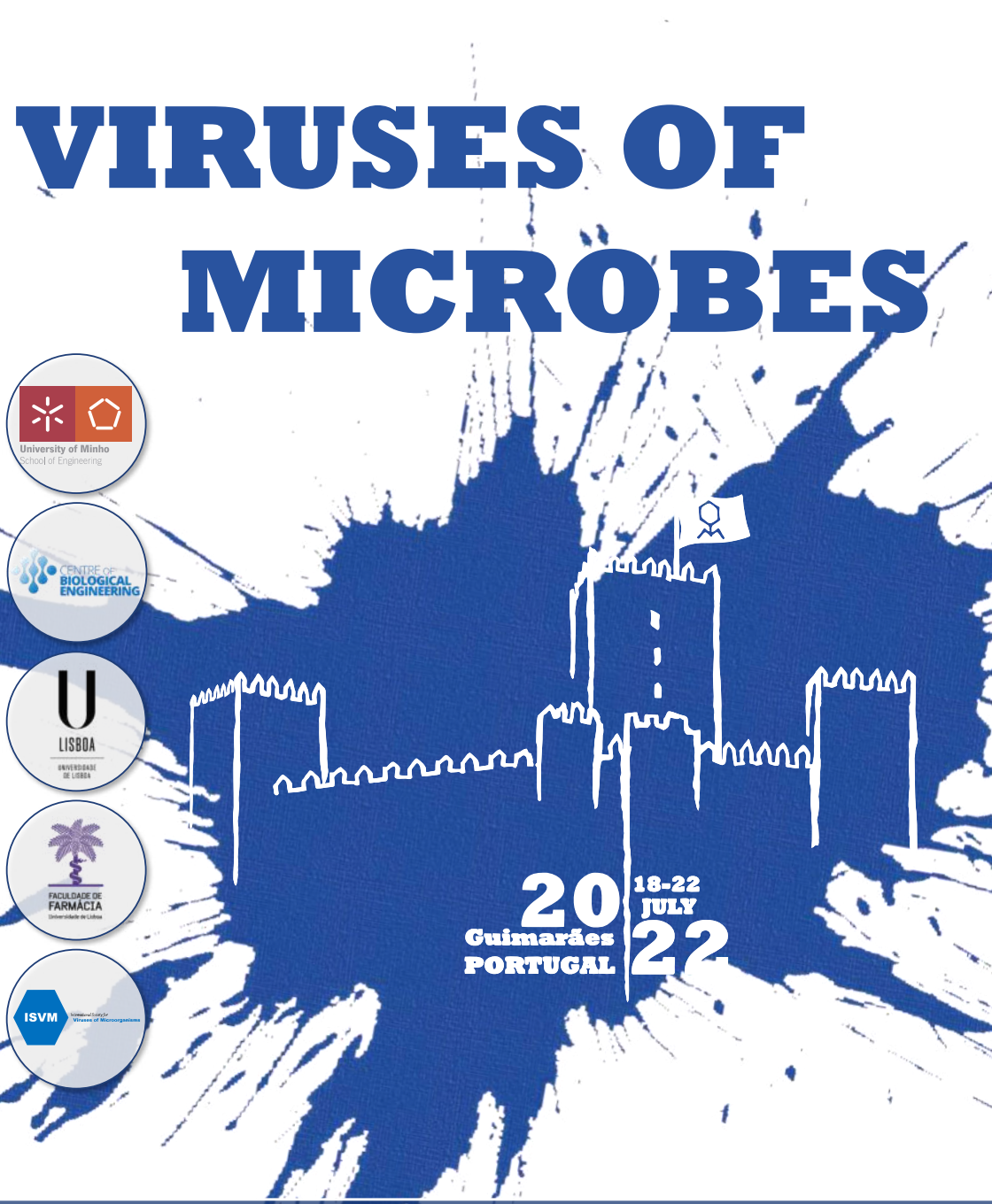
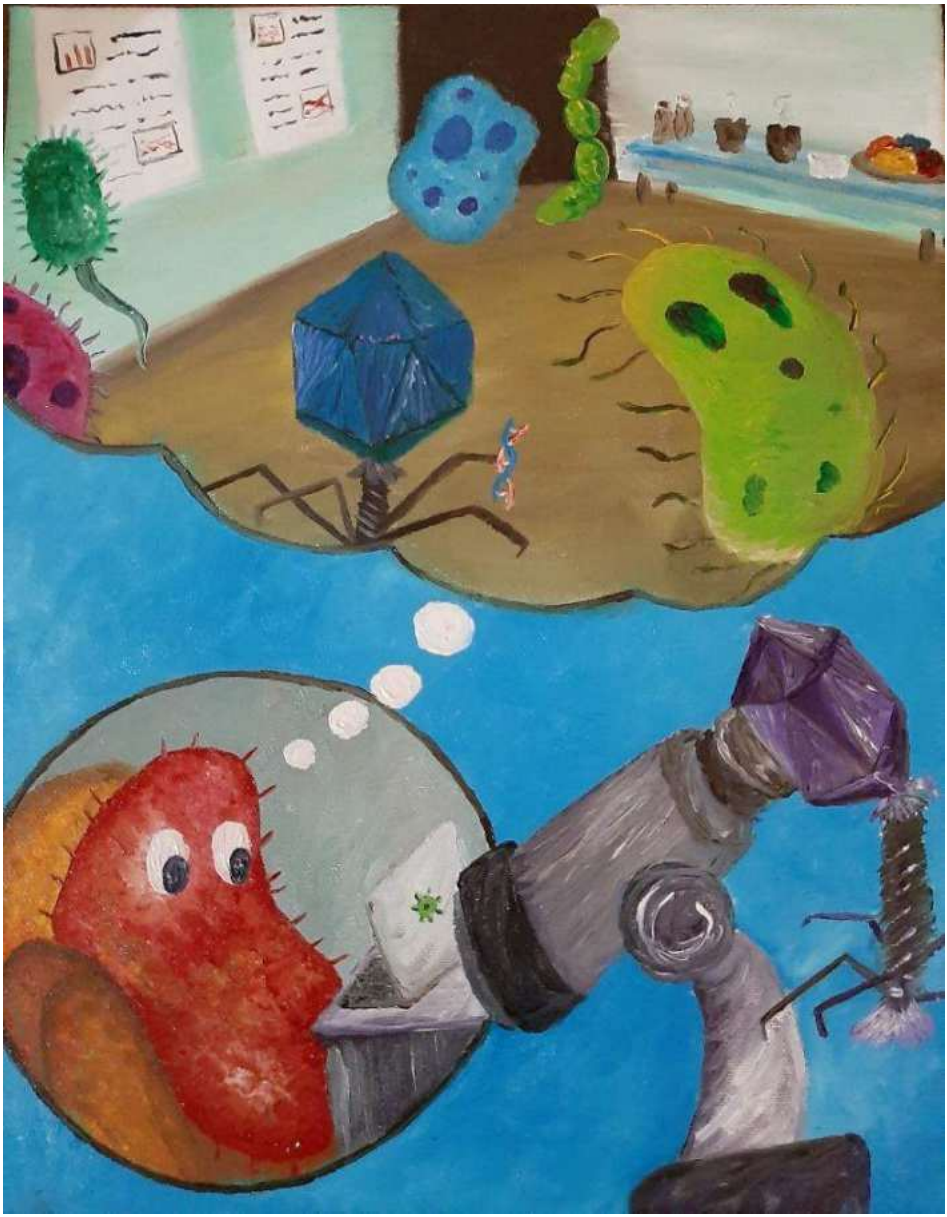
VIRUSES OF MICROBES



THE LATEST CONQUESTS

PROGRAM AND ABSTRACT BOOK



iVoM2020 winning drawing (by Carlos Lomelí-Ortega

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I. WELCOME MESSAGE

Welcome to Viruses of Microbes 2022!

We are very pleased to welcome you in Guimarães for a face-to-face meeting, particularly after two years of pandemic. The central theme of VoM 2022 is "The latest conquests on viruses of microbes". This theme is allusive to the enormous scientific advances, both in fundamental knowledge and biotechnological applications of viruses of microbes that we are recently witnessing, and at the same time makes a parallel to the city of Guimarães, a symbol of the Portuguese conquests.

The scientific program was planned to spotlight the most recent advances in ecology and evolution of microbial viruses, virus structures and function, virus-host interaction, agro-food, veterinary and environmental biotechnology applications and phage therapy. We thank the speakers and poster presenters for helping us in constructing this exciting and attractive program, covering fundamental and applied aspects of virus exploitation.

We are deeply grateful to the organizers and to the scientific committee for accepting the challenge to join us in this adventure and for all the support to make VoM2022 an in-person meeting. We are also grateful to the International Society of Viruses of Microbes (ISVM) and Phage Directory for their valuable support.

We would like to address a warm welcome to our 700 participants that enthusiastically registered to our event, reinforcing the importance of viruses of microbes' science.

We wish you a pleasant stay in Guimarães!

Joana & Madalena

II. ORGANIZING COMMITTEE

Organizers



Joana Azeredo



Madalena Pimentel

Co-organizers



Carlos São-José



Krystyna Dabrowska



Rob Lavigne



Laurent Debarbieux



Ana Oliveira



Hugo Oliveira



Ivone M. Martins



Luís Melo



Diana Priscila Pires



Sílvio B. Santos

III. SCIENTIFIC COMMITTEE

Andrew Kropinski (University of Guelph, Canada)

Ana Oliveira (University of Minho, Portugal)

Carlos São-José (University of Lisbon, Portugal)

Curtis Suttle (The University of British Columbia, Canada)

David Prangishvili (Institute Pasteur, France)

Diana Priscila Pires (University of Minho, Portugal)

Douwe van Sinderen (University College Cork, Ireland)

Hugo Oliveira (University of Minho, Portugal)

Ivone M. Martins (University of Minho, Portugal)

Joana Azeredo (University of Minho, Portugal)

Jeremy Barr (Monash University, Australia)

Krystyna Dabrowska (Polish Academy of Sciences, Poland)

Laurent Debarbieux (Institute Pasteur, France)

Luís Melo (University of Minho, Portugal)

Madalena Pimentel (University of Lisbon, Portugal)

Martha Clokie (University of Leicester, United Kingdom)

Martin Loessner (ETH Zurich, Switzerland)

Peter Fineran (The University of Otago, New Zeland)

Pilar Garcia (Spanish National Research Council, Spain)

Rob Lavigne (KU Leuven, Belgium)

Ruth-Anne Sandaa (University of Bergen, Norway)

Sílvio Santos (University of Minho, Portugal)

Zuzanna Drulis-Kawa (University of Wroclaw, Poland)

IV. PARTNERS





















ADEIM

Associação para o Desenvolvimento do Ensino e Investigação da Microbiologia









V. SPONSPORS

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1. GENERAL INFORMATION

Venue

The Vila Flor Cultural Center (CCVF) is a cultural facility located on Avenida D. Afonso Henriques, in Guimarães. The complex was completed in September 2005 and was born from the restoration of the Vila Flor Palace and surrounding spaces. Guimarães is one of Portugal's UNESCO World Heritage cities. This city of medieval origin is also known to be the cradle of the nation. With its roots in the remote 10th century, the history of Guimarães is closely associated with the establishment of the Portuguese identity and the Portuguese language in the 12th century.





