organization of its capsid.

Elucidating the molecular mechanisms by which the HNH endonuclease gp74 activates the terminases in bacteriophage HK97

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The last gene in the genome of the bacteriophage HK97 codes for gp74, an HNH endonuclease. HNH endonucleases digest DNA in the presence of metals and are characterized by two highly conserved His residues and an Asn residue. Gp74 is essential for phage head morphogenesis, likely because gp74 enhances the activity of the HK97 terminase enzymes toward the cos site. Enhancement of terminase-mediated cleavage of the phage cos site requires the presence of an intact HNH motif in gp74. Mutation of the canonical metal binding His in the HNH motif abrogates gp74 mediated-enhancement of terminase activity. Although phages are widely studied, there is no definitive structural or mechanistic evidence as to how the HNH endonuclease within gp74 functionally interacts with the terminase enzymes to facilitate phage morphogenesis. Further, gp74 possesses very low sequence similarity to HNH proteins for which the structure has been determined, making structural studies of gp74 necessary.

To gain structural information of gp74 we use nuclear magnetic resonance (NMR) spectroscopy. Current work reports NMR resonance assignments of 80% of the backbone and side chain Cb resonances of gp74. Analysis of the chemical shifts indicates that, as expected, gp74 contains the characteristic ββα motif found in HNH endonucleases. However, there are additional structural elements in gp74 outside the ββα motif that are not present in other HNH endonucleases, including the HNH endonuclease Gmet_0936 that is predicted to be structurally similar to gp74. In addition, NMR studies have elucidated residues within gp74 required for metal binding and terminase activity. These data are being used to assess the role of specific gp74 residues in phage morphogenesis. Together, this work will identify how metal binding to the HNH endonuclease gp74 is crucial in the replication and morphogenesis of phages.

Anti-phage antibody response in intensive care patients receiving phage therapy against hospital acquired infections

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To evaluate the influence of humoral anti-phage immunity on the efficiency of phage therapy against hospital acquired infections (HAIs). Bacteriophage cocktails were administered per os in 20-ml doses once a day for 3 consecutive days to 42 patients supported by prolonged mechanical ventilation at a neurological intensive care unit (ICU). Six of the patients received the phage treatment 3 to 5 times. To monitor the efficiency of phage therapy, endotracheal aspirate, blood, urine and fecal samples were collected before and after the treatment and cultured for HAI-causing bacteria. Anti-phage immunity was evaluated by ELISA before and after the treatment.

The bacteriophage cocktail contained eight phages specific for *A. baumannii*, *K. pneumoniae*, *P. aeruginosa and S. aureus*, two against each species. These phages had an aggregate lytic spectrum of 73% against antibiotic-resistant strains isolated from the ICU patients in Russia. The sensitivity of German and Finnish clinical strains to the phages will be reported. *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa* were isolated from 87.5% of the patients' pre-treatment samples. After first round of phage therapy efficient elimination of the pathogens took place in 54-62.5% of cases. We were able to detect phages in blood, urine, endotracheal aspirate and feces samples thus confirming a systemic distribution of orally administered bacteriophages. Two to three weeks after oral phage administration specific anti-phage IgG-antibodies were detected from the patient blood samples with the titres of 1:16-1:4096 and the levels were retained for at least one year. Repeated phage treatments were accompanied by a faster rise of the specific anti-phage antibodies. While the first round of phage therapy gave a clear clinical response in the study group evidenced for example by sanitation of the loci initially infected by HAI agents, repeated rounds of phage therapy with the same phages were not as successful. The efficiency of phage therapy may be compromised by repeated rounds of treatment with the same phage due to formation of anti-phage antibodies. Therefore, when used repeatedly to treat a single patient the composition of phages in the phage cocktail should be changed and this is even valid for orally administered phages.

Investigating the diversity of mycobacteriophages in India and exploring the potential of endolysins as anti-bacterials

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Background: With the wide-spread emergence of drug resistance in *Mycobacterium tuberculosis* (Mtb) and a slow progress in finding new TB drugs, mycobacteriophages and their endolysins appear to be promising as anti-TB resource. Also information on Mycobacteriophages isolated from India and on their endolysins is scarce.

Methods: Hundred and fifty soil and water samples were collected for the isolation of mycobacteriophages and *M.smegmatis* mc2155 was used for screening the samples. The purified mycobacteriophages were tested for lytic activity against H37Rv strain of Mtb by spot test. Morphology of the purified phages was analyzed by transmission electron microscopy and sub-cluster classification was carried out by PCR analysis of Tape Measure Protein gene. Further, whole genome sequencing and analysis of one mycobacteriophage (PDRPxv) was carried out and Lysin A and B were purified and assayed.

Results: Eighteen new mycobacteriophages have been purified. Significantly, 9 were found to be capable of infecting H37Rv strain. Cluster classification suggests mycobacteriophages to belong to B1 sub-cluster (except for two phages), which is currently the most populous sub-cluster. Genome size of PDRPxv is 69171 bp, with ~66% GC content. Based on the functional annotation, Lysin A and Lysin B have been cloned, expressed and purified.

In silico analysis shows Lysin A to be modular in structure, with a M23 peptidase domain at the N-terminal, a central amidase domain and the peptidoglycan recognizing protein domain at the C-terminal. In Lysin B, the presence of a glycine rich region at the C-terminal is observed which is found only in Lysin B from B1sub-cluster mycobacteriophages. Interestingly, we found lysis of *E. coli* host from within due to over-expression of Lysin A evident by decrease in the absorbance of the induced culture as compared to the un-induced culture over time. Turbidimetric assay using purified Lysin A and Lysin B showed inhibition of the growth of *M. smegmatis* by 43%.

Conclusion: Mycobacteriophages are found at a high frequency in India. Anti-Mtb activity of phages and their endolysins signify their potential applications in managing drug resistant tuberculosis.

Inverstigation of *Phikz* Phage Therapy Against *Pseudomonas aeruginosa* Mice Pneumonia Model

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Background: *Pseudomonas aeruginosa* is one of the most common causes of hospital-acquired pneumonia.

It cause a pneumonia with high morbidity and mortality especially in immuno-compromised and cystic fibrosis patients. As a result of inadequate treatment with existing antibiotics due to increasing resistance have been made to search for new treatments such as phage therapy.

Aim of the study was evaluate the therapatic effect of Phikz phage against *Pseudomonas aeruginosa* PAO1 in mice pnoeumonia model. Systemic and organ specific inflammation level and glycocalix (hyaluronan) damage was also studied to inverstige cross-talk between lung and kidney.

Material/methods: In this study, systemic and organ (lung and kidney) specific inflammation level, bacterial load in lung tissue and the effect on the glycocalyx injury was investigated after treatment of phiKZ and meropenem alone or combined treatments in pneumonia C57 / BL6 mice model created with *P. aeruginosa* PAO1 strain. All mice except the control group were infected with *P. aeruginosa* PAO1.

Treatment was started 2 hours after infection and the bacterial load count in samples of lung tissue was carried out after 24 hours. IL-6, TNF-α, IL-1β and hyaluronan (component of glycocalix) levels also measured in sera, kidney tissue and Bronchoalveolar lavage (BAL) samples in order to investigate the treatment effectiveness.

CRP, ALT, AST, glucose, urea, lactate, creatinine measurements in serum samples were also made.

Results: The differences of serum CRP, lactate, hyaluronan and IL-6 levels between patients and control group were found statistically significant. Bacterial burden in lung were significantly decreased in the treatment group. IL-6 levels in the BAL were different between the control group and the patient group. In the kidney homogenates, the differences of IL-6 and TNF-alpha levels between the patient and control groups were statistically significant.

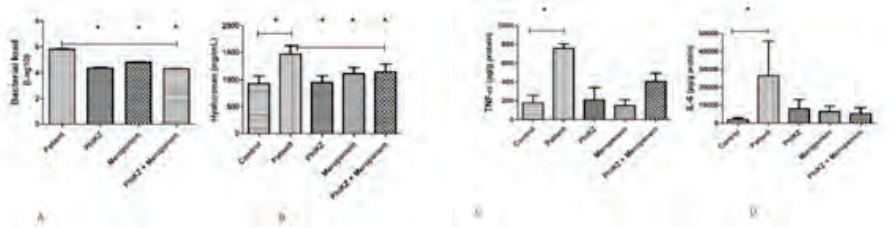

Figure 1. Bacterial load and ELISA results: A) Bacterial load in lung homogenate B) Hyaluronan levels of sera C) TNF-α levels of kidney homogenate D) IL-6 levels of BAL

Conclusions: The greatest reduction in pulmonary bacterial load were obtained by the combined treatment with meropenem and phikz phage. Elevated proinflammatory cytokines after infection, have declined due to a reduction in bacterial load with treatment. An increase of IL-6 and TNF-alpha levels in kidney tissue indicate the cross-talk between the kidney and the lung.

A rare case of lysogeny in *Streptococcus thermophilus*

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The sustained use of starter cultures in dairy fermentation has resulted over time in the appearance and spreading of bacteriophages (phages) that are specific to the strains composing these starter cultures. The epidemiology of dairy phages is such that the use of selected strains becomes problematic in industrial settings. Hopefully, the exploitation of active CRISPR-Cas systems in *Streptococcus thermophilus*, providing adaptive immunity against phages, has opened new avenues for the selection of phage-resistant strains, notably through the recycling of frequently attacked strains.

Phage-resistant variants can be naturally selected following phage challenge in controlled conditions.To render *S. thermophilus* strain DGCC8846 resistant to its known phages, it was first challenged with phage D6193. Among 96 surviving isolates, none had acquired additional repeat-spacer unit in their CRISPR1 or CRISPR3 loci. Instead, the phage genome was found to be integrated into the bacterial chromosome. Similar results were obtained for challenges made with phages D6268 or D6269. Further molecular analyses of the phages showed that all three belong to the *cos* type, and that they all possess an integrase gene. DGCC11909, a lysogenic variant of DGCC8846 containing phage D6193 genome integrated in its chromosome, was found to be insensitive to the three phages. Excision of DGCC11909 prophage (10^3 pfu/mL) was spontaneously observed during cultivation in optimal conditions. Higher induction of the prophage was observed when the strain was cultivated in stressful conditions such as a limiting carbon source (5 g/L lactose, leading to 4.10^4 pfu/mL), or in the presence of hydrogen peroxide (1.5 mM, leading to 5.10^5 pfu/mL) or mitomycin C (0.6 µg/mL, leading to 4.10^6 pfu/mL).