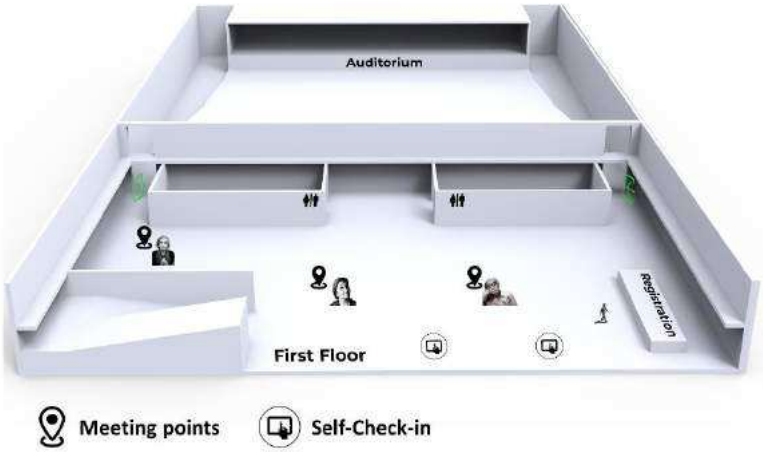
Vila Flor Cultural Centre
Av. Dom Afonso Henriques 701, 4810-431 Guimarães, Portugal



Venue map

Registration Desk

The registration desk is located in the first floor of the Grand Auditorium. Reception is open from 12:30 to 17:30 (18th July), 8:00 to 18:00 (19th-21st July) and from 8:00 am to 17:00 (22th July). To fasten the check-in process, self-check-in points are available close to the registration desk for all participants. Participants have the possibility to register at the registration desk or using the self-check-in points, with the exception of grantees that need to register at the desk.



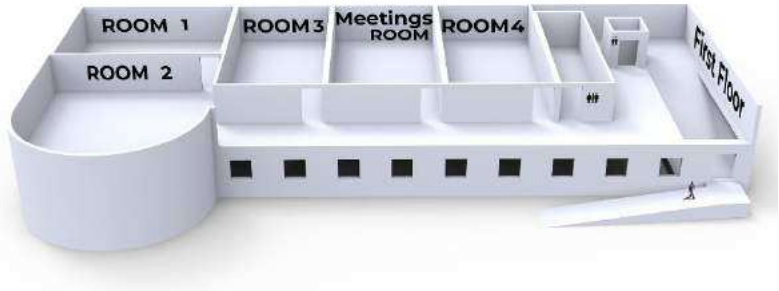
First floor of the Grand Auditorium

Oral & Poster presentations

Slide presentations of talks must be handed in an USB memory disk to a member of the staff the day before (except on Monday, in which the presentations should be delivered before the session). Presentation files should have the speaker's name.

Posters will be displayed in different locations of the venue, and their presentation will be distributed by 3 poster sessions (3 different days). Please refer to the poster ID to see where and when it will be presented. As an example, poster ID **EcoE.012(PS3-R4.05)** stands for ThematicArea.PosterNo(PosterSession-Room.BoardAffixationPosition), that is, a poster on the Thematic Area **Ecology and evolution of microbial viruses**, Poster No **12**, (Poster Session **3** – Room **4**, Board Affixation Position **05**). For more details about the location of the posters

at the venue, please check the abstract poster communications section. For poster award eligibility, it is required that presenting authors are near their posters and available for discussion during the corresponding poster session.



Poster rooms 1-4 and Meetings room at the first floor of the Palace



Poster room 5 on the floor -1 of the Small Auditorium

Awards

With the support of our partners and sponsors, VoM2022 will be awarding 1 best oral (500 euros) and 8 best poster communications (250 euros, for each of the 7 thematic areas plus a best phage therapy poster). Please visit our website for more information: https://vom2022.org/awards.php

Lunch & coffee-breaks

Lunch and coffee breaks will be outdoors and are included in the congress fee. Please see location on the “Venue map” above.

Internet access

A Wi-Fi connection will be available in the auditoriums and Palace. To have access to the internet you have to choose the SSID: CCVF_EVENTOS and the password: VOM2022#

Certificates of attendance

Certificates for participants will be sent automatically by email after the conference.

Social media

Follow us in Facebook, Instagram, Twitter and YouTube!

Facebook: @VirusesOfMicrob2022

Instagram: @VirusesOfMic22

Twitter: @VoM_2022

YouTube: www.youtube.com/channel/UCGOi4OtFWaRG_giXYa23mXQ

Special issue and Research Topic

Viruses - "Viruses of Microbes 2022"

https://www.mdpi.com/journal/viruses/special_issues

Frontier in Microbiology - "The Latest Conquests of Viruses of Microbes"

https://www.frontiersin.org/journals/microbiology/research-topics

Public transports

Participants will have a reduced fee in the urban transport GuimaBus. It is mandatory to show your badge at the bus entrance. Please note that face masks are also mandatory inside public transports.

Touristic information

Participants have free access to the following museums: "*Casa da Memória de Guimarães*" (https://www.casadamemoria.pt/) and, "*Centro Internacional de Artes José de Guimarães – Plataforma das Artes*" (https://www.ciajg.pt). Participants have also free access to the Guimaraes cable car (*Teleférico de Guimarães,* https://turipenha.pt/media/videos/Teleferico.mp4) to visit the beautiful Penha hilltop at 617 meters. **It is mandatory to show your badge at the entrance**. During the Conference week the City of Guimarães offer a rich cultural agenda with street exhibitions of performing arts, please visit the cultural agenda (https://em.guimaraes.pt).

APP

There is a mobile App for the conference available under the name VOM2022, in Google Play Store or Apple App Store. The VOM2022 App allows the users to be informed about the scientific and social programs, as well as facilitate networking by setting personal meetings. There are

available different meeting points named with Portuguese notable persons on the first and second floor of the Grand Auditorium. Most of the posters can also be viewed in the App. Participants will use the App to rate abstracts, upon which the best oral and poster communications will be selected. Important updates will also be sent through the VOM2022 App. Log in information to access the VOM2022 App was given by email a day before the event.

Emergency information

In case of emergency, dial 112 for general emergency assistance. Hospital (+351 253 540 330) and firefighters (+351 253 515 444).

Contact us

Our Staff is wearing blue T-shirts, so they are easy to identify. If you have any question or request during the meeting, please contact our staff. They are there to help you.

2. SOCIAL EVENTS

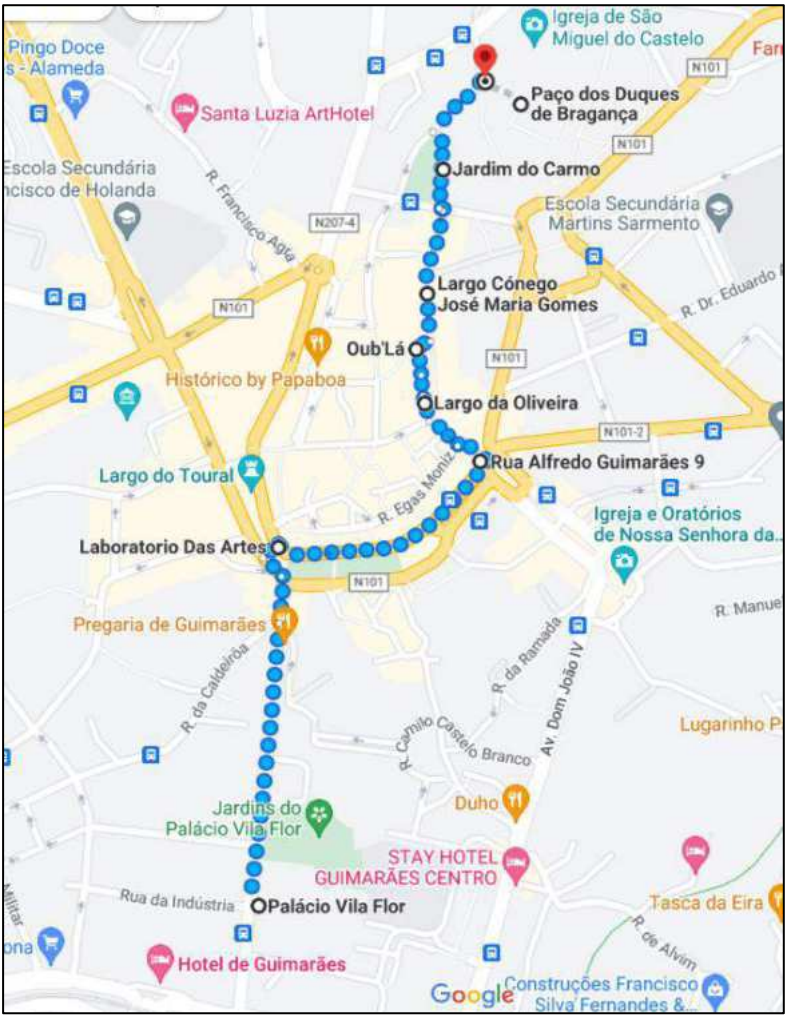
All conference participants are invited to participate in the social events free of charge (badge required!).

Opening Ceremony

The Opening Ceremony It will take place at the Grand Auditorium of Vila Flor Cultural Centre at 14:45 on the 18th of July. The opening ceremony will start with a musical moment played by "*Quarteto ao Luar*" a group of young musicians from the Conservatorium of Music of Guimarães and a Duet of ballet dancers (world cup finalists and Got talent finalists), from the Backstage *Escola de Dança e Artes Performativas*.

Welcome reception - Ducal Palace of Bragança

The Welcome Reception, with a welcome cocktail and a musical moment of traditional Portuguese Music interpreted by *Orfeão de Guimarães* will take place on the 18th of July (18:00 - 20:00) at The Ducal Palace of Bragança (*Paço dos Duques de Bragança*). Participants will be guided through the beautiful city of Guimarães, from the venue place (departure at 17:00) to The Ducal Palace (arrival at 18:00).



Map of the walking tour from *Centro Cultural Vila Flor* to the Ducal Palace (https://goo.gl/maps/7vFQtZnWNYxE7DWF6)

Conference Dinner – MitPenha

The conference Dinner on the 20th of July (20:00), will be held in MitPenha, a contemporary space, located at 607 meters high, on Penha's hill. It is embedded in the mountain, on a balanced integration with the surrounding ecosystem, enhancing the beauty of nature. MitPenha presents a privileged scenery, as it provides a panoramic view of the region, especially of the urban site. Buses will be available for transportation to and from MitPenha. All participants are invited to gather at MitPenha, for a group photo.

Wine tasting

On the 21st of July (19:00), a wine and local product tasting will take place at the venue.

3. SCIENTIFIC PROGRAM

Detailed Program

For an updated program please visit our website at: https://vom2022.org

Monday, 18th July		
12:30	**Registration**	
14:45	Welcome	
Bird's eye view		
Chairs: Joana Azeredo and Madalena Pimentel		
15:20	Corina Brussaard	Algal ecology from a virus's View
16:15	Sylvain Moineau	Sparrow's view on phage research
17:10	**Opening Reception**	
Tuesday, 19th July		
Session 1: Ecology and Evolution of microbial viruses I		
Chairs: Cristina Moraru and Corina Brussaard		
08:30	Curtis Suttle	Sequencing ribosomal RNA to infer taxon-specific lysis in microbial communities
08:55	Stineke van Houte	Evolutionary and ecological aspects of CRISPR-Cas – phage interactions
09:20	Matthias Fischer	Uncultured giant viruses in soil exhibit previously unseen capsid morphologies
09:35	Leonard Bäcker	Phage superinfection exclusion factors against host cell scarcity
09:50	Nikhil George	Virus-host interactions in a municipal landfill include non-specific viruses, hyper-targeting, and interviral conflicts
10:05	Pedro Leão	Asgard archaeal viruses
10:20	**Coffee Break**	
Session 2: Virus structure and function I		
Chairs: Douwe van Sinderen and Deborah Hinton		

10:50	Alfred Antson	DNA recognition and capsid expansion during virus assembly
11:15	Mart Krupovic	The secrets of unique lemon-shaped viruses of Archaea
11:40	Ian J. Molineux	Towards a complete molecular description of infection initiation by bacteriophage T7
11:55	Miroslav Homola	Mechanism of virion formation of the *Emiliania huxleyi* virus 201 enveloped by two membranes
12:10	Frank Oechslin	Implications of endolysin diversity on phage-host adaptation and evolution
12:25	**Lunch**	
13:30	**POSTERS SESSION I**	
Session 3: Agro-food, Veterinary and Environmental Biotechnology Applications I		
Chairs: Zuzanna Drulis-Kawa and Hugo Oliveira		
15:00	Jennifer Mahony	Cell wall polysaccharides - the gate-keepers of phage infection in *Streptococcus thermophilus*
15:25	Lars Fieseler	Infection of *Erwinia amylovora* by the phages S6 and M7 depends on bacterial cellulose
15:50	Cedric Woudstra	How to produce phage tail-like particles from phages
16:05	Kiandro Jeroen Fortuna	Phage biocontrol of rhizogenic *Agrobacterium*: solving the root of the problem
16:20	Carlos Soto	SMART design of a multi-receptor phage cocktail against *Salmonella* and impact of phage resistance on bacterial fitness
16:35	**Coffee Break and Group Photo**	
Session 4: Biotechnology applications in health care I		
Chairs: Jeremy Barr and Ivone Martins		
17:10	Paul Bollyky	Identification of Bacterial Pathogens in Sepsis Using Bacteriophage Present in Circulating Free DNA
17:35	Yves Briers	Phages playing molecular Lego
18:00	Luís Melo	Efficacy against dual-species biofilms using phage-antibiotics combinations is independent of the biofilm model
18:15	Christian Röhrig	Chimeric endolysins selectively kill *S. aureus* in vitro, on reconstituted human epidermis, and on murine skin

18:30	Anja Keller	Engineering of endolysins for the targeted treatment of localized *Staphylococcus aureus* infections
18:45	Roberto Vázquez	From biological data to new phage-based antimicrobials: the case of AMP-like regions in Gram-negative phage endolysins
19:00	**ISVM General Assembly**	
Wednesday, 20th July		
Session 5: Virus-host interaction: host defense and viral evasion mechanisms		
Chairs: Sylvain Moineau and Peter Fineran		
08:30	Karen L. Maxwel	Pseudomonas prophages exploit bacterial signalling to modulate phage defence
08:55	Rotem Sorek	New mechanisms of anti-phage defense
09:20	Gaëlle Hogrel	Cyclic nucleotides in bacterial immune response: a glue for deadly molecular assemblies
09:35	Witold Kot	Biological consequences of 7-deazaguanine hypermodifications in phage genomes
09:50	Leah M. Smith	Discrimination of phages and plasmids through inverse regulation of surface-based and CRISPR-Cas immune strategies
10:05	Alice Maestri	CRISPR and non-CRISPR defences in *Pseudomonas aeruginosa* act synergistically against phage infection
10:20	**Coffee Break**	
Session 6: Virus-host interaction: overcoming cell barriers		
Chairs: Yves Briers and Sílvio Santos		
10:50	Lone Brøndsted	Phage strategies for overcoming diverse cell barriers in *E. coli*
11:15	Zuzanna Drulis-Kawa	Phage-derived carbohydrate depolymerases - from structure to function and exploitation
11:40	Olaya Rendueles	Bitter sweet symphony: Coevolutionary dynamics and diversity of resistance mechanisms against a polylysogenic competitor
11:55	Hugo Oliveira	Understanding the complete reservoir of bacteriophage depolymerases against *A. baumannii* capsules
12:10	Yang Shen	The interplay between phages, *Listeria* and host cells

12:25	**Lunch**	
13:30	**POSTERS SESSION II**	
Session 7: Virus structures and function II		
Chairs: Mart Krupovic and Priscila Pires		
15:00	Deborah Hinton	The T4 MotB protein improves infection in various, unexpected ways
15:25	Douwe van Sinderen	Lactococcal phage-host interactions at a molecular level: a cheesy story
15:50	Mateo Seoane-Blanco	*Salmonella* virus Epsilon15 tailspike has multiple binding sites and two catalytic activities
16:05	Anders Nørgaard Sørensen	Diversity and evolution of tail spike proteins of *Ackermannviridae* phages
16:20	Shin-Yae Choi	Insertion tolerance regions for synthetic RNA phage engineering
16:35	**Coffee Break**	
Session 8: Ecology and evolution of microbial viruses II		
Chairs: Kelly Williams and Curtis Suttle		
17:10	Cristina Moraru	Using cultivations to discover new phage diversity in the marine environment
17:35	Valerian V. Dolja	Global metatranscriptome analysis reveals vast diversity of novel RNA viruses in protists and other eukaryotes
17:50	Cynthia Silveira	Marine viral infection networks revealed through proximity-ligation sequencing
18:05	Karin Holmfeldt	Seasonality and transcriptomic responses of the *Rheinheimera* phage-host system from the Baltic Sea
18:20	Yichang Zhang	The influence of early life exposures on the infant gut virome
18:35	Marianne De Paepe	Intestinal virome in Crohn's disease patients
20:00	**Conference Dinner and Group Photo**	
Thursday, 21st July		
Session 9: Virus-host interaction: molecular mechanisms I		
Chairs: Laurent Debarbieux and Luís Melo		
08:30	Julia Frunzke	Phage defense at the multicellular level – from small molecules to cellular development

08:55	Daniella Schatz	Ecological significance of extracellular vesicles in modulating host-virus interactions during algal blooms
09:20	Ombeline Rossier	Genetic dissection of host takeover by phage T5: developing tools for genome engineering of large virulent phages
09:35	Chaffringeon Lorenzo	The gut environment regulates bacterial gene expression which modulates susceptibility to bacteriophage infection
09:50	Julián Bulssico	Phage-Antibiotic Synergy for filamentation-inducing antibiotics
10:05	Hanne Hendrix	Exploring protein lysine acetylations during phage infection in *Pseudomonas aeruginosa*
10:20	**Coffee Break**	
Session 10: Agro-food, veterinary and environmental biotechnology application II		
Chairs: Martin Loessner and Ana Oliveira		
10:50	Mathias Middelboe	Phages select for non-virulent variants of *Flavobacterium* pathogens: implications of pathogen control in aquaculture
11:15	Sanna Sillankorva	Ultrasonic spray deposition of a bio-based coating loaded with the *Salmonella* phage Felix 01 on food contact materials
11:40	Graça Pinto	Unveiling the impact of STEC infecting phages on the colon microbiota using an in vitro fermentation model
11:55	Ewelina Wójcik	Multi-aspect safety assessment of bacteriophage products dedicated for animal health
12:10	Golam Shaharior Islam	Evaluation of spray-dried microencapsulated phage cocktail to control *Salmonella* carriage and shedding in weaned pigs
12:25	**Lunch**	
13:30	**POSTERS SESSION III**	
Session 11: Virus-host interactions		
Chairs: Karen Maxwel and José Penades		
15:00	Tessa Quax	Infection mechanism of haloarchaeal viruses
15:25	Stefanie Barbirz	A sweet key to bacteriophage infection: Polysaccharide interactions at Gram-negative

		bacterial surfaces and in extracellular glycan matrices
15:50	Véronique Ongenae	Reversible bacteriophage resistance by shedding the bacterial cell wall
16:05	Patrick Arthofer	Abortive infection and defensive symbiosis - novel defense strategies against giant viruses
16:20	Ana Gouveia	Unraveling bacterial determinants of tolerance to endolysin lytic action
16:35	**Coffee Break**	
17:10	**Andrew Kropinski Evelien & Adriaenssens**	**Workshop on Viral Genomics and Taxonomy - from isolate to GenBank and beyond**
Friday, 22nd July		
Session 12: Biotechnology applications in health care II		
Chairs: Krystyna Dabrowska and Martha Clokie		
08:30	Graham Hatfull	Discovery, dynamics, engineering, and the therapeutic opportunities for phage treatment of *Mycobacterium* infections
08:55	Jeremy Barr	Fundamentally applied phage biology
09:20	Sarah Djebara	Phage therapy: a Belgian experience
09:35	Alexandra von Strempel	Targeted design and manipulation of defined microbial consortia by bacteriophages
09:50	Luca Ulfo	Selective photokilling of colon cancer cells using a spheroid-penetrating phage retargeted to the fibronectin receptor
10:05	Kevin Champagne-Jorgensen	Phages promote polarization of M1 macrophages
10:20	**Coffee Break**	
Session 13: Round Table: Current state and latest developments of phage therapy		
Chair: Tobi Nagel		
10:50	Jean-Paul Pirnay, Patrick Soentjens, Ran Nir-Paz, Martha Clokie, Sofia Corte-Real	
12:25	**Career Award**	
13:00	**Lunch**	
Session 14: Virus-host interaction molecular mechanisms II		
Chairs: Lone Brøndsted and Carlos São-José		