Temperate phages have only been described occasionally in the *S. thermophilus* species, which is a chance for starter culture producers. Indeed, temperate phages impair the possibility to select CRISPR-improved variants from sensitive strains. In addition, as prophages appear to be instable once integrated into the *S. thermophilus* chromosome, it is recommended to carefully select prophage-free strains for starter culture development.

Explorations into Spirochete phage space: *Borrelia* bacteriophages for the diagnosis and treatment of Lyme disease

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Bacteriophages have been used to effectively target and kill many bacterial species that cause humans infections, and there is currently a revival of research in this area in the Western world. Despite its increasing prevalence, serious nature, and difficulty to diagnose and treat, very little research has been carried out on phages that infect *Borrelia*, the Spirochete bacterium that is the causative agent of Lyme disease. *Borrelia* are carried by ticks which cause bacterial infection when following the biting of humans and subsequent consumption of a human blood meal. Both treatment and disease diagnosis would potentially benefit from a better understanding of phages. The current primary treatment for Lyme disease (LD) is antibiotic therapy but complete *Borrelia* eradication is difficult, and the current diagnosis of LD is problematic. In this project, we aim to characterise *Borrelia* phages in order to develop obtain a better understanding of their biology and improve both diagnostics and therapeutics.

To redress the lack of study of *Borrelia* phages we have pursued four research strands, these are;

1. An analysis of *Borrelia* phage genomes that was used to design phage-based detection assays. These assays have been extensively validated on patient and healthy volunteer's samples in our laboratory and shown to be significantly more sensitive and selective than existing tests.
2. We have established how to culture *Borrelia* on plates and organised a large network of tick samplers throughout the UK from which we have screened 100s of ticks for *Borrelia* and phages. We have established how to isolate the bacteria, and optimised the assay for lytic phage detection.
3. We have induced phages from within *Borrelia* genomes using antibiotics and characterised the resultant phages that are induced.
4. Phage-encoded holins (enzymes that rupture bacterial cytoplasmic membranes) and endolysins (enzymes that break down bacterial cell walls) have been investigated to establish their 'anti-*Borrelia*' characteristics. These proteins have been over-expressed in a yeast-based protein expression system and we are currently working on their purification and on efficacy studies.

Data will be presented from these four strands of this newly explored Spirochete phage space.

Phage therapy efficiently treats acute *Pseudomonas aeruginosa* lung infection in mice

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Introduction: *Pseudomonas aeruginosa (Pa)* lung infections are increasingly difficult to treat due to evolving antibiotic resistance; bacteriophages (phages) are part of the foreseen therapeutic alternatives. In this study, we assessed the efficacy of a phage cocktail delivered through the airways in a mouse model of acute lung infection.

Materials and methods: The selection of therapeutic phages was based on the activity of an anti-PA phage collection on an international reference panel of *Pa*[2], as well as, clinical strains from the major clinical serotypes. A purification process has been developed to produce pure phages at a high titer.

Their efficacy has been tested in an acute model of respiratory infection with a bioluminescent strain. Two hours post-infection (p.i.), phages were delivered by intravenous or pulmonary route, at an MOI of 0.1, 1 and 10. The infection was monitored by bioluminescence, survival, as well as lung/blood bacteria and phage counts at various time points. Phage efficacy was then compared to amikacin's.

Results: Nine phages were selected. After purification, their titer reached 10^{12}pfu/mL with undetectable levels of host cell proteins and host cell DNA and low endotoxin levels (<50 EU/mL for 10^{11}pfu/mL).

Pulmonary rather than IV phage administration resulted in a significant decline of lung infection, and reduced the 24h-mortality. The dose-response study demonstrated the best 48h-efficacy for MOI 10 (equal to amikacin's). Phage therapy also allowed a rapid decline of pulmonary bacterial load, which was undetectable at 96h p.i.

Conclusion: Herein, a unique pulmonary administration of phages dramatically reduced lung bacterial load and mortality. These data support that topical phage therapy is relevant to treat acute *Pa* lung infections.

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Comparison of two phages for potencial comercial use – genomes, host spectrum and declaration of nontoxicity

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The bacterial species of the *Staphylococcus* genus are important human and animal pathogens which cause severe infectious diseases. The most pathogenic species *Staphylococcus aureus* is the major causative agent of numerous hospital- and community acquired infections. The increasing number of pathogenic strains resistant to antimicrobial drugs is a serious public health problem that can be solved by applications of phage therapy as a suitable alternative to antibiotics treatment. One of the disadvantages of phages is that compared to wide-spectrum antibiotics phages act on species or strains only. Using polyvalent bacteriophages or preparation of phage cocktails with broad host range is asuitable solution for this problem of fighting unwanted bacteria in medicine, food industry, biotechnology and agriculture.

Two anti-staphylococcal phages MB401 and MB402 were chosen for a potential synergic use in a cocktail. Genomes of these phages were sequenced and alignment shows they share more than 97% of homology. Only few rearrangements in genome influenced host spectrum of these phages. Lytic spectrum of bacteriophages was tested on 53 clinical isolates of *S. aureus* from Czech hospitals including MRSA strains. 24 strains were sensitive due to synergic effect of both phages. In the future we are planning to isolate more phages which could extend host spectrum and add them into the phage cocktail.

A number of analyses is required for commercial products especially in veterinary and human medicine. An important feature is declaration of nontoxicity of phage lysate.

Phages MB401 and MB402 are propagated on well defined bacteria strain which does not contain any known prophages, genes for toxins, virulence factors etc. and that increases probability of safe final product. Local dermal tolerance and acute dermal toxicity studies were performed with phage lysate in rats and rabbits, results confirm that phage lysate is nontoxic.

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Isolation of phages from the female genital tract targeting *E. coli*

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Background: *E. coli* in the female genital tract is responsible for a range of pathogenic conditions, including urinary tract infections (UTI), chorioamnionitis and preterm labour, and neonatal sepsis. With increasing antibiotic resistance owing to integrons and other resistance genes, as well as the ability for *E. coli* to form biofilms, alternative treatment strategies, such as phage therapy, should be explored.

Methods: Lateral vaginal wall swabs were collected from South African adolescents at high risks of HIV infection and stored in Amies medium. The medium was chloroformed and the supernatant was stored at 4°C treatment. After *in vitro* serial transfers in Luria broth (LB) using *E. coli* MG1655 as target, we screened for the presence of bacteriophages by plaque assays. For each sample presenting plaques, we isolated several plaques with different phenotypes and re-amplified them individually in *E. coli* MG1655. After two isolation cycles, we examined the bacteriophage lysates with electron microscopy and tested their host-range.

Results: So far, 88 vaginal samples were screened. Our preliminary results show that several samples contained bacteriophages (*Siphoviridae, Podoviridae* and *Microviridae* – based on their morphology).

We tested the activity of these bacteriophages against a collection of (i) Shigatoxigenic *E. coli* (STEC) isolated from feces of individuals presenting symptoms of diarrhea but also isolated from environmental samples, as well as (ii) *E. coli* isolated from female genital tracts presenting with an UTI. The results will be presented at the conference.

Discussion-perspectives: To our knowledge, our study is the first to report functional bacteriophages isolated directly from female genital tract samples. Further investigations are needed to characterize the isolated bacteriophages, such as the determination of their full genome sequence as well as phenotypic traits that would be interesting for phage therapy applications, for example their ability to reduce biofilms caused by *E. coli* in UTIs.

PhageTerm: a Fast and User-friendly Software to Determine Bacteriophage Termini and Packaging Mode using randomly fragmented NGS data

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Bacteriophages are the most abundant viruses on earth and display an impressive genetic as well as morphologic diversity. Among those, the most common order of phages is the Caudovirales, whose viral particles packages linear double stranded DNA (dsDNA). In this study we investigated how the information gathered by high throughput sequencing technologies can be used to determine the DNA termini and packaging mechanisms of dsDNA phages. The wet-lab procedures traditionally used for this purpose rely on the identification and cloning of restriction fragment which can be delicate and cumbersome. Here, we developed a theoretical and statistical framework to analyze DNA termini and phage packaging mechanisms using next-generation sequencing data. Our methods, implemented in the PhageTerm software, work with sequencing reads in fastq format and the corresponding assembled phage genome. PhageTerm was validated on a set of phages with well-established packaging mechanisms representative of the termini diversity: 5'cos (lambda), 3'cos (HK97), pac (P1), headful without a pac site (T4), DTR (T7) and host fragment (Mu).

In addition, we determined the termini of 9 *Clostridium difficile* phages and 6 phages whose sequences where retrieved from the sequence read archive (SRA). A direct graphical interface is available as a Galaxy wrapper version at https://galaxy.pasteur.fr and a standalone version is accessible at https://sourceforge.net/projects/phageterm/.

Formulation and encapsulation of bacteriophages in pH responsive microparticles for gastrointestinal delivery

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Increasing antibiotic resistance in pathogenic microorganisms has led to renewed interest in bacteriophage therapy both in humans as well as in animals. A number of phage therapy studies in small vertebrate animals have demonstrated reduction in the intestinal concentration of pathogenic bacteria including E. coli [1], Salmonella [2], Campylobacter [3] and C. difficile [4].

A recent randomised clinical trial in children (male, aged 6 – 24 months) looked at the potential of E. coli phage to treat acute diarrhoea [5]. The study was unable to demonstrate efficacy in reducing diarrhoea. This was attributed to low intestinal E coli concentrations resulting in lack of significant in situ phage amplification. An additional factor responsible for poor phage amplification could have been a low concentration of phage dose delivered at the site of infection due to phage inactivation upon exposure to stomach acidity and enzymes during transit (no antacid was given during oral phage delivery). The punitive environment of the stomach poses a challenge in orally delivering viable phages to the gastrointestinal tract. Without protection, the amount of phage reaching the target site is variable and potentially significantly lower than the dose needed to eradicate the bacterial load. Modelling studies of phage-bacterium population dynamics suggest both high phage and bacteria concentrations are needed to effectively reduce bacteria numbers such that the host immune system is able to eradicate the infection [6]. A number of recent studies have evaluated the prospect of encapsulating phage in biopolymers e.g. alginate [2], polyesters [7] and liposomes [8]. Previous studies have used fairly crude extrusion techniques to encapsulate the phage with variable particle size and encapsulation yield. The aim of the present study was to evaluate the use of novel microfluidic and membrane based microencapsulation techniques.

Phages CDKM9 (temperate, C. difficile myovirus) and Felix O1 (lytic, Salmonella myovirus) were used in the study. Both phages were found to be rapidly inactivated upon exposure to an acidic solution (pH 2). A formulation containing a pH responsive methacrylate polymer (Eudragit® S100) mixed with alginate was evaluated to microencapsulate the phage and protect them from stomach acidity. The polymer is insoluble at low pH however, it dissolves at elevated pH found in the lower GI tract. The microfluidic technique permitted exquisite control of the encapsulated phage dose, resulted in uniform particle size and allowed careful tailoring of the phage release kinetics demonstrated in vitro in simulated intestinal fluid (SIF).

The membrane emulsification process allowed scale-up of the phage formulation. Encapsulation was shown to protect phage upon exposure to pH 2 (exposure time ~ 2h) followed by release in SIF.

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Podoviridae bacteriophages as surrogates for validating efficacy of membrane filters

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Ensuring the availability of affordable and safe drinking water is one of the great global challenges. The bacteriophages are uniquely suitable as surrogates for enteric human viruses and can be used to test and optimize water treatment systems to improve their ability to remove viral pathogens. Here, we present results of a study where in fifty new phages (42 *E. coli* and 8 *P. aeruginosa* specific) isolated from various water bodies in the Georgia and the U.S. and evaluated for their suitability as surrogates for human adenovirus 40.

The phage isolates were purified and concentrated, and the morphology of phages was assessed by TEM. Ten Podoviridae phages, characterized by short tails and showing morphological similarity with enteric viruses, were further purified using CsCl density gradient centrifugation and characterized in terms of the hydrodynamic diameter and zeta potential. The phages were then employed in cross-flow ultrafiltration studies with 100kDa membranes to quantify their fate during membrane separation.

The average hydrodynamic size of the phages ranged between 50 and 75nm and the zeta potential between -16 and -26 mV. The cross- flow filtration experiments showed that the total (i. e. post-elution) phage recovery was highly variable ranging from 21,2 to 92,9% for different phages. Phage loss to the permeate was insignificant, resulting in high virus rejection (1.55 Log < <4.35 Log; 2.8 Log). Thus the main factor limiting phage recovery was virus loss to the membrane. This conclusion is corroborated by the fact that elution was relatively ineffective contributing <4% to the total recovery.

Correlation analysis demonstrated that virus size and charge alone are poor predictors of virus recovery by crossflow ultrafiltration. We anticipate that virus hydrophobicity plays a role in determining virus adhesion to the membrane. Quantification of phage surface energies and hydrophobicities are underway in our laboratories. We tentatively conclude that Podoviridae phages could be valuable as test microorganisms in membrane clearance tests but a more comprehensive analysis of virus-membrane interactions is required to inform surrogate selection.

The perfect cocktail: Developing a phage cocktail against *Escherichia coli* and *Shigella* ssp

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Shigella ssp. and enterotoxigenic *Escherichia coli* are the most common etiological agents of diarrhoeal diseases in malnourished children under five years of age in developing countries. Continued development of antibiotic resistance in pathogens and the potential negative impact on the development of the infant commensal microbiota are significant drawbacks of antibiotic therapies.

Bacteriophages (or phages) represent an alternative tool which can be used to specifically treat bacterial infections. Here, we screened water samples for the presence of phages and managed to isolate 28 individual phages infecting *Shigella sonnei* 53G. All isolated phages were deemed to be unique through determination of their host range against 72 strains of the ECOR collection. Genome sequencing of 10 chosen phages revealed three distinct groups of phages, with significant diversity evident in the receptor binding protein region of phages. A cocktail of these 10 genetically distinct phages was designed. Phage therapy using this cocktail was tested *in vivo* using the *Galleria mellonella* (wax moth) larvae model. A single dose of the phage cocktail administered two hours before (prophylaxis model) or after (remedial model) infection with *E. coli* ECOR62 successfully improved survival rate of the larvae. On the other hand, the phage cocktail applied against a mixture of five ECOR strains increased the larvae survival rate in the prophylaxis model only.

Isolation and genome analysis of bacteriophages infecting hypermucoviscous highly virulent strains of *Klebsiella pneumoniae* K1 and K2 serotypes

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Backgrounds: *Klebsiella pneumoniae* strains of K1 and K2-serotypes overproducing capsular polysaccharides are most virulent human pathogens. They cause nosocomial and community-acquired infections such as primary pyogenic liver abscess, meningitis, endophthalmitis, as well as respiratory and urinary tract infections. These strains are additionally tending to acquire antibiotic resistance determinants. Lytic bacteriophages with polysaccharide depolymerization (PS-dep) activity are a potential remedy to control hypermucoviscous *K.pneumoniae*.

Objectives: The purpose of this study is the isolation and characterization of bacteriophages and their enzymes active against hypermucoviscous *K.pneumoniae* strains K1 and K2 serotypes.

Methods: Bacteriophages specifically lysing *K.pneumoniae* K1 and K2 capsular serotypes were isolated from clinical material and sewage. Bacteriophage genomes of four K1-specific phages and two K2-specific phages were sequenced using Ion Torrent technology and assembled with Newbler (v2.9). The complete genomes were annotated using GeneMark.hmm, RAST, HHPred, and the BLAST database suite. Phage PS-dep genes of bacteriophages KpV71 (K1-specific) and KpV52 (K2-specific) were identified, amplified and cloned into expression vectors to detect the PS-dep activity. The activity and specificity of the phages and the enzymes were demonstrated on a collection of a *K.pneumoniae* different capsular type as well as on *K.pneumoniae* capsular polysaccharides.

Conclusions: We have identified and characterized bacteriophages and recombinantly expressed phage polysaccharide-degrading enzymes specific to *K.pneumoniae* K1 and K2 serotypes. Results obtained are a basis for designing specific formulations based on bacteriophages and phage enzymes to diagnose and to cure infections associated with *K.pneumoniae* K1 and K2 serotypes.

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Additional confirmation of lytic nature of therapeutic Staphylococcal bacteriophage Sb-1

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G. Eliava Institute of Bacteriophages, Microbiology and Virology have elaborated the phage preparation – Staphylococcal bacteriophage that is successfully used for treatment of infectious diseases caused by *S. aureus*, including methicillin-resistant (MRSA) bacterial strains.

To increase efficacy of therapeutic phage preparations that is based on expanding host range of lytic activity, various clinical bacterial strains (including antibiotic-resistant strains) are used. Sb-1 phage genomes from four commercial batches were examined by PCR for presence of genes coding erythromycin-resistance. The reaction conditions for the PCR and relevant specific primers were kindly provided by Shawna McCallin (UNIL, University of Lausanne, Switzerland). As a positive control, genomic DNA of erythromycin-resistant bacterial strain *S. aureus* 164 was used.

PCR results revealed the absence of erythromycin-resistant genes in the genomes of commercial preparations of staphylococcal bacteriophage Sb-1. PCR product with length ≈ 800 bp, was synthesized only in reactions with genomic DNA of *S. aureus* 164.

One of the main evaluation criteria for phage preparations used in therapy is phage virulence and reproduction peculiarities of phage-host bacteria interactions. Lack of the ability of phage for transduction is the most important condition for therapeutic preparations.

We investigated the transduction ability of bacteriophage Sb-1, which is the active ingredient of the commercial staphylococcal bacteriophage produced by the “Eliava Biopreparations“Ltd.

For determination of possible transfer of erythromycin resistance determinants by phage from a donor bacterium to a recipient cell, several commercial batches of phage Sb-1 were examined. Bacterial strain *S.aureus* 164 resistant to erythromycin (ermR) was used as a donor, and the strains *S.aureus* 142 and *S.aureus* 148 were used as recipient erythromycin sensitive strains. Experiments were carried out according to the Richardson Lab Protocols.

The results of several rounds of transduction experiments showed that *S. aureus* recipient cells remain sensitive to erythromycin. Bacteriophage Sb-1 does not transfer bacterial genes from the host strain to another.

The obtained date demonstrates once again the strictly virulent nature of commercial staphylococcal bacteriophage Sb-1 that correlates with our previously published results regarding the basic biological and molecular properties of this bacteriophage.

Klebsiella podovirus KP32 produces two polysaccharide depolymerases, promising anti-virulence tools

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Bacteriophages (phages) as natural enemies of bacteria have a huge ecological impact on the dynamics of bacterial populations. To overcome the capsule barrier, some phages are equipped with a virion-associated depolymerase – highly specific enzymes depolymerizing capsular polysaccharides (CPS), thus also responsible for host cell recognition and receptor binding. Bacteriophages possessing proteins with such activity are also able to better diffuse in the biofilm, gaining access to the present microcolonies, and infecting embedded bacteria. Depolymerization of CPS results in the increasing susceptibility to chemical and physical agents as well as to host defences such as phagocytosis or complement-mediated killing. Although depolymerases do not kill the bacterium, they have special interest as anti-virulence compounds that disarm the bacterium, reducing or even avoiding the infection process.

In the presented study two genes encoding putative depolymerases have been identified in the genome of *K. pneumoniae* specific KP32 podovirus. According to *in silico* prediction (PHYRE2), the β-helical structure is highly probable for both proteins. Selected gene products have been produced as recombinant proteins. Zymographic analysis using the crude exopolysaccharides extracted from bacterial strains, confirmed their enzymatic activity. Both depolymerases, one of which has been annotated as a tail fiber with similarity to T7 tail fiber region, while the other as hypothetical protein, recognize and cleave CPS of different *Klebsiella* serotypes. They possess mainly β-conformation, according to circular dichroism (CD) spectroscopy, what explains their stability at high temperature and wide range of pH. The oligomeric state of all depolymerases is trimeric, according to multi-angle static light scattering (MALS) spectroscopy. Microbiological results showed that these depolymerases by modifying the phenotype of the bacterial cells without affecting their viability, make the bacteria more easy to eradicate by immune response mechanisms.

Salmonella treatment by a bacteriophage cocktail: from petri-dish to farm

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In 2011 PTC GmbH developed a broad range bacteriophage cocktail targeting *Salmonella*, in order to provide the poultry industry with novel means to comply with European law. The directive 2160/2003, which took effect in November 2010, required a complete absence of *Salmonella* in 25 grams of fresh poultry meat. In the laboratory a six component bacteriophage cocktail showed efficiency against all *Salmonella* strains relevant in poultry, both in liquid and on plates. *In vitro* experiment with artificially contaminated meat surfaces showed that the treatment with the cocktail can eliminate up to 1000 *Salmonella* cells per square centimeter. A small scale trial with 200 birds confirmed that the addition of the bacteriophage cocktail to the chicken diet can keep birds *Salmonella*-free. However, large scale trials in commercial sheds with 40 000 chicken showed, that converting a bacteriophage cocktail into a commercial product is not trivial and should be considered as a separate step in the product development, requiring extra efforts and considerations.