14:30	José Penadés	Bacteriophages mobilise pathogenicity islands encoding immune systems as weapons to eradicate competitors
14:55	Alexander Hynes	Biasing bacteriophage behaviours
15:10	Edze Westra	Regulation of prophage induction and lysogenization by phage communication systems and the SOS response
15:25	Audrey Labarde	Compartmentalization of bacteriophage SPP1 replication and assembly in the Gram-positive bacterium *Bacillus subtilis*
15:40	Raphael Laurenceau	Diversity generating retroelements: from phage host-range evolution to targeted mutagenesis tools
15:55	John Chen	Dual pathogenicity island transfer by a novel form of lateral transduction
16:10	**Coffee Break**	
Session 15: New horizons, new conquests!		
Chairs: Julia Frunzke and Rob Lavigne		
16:25	David Bikard	Phages and their satellites encode hotspots of antiviral systems
16:50	Peter Fineran	Type III CRISPR-Cas provides resistance against nucleus-forming jumbo phages via abortive infection
17:15	Carlos São-José	On the occurrence and multimerization of two-polypeptide phage endolysins encoded in single genes
17:30	Samuel Kilcher	Enhancing bacteriophage therapeutics through in situ production and release of heterologous antimicrobial effectors
17:45	Katharina Höfer	How to connect RNAs with proteins? - RNAylation of proteins
18:00	Carina Büttner	Structural insight into giant virus infection of marine picoplankton
18:15	**Closing session and Awards**	

ORAL COMMUNICATIONS

ABSTRACTS

4. ORAL COMMUNICATIONS - ABSTRACTS

July 18

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Algal Ecology from a Virus's View 35

Sparrow's View on Phage Research 36

July 19

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How to produce phage tail-like particles from phages. 50

Phage biocontrol of rhizogenic *Agrobacterium*: solving the root of the problem. 51

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From biological data to new phage-based antimicrobials: the case of AMP-like regions in Gram-negative phage endolysins .58

July 20

Session 5: Host defence and viral evasion mechanisms

Pseudomonas prophages exploit bacterial signalling to modulate phage defence 59

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July 21

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The gut environment regulates bacterial gene expression which modulates susceptibility to bacteriophage infection 84

Phage-Antibiotic Synergy for filamentation-inducing antibiotics 85

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Phages select for non-virulent variants of *Flavobacterium* pathogens: Implications of pathogen control in aquaculture 87

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marine ecosystem | phytoplankton | Virus-host interactions

Algal Ecology from a Virus's View

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With more than 70% of the living biomass in the oceans being microbial, marine viruses have plenty of unicellular hosts to choose from. Forming the base of virtually every pelagic marine food web, key for taking up carbon dioxide (via photosynthesis) and responsible for half the oxygen production on Earth, phytoplankton are important microorganisms. Viruses for all taxonomic groups have been reported but a more detailed comprehension of their impact on population dynamics of the various phytoplankton under natural conditions, the related carbon cycling and hence the productivity of the ecosystem, is still limited.

Using methods developed to enumerate aquatic viruses and determine the host specific mortality rates in the field, we show that viral lysis is a highly important loss factor for marine microorganisms with average rates comparable to the more traditional losses by predation. We translate our findings to biogeochemical fluxes to understand how viral activity affects the mass balance differently than thus far thought and modeled. Dependent on their hosts' metabolism, viral production is influenced by environmental factors affecting host growth and viability. At the same time, environmental variables regulate viral abundance through particle decay and loss of infectivity. Considering the increasing pressure of global climate change on aquatic systems, it is timely to study also virus-host interactions under different environmental conditions. Well-controlled experimental studies using key virus-host model systems are used to unravel underlying mechanisms. During this presentation, I will illustrate the ecological importance of marine viruses and highlight some of the environmental controls on virus production and availability.

bacteria-phage interaction Bacteriophage Bacteriophage collection

Sparrow's View on Phage Research

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It is now well-recognized that phages can influence the balance of microbial ecosystems, including our microbiota. Phages are also excellent viral models applicable to various research areas, including as surrogates in aerovirology studies related to the ongoing pandemic. Phages can also be both friends and foes. Friends since they suppress bacterial populations with harmful properties, including pathogenic bacteria. Thus, phages can have industrial and medical applications as biocontrol agents. Foes because they can destroy bacteria that play key roles in fermentation and biotechnology processes. Understanding their biology, ecology and evolution is still of utmost importance to develop new defence strategies to control them in fermentation processes as well as to optimize their use as antibacterial agents in food safety and public health. Finally, the last decade has seen a heightened awareness of the value of collections of viruses for the conservation of genetic resources and biodiversity. Phage collection initiatives have increased lately but long-term support will be essential to maintain these infrastructures.

Extracellular rRNA | Taxon-specific lysis | Microbial mortality

Sequencing ribosomal RNA to to infer taxon-specific lysis in microbial communities

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Viruses of marine microbes are the most abundant biological entities in the oceans, where each day they are estimated to kill about 20% of the living material, by weight, and in the process shape microbial communities and drive biogeochemical cycles. Yet, despite their critical role in marine ecosystems, the relative amount of lysis experienced by different microbial taxa has remained unknown. By sequencing extracellular ribosomal RNA ($rRNA_{ext}$) produced by cell lysis we are able to quantify those taxa in which lysis has occurred. In ten seawater samples collected at five depths between the surface and 265 m during and following a phytoplankton bloom, lysis was detected in about 15% of 16,946 prokaryotic taxa, identified from amplicon sequence variants (ASVs), with lysis occurring in up to 34% of taxa within a water sample. The ratio of $rRNA_{ext}$ to cellular rRNA ($rRNA_{cell}$) was used as an index of taxon-specific lysis, and revealed that higher relative lysis was most commonly associated with copiotrophic bacteria that were in relatively low abundance, such as as members of the Bacteriodetes; whereas, relatively low lysis was more common in taxa that are often relatively abundant, such as members of the Pelagibacterales (i.e. SAR11 clade), cyanobacteria in the genus Synechococcus, and members of the phylum Thaumarchaeota. These results provide an explanation as to why highly productive bacteria that are readily isolated from seawater are often in low abundance. The ability to estimate taxon-specific cell lysis adds an important tool in our quest to explain the distribution and abundance of specific microbial taxa in nature, as well as allows how lysis in a specific taxon can be affected by environmental conditions.

CRISPR-Cas bacteria-phage interaction evolution coevolution anti-CRISPR

Evolutionary and ecological aspects of CRISPR-Cas – phage interactions

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Many bacteria rely on CRISPR-Cas immunity to defend themselves against infections with bacteriophages. I will share our latest insights on the (co-)evolutionary and ecological consequences of CRISPR-Cas immunity for both hosts and phages, and how this is shaped by phage-encoded counterdefenses.

Giant Viruses | viral diversity | cryo-electron microscopy | soil microbiology

Uncultured giant viruses in soil exhibit previously unseen capsid morphologies

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Giant viruses of protists reach bacterial dimensions in capsid size and genome complexity. Metagenomic surveys have revealed an unexpected degree of genetic diversity among giant viruses, yet based on the limited number of cultured virus-host systems, little is known about their biological properties.

Here, we use electron microscopy (EM) to survey the diversity of giant viruses from a different angle. We explore the shapes of giant virus-like particles (gVLPs) in forest soil using high-resolution negative-stain EM.

After enrichment for particles in the 0.2 - 1.2 μm size fraction we analysed more than 600 EM images and measured bacteriophages and gVLPs.

We found a diverse array of gVLPs in organic horizon soil, whereas diversity was noticeably lower in mineral horizon soil. Icosahedral capsids with a diameter of 300-500 nm were often decorated with surface modifications such as fibers, tubes, and unique vertex structures, including many previously unseen morphotypes.

Non-icosahedral, ovoid particles with features reminiscent of pandoraviruses were also present, although a clear viral affiliation was more difficult to determine in these cases.

Our results show that the diversity of giant virus morphotypes in forest soil is comparable to that of viral genomes, encouraging further studies to isolate and characterize novel virus-host systems in culture.

Phage infection | population dynamic | Phage-host interactions

Phage superinfection exclusion factors against host cell scarcity

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Temperate bacterial viruses spread through host populations using both horizontal and vertical transmission routes that are typically supported by lytic or lysogenic development at the cellular level. Upon entering a host cell, viral commitment to either lysis or lysogeny is often affected by cues relaying information on the surrounding virion-to-host cell ratio. For several temperate model systems studied, these cues favor lysogenic development when this ratio is high and available host cells become scarce. Through anomalies observed in *Salmonella* Typhimurium chemostat populations infected by temperate bacteriophage P22, we discovered that subpopulations of *S.* Typhimurium become lytically consumed despite high phage-to-host ratios that would normally favor lysogeny. We found that these subpopulations originate from the proliferation of P22-free siblings that spawn off from previously reported P22 carrier cells from which they cytoplasmically inherit P22-borne superinfection exclusion factors (SEFs). We demonstrate that the gradual dilution of these SEFs in the growing subpopulation of P22-free siblings limits the number of incoming phages, thereby imposing the (intracellular) perception of a low phage-to-host ratio that favors lytic development and boosts phage production. While SEFs have so far been regarded as protecting established lysogens from superinfection, our data indicate that they can spur complex infection dynamics in the face of host cell scarcity.

metagenomics bacteriophage Contaminated sites

Virus-host interactions in a municipal landfill include non-specific viruses, hyper-targeting, and interviral conflicts