Characterization of a Twort-like *Myoviridae* phage used for phage therapy

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Staphylococcus aureus is a commensal and pathogenic bacterium that causes infections in humans and animals as well as food poisoning. Currently the antibiotic resistance of this species poses a threat to public health, with the methicillin-resistant *S. aureus* (MRSA) being a major cause of nosocomial infections worldwide. The emergence of multidrug resistance results in difficulties in eradication of the pathogen with the use of conventional therapies. In this respect staphylococcal myoviruses with broad host range have been demonstrated to be excellent candidates for phage therapy, an alternative to conventional antibiotic treatment.

Here we present the characterization of bacteriophage fRu-Sau02 that we isolated from a commercially available Staphylococcus Bacteriophage cocktail produced by MicroGen. The genomic analysis revealed that fRu-Sau02 is very closely related to phage MSA6 and to some other Twort-like viruses, namely A5W, Staph1N, and Fi200W. Bacteriophage fRu-Sau02 possesses a large genome (148,464 bp) with typical modular organization and a low G+C (30.22%) content and therefore can be classified as a new virus among the genus *Twortlikevirus*.

Altogether 236 predicted genes were identified from the phage genome; four predicted genes were interrupted by different insertion sequences. Altogether 78 different structural and virion-associated proteins were identified from purified phage particles by LC-MS/MS. The host range of fRu-Sau02 was tested with 135 strains, including 51 human *S. aureus* isolates, 54 *S. aureus* isolates of pig origin and 30 coagulase-negative *Staphylococcus* strains of human origin. fRu-Sau02 was able to infect 49 (96%) coagulase-positive and 15 (50%) coagulase-negative strains of human origin, whereas the rate of infection of pig isolates was much lower with only 18 (33%) strains infected. Importantly, none of the *S. aureus* human isolates was fully resistant to fRu-Sau02 and it showed infectivity against such species as *S. intermedius, S. lugdunensis, S. epidermidis, S. haemolyticus, S. saprophyticus* and *S. pseudointer.* We conclude that phage fRu-Sau02, a successful phage therapy agent in Russia, would also serve human phage therapy excellently in Finland. However, it shows limited usefulness for the prevention and control in the animal husbandry and food industry.

Modelling the dynamics of bacteria, phage, and innate immunity in acute respiratory infections

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The rise of antibiotic resistance in pathogenic bacteria has led to renewed interest in the use of phage as a treatment for bacterial infections [1]. However, there is a lack of understanding of the mechanism(s) that make phage effective as therapeutic agents. For example, mathematical models show that combining phage and bacteria often leads to coexistence of phage and bacteria. Therefore, a potential resolution to the tension between the clinical aims of phage therapy and models of phage-bacteria interactions is the hypothesis that phage works synergistically with host immunity to eliminate pathogenic bacteria. To study the effects of host immune contribution to the success of phage therapy, we recently adapted a mathematical model of phage therapy [2] to *in vivo* experiments.

The experiments measure dynamics of bacterial burden in a murine model of acute respiratory infection by *Pseudomonas aeruginosa* in both immunocompetent and immunodeficient hosts. Our mathematical model accounts for the interactions between pathogenic bacteria, phage and innate immunity of the mammalian host. We find that phage adsorption rate measured *in vitro* grossly overestimates the phage killing rate *in vivo*, likely a result of spatial heterogeneity of the lungs or saturation of phage infection at high densities. We show that these effects can be modeled by phage lysis rates that depend nonlinearly on phage densities. Our model predicts that deficiency in innate immune activation is detrimental to the efficacy of phage therapy. In addition, the model shows that synergy between phage and neutrophils is necessary for effective phage therapy. Both predictions are in accordance with observed phage therapy efficacies in murine models of pneumonia. This phage-immune synergy is caused by the phage reducing bacterial densities to levels manageable by host immunity, as well as host immunity preventing the outgrowth of phage-resistant bacteria. We also investigate the level of host immunity required to achieve phage-immune synergy, and show that phage therapy may still work in hosts with limited immunodeficiency even with emerging phage resistance.

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Sensors going viral

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Due to its simplicity and stability, the filamentous M13 bacteriophage has been a widely used as part of the synthetic biology toolkit. It is a powerful, programmable bionanomaterial with multiple nanotechnology applications. Our group has focused on chemically modifying the M13 to use it as a scaffold for novel biosensing methods. These approaches exploit the M13 structural features – a long, thin molecule that can be aligned under flow. Using this principle, two technologies were designed – a diagnostic platform harnessing linear dichroism (LD) spectroscopy, and a microscopy-based method for studying cardiovascular phenomena (*e.g.* atherosclerosis).

The diagnostic assay is based on a modified version of the M13 phage that is capable of binding to a target of interest (*e.g.* pathogenic bacteria). When the target pathogen is present in a sample, this will interfere with the phage alignment giving a spectroscopic signal that allows for the detection and quantification of the target. The reader for the assay is a small, handheld device that is easy to use and can give results in less than 10 minutes. This is a versatile tool that can be utilized in a wide range of sectors including medical, environmental, defense and security, veterinary, food and agriculture.

For the microscopy-based application, we constructed a nanosensor that can detect variations in flow intensity, envisioning studying blood circulation in heart diseases. The M13 phage was decorated with a fluorescent dye and anchored to a surface, and its dynamic behavior was visualized on a microscope. This proof-of-concept demonstrated that this system can be adapted for *in vivo* studies, giving exciting results that show shear stress in the micro-domains of endothelium cells.

Activity of bacteriophages against clinical *Salmonella* strains isolated in Armenia and Georgia

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Salmonella is recognized as a major food-borne pathogen worldwide, which rapidly becomes resistant to antibiotics and presents a serious threat to global public health. Therefore, characterization of clinically problematic non-typhoid *Salmonella* strains, investigation their drug-resistance profiles, genetic particularity, and learning possibilities of bacteriophage therapy against MDR *Salmonella* strains as an alternative of antibiotics is of a great interest.

Sixty two clinical isolates of non-typhoid *Salmonella* isolated in 2012-2016 from patients with salmonellosis in Armenia (37) and Georgia (25) were studied. The isolates were provided by the “Nork" Clinical Hospital of Infectious Diseases (Yerevan, Armenia) and the Referral Laboratory at the Republican Clinical Hospital of Infectious Diseases (Tbilisi, Georgia). Only the samples obtained from the patient prior to the start of medication were chosen.

The isolates were identified at the clinical settings by standard biochemical procedures and plating on the selective media.

Serotyping of isolates were performed by testing agglutination with polyvalent antisera for flagellar (H) and lipopolysaccharide (O) antigens. Antimicrobial (AM) susceptibility of isolates was tested against 12 AMs agents, belonging to 9 different classes. The SOPs were performed in accordance with the CLSI guidelines for standard disk diffusion assays. The phage susceptibility test was performed using spot-test method. For this purpose 19 *Salmonella* specific phage lines isolated from the sewage and environmental water samples in 2012-1016 were used. Eleven isolates out of 62 showed resistance to all phage clones. Therefore, all 62 strains underwent additional identification using MADI-TOF analysis. The results approved that none of the phage-resistant strains belonged to *Salmonella* spp., which correlates with the phage-typing results and indicates the effectiveness of phages for diagnostic purposes.

Two phage clones out of 19: Ns7 and BS, appeared to have the best strain coverage (94.12%), but different host profiles. In particular, a wide range of MDR phenotypes demonstrated susceptibility to phage BS, while the drug-sensitive strains appeared to be susceptible to phage Ns7 to? The phage- and antibiotic- resistance profiles showed a week correlation (PCC =0.2). An explanation of these results may be possible after sequencing and annotation of the strains and phages which is planned in the nearest future.

A scalable continuous ultrafiltration process for phage purification

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There is increasing interest in utilising the potential of bacteriophages, not only for phage therapy but also for other applications such as biocontrol, using phage immobilised in packaging films to control foodborne pathogens and prevent food spoilage [1-2], and or as a sensing element for low cost diagnostics. Bacteriophages are capable of selectively killing pathogenic bacteria, even antibiotic resistant ones. Phages, as novel antimicrobials, are a logical alternative approach to antibiotics [3]. The manufacture of phages is still carried out using poorly scalable techniques; this is particularly the case for downstream purification of phages. Crude lysates requiring purification contain live bacterial cells, lysed cell debris, media components, cellular proteins and DNA as well as secondary metabolites. Scalable and economic manufacture of bacteriophages requires the development of robust separation and purification strategies. Development of a scalable manufacturing platform for phages for therapeutic purposes would involve a fermentation step for amplification, followed by separation and purification steps with the required final purity determined by the regulatory requirements [4].

Achieving high volumes of concentrated (i.e. high phage titres) and purified product is the ultimate goal and main challenge for using phages as therapeutics. Previously published studies on purification of phages [5–7] have dealt with small volumes suitable for laboratory scale usage. Here we present a comparative study on a scalable purification and concentration step using ultrafiltration for downstream phage processing. A comparison between dead-end stirred cell filtration (SC) and continuous cross-flow filtration (CFF) is presented and discussed.

During dead-end filtration, membrane fouling is unavoidable at high Peclet numbers (Pe), which is quite common of such systems, resulting in a significant decrease in permeate flux rates. Shear induced surface regeneration is the most common method to reduce membrane fouling, however, this imposes a high shear stress that damages the tailed phages and results in loss of their titre. The focus of this work is on phage purification using ultrafiltration as an alternative to the dead-end filtration. Particular attention is dedicated to the effect of the key processing parameters such as transmembrane pressure, membrane cut-off, buffer exchange etc. on phage purity, yield and production output.

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Use of bacteriophages for control of AHPND associated *Vibrio parahaemolyticus* strains

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In the last years a new and highly dangerous disease known as Early Mortality Syndrome (EMS) or Acute Hepatopancreatic Necrosis Disease (AHPND) has appeared and caused devastating effects to shrimp farming worldwide. This infectious disease is caused by bacteria of genus Vibrio and there is no available or known cure for it so far. As a safety and selective alternative, we explored the use of lytic bacteriophages as means for control of its causative agent, V. parahaemolyticus-AHPND (VPAHPND). The effect of a cocktail of two phages (T2A2 and VH5e) on the survival ratio and bacterial concentration was evaluated using the Artemia model. The results show that there was a significant reduction in CFU numbers of VPAHPND when treated with phages compared to control groups. Also an increase in phages numbers (PFU) was observed at the end of the experiment. As a consequence, Artemia nauplii infected with VPAHPND that received phage treatment showed a significantly higher survival ratios than control groups. Untreated groups were characterized by high mortalities which confirmed the virulence of VPAHPND strains. The results suggest that the use of a cocktail of phages can be a viable alternative for the treatment and control of the AHPND disease in shrimp. The phage cocktail showed good potential using Artemia model, however more studies and especially on shrimp should be performed to further validate the viability of this promising alternative.

Comparative analysis of antimicrobial resistance gene packaging frequencies of staphylococcal Twort-like viruses propagated on various host strains

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The drug resistant *Staphylococcus aureus* strains cause a variety of life threatening diseases in hospitals and community. The emergence of antibiotic-resistant bacterial strains increased interest in bacteriophage-based therapy as a promising alternative to antibiotic treatment. Approval of phage-based therapeutics for clinical settings requires detailed understanding of molecular mechanisms of the phage impact to bacterial cells and to the macroorganism, which is facilitated by recent advances in basic and applied research. A large number of polyvalent bacteriophages infecting clinically relevant strains of the genus *Staphylococcus* and employed for phage therapy belong to the genus Twort-like (family *Myoviridae*, subfamily *Spounavirinae*, genus *Kayvirus*). These phages were previously evaluated with regard to the host range, the absence of genes for toxins, safety, but their abilities to package antimicrobial genes were not yet evaluated.

In this work we carried out a comparative analysis of bacterial antimicrobial resistance gene packaging frequencies of three bacteriophages isolated from commercially available preparations STAFAL® (Bohemia Pharmaceuticals, Czech Republic), Pyo-Phage (G. Eliava Institute of Bacteriophages, Microbiology and Virology, Georgia), Staphylococcal Bacteriophage (Microgen, Russia) and of the well characterized bacteriophage K. The transducing siphophage 80alpha was used as a positive control. Phages were propagated on archaic MRSA strain COL, strain USA300, and strain RN4220 as prophage-less and methicilin-susceptible control strain. Real-time PCR method targeting plasmid genes *tetK* and *blaZ* and chromosomal gene *mecA* was used to prove the ability of phages to package bacterial genes into virions. The frequencies of packaging were compared using statistical methods and data were related to various phages, different genes and host strains. Genes located on plasmids were packaged with higher frequencies than chromosomal genes. No difference in the packaging frequency depending on the propagation strain was observed. The results confirmed that the polyvalent staphylococcal myophages package bacterial genes at significantly lower frequencies compared to siphophages and thus are safe for phage therapy.

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A Phase 1 study to evaluate the safety, tolerability and preliminary effectiveness of AB-SA01 in patients with chronic rhinosinusitis associated with *Staphylococcus aureus* infection

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Chronic rhinosinusitis (CRS) is a debilitating inflammatory and infection based condition, affecting up to 9%, 10% and 12.5% of the adult population in Australia, Europe and the United States respectively. Currently available therapies to treat this condition include steroids, antibiotics and surgical intervention. Unfortunately, there remains a cohort of patients that are resistant to both medical and surgical interventions who experience persistent CRS symptoms, termed recalcitrant CRS (rCRS). *Staphylococcus aureus* infections are associated with rCRS and the emergence of multidrug resistant *S. aureus* stresses the need for the development of new antimicrobial therapies. Bacteriophage (Phage) therapy has gained significant interest in recent years as an alternative to antibiotics. Thus, the safety and preliminary effectiveness of a novel bacteriophage-based treatment, AB-SA01, was evaluated in rCRS patient.

Three patient cohorts (n=3 patients/ cohort) were dosed with twice daily (i) lower concentration phage for 7 days; (ii) lower concentration phage for 14 days; and (iii) higher concentration phage for 14 days. Safety observations included vital signs, physical examinations, clinical laboratory tests and adverse event (AEs) reporting. Preliminary efficacy was assessed comparing pre- and post-treatment microbiology results, endoscopic Lund Kennedy Scores (LKS) and symptom scores using Visual Analogue Scale (VAS) and Sino-Nasal Outcome Test (SNOT-22).

AB-SA01 was safe and well tolerated when administered to participants and preliminary efficacy data were suggestive of proof-of-concept. Results support decreased bacterial load following treatment, and improvements in endoscopic findings and symptoms in participant-reported outcomes. A phase 2a, multicenter, randomized, double-blind, parallel-group, placebo-controlled study to evaluate the safety and efficacy of AB-SA01 is currently being planned.

Very active broad-range lytic bacteriophages kill Staphylococcus aureus local field strains in Argentina

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Staphylococcus aureus is a very successful opportunistic pathogen capable of causing a variety of diseases ranging from mild skin infections to life-threatening sepsis, meningitis and pneumonia. Its ability to display numerous virulence mechanisms matches its skill to display resistance to several antibiotics, including β-lactams, underscoring the fact that new anti-*S. aureus* drugs are urgently required. In this scenario, the utilization of lytic bacteriophages that kill bacteria in a genus -or even species- specific way, has become an attractive field of study. In this study, we report the isolation, characterization and sequencing of phages capable of killing *S. aureus* including methicillin resistant (MRSA) and multi-drug resistant *S. aureus* local strains from environmental, animal and human origin. Genome sequencing and bio-informatics analysis showed a very similar conserved gene organization in the three cases, as well as the absence of genes encoding virulence factors, toxins or antibiotic resistance determinants. Of note, there was a high similarity between our set of phages to others described in the literature such as phage K. However, we found that they lacked introns previuosly described as present in some genes while contained introns in genes for which no introns have previously been described. Considering that our phages and the phages reported in the literature were obtained in different continents, it seems plausible that there is a commonality of genetic features that are needed for optimum, broad host range anti-staphylococcal activity of these related phages. Importantly, the high activity and broad host range on a large number of local S.aureus strains of one of our phages underscores its promising value to control the presence of *S. aureus* in fomites, industry and hospital environments and eventually on animal and human skin. The development of a cocktail of the reported lytic phages active against *S. aureus* –currently under way for the first time in this country- is thus, a sensible strategy against this pathogen.

Genome analysis of 32 lytic bacteriophages active against *Shigella* pathogens

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Bacillary dysentery, or shigellosis, is a severe diarrheal disease with global spread. The rapid dissemination of multidrug resistance among *Shigella* pathogens is becoming a more serious public health problem that demands the urgent development of alternative antibacterial agents such as bacteriophages (phages).

Though phages have been used for prophylaxis and therapy of shigellosis in the USSR and Former Soviet countries for decades, there are currently no licensed phage therapeutics available in the West. Previously WRAIR isolated and partially characterized thirty-two therapeutic phage candidates active against *Shigella* pathogens. Host ranges of individual phages and 3-phage mixes tested on 97 global clinical isolates of *Shigella* showed significant variation. An optimal 3-phage cocktail was active against 90% of strains including *Shigella flexneri* (15 serotypes), *Shigella dysenteriae* (two serotypes) and *Shigella sonnei* (Sergueev *et al.,* manuscript in preparation).

The objective of this effort was to analyze genomes of these 32 *Shigella* phages in depth. The genomes were sequenced using the Illumina MiSeq desktop sequencer (Illumina Inc., CA, USA) with 600-cycle v3 reagents. Sequencing libraries were prepared with the KAPA HyperPlus PCR-free NGS kit (KAPA Biosystems, MA, USA) containing 550bp inserts. Paired end sequencing reads were assembled using Btrim, FLASH 1.2.6, Newbler 2.7, and genomes were finished with Geneious 8.0.2. Genome sequences were clustered using Gegenees_v2.2.1, SplitsTree4, Gepard 1.30, Phamerator, and the get_homologues.pl Perl script. Protein sequences were annotated with HMMER 3.1b1 and the vPOG database (downloaded December 22, 2016), as well as dplyr, ggplot2, tidyr, and Phyre2. The 32 phages were shown to belong to seven clusters, T4-, T7-, T3-, Felix O1-, pSF-1-, Dev2- and vB_EcoM_JS09-like, with genome sizes varying from 38,928 to 170,746 nt and containing from 31 to 231 predicted coding sequences. The largest cluster is represented by T4-like phages with the broadest host ranges toward *Shigella* strains, while T3- and T7-like phages have narrower host ranges but the highest lytic activities. Phages ESh19-22 that belong to the Felix O1-like group are likely able to mobilize bacterial genetic elements and thus are not suitable candidates for phage therapy.

Synergistic interaction between phage therapy and antibiotics clears *Pseudomonas aeruginosa* infection in endocarditis and reduces virulence

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The continuing development of antibiotic resistance stresses the need for alternative treatment. Infective endocarditis due to *P. aeruginosa* is an archetype of highly lethal valve infection in human. Therefore we used in vitro and in vivo models of *P. aeruginosa* experimental endocarditis (fibrin clots and rats with catheter-induced aortic vegetations, respectively) to study the efficacy of an anti-pseudomonas phage cocktail and ciprofloxacin administered alone or in combination.

In fibrin clots, phage therapy decreased bacterial density by 6 log10 CFU/g in 6h. Bacterial regrowth due to phage resistance was observed after 24h, but was prevented by addition of ciprofloxacin (2x MIC). In rats, phage therapy alone decreased vegetation bacterial density by 2.5 log10 CFU/g after 6h ($P<0.001$), compared to 2.2 log10 CFU/g with ciprofloxacin ($P<0.05$). Moreover, combining phages with ciprofloxacin appeared highly synergistic with a >6 log10 CFU/g decrease in 6h, and successful treatment in 64% (7/11) of the animals. Phage-induced killing correlated with in situ phage multiplication - as also confirmed by histology and transmission electron microscopy examination of the vegetations – and cytokines production compared to antibiotherapy alone. Importantly, no phage-resistant mutants were detected in vivo, which was most likely due to altered fitness. Indeed, two phage-resistant clones isolated in vitro were 50-70% less infective in rats with experimental endocarditis ($P<0.01$ versus parent strain). This infectivity decrease was either due to a 362 kb deletion encompassing the *galU* gene, resulting in impaired LPS synthesis, or a 15 bp deletion in the *pilT* gene resulting in impaired motility (Oechslin et al. The Journal of infectious diseases, 2016).

In conclusion, phage therapy significantly reduced *P. aeruginosa* experimental endocarditis and was highly synergistic with ciprofloxacin. Phage-resistant mutants selected in vitro resulted in impaired infectivity, due to reduced in vivo fitness. Phage therapy alone or combined with antibiotics represents a promising alternative in the treatment of *P. aeruginosa* infections and merits further consideration (Stratton. The Journal of infectious diseases, 2016).