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Viruses are the most abundant microbial guild on the planet and impact microbial community structure and ecosystem services. Viruses are underrepresented in databases and specifically understudied in engineered environments. Using metagenomics, we examined host-associated viruses from a municipal landfill across two years via CRISPR spacer to viral protospacer mapping. Approximately 46,000 viral elements were identified using VirSorter. 1,858 Metagenome-Assembled Genomes (MAGs) were identified using three automated algorithms (CONCOCT, MetaBAT2, and Maxbin2), DAS Tool for consensus-based binning, and CheckM for quality filtering. Viruses comprised ~5% of the assembled community DNA sequence. A total of 506 unique virus-host connections capture hyper-targeted viral populations and host CRISPR array adaptation over time, as well as viruses predicted to infect across multiple phyla, suggesting that some viruses may be far less host-specific than is currently understood. Hyper-targeted viral elements showed a relative depletion in Chi sites which we predict influences host CRISPR-Cas systems' disproportionate recruitment of spacers from these genetic elements. We detected 190 viral elements that encode CRISPR arrays, including the longest virally-encoded CRISPR array described to date, with 187 spacers. CRISPR-encoding proviruses integrated into host chromosomes were observed, as examples of CRISPR-immunity-based superinfection exclusion. Our networks highlight virus-host interactions that are rare or have not previously been described, but which influence the ecology of this dynamic engineered system. We intend to use these networks as a framework to understand how virus-induced host mortality may be influencing landfill processes such as nutrient cycling and contaminant degradation.

viral metagenomics Archaeal Virus environmental

Asgard Archaeal Viruses

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Asgard Archaea are proposed to be the closest descendants of the archaeal cell that gave rise to the first eukaryotic cells. Viruses have been predicted to play a role in the origin of eukaryotes through the viral eukaryogenesis (VE) hypothesis, which postulates that the eukaryotic nucleus originated from an ancient virus with replication machinery akin to nucleocytoplasmic large DNA viruses (NCLDVs). In this work, we characterize the genomes of 6 Asgard viruses capable of infecting representatives of Lokiarchaeota (Fenrir and Sköll viruses) and Helarchaeota (Nidhogg and Ratatoskr viruses) phyla. Asgard virus-host predictions were determined by matching CRISPR spacers from metagenome-assembled genomes (MAGs) to uncultivated virus genomes (UViGs) recovered from hydrothermal vent-associated sediments in Guaymas Basin. Initial genomic analyses of these viruses reveal a mix of prokaryotic and eukaryotic viral features, with predominantly *Caudovirales*-like viral hallmark proteins. 1-5% of Asgard viral proteins are similar to those in NCLDVs. These viruses are predicted to have the ability to perform semi-autonomous genome replication, repair, epigenetic modifications, and transcriptional regulation. Phylogenetic analysis of Asgard viruses DNA polymerase B reveals a diverse origin of the polymerase within this group.

In addition, we have developed a novel approach for the detection of Asgard proviruses that has identified 41 in Asgard genomes (13 complete). This expanded database of Asgard viruses will allow a more thorough exploration of the VE hypothesis, such as identifying Asgard virus NCLDV-like proteins involved in the segregation of transcription and translation. This work significantly expands our knowledge of Asgard viruses and thus will help elucidate the potential role of these viruses in the VE hypothesis as well as the evolutionary history of eukaryotes.

Bacteriophage structural biology capsid DNA packaging

DNA recognition and capsid expansion during virus assembly

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Tailed bacteriophages utilise sophisticated machinery to recognise, process and translocate their genomic DNA into preformed empty capsids. The small terminase is an essential part of this machine. This protein recognises viral genome and recruits large terminase for subsequent translocation of DNA into the capsid. Structures of small terminase proteins from different bacteriophages reveal similarities, but also certain differences, indicating potential mechanisms of DNA recognition.

To understand changes in capsid structure during virus assembly, we determined structure for the P23-45 bacteriophage capsid in immature and mature states. Cryo-EM reconstructions reveal conformational changes associated with capsid expansion. Capsomer interactions in the expanded capsid are multi-layered, being reinforced by formation of additional interactions in the mature state. Despite a two-fold increase in the internal volume of the mature capsid, its structural integrity is maintained at extreme temperatures.

Reconstructions of the procapsid and the expanded capsid defined the structure of the unique portal-containing vertex and indicated how translocation of DNA into the capsid could be modulated by a changing mode of protein–protein interactions between portal and capsid, across a symmetry mismatched interface. Structural data also indicated how portal protein may work as a one-way valve, naturally safeguarding virus against genome loss.

Archaeal viruses | virus evolution | major capsid protein

The secrets of unique lemon-shaped viruses of Archaea

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Over billions of years of evolution, viruses have ‘‘invented’’ capsids on multiple independent occasions from nonhomologous and structurally unrelated proteins. Nevertheless, the majority of known viruses package their genomes into icosahedral or filamentous helical protein capsids or nucleocapsids. Viruses infecting archaea notoriously deviate from this general paradigm by producing virions with unique, odd-shaped morphologies, which are not observed among bacterial or eukaryotic viruses. Such archaea-specific virion architectures resemble droplets, champagne bottles, or spindles. Viruses with spindle-shaped (or lemon-shaped) virions are particularly common in diverse extreme and moderate environments and infect a wide range of archaeal lineages. During my talk I will present our recent findings on the structural organization of lemon-shaped virions and discuss how this information explains the emergence of this unique virion morphology.

adsorption process cryo-EM DNA-ejection Phage T7

Towards a complete molecular description of infection initiation by bacteriophage T7

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T7 gene 7.3 deletion mutants fail to adsorb to cells. Our study of second-site revertants that partially restore infectivity has revealed intermediates of the molecular mechanism of T7 infection. Using single-particle cryo-EM analyses of both mature phage and infection intermediates we have elucidated how tail fibers rotate from a capsid-bound conformation to an extended state, revealed how the genome terminus is held by the portal in a conformation that allows the essential internal core proteins to be ejected into the infected cell, and show two of those proteins in a partially ejected state. We further delineate a DNA sequence motif in T7 that is bound by the portal as corresponding to the genome terminus. This motif is conserved in the Autographivirinae and some other cos-type phages

assembly Giant Viruses Emiliania huxleyi virus 86 cryo-EM

Mechanism of virion formation of the *Emiliania huxleyi* virus 201 enveloped by two membranes

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Emiliania huxleyi is a worldwide distributed unicellular marine alga whose cells are covered by calcite disks called coccoliths. By reflecting light, the coccoliths influence retention of heat in oceans, which impacts planetary climate [1]. *Emiliania huxleyi* virus 201 (EhV-201) and related nucleocytoplasmic large DNA viruses limit the population growth of *E. huxleyi* [2].

Virion of EhV-201 is pleiomorphic in shape, therefore we used localised reconstruction of small fractions of the virion edges to elucidate its complex ultrastructure, comprising an inner membrane, capsid, outer membrane, and surface protein envelope. Furthermore, we used focused ion beam milling and cryo-electron tomography to characterize the formation of EhV-201 virions in *E. huxleyi* cells. The particle assembly is initiated on membrane fragments, which separate from the endoplasmic reticulum. Assembly of the capsid proteins at the outer surface of the membrane fragment induces its bending and gradual formation of capsids containing a membrane sack. Virus DNA is packaged into the pre-formed particles through an opening in the capsid and inner membrane. The genome-filled intermediates bud into intracellular vesicles, and in this process, acquire the outer membrane and protein envelope. Virions are released from the cell by exocytosis or lysis of the infected alga. Our results give structural insight into the formation of EhV-201 – a pathogen that influences the Earth's climate.

endolysins | CRISPR-Cas9 genome edditing | phage-host adaptation | enzyme evolution | Lactococcus lactis

Implications of endolysin diversity on phage-host adaptation and evolution

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Endolysins are produced by (bacterio)phages to rapidly degrade the bacterial cell wall and release new viral particles. Despite sharing a common function, endolysins present in phages that infect a specific bacterial species can be highly diverse and vary in types, number, and organization of their catalytic and cell wall binding domains. While much is now known about the biochemistry of phage endolysins, far less is known about the implication of their diversity on phage-host adaptation and evolution.

Using CRISPR-Cas9 genome editing, we could genetically exchange a subset of different endolysin genes into distinct lactococcal phage genomes. Regardless of the type and biochemical properties of these endolysins, fitness costs associated to their genetic exchange were marginal if both recipient and donor phages were infecting the same bacterial strain, but gradually increased when taking place between phage that infect different strains or bacterial species. From an evolutionary perspective, we observed that endolysins could be naturally exchanged by homologous recombination between phages coinfecting a same bacterial strain. Furthermore, phage endolysins could adapt to their new phage/host environment by acquiring adaptative mutations in regions that interacts with peptidoglycan. Deletion of entire domains, variation in the length of the linker connecting them and modification of parts involved in the regulation of the enzymes was also observed.

These observations highlight the remarkable ability of phage lytic systems to recombine and adapt, and therefore explain their large diversity and mosaicism. It also indicates that evolution should be considered to act on functional modules rather than on bacteriophages themselves. Furthermore, the extensive degree of evolvability observed for phage endolysins offers new perspectives for their engineering as antimicrobial agents.

dairy fermentations | host interactions | Phage receptor

Cell wall polysaccharides- the gate-keepers of phage infection in *Streptococcus thermophilus*

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The dairy fermentation industry profoundly depends on reliable, technologically favourable and robust starter cultures. *Lactococcus lactis* and *Streptococcus thermophilus* strains are the most widely applied starter cultures in the dairy industry globally. One of the most significant and consistent threats to these fermentation processes is infection of these starter bacteria by bacterial viruses, called (bacterio)phages. Five groups of dairy streptococcal phages are currently described. Recently, the Rgp (rhamnose glucose polymer) and EPS (exopolysaccharide) components, which present saccharidic polymers around the *S. thermophilus* cell surface have been implicated in host recognition and binding by phages of this species. The gene clusters that underpin the biosynthesis of these structures have been compared and analysed using hierarchical clustering and functional annotation tools to establish the diversity and functionalities associated with the encoded dairy streptococcal Rgp structures. Furthermore, the chemical structures of a selection of strains representing distinct Rgp genotypes have been elucidated and the structural diversity correlates well with the genetic diversity within the Rgp gene clusters. We postulate that the diversity of Rgp structures accounts, at least in part, for the high degree of specificity of dairy streptococcal phages.

bacterial cellulose | Erwinia amylovora | phage encoded cellulase

Infection of *Erwinia amylovora* by the phages S6 and M7 depends on bacterial cellulose

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Erwinia amylovora is a member of the *Erwiniaceae* and the causative agent of fire blight, a severe disease of *Rosaceae* plants. To prevent infection, streptomycin is usually applied during the flowering period. However, in some countries use of streptomycin was banned due to the spread of antibiotic resistance. *E. amylovora* usually infects host blossoms via the stigma and invades the ovary. Later it spreads through the xylem vessels of an infected plant. In the xylem vessels *E. amylovora* produces high amounts of exopolysaccharides (EPS), e.g., a capsule, which leads to ooze formation and canker development. The capsule is composed of different carbohydrates such as amylovoran, levan, and cellulose, respectively.