Inhibition of foreign microflora growth during *in vitro* plant propagation with lytic bacteriophages

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Micropropagation is a method of plant multiplying with allow rising new plants in an artificial medium. Nowadays this method is widely used in propagation and conservation of many plants. Microbial contamination is a constant problem, which often compromise development of all *in vitro* techniques. Control of microbial contaminants mainly relies on application of bactericidal compounds and antibiotics that often display phytotoxic effects.

Due to limitation of chemical methods of contaminant eradication, new environmentally friendly approaches need to be developed. Bacteriophages as specific bacterial viruses comprising potent agents capable to inhibit microorganisms during plant micropropagation. The aim of our work was to investigate the influence of bacteriophages on the development of *Pseudomonas siringae* during micropropagation of tomato plants.

Specific bacteriophage capable of lysing *P. siringae* pv. tomato was isolated from plant material, purified and amplified. The morphological features of the selected bacteriophages were studied using electron microscopy. *In vitro* tomato seedlings were produced on MS medium from mature seeds after surface sterilization. To imitate bacterial infection aseptic plantlets were dipped into bacterial suspension and then transferred on culture medium. The bacteriophages were added into culture medium before its solidification and applied on the surface of the medium immediately prior to planting explants.

As a result, in control flaks the microbial contamination at the base of the explants and on culture medium was observed within 7 days after inoculation. Application of bacteriophages completely inhibited pathogenic bacteria and promoted the plant survival under experimental condition. Thus, our results revealed the possibility of bacteriophage employment to control microbial contaminants during plant micropropagation.

The antimicrobial Activity of Bacteriophages against *Acinetobacter baumannii*

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Acinetobacter baumannii; a recalcitrant infectious agent is the top most health threat worldwide. It is a well-known potential nosocomial pathogen, increasingly being isolated in critically ill patients especially in ICUs. It is one of the most communal source of nosocomial acquired ventilator associated pneumonia, bacteremia, urinary tract infections and wound infections. It is one of the difficult to treat pathogen and exhibit resistance to wide numbers of antibiotics. Increasing antibiotic resistance and dry pipeline for new antibiotics has revitalized the traditionally neglected property of phages to kill the bacteria. Present study aimed to add up our efforts in search of promising lytic phage against *Acinetobacter baumannii.* Firstly, antibiotic susceptibility profile of 32 *Acinetobacter baumannii* isolates was carried out by Kirby-Bauer disc diffusion method in accordance to CLSI guidelines 2015. Eighty-seven percent of the isolates were MDR while XDR contribute 60% of isolates. It is unfortunate that only polymyxin b and colistin is susceptible in 100% of cases but they have unavoidable nephrotoxicity and neurotoxicity. All other tested antibiotics were sensitive in less than 30% of isolates. Three different bacteriophages were isolated from three different sewage samples against environmental isolate of *Acinetobacter baumannii* and named as; TAC1, TAC2 and JAC5. Their stability at various pH and temperatures were assessed by double layer agar technique. Phages TAC1 and TAC2 revealed optimum activity at pH 7, while phage JAC5 was more active at pH 8. However, marked decline in titer was observed at pH 5 and 9. All phages survived well after 1-hour treatment at different temperatures however, marked decline in titer was observed after treatment at 60^{o}C in phage TAC1 and JAC5 excluding TAC2. All phages were remained stable when stored at -80, -20 and 4^{o}C for one-month storage. All isolated bacteriophage showed narrow host range and infected only *Acinetobacter baumannii.* Bacterial growth reduction assay revealed TAC1 as very promising phage as it controlled growth for entire 24-hours at MOI 1 and 100 in comparison of control TAC2 was more active at MOI 100 and controlled bacterial growth for 10 hours while JAC5 phage was effective at only MOI 1000 and controlled the growth for only 6 hours. Growth controlling ability of all isolated phages in comparison of control produced statistically significant results. Gel electrophoresis revealed that all isolated phages have ds DNA genome with a size more than 12kb. Further study of such promising phages can further enlighten the concept of phage therapy.

Evaluation of a new bacteriophage cocktail for the treatment of *Acinetobacter baumannii* associated experimental bacteremia

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Bacterio(phages), i.e. specific bacterial viruses, are therapeutically interesting since they are able to kill multidrug resistant (MDR) pathogens. Therefore, phage therapy is currently reconsidered as a realistic alternative for patients in therapeutic dead-ends. *Acinetobacter baumannii* is a member of the ESKAPE family grouping main pathogens responsible for nosocomial infections worldwide. Especially, MDR *A. baumannii* (MDRAB) is an emerging pathogen in the intensive care setting, which is largely explained by its capacity to easily acquire foreign genetic material and survive for long periods on surfaces. In an effort to develop a phage cocktail with potential therapeutic application, 17 different *A. baumannii* phages were isolated from environmental samples. Phages from the Myoviridae, Podoviridae and Siphoviridae families harboured genomes between 41.2 to 193.3 kb in length. From full genome sequencing, four phages were temperate and two lytic phages harboured antibiotic resistance determinants preventing them to be included into the cocktail. A cocktail of two lytic phages, vB_MAba18 and vB_SAbaSLO, was found to cover 65% of a collection of 170 *A. baumannii* strains with no significant extent when other phages were considered. Strong antibacterial activity against the MDRAB clinical isolate Ab20 was observed *in vitro* for both phages alone or in cocktail. Accordingly, a single i.p. injection of vB_MAba18 (MOI 0.3) 2h post-infection (10^9 CFU/mouse) significantly improved mouse survival (ca. 80% survival at day 6 versus ca. 20% at day 2 for non-treated animals, $p<0.0001$). A similar protocol failed to show significant survival improvement with vB_SAbaSLO (20% survival at day 6). Combination of both phages decreased significantly the efficacy of the vB_MAba18 alone (ca. 50% survival at day 6). This observation suggesting negative interaction between both phages is under detailed investigation in a model of infection in Galleria mellonella before going back to mice and further efforts are performed to isolate new phages that could increase the coverage of the cocktail. Altogether, this work is contributing to the development of *A. baumannii* phages in particular and phages in general as antibacterial agents.

Characterization of chitosan and *Salmonella* phages biocompatibility as a biomaterial to be used in protective and edible films

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Introduction: *Salmonella* is a widely distributed zoonotic pathogen that is mostly transmitted to humans as foodborne. At the global level, the serovar that produces the largest number of outbreaks is *S.* Enteritidis. Different countries have approved the use of phages to be considered safe as food additive. Usually been suggested that phages can be added by dipping or spraying or as a liquid to a large volume of food. These methods may not be adequate, as they could be wasteful and lead to the potential inactivation of the phage cocktail. Then, the selection of a phage immobilization method depends on the nature and type of application of the bioactive material. Chitosan, is a natural and biodegradable biopolymer, which as antimicrobial activity. Even though chitosan is used to encapsulate phages, biocompatibility between chitosan and bacteriophages has not been described, accurately.

Purpose: The aim of this study was to evaluate the biocompatibility of chitosan and phages by the generation of chitosan-phages films that reduces effect against *S. Enteritidis*.

Methods: A purified stock of a cocktail of 5 wide host range phages, amplified on *S. Enteritidis*, was obtained using PEG precipitation and successive centrifugation and filtration passages. Medium molecular weight chitosan with 75-85% degree of deacetylation and 200-800 cps of viscosity was used. Different mixtures of phage and chitosan concentration at 1% and 2% were performed. Were tested phage viability using the spot test on *Salmonella Enteritidis* host. Subsequently, 1% and 2% chitosan films immobilized on surface were dried at 30ºC by the solvent evaporation technique. The assays were performed in duplicate considering a negative control (no phage added).

Results: All concentrations of chitosan (0.5-1 and 2%) facilitated the growth of Salmonella Enteritidis phage. The performance of chitosan films was successful and demonstrated that the chitosan and phages mixture is effective to reduce phage (MOI 1 approximately), In the model Salmonella Enteritidis.

Significance: This easy-way method for producing biofilm of chitosan could be representing a key strategy to control or use as suitable method for leading a potential inactivation of *Salmonella* on food production.

Basic characterization of two newly isolated bacteriophages infecting *Staphylococcus aureus*

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Staphylococcus aureus is commensal and opportunistic pathogen colonizing humans and animals, but it is also a major causative agent of hospital-associated infections. Antibiotic resistant strains have emerged as a significant threat in hospital and community environment. A method of choice in curing such infections might be phage therapy.

In this work we describe two newly isolated phages infecting *S. aureus*. Phages were isolated from samples of waste water originating from Brno and České Budějovice. Both phages were propagated on prophage-less *S. aureus* strain and showed clear plaques. Using PCR (Pantůček, *et al.* 2004) the phages were defined as members of the *Myoviridae* family, Twort-like, belonging to serological group D. The morphology of bacteriophages was determined by negative-staining and electron microscopy. Genomic DNA of the phages was digested with restriction endonucleases and compared to restriction patterns of previously characterized Twort-like phages (K, 812, G1 and A5W). Size of the phage genomes was estimated according to restriction profiles. Virion proteins of the newly isolated phages were compared using SDS-PAGE.

We analyzed the lytic host range and lytic ability of both phages and phage 812 using spot tests of a collection of laboratory strains and clinical isolates including antibiotic resistant strains. Lytic ability of the newly isolated phages was compared to the polyvalent staphylococcal phage 812, which is known for its wide host range. Both new phages proved to be promising candidates for the therapeutic use. Our aim is to create a phage cocktail for curing staphylococcal infections in humans and animals.

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Bacteriophage encapsulation for gastrointestinal tract delivery

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Potential applications of bacteriophages for therapeutic use are nowadays receiving increasing attention, as phages reveal valuable properties: Phages are i) ubiquitous in the environment, ii) can be easily isolated and propagated and iii) have narrow host ranges. Therefore, they may be suitable means to control and minimize undesirable bacteria. Phages have previously been used to control bacterial diseases, and more recently the application of phages in food processing to reduce pathogens and spoilage bacteria has been approved.

The human gut contains about 10^{15} individual phage particles, and the gut phage populations of individuals vary significantly in terms of phage biodiversity and phage titers. Various studies have proposed a correlation between the gut phagenome, human health and diseases. Nevertheless, the targeted application of phages in the human gastrointestinal tract faces numerous challenges, i.e., their limited host ranges, bacterial resistances to phages, manufacturing issues, delivery systems and sensitivity to gastrointestinal conditions.