As an alternative to antibiotics, the application of bacteriophages for fire blight control is a promising alternative. Transposon mutagenesis of *E. amylovora* revealed that adsorption of phage S6 (*Schitoviridae*) and M7 (*Myoviridae*) depends on the bacterial cellulose synthesis (*bcs*) operon. Deletion of the *bcs* operon or associated genes (*bcsA*, *bcsC*, and *bcsZ*) verified the crucial role of bacterial cellulose for S6 and M7 infection. Application of the cellulose binding dye Congo Red blocked infection by both phages. In addition, we demonstrate that infective S6 virions degraded cellulose to glucose molecules and that Gp95, a phage encoded cellulase, is involved to catalyze the reaction. On detached blossoms the phages did not significantly inhibit fire blight symptom development. Moreover, deletion of *bcs* genes in *E. amylovora* did not affect bacterial virulence in blossom infections, indicating that sole application of cellulose targeting phages is less appropriate to bio-control *E. amylovora*. Finally, the interplay between cellulose synthesis, host cell infection, and maintenance of the host cell population is discussed.

cell-puncturing device | phage engineering | phage tail-like bacteriocins

How to produce phage tail-like particles from phages.

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Bacteriophages (phages) are promising antimicrobials to be used to combat life-threatening multiresistant bacterial infections. Yet, the use of phages as therapeutics also present drawbacks, as they may be inactivated by the numerous defense systems developed by bacteria. Phage tail-like particles are high-molecular-weight bactericidal protein particles that resemble tail structures of phages, allowing them to kill bacteria by puncturing and disrupting their membrane potential. Phage tail-like particles are made of a contractile (or non-contractile) tail, consisting of a central spike-carrying tube surrounded by a sheath and the baseplate with associated tail fibers that work as receptor binding proteins (RBPs). In nature, they allow bacteria to compete for ecological niches and are produced by many different species.

While many studies have focused on engineering RBPs to change the host range of a few sets of well characterized phage tail-like particles, another strategy is to turn phages already having the desired host range into new phage tail-like particles by removing the phage head. This strategy is supported by the observation that a T4 phage mutant without the major capsid protein retains its killing properties.

Here, we present different strategies used to produce phage tail-like particles. These include bursting the phages head off in a suspension using a hypotonic shock and overproduction of tail particles during a high MOI infection cycle. We will furthermore demonstrate how lytic phages can be engineered into tail-like particles by removing genes essential for capsid formation using CRISPR-cas. Finally, we will show the potential of such phage-based tail-like particles as an alternative therapeutic strategy.

Agrobacterium | bacteriophage biocontrol | Ecology

Phage biocontrol of rhizogenic *Agrobacterium*: solving the root of the problem.

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Hydroponically grown tomatoes, bell pepper, cucumber and eggplant suffer from ‘crazy roots disease’, caused by rhizogenic *Agrobacterium* biovar 1 (Ab1). This disease causes an abnormal root proliferation with associated yield losses of 10% and higher. Currently, mitigation strategies include disinfecting irrigation water using hydrogen peroxide and/or UV light, but the number of infected greenhouses has drastically increased over the last decade. Therefore, an integrated pest management (IPM) is urgently needed to tackle this disease. Bacteriophages could be an important element in IPM, because they have a specific activity range and can often break down biofilms, allowing them to kill the bacteria colonizing the irrigation tubing.

Over the past three years, five different Ab1-specific phage species have been isolated from different Flemish greenhouses: a collection that can infect 86% of a representative bacterial collection consisting of ten genomospecies, sampled over the past decade. These phages were sequenced and their tolerance to the different conditions to which they are exposed in greenhouses was assessed. The ability of the phages to kill *Agrobacterium* in rich medium and greenhouse nutrient solution was assessed. During this treatment, phage resistant mutants were isolated, analyzed by whole genome sequencing and assessed for virulence. Based on these results, phages were selected for an ongoing long-term greenhouse trial. Here, samples are taken on a regular basis to determine the amount of active phage particles present, the concentration of virulent *Agrobacterium* and for metataxonomic analysis of the microbial composition in the rockwool mats. As such, the effectivity of the phages to tackle this notorious disease is addressed while investigating the influence of phage biocontrol on the microbiome residing in the greenhouse.

bacteriophage | bacteriophage biocontrol | Phage resistant mutants

SMART Design of a Multi-Receptor Phage Cocktail Against *Salmonella* and Impact of Phage Resistance on Bacterial Fitness

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Salmonella is one of the most prevalent food-borne bacterial pathogens around the world causing salmonellosis in humans. Several outbreaks and recalls have been associated with the consumption of contaminated poultry products. The use of broad host range lytic bacteriophages (phages) as biocontrol agents has recently emerged as a novel approach to enhance food safety. However, emergence of phage resistance remains one of the major challenges of this technology. Hence the objective of this study was to overcome this hurdle through formulating a multi-receptor phage cocktail. The developed phage cocktail in this study targets four different receptors on the bacterial surface: O-antigen, BtuB, OmpC, and rough *Salmonella* strains. The host range, morphotype, receptor, infection kinetics, genome sequence, temperature and pH stability of all phages comprising the cocktail was characterized. The phage cocktail significantly inhibited the growth of 66 *Salmonella* strains representing 22 serovars. Moreover, the efficacy to control the growth of *Salmonella* on chicken skin was assessed. Phage cocktail application at 7 $\log_{10}$ PFU/mL showed a bacterial count reduction of around 3.5 $\log_{10}$ CFU/cm^2 after 48 hours at 25 and 15°C, and 2.5 $\log_{10}$ CFU/cm^2 at 4°C when compared to the control. Lastly, the emergence of bacteriophage resistance mutants (BIM) against the phage cocktail was studied in *Salmonella* Enteritidis. The average BIM frequency against the cocktail was calculated to be 6.22×10^{-6}, significantly lower compared to single phage ($P<0.05$). A BIM strain showing cross-resistance to all phages was isolated to study the impact of phage resistance development. A genome sequence analysis revealed mutations in genes encoding for tRNA ligase (*thrS*), vitamin B12 receptor (*btuB*), and O-antigen biosynthesis (*rfbK* and *rfbP*). More experiments are in progress to understand the cost of phage resistance, including antibiotic susceptibility assays, phenotypic microarrays, and transcriptomics.

critical infections | functional metagenomics | Bacteriophage ecology

Identification of Bacterial Pathogens in Sepsis Using Bacteriophage Present in Circulating Free DNA

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We have asked whether bacteriophage, viruses that parasitize bacteria, might enable species and strain-level insights into the identity of bacterial pathogens in sepsis. To this end, we generated a computational pipeline for identifying bacterial hosts associated with bacteriophage. In two independent cohorts of septic patients and asymptomatic controls, we demonstrate that bacteriophage are present in plasma circulating free DNA (cfDNA) sequenced using conventional next generation sequencing. We find that healthy individuals have a circulating phageome and that this is disrupted in sepsis, allowing for the identification of bacterial pathogens. This approach can distinguish between pathogenic and commensal *Escherichia coli* strains and between *Staphylococcus aureus* and coagulase-negative *Staphylococcus* infections. Circulating phage DNA may have utility in identifying bacterial pathogens and distinguishing them from commensal flora in sepsis and other settings.

Horizontal transfer Endolysin Receptor-binding Proteins Modular protein Engineering

Phages playing molecular Lego

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Phages are subject to rapid evolution given their short replication cycle and high turnover rate. While the accumulation of small genetic changes (vertical transfer) allows for gradual adaptive evolution, horizontal transfer, or the exchange of genetic fragments, is the most disruptive evolutionary force driving this process. Phages encode at least two key proteins that are featured by a high modularity and that are shaped by intense horizontal transfer events: receptor-binding proteins to initiate infection and endolysins to achieve cell lysis. Their modules have an autonomous folding and dedicated function, and they are steadily reused. While the natural collection of available modules allows for a practically infinite number of modular combinations, the Darwinian selection process has withheld the best adapted modular proteins in view of their natural role in phages. We study the modularity of these modular phage proteins and apply the recently developed VersaTile technique to mimic horizontal transfer with an unprecedented throughput on a lab scale for directed evolution of receptor-binding proteins and endolysins. As such we develop synthetic phages with swapped receptor-binding proteins and tailor-made endolysins to be used as enzyme-based antibiotics.

biofilms | Pseudomonas aeruginosa | Staphylococcus aureus | ex vivo models | phage/antibiotic combinations

Efficacy against dual-species biofilms using phage-antibiotics combinations is independent of the biofilm model

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Pseudomonas aeruginosa and *Staphylococcus aureus* are opportunistic pathogens commonly found in polymicrobial infections, namely in wounds and in respiratory tract infections. Both organisms frequently cause chronic biofilm infections and due to their antibiotic tolerance are very challenging to control. We have previously shown that the combined treatments of *Pseudomonas* phage EPA1 and gentamicin have increased anti-biofilm activity against mono and dual-species biofilms formed in microtiter plates.

Here, we developed an innovative approach to study the efficacy of phage-antibiotic combinations in two *in vivo*-like models: a three-dimensional lung epithelial model that mimics aspects of the parental tissue and an artificial wound model. The efficacy of single, simultaneous and sequential treatments were compared. In the lung model, the sequential treatment of phages and gentamicin resulted in *P. aeruginosa* biofilm eradication. In artificial dermis, sequential treatment was also the treatment where higher reductions of culturable cells was observed in dual-species biofilms. Globally, our data suggests that the sequential phage treatment causes an adjuvant effect by lowering the MIC value of the phage-surviving population.

LDH test showed that this sequential application of phages and antibiotics is not cytotoxic to lung cells. In addition, we observed that on the lung model the 3-D cell integrity was not affected by sequential treatments.

We also demonstrated that the order in which phages and antibiotics are applied lead to different efficacy outcomes, showing that in clinical practice the timing to apply antibiotics will be very crucial for the success of treatment.

The sequential application of phages and ciprofloxacin was shown to be safe and very efficient against dual-species biofilms formed in different models simulating different types of infection and opening new perspectives for their clinical application.