Our hypothesis is that phages that are specifically integrated into food matrixes may shape and modulate the microbiota associated with the human gastrointestinal tract. Hence, a preliminary research study aimed at encapsulating phages to improve their viability under gastrointestinal acid conditions and, hence, delivering them to the intestine in an active form. *Lactococcus lactis* phages were selected as a simple model system. The effects of different encapsulation techniques, gastrointestinal pH and enzymes were investigated in *in vitro* experiments simulating human digestive conditions. The data obtained showed that encapsulated phages have a significantly higher survival rate in acidic stomach conditions compared to free phages. Furthermore, a fast release of phages from the capsules was achieved in simulated intestine conditions. In a subsequent study, the stability of encapsulated and non-encapsulated phages will be analyzed in a dynamic *in vitro* gastrointestinal model simulating conditions of the human upper gastrointestinal tract. First results of this study will be presented and discussed.

Investigation into the genomic structure and bactericidal effect of phages active against *Propionibacterium acnes*

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Acne vulgaris, an often underestimated disease, ranges from a self-limiting condition to something more devastating and chronic leaving behind physical and emotional scars on sufferers. The current research into acne implicates *P. acnes* as playing a major role in the pathogenesis of the disease, furthermore, a range of infections in surgical implants and the prostate show *P. acnes* as a severe pathogen. Current treatments for acne, and the use of antibiotics for *P. acnes* infections, show variable levels of success, with adverse side effects, the rise of antibiotic resistance and the difficulties of clearing biofilm related infections evident. Typing methods have allowed some clarification of the differences between strains involved in pathogenic infection compared to background commensal strains. The possibility of using bacteriophage to treat bacterial infections is an exciting prospect that shows promising preliminary results, particularly in addressing antibiotic resistant infections. Phage therapy is not without complications and requires a careful approach considering the problems of pseudolysogenic phage. Utilising endolysins from phage lysis mechanisms may prove to be an effective treatment that is adaptable (in the form of artilysins) to suit a range of functions.

P. acnes strains were characterised by RAPD and MLST and SEM imaging was used to visualise biofilm growth on the surface of plastics and hydroxyapatite (a material commonly used in surgical implants).

Bacteriophage B1, a virulent Siphoviridae phage, is lytically active against ATCC6919 and nearly all isolated *P. acnes strains*. The 29 kb phage genome was fully sequenced using Sanger sequencing which provided a scaffold genome for high coverage data produced using next-generation nanopore technology. 45 putative genes were identified and explored using bioinformatic analysis. Structural proteins were identified and compared to proteins from phage with similar morphological and genetic identity. The lytic cassette, consisting of a holin and endolysin, and DNA regulatory genes were all identified.

This work supports the application of bacteriophage and phage derived products in their application to *P. acnes* infections and explores the use of new technologies to further develop this concept.

The Efficacy of Microaerosol of Bacteriophages for Decontamination of different surfaces

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Introduction: The development of alternative anti microbial remedies has become one of the highest priorities of modern medicine and biotechnology. One of such alternatives might be bacteriophages. Recently, volumetric decontamination of confined environment, such as hospitals, transportation units, and public buildings raised substantial interest because it allows to simultaneously decontaminating pathogens, including in hardly accessible sites, and pathogens resided on inanimate surfaces within confined environments, that is impossible with conventional manual protocols widely accepted and practicing today.

Aim: Our aim was exploration of bacteriophage-containing microaerosol for reduction of high load of *Salmonella* cells on inanimate surfaces. In this work we limitedly explored volumetric disinfection of *S. typhimurium* cells inoculated on hard surfaces and on the surfaces of rich organic medium, using microaerosol containing *Salmonella* phage.

Materials and Methods: Fresh *S. typhimurium* cells were inoculated on a surface of nutrition medium and also, were inoculated and predried on microscopic glass. All coupons were placed to enclosed plastic chamber and microaerosol composed of *Salmonella* bacteriophage *Sal.phi18* was fumigated to the chamber using nebulizer attached to the chamber. Multiple controls were applied, such as cells survival on the coupons that had not been exposed to bacteriophage, fumigation of microaerosol not-containing phage, etc.

Results Discussion: The results of the trial illustrated above represent unambiguous evidence of the efficacy of the microaerosol composed of bacteriophage to reduce high load of *Salmonella* cells on glass surface and on rich nutrition medium below detection limit. Predictably, exposure of the coupons to microaerosol containing no phages had neither positive, nor negative effect to viability of the cells inoculated on glass surface and on nutrition medium.

Summary: Successful results of such microaerosol application presumably will expand bacteriophage application as environmentally safe disinfectants. It might be specifically effective to prevent cross-contamination with airborne pathogens, such as *M.tuberculosis*, *Legionella*, etc., and it might find an application for preventing airborne pathogens dissemination.

Bacteriophage Preparation to Prevent Intestinal Infections in Poultry

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Introduction: Poultry is known to be the main reservoir for food-borne pathogens. The main problem at the poultry farms is bacterial infections coused by salmonella, E.coli, St.aureus. In many cases, these bacterial agents characterize high resistance to antibiotics and sulfonamides. Existence of such pathogens is problematic not only for animal health, but also because of possible transmission of antibiotic resistant bacteria from animals to humans through the food supply. Recently, the legislation of Europe has established some restrictions on the use of antibiotics and sulfonamides in agriculture, including poultry.

Aim: The aim of our work aim was to study the effectiveness of polyvalent bacteriophage preparation –"Zoovetphage", as an alternative remedy to prevent intestinal infections in poultry. The preparation is produced on the base of Salmonella, pathogenic E. coli and St. aureus phages.

Materials and Methods: Experiments with broiler chickens were carried out at the poultry farm, near Tbilisi. In the first group, 50 one-day old broiler chickens were fed a combined food, free of antibiotics or other antibacterial preparations. Chickens in this group were treated "Zoovetphage" preparation added in water at days1-3, 11-13, and 25-27, a total of nine days. In the second group, 50 one-day old broiler chickens were fed daily with food containing antibiotic (Enrofloxacin).

Results: In the group treated with phage preparation, none of chicken had developed colibacteriosis or other infectious disease during the experiment. In the second antibiotic treated group, 8 chicken died (developed colibacteriosis). On day 40, all alive chicken of both groups have been euthanized. The gain of weight of chicken of phage treated group was 70-100 grams more than in antibiotic treated group.

Summary: Our experiment proves that the use of phage preparations in broiler chickens provides a healthy growth of the poultry throughout the cycle. Prevention of bacterial colonization of poultry at the farm level could minimize the risk of transmission and spread of bacterial infection in human.

Leading bacterial pathogens from three acute care hospitals in Georgia show high susceptibility to therapeutic phages

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Studies on the prevalence and cause of health care associated infections (HAI) in acute care hospitals, also susceptibility of related pathogens to antimicrobials is of high importance for development of proper strategy for elimination of these infections. Bacteriophages have been considered as efficient and safe tool for control of multidrug resistant bacterial infections.

The study was undertaken in three Georgian clinics: the respiratory department at the Central Children's Hospital (CCH) and Center for Thermal Injuries (CTI) in Tbilisi, also the multiprofile hospital (GMH) in the regional town Gori, with the aim to identify prevalent bacterial pathogens and to determine their antibiotic- and phage susceptibility profiles.

Up to 300 various clinical samples from patients and fomites were collected during 8 months period in 2016-2017. The obtained isolates were studied by conventional bacteriology and identified using specific API systems followed by PCR confirmation.

Out of total 340 bacterial isolates collected, 193 were Gram positive bacteria, with prevalence of *S.aureus* and coagulase –negative staphylococci (39.8%), while among Gram negative bacteria *P. aeruginosa* was the most frequently isolated (49.6 %), followed by *Klebsiella pneumoniae* and *K. oxytoca* (13.6%), and *Acinetobacter baumanii/calcoaceticus* (8.8%).

Higher bacterial diversity was revealed at the CCH, with frequent isolation of polymicrobial cultures. CTI and GMH populations were dominated by a couple of pathogens, showing characteristics of HAI infections. Leading bacterial pathogens were studied for susceptibility to antibiotics by disc diffusion method and were screened against commercial phages of "Eliava Biopreparations' and individual phages from the lab collection.

Majority of clinical isolates, especially *P. aeruginosa*, appeared to be multidrug resistant, with resistance patterns changing depending on the hospital. The phage susceptibility was varying by pathogen, with highest susceptibility for *S. aureus* (92.2% of strains) and *P. aeruginosa* (90.9 %), and slightly less for *Acinetobacter spp.* (81.8%) and Klebsiella *spp*. (80%). No correlation was observed between phage and antibiotic resistance.

The studies demonstrated high potential of therapeutic phages for elimination of bacterial infections in health cares settings. The necessity of enrichment of commercial preparations with newly isolated and characterized phages to extend their lytic spectrum was shown as well.

Evaluation of alternative animals models for testing the efficacy of phages as therapeutic agents

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Avian colibacillosis, induced by extraintestinal pathogenic *Escherichia coli* strains, is the major bacterial disease in poultry and is mainly treated by antibiotics. Increasing resistance to different antibiotic classes and restrictions on the use of antibiotics by the European Union led to the search for antibiotics alternative such as bacteriophage therapy. Before investigating the potency of phages to control avian colibacillosis in experimental model of reproduction of the disease in chicken, we evaluated alternative *in vivo* models more ethically acceptable. The use of insect models and embryonated eggs appears to be of interest, providing rapid and cost effective alternative to host models. Two insects' models (*Manduca sexta*, the tobacco hornworm and *Galleria mellonella*, larvae of the greater wax moth) and embryonated eggs have been evaluated.

First, we assessed in these 3 models the virulence potency of three well characterized avian *E. coli* strains: the virulent strains BEN5202 and BEN5217 and the non pathogenic strain BEN5048. *Manduca sexta* appears to be highly resistant to *E. coli* infection since no dead was observed when inoculated with the virulent strains at high dose (10^8 per caterpillar). BEN5202 and BEN5217 caused 80% and 70% of mortality in *Galleria mellonella* and 91.6 and 88.3% in embryonated models, respectively. However, the non pathogenic strain BEN5048 induced 60% mortality of *Galleria* while inducing no embryo mortality. Then, the efficacy of phage ESCO5, a myoviridae closely related to phAPEC8, has been evaluated. *Galleria mellonella* and embryonnated eggs were inoculated with strain BEN5202. Two hours post infection, ESCO5 phage was injected at 3 different MOI. Melanization or embryo death was monitored daily during 48h and 7 days, respectively. A decrease of melanization or embryo mortality has been observed in groups that received the phage. These observations can be correlated to the number of injected phages.