Endolysin skin microbiome lysins

Chimeric endolysins selectively kill *S. aureus* in vitro, on reconstituted human epidermis, and on murine skin

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Staphylococcus aureus causes a broad spectrum of diseases in humans and animals. It is frequently associated with inflammatory skin disorders such as atopic dermatitis, where it aggravates symptoms. Treatment of S. aureus-associated skin infections with antibiotics is discouraged due to their broad-range deleterious effect on healthy skin microbiota and their ability to promote the development of resistance. Thus, novel S. aureus-specific antibacterial agents are desirable. We constructed two chimeric cell wall-lytic enzymes, Staphefekt SA.100 and XZ.700, which are composed of functional domains from the bacteriophage endolysin Ply2638 and the bacteriocin lysostaphin. Both enzymes specifically killed S. aureus and were inactive against commensal skin bacteria such as S. epidermidis, with XZ.700 proving more active than SA.100 in multiple in vitro activity assays. When surface-attached mixed staphylococcal cultures were exposed to XZ.700 in a simplified microbiome model, the enzyme selectively removed S. aureus and retained S. epidermidis. Furthermore, XZ.700 did not induce resistance in S. aureus during repeated rounds of exposure to sublethal concentrations. Finally, we demonstrated that XZ.700 formulated as a cream is effective at killing S. aureus on reconstituted human epidermis, and that an XZ.700-containing gel significantly reduces bacterial numbers when compared to an untreated control in a mouse model of S. aureus-induced skin infection

Endolysin Staphylococcus aureus Tissue-targeting

Engineering of endolysins for the targeted treatment of localized *Staphylococcus aureus* infections

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Staphylococcus aureus is a leading cause of a variety of systemic and local bacterial infections, including osteomyelitis. Whereas the current standard of treatment for staphylococcal osteomyelitis is heavily dependent on antibiotic therapy, intracellular persisters and the widespread occurrence of antibiotic resistance contribute to therapeutic failure. Moreover, systemic drug application results in low concentrations of the therapeutic at local infection sites due to the general distribution throughout the organism. Thus, novel antimicrobials and therapeutic approaches that efficiently target extra- and intracellular *S. aureus* and infected tissues are urgently needed. Phage-derived endolysins and other peptidoglycan hydrolases (PGHs) constitute a novel class of antimicrobials of vast potential, as they cause rapid cell lysis upon cleavage of highly conserved target bonds in the bacterial cell wall. We have explored a novel treatment approach for staphylococcal osteomyelitis that utilizes highly staphylolytic chimeric PGHs modified with cell-penetrating homing peptides (CPHP). Such functional peptides mediate the tissue-specific accumulation of associated therapeutics, thus increasing their local concentration and enhancing treatment efficacy. Additionally, their cell-penetrating characteristics allow for the targeting of intracellular persisters. We have identified CPHPs with specificity for osteoblasts using phage display and next generation sequencing. Further, the modification of PGHs with osteoblast-specific CPHPs led to the accumulation of the therapeutic in murine bones upon intravenous administration. This translated to an increased treatment efficacy in a murine model of staphylococcal osteomyelitis. Our findings show that the modification of PGHs with tissue-specific CPHPs presents a viable approach for the systemic treatment of localised infections associated with intracellular bacteria.

endolysins AMP Phage Lytic Proteins

From biological data to new phage-based antimicrobials: the case of AMP-like regions in Gram-negative phage endolysins

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(Bacterio)phages and their products, such as (endo)lysins, are some of the most promising alternative antimicrobials currently under development. Lysins are the enzymes responsible for the degradation of bacterial peptidoglycan to allow the release of the viral progeny. The exogenous application of lysins against Gram-positive bacteria has been extensively proven as a viable alternative to antibiotics. In the case of Gram-negatives, the presence of an outer membrane has been traditionally assumed to hinder the efficacy of lysins. However, many developments have called this into question. For example, more and more evidence is being gathered on many lysins being intrinsically able to interact with the outer membrane and even permeabilize it. This has been linked to the presence of antimicrobial peptide-like (AMP-like) regions within the proteins. In this work, more than 2000 lysin sequences have been analyzed to describe their possible structural adaptations to the phage bacterial host architectures [1]. Specifically, the spread of AMP-like elements was studied to conclude that they are a relatively general feature among Gram-negative endolysins. The physicochemical features of such elements were used to select intrinsically active lysins against Gram-negative pathogens [2], and one of them (Pae87) was structurally and functionally characterized, emphasizing the role of its C-terminal AMP-like peptide (P87) [3]. In this way, a proof of concept is presented on how a biodata-driven pipeline can be built up to generate novel phage-based protein antimicrobials tailored to specific infections.

[1] Vázquez R, García E, García P (2021). J Virol 95(14): e0032121 https://doi.org/10.1128/jvi.00321-21

[2] Vázquez R, Blanco-Gañán S, Ruiz S, García P (2021). Front Microbiol 12: 660403 https://doi.org/10.3389/fmicb.2021.660403

[3] Vázquez R, Seoane-Blanco M, Rivero-Buceta V, Ruiz S, van Raaij MJ, García P (2022). Acta Cryst D78 https://doi.org/10.1107/S2059798322000936

anti-phage | type IV pilus | Pseudomonas aeruginosa

Pseudomonas prophages exploit bacterial signalling to modulate phage defence

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Bacteria possess a diverse array of anti-phage defences, many of which are encoded in prophages and other mobile genetic elements. While these defences provide a selective advantage in the presence of phage challenge, this is balanced by a potential fitness cost. We recently discovered that *Pseudomonas* prophages encode a protein that modulates the activity of the type IV pilus through an interaction with the pilus assembly protein PilZ. This protein, known as Zip for Pil**Z** **i**nteracting **p**rotein, does not abrogate pilus assembly, but fine tunes its activity, providing strong phage resistance without the evolutionary cost associated with loss of twitching motility. Like CRISPR-Cas defence, Zip expression from the prophage is controlled by quorum sensing. This allows the prophage to finely tune expression in concert with bacterial cell density, ensuring maximal protection when bacterial populations are at the highest risk of phage infection.

anti-phage Bacillus subtilis Defense mechanism

New mechanisms of anti-phage defense

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The arms race between bacteria and phages led to the development of sophisticated anti-phage defense systems, including CRISPR-Cas and restriction-modification systems. There have recently been a flurry of discoveries showing that the microbial pan-genome contains many new defense systems whose function was so far largely unexplored. The talk will present progress in understanding the mechanisms of action of new defense systems, and will highlight cases in which bacterial defense from phage gave rise to key components in the eukaryotic innate immune system.

antiphage defense system | cyclic nucleotide | abortive infection | CBASS | TIR domain

Cyclic nucleotides in bacterial immune response: a glue for deadly molecular assemblies.

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In recent years there has been a revolution in our understanding of prokaryotic antiviral immune systems, in particular CRISPR and CBASS (Cyclic nucleotide Based Antiphage Signalling Systems). Studies of CBASS have uncovered the prokaryotic roots of eukaryotic innate immunity via the cGAS-STING pathway. Many of these systems use cyclic nucleotides as second messengers of infection, triggering effector protein function. Here, we unveil the action of a two-domain TIR-SAVED effector in bacterial immunity. The SAVED domain binds cyclic triadenylate (cA3), resulting in the activation of the catalytic TIR (Toll/Interleukin 1 receptor) domain which degrades NAD+ leading to cell death. By cryo-electron microscopy, we revealed a remarkable new structure, an extended superhelical filament (colloquially, a “Slinky”) that is the active form of the effector protein. Our study illuminates a striking example of large-scale molecular assembly controlled by cyclic nucleotides and reveals key details of the mechanism of TIR enzyme activation. The use of this NAD consuming enzyme as a system to abort phage infection by suicide, is a reminder of the evolutionary conservation of TIR domain in defence systems or programmed cell death.

nucleotide modification | Phage-host interaction | Phage-encoded defence systems

Biological consequences of 7-deazaguanine hypermodifications in phage genomes.

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Although 7-deazaguanine derivatives are commonly found in tRNA molecules, these nucleotide hypermodifications were recently detected in DNA. The genomic DNA from several bacteriophages was modified with either 2'-deoxy-7-amido-7-deazaguanine (dADG), 2'-deoxy-7-cyano-7-deazaguanine ($dPreQ_0$), 2'-deoxy-7-aminomethyl-7-deazaguanine ($dPreQ_1$) or 2'-deoxyarchaeosine (dG^+). Furthermore, the presence and motifs of these modifications could be detected using Oxford nanopore sequencing[1] and was linked with a high level of protection towards diverse restriction endonucleases[2].

Using CRISPR-Cas9-mediated directed mutagenesis we have constructed 7-deazaguanine deficient mutants of *E. coli* phages that utilize 7-deazaguanine modifications in their genomes. These mutants were afterwards tested regarding their general fitness and interactions with several recently published anti-phage mechanisms[3]. Our results suggest that 7-deazaguanine hypermodifications provide increased fitness and a broad spectrum of protection towards not only restriction enzymes but also other phage-resistance mechanisms including BREX, RADAR, SIR2, AVAST and DRT systems, many of which are involved in abortive infection upon phage infection. These results highlight the complexities of interactions occurring between host defense and viral evasion mechanisms.

References:

1 Kot W. at al. *Nucleic Acids Res*. 2020;48(18):10383-10396. doi:10.1093/nar/gkaa735

2 Hutinet G. at al. *Nat Commun*. 2019;10(1):1-12.

3 Gao L. at al. *Science (80-).* 2020;369(6507):1077-1084. doi:10.1126/science.aba0372

Tn5 CRISPR-Cas Phage receptor phage resistance transcription regulation

Discrimination of phages and plasmids through inverse regulation of surface-based and CRISPR-Cas immune strategies

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Bacteria harbor multiple lines of defense against bacteriophages and mobile genetic elements. CRISPR-Cas systems represent the only known prokaryotic adaptive immune response, during which 'memories' of past infections facilitate future immunity against the same foreign invader. While beneficial for defense, CRISPR-Cas systems can impose fitness costs on the host, including self-targeting, as well as representing a potential barrier to beneficial horizontal gene transfer. Identifying regulatory networks controlling CRISPR-Cas immunity is crucial to understanding when adaptive defense is favored or dispensable, in order for cells to balance fitness costs with defense. To systematically uncover regulators of CRISPR-Cas immunity, we first had to overcome a lack of suitable high-throughput genomic tools. We developed the SorTn-seq method, which employs fluorescence activated cell sorting to enrich mutants with altered fluorescent reporter activity from within a saturated transposon mutant pool. Sorted cells are then deep sequenced to locate transposon insertion sites to identify putative regulators of the gene of interest. We applied SorTn-seq to assess *csm* (type III-A CRISPR-Cas) gene expression in ~300,000 unique mutants of the enterobacterium *Serratia* sp. ATCC 39006. We identified several genes implicated in regulation of the type III-A CRISPR-Cas system, including those involved in resource utilization, motility, and stress response. Activation of the Rcs outer-membrane stress response system repressed adaptive immunity by three distinct CRISPR-Cas systems, while coordinately promoting cell surface-based immunity against diverse phages. Our results suggest that cell stress can differentially control bacterial immune strategies, which has important consequences for horizontal gene transfer.

anti-phage defence systems | coevolution | P. aeruginosa

CRISPR and non-CRISPR defences in *Pseudomonas aeruginosa* act synergistically against phage infection

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The constant arms race between phages and bacteria results in great diversity in the type and distribution of bacterial defences, with some strains carrying one system, while others have multiple layers of protection. However, how and to what extent these different mechanisms interact with one another is still unclear.