We thus showed that administration of phage ESCO5 has induced protection against infection in *Galleria mellonellla* and embryonated eggs models. However, embryonated eggs model is a more relevant model since the chicken embryo lethality assay has the capability to delineate virulent from non-virulent strains of *E. coli* at the opposite of *Galleria melonella* assay.

Multidrug resistant *K. pneumoniae* and its bacteriophages

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Understanding the mechanisms shaping the evolution of bacterial populations is paramount for any practical microbiology application from biocontrol, food and biofuel production to disease management and treatment. *Klebsiella pneumoniae* (*Kp*) is an important ubiquitous Gram-negative species capable of both causing disease in mammals and establishing mutually beneficial associations with plants [1]. The rise in recent decades of *Kp* resistant to multiple antibiotics (MDR) has resulted in the classification of this species as an urgent threat by health agencies worldwide and its recognition as an important antimicrobial resistance reservoir [2,3]. Antibiotics are heavily relied upon to treat *Kp* infections, though they are not recommended in the management of some forms of animal disease and, due to the rise in MDR strains, not always effective [1,2]. Alternative or adjuvant therapies to antibiotics are therefore urgently needed. We are currently exploring bacteriophage therapy and decontamination strategies against MDR *Kp* isolated in Australia from humans and animals and belonging to genetically diverse clonal lineages, including CG258. As part of this project, we have fully characterized the bacterial genomes of 20 target strains producing extended spectrum β-lactamases (NDM-1, KPC) and tested the infectivity of 20 novel bacteriophages selected from our extensive library. We found that the genetic diversity of the bacterial population was primarily associated with differences in cell surface structure components and this was reflected in the phage susceptibility profiles. These findings will allow for speedier identification and selection of a contained number of phages to be combined in a therapeutic mix targeting these specific pathogenic types.

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Optimisation of phage therapy against leek blight and black rot

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Every year, agriculture suffers from huge economic losses due to plant infectious diseases like leek blight in leek or black rot and leaf spot disease in cabbage.

These bacterial infections are caused by *Pseudomonas syringae* pv. *porri* (Pspo) and *Xanthomonas campestris* pv. *campestris* (Xcc), respectively. Until recently, infected crops were still treated with copper or antibiotics like streptomycin. However, since bacteria are becoming more and more resistant to these chemical treatments and because of their risk for nature, alternatives are necessary. One possibility would be phage therapy.

Therefore, we first isolated five phages KIL1 to KIL5 and six phages SoPhi1 to SoPhi6 against Pspo and Xcc, respectively. These phages were characterized both genomically and microbiologically (Rombouts *et al.*, 2016). Moreover, one host range mutant KIL3b was created, that infects 36 different isolates of Pspo. All phages were subsequently used in preliminary field phage therapy trials in leek and cabbage. A significant reduction (e.g. from 63% to 38.5% symptomatic leek plants) of the disease symptoms was observed after application of the cocktail, which is promising.

However, to be more effective, further research is needed. First of all, the application method has to be optimized (so far, only one method was tested) and the composition of the phage cocktails has to be further improved, especially for Xcc where the strains are very diverse. One other important shortcoming that makes phage therapy commercially less attractive is the fact that the identified phage cocktail often cannot be produced in an industrially applicable way. Therefore, we optimized the production of the phage cocktails by using an automated anion-exchange chromatography to purify the phages on CIM disks (BIA Separations). This resulted in a fast method for the purification which is also suitable for large scale production. Up to 80% recovery of the phages could be observed.

Intratracheal application of lytic phages against *Acinetobacter baumannii* in an *in vivo* mouse lung model

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Introduction: Chronic lung diseases are frequently complicated by lung and airway infections and the increase of multidrug-resistant (MDR) bacteria including *Acinetobacter baumannii* is alarming. The high specificity of bacteriophages for their bacterial hosts and their effectivity in lysis makes phage-therapy attractive for medicine.

However, phages need to be properly chosen and characterized according to scientific state-of-the-art technology. For safe application, the preparations must be highly purified and free of contamination. Processing methodology according to Good Manufacturing Practice (GMP) guidelines should route new approaches to foster clinical studies.

Objectives: In anticipation of a future clinical trial applying aerosolized lytic phages against gram-negative bacteria in patients with chronic airway infection, this preclinical pilot study aims at determining the efficacy, safety and tolerability of a GMP-like phage preparation.

Materials & Methods: Mice were transnasally infected with *A. baumannii* and 12h p.i. treated with specific phages or solvent intratracheally. 24h after infection bacterial burden in lung, bronchoalveolar lavage fluid (BALF), blood and spleen was analyzed. Leukocytes in blood, lung and BALF were differentiated and clinical parameters measured. Furthermore, phage titers were quantified in plasma, BALF, and lung. The used bacterial strain was highly pathogenic and resistant to various antibiotics. The specific phage Acibel004, a myovirus, was produced as high-titer suspension subjected to final GMP-like processing including column affinity chromatography.

Results: The optimal infection dose of *A. baumannii* inducing a clinically apparent pneumonia within 24h p.i. was determined. Endotoxin levels were reduced from crude lysate phage suspensions at a scale of approximately 100,000 EU/ml by several affinity column cycles. Bacterial burden in lung and BALF was reduced by trend after phage treatment 24h p.i.. Phages were detected in BALF, lung and plasma of the infected and treated mice. Phage treatment did not affect the number of leukocytes in BALF, lung and blood, but treated mice recovered faster from infection-associated decrease in body temperature.

Conclusion: A clinically relevant model of *A. baumannii* pneumonia was established and phages for intratracheal application were produced under GMP-like conditions. This preclinical study generated data enabling for the design of a Proof of Concept study for clinical use of phages.

Efficacy and safety of a *Staphylococcus aureus* phage cocktail in a murine model of bovine mastitis

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The aim of this study was to evaluate the efficacy of a purified phage cocktail for treatment of bovine *Staphylococcus aureus* mastitis in a well-defined mouse model. Candidate phages were selected based on their performance in a series of *in vitro* assays designed to test various phage characteristics. Phages were tested individually and as cocktails to determine the most optimal cocktail composition, a process referred to herein as phage scoring. The phage score incorporates quantitative measures of killing efficiency in broth, time to resistance, host range, and other factors predictive of a successful cocktail. The highest scoring phages were further tested for efficacy and resistance suppression in broth and raw milk with and without supplemental IgG, and significant decreases in CFU were observed. Based on these *in vitro* results the cocktail was then purified for testing in the *in vivo* model.

Mammary glands were experimentally infected with *S. aureus* N305 (ATCC 29740), a clinical bovine mastitis isolate that has been widely used for experimental infection of dairy cows and mice. Lactating mice mammary glands were inoculated by intramammary infusion with *S. aureus* and the phage cocktail treatment was applied via the same route four hours post infection. Treated mammary glands were graded for gross pathological appearance and excised for bacterial load (CFU) and phage load (PFU) quantification, and were compared to both sham- or antibiotic-treated (the current standard of care) infected glands and non-infected controls.

Observation of clinical and gross macroscopic changes as well as quantification of CFU demonstrated that the phage cocktail treatment significantly improved gross macroscopic clinical observations, and significantly decreased intramammary bacterial loads. PFU indicated that the tested phage cocktail treatment was able to maintain high local phage titers in the mammary gland without spreading systemically. Further analyses (such as inflammatory cytokine profiling and histopathology) will document the safety and efficacy of this candidate novel curative phage cocktail mastitis treatment in the elegant preclinical model. These results present promising data for phage therapy as an alternative to antibiotics for the treatment of bovine mastitis.

The first two and the last two authors contributed equally to this work.

Vibriophage endolysins as a bacterial control agent

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Vibriosis is a set of diseases of high incidence in the aquaculture sector, responsible for massive mortalities and economic losses worldwide. Currently there are severe restrictions on the use of antibiotics, so lytic enzymes encoded by bacteriophages could be an effective and safe antibacterial alternative to reduce the risks in this industry. In this study the recombinant endolysin of a marine vibriophage was obtained, and its lytic activity against different species of the genus *Vibrio* was evaluated. The enzyme produced has a lytic effect against virulent strains of *V. parahaemolyticus*, *V. alginolyticus*, *V. harveyi*, *V. campbelli*, and other non-Vibrio Gram-negative bacteria. Its spectrum does not include probiotic Gram-positive strains used in the white shrimp culture, its maximum lytic activity is around pH 6 and retains its lytic activity after being exposed at 50 °C for 30 min. These findings show that it would be feasible to use it in aquaculture due its broad-spectrum against various *Vibrio* strains and their inability to destroy probiotic bacteria frequently applied in aquaculture production. The next step will be to evaluate their ability to prevent or control vibriosis under in vivo conditions.

Phage-induced bacteriolysis is not more inflammatory than antibiotics

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Background: Beside its efficacy, bacteriophage (phage)-therapy safety has not be fully addressed. In particular, as phages infectious cycle can last less than 10 minutes, the associated endotoxin release by lysed bacteria, and consequently the immune proinflammatory response, could be a matter of concern.

Methods: First, *in vitro*, two virulent phages of therapeutic value (one *Myoviridae* and one *Podoviridae*) and four reference antibiotics (amikacin, cefoxitin, ceftriaxone and imipenem) were studied for their ability to kill two pathogenic strains of *Escherichia coli* and generate a bacterial endotoxin release. The early interaction between these actors was assessed over time by studying the CFU count, the concentration of free endotoxin released and the cell morphology under light microscope. Second, we measured cytokines production in mice lungs during experimental pneumonia treated by either phages of antibiotics. Twenty cytokines and chemokines were quantified from lungs homogenates at two time points, 10 and 22 hours post-infection, by multiplex assay.

Results: *In vitro*, phages killed bacteria faster than betalactams. When considering the fastest phage the concentrations of phage-induced endotoxin release by bacteria never reached the highest values, which were recorded with antibiotic treatments. Cumulated concentrations of endotoxin over time in phage treated conditions were lower than those observed with betalactams. With the experimental model of pneumonia, we observed a higher level of cytokines in *E. coli* infected lungs compared to not infected controls. Treated mice, either by phage or antibiotic, had a lower level of cytokines than infected animals, which nevertheless stay higher than in not infected controls. No significant difference was observed in cytokines levels from mice treated by either phage or antibiotic, except for interferon gamma, which was lower in the phage treated groups at 10h.

Conclusions: This work provides important and comforting data regarding the safety of phage therapy. Therapeutically relevant phages, with their low endotoxin release profile, their fast bactericidal effect and their low cytokine release profile, are not inferior to betalactams.

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