Here we show that, in the clinical isolate of *Pseudomonas aeruginosa* strain SMC4386, a type I-E CRISPR-Cas system and a previously undescribed genomic island, which we call “Maestri”, act synergistically to fight phage infection.

The Maestri system is composed of 8 genes that encode a serine-threonine kinase, an ATPase, a DNA methylase, and hypothetical proteins. Infection assays suggest the activity of Maestri eventually leads to an abortive infection response. Further assessment of gene functions is ongoing through bioinformatics and experimental approaches.

To understand how coexistence of Maestri and CRISPR systems shape phage population dynamics, we co-cultured phage DMS3vir, SMC4386-wt and isogenic mutants lacking either defence or both defences. Interestingly, phage DMS3vir, despite carrying the anti-CRISPR gene *acrIE3*, is quickly driven extinct if both defence systems are present. Deletion of the CRISPR-Cas system enables the phage to overcome the Maestri system. Multiple passages of these escape phages on the alternative *P. aeruginosa* strain PA14, which naturally lacks Maestri, shows that the capacity to escape is lost, whereas it is maintained after passages on SMC4386-wt, suggesting the modification that allows phages to overcome Maestri is epigenetic. Curiously, phages lacking an Acr do not evolve the ability to escape CRISPR-Cas when Maestri is absent, possibly suggesting that additional interactions are at play and remain to be investigated. This work will help to provide insights on the phage-bacteria coevolutionary dynamics, unveiling multi-layer interactions of defence and anti-defence systems in a clinical strain of *P. aeruginosa*.

Receptor binding proteins | Bacteriophage collection | bacteriophage diversity

Phage strategies for overcoming diverse cell barriers in *E. coli*

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Escherichia coli is a versatile and genetically diverse species inhabiting the gut of animals and humans as commensal or pathogenic strains. While the average *E. coli* genome carry ~4700 genes with ~2000 genes belonging to the core genome, the pan genome consists of more than 180.000 genes. This huge genetic diversity is reflected in more than 180 different O-antigen in *E. coli*. Given this enormous genetic diversity, *E. coli* phages are highly diverse and are currently found in 11 phage families and 111 genera. In this talk, I present our recent work demonstrating phage strategies for overcoming the diverse cell barrier of *E. coli*. For example, Kuttervirus phage S117 encode four different tail spike proteins (TSPs), each responsible for binding and degrading different O-antigen receptors of *Salmonella* and *E. coli*. Similarly, four unique TSPs of a novel Agtrevirus phage AV101 allow this phage specifically to infect Extended-Spectrum-β-Lactamase-producing *E. coli* (ESBL). Interestingly, both phages belong to *Ackermannviridae* that in total encode for more than 96 different and interchangeable TSPs allowing the members to adjust to diverse host O-antigen receptors. While Agtrevirus AV101 has a narrow host range infecting only 10 out of more than 300 *E. coli* and *Salmonella* strains tested, two novel phages belonging to *Phapecoctavirus* showed a broad host range infecting 80 out of 198 diverse ESBLs in our collection. Based on *in silico* analysis, these phages seem to encode multiple receptor binding proteins (RBPs) which may explain their broad host range. We are currently investigating these RBPs and the cognate receptors to understand their nature. In summary, the huge diversity of RBPs and TSPs may allow phages overcoming diverse *E. coli* cell barriers found in nature and may be explored in engineering of phages for specific therapeutic purposes.

Bacterial fitness Bacteriophage capsule degrading depolymerase antivirulence cell barriers

Phage-derived carbohydrate depolymerases - from structure to function and exploitation

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Bacteria evolved to display diverse carbohydrates at their surface to protect themselves from harsh conditions as well as predators. In turn, co-evolving bacteriophages developed specialized enzymes termed depolymerases allowing recognition and degradation of these carbohydrates barriers. On the other hand, bacterial surface glycans, as key virulence agents, serve as molecular patterns for recognition by the immune system, thus their enzymatic digestion or modification might lead to bacteria sensitization to host response. I will discuss the role of phage-borne carbohydrate depolymerases in bacteriophage-bacteria-host interactions with some examples of how depolymerases can be exploited. With the advances in synthetic biology tools, we could develop new diagnostics and therapeutic approaches against infectious diseases.

capsule coevolution polylysogeny

Bitter sweet symphony: Coevolutionary dynamics and diversity of resistance mechanisms against a polylysogenic competitor

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Many bacterial species are polylysogenic, and despite the potential cost, which includes cell death upon phage induction, prophages can also provide the host multiple fitness advantages, including infection of direct competitors. Moreover, little is known about coevolutionary dynamics between a polylysogen and a phage-sensitive strain. We tested this by experimentally coevolving two *Klebsiella pneumoniae* strains for 30 days under different degrees of phage pressure. At the end of the experiment, the phage-sensitive strain was still present in all mixed populations, but in a larger proportion with greater phage pressure. We then determined the diversity of the resistance mechanisms and its evolution over time. At lower phage pressures and at the beginning of the evolution experiment, most resistant clones were non-capsulated due to mutations in *wcaJ* gene, which codes for first enzyme of the capsule biosynthesis pathway. At higher phage pressures, other mutations in the capsule operon emerge, diminish capsule production and limit phage adsorption. These mechanisms are generic and also provide resistance against other polylysogenic lysates produced by other *K. pneumoniae* strains. We then tested the frequency of lysogenic conversion, predicted to be a common strategy to escape phage killing. During coevolution, this is rare and new lysogens are easily outcompeted as they are unstable and exhibit exacerbated death rates. Finally, we also show that many clones do not rely on genetic mutations for resistance. These are only transiently resistant to phages and become sensitive after regrowth in the absence of phage pressure.

Taken together, our results provide a portrait of the coevolutionary dynamics under different degrees of parasitic pressure, as well as the diversity of resistance mechanisms and the underlying genetic basis.

Bacteriophage | Acinetobacter | capsule degrading depolymerase

Understanding the complete reservoir of bacteriophage depolymerases against *A. baumannii* capsules

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A. baumannii is an important nosocomial and drug-resistant pathogen. The capsule is a major virulence factor that helps bacteria to avoid host immunity and viral predation. *Acinetobacter* phages can bind and degrade host capsules through capsular depolymerases with proven anti-virulence activity (1-3). Understanding the full reservoir of phage depolymerases against *A. baumannii* capsules, as well as relevant capsular types in clinical isolates, is crucial for developing depolymerase-based treatments.

In this work, we 1) characterized 94 carbapenem-resistant *A. baumannii* Portuguese isolates; 2) isolated phages for relevant capsular types and characterized their depolymerases and 3) developed bioinformatic tools to collect the diversity of phage depolymerases.

We show clonal shifts of *A. baumannii* KL2, KL7, KL9 and KL120 serotypes over time, with different virulence assessed in *G. mellonella*. *Acinetobacter* phages specific for particular k-types were isolated and several depolymerases (for KL1, KL2/KL19, KL9, KL30/KL45, KL32, KL38, KL44, KL67 types) characterized. We also demonstrate that most *Acinetobacter* phages encode capsular depolymerases (from 134 deposited in 2021, 73 contain capsular depolymerases), exclusively located in small viruses (<90 kb).

To disclose the full genetic diversity, we developed PhageDPO (available in Galaxy uminho.pt server), a machine learning tool that identifies depolymerases in phages and bacteria genomes (prophages). We also present PhageKDB, a database that compiles available information of capsular depolymerases, retrieved through both manually and text-mining approaches, serving as an open portal to phage community.

Overall, we present novel insights into *A. baumannii* isolates and phage depolymerase diversity and a collaborative tool to advance research in the field.

(1) Oliveira *et al* (2017). EM. PMID: 34185951

(2) Oliveira *et al* (2019). JV. PMID: 30463964

Cell wall glycosylation receptor-binding protein host cell interaction predation phage resistance

The interplay between phages, Listeria and host cells

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Bacteriophages (or phages) recognize specific cell wall receptors of host bacterium upon adsorption, the first step of phage infection. In response to the selective pressure from phage predation, bacteria have evolved to modify the phage-targeting receptors to prevent the initial contact, therefore becoming resistant to phages. Interestingly, phage resistance often comes at a cost of fitness or virulence due to the alteration of the bacterial cell wall. Using the opportunistic foodborne pathogen *Listeria monocytogenes (Lmo)* as a model organism, we show that challenge by phages selects for surviving clones that feature loss of galactose from the surface-associated wall teichoic acid (WTA) molecule by mutations in genes involved in WTA galactosylation. Such loss not only prevents phage adsorption, but renders the bacterium non-invasive and avirulent due to diminished surface retention of the canonical invasion protein InlB. We show that both the phage receptor-binding protein A500-Gp19 and *Listeria* InlB specifically recognizes and attaches to galactosylated WTA in a micromolar affinity. Collectively, our findings paint a vibrant picture of the interplay between phages, *Listeria* and host cells, demonstrate a trade-off between phage resistance and virulence, as well as support further investigation of phages as antivirulent agents.

T4 phage | Host take-over | fitness

The T4 MotB protein improves infection in various, unexpected ways

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The phage biosphere includes many genes that do not have homologs outside of phage. Consequently, these genes may represent a unique repository of genetic information with unknown functions and mechanisms. For bacteriophage T4, most of these genes encode non-essential, early products, which are thought to be involved in host takeover. We have characterized one such gene, *motB*, by using RNA-seq analyses to examine the effect of *motB* expression and by investigating the functions of purified MotB *in vitro* (DOI:10.3390/v10070343; DOI:10.3390/v13010084; DOI:10.1093/nar/gkab678). We find that MotB is a highly abundant (~50,000 molecules per cell), nonspecific DNA-binding protein that generates compacted protein/DNA complexes resembling those formed by the bacterial Nucleoid Associated Protein (NAP) Dps and by yeast cohesion. MotB expression *in vivo* is toxic to *E. coli*, yields a condensed host nucleoid, and leads to the dysregulation of host genes regulated by the *E. coli* NAP H-NS. Thus, MotB appears to globally change host gene expression by its alteration of chromosome topology. In contrast, T4 gene expression is minimally affected. In *E. coli* B, *motB* overexpression during infection also down-regulates 21/84 host tRNAs, many of which are used less frequently by T4 or have a counterpart encoded within the T4 genome. This suggests that in this case, *motB* is involved in establishing a more suitable tRNA pool for the phage. Finally, *in vitro* T4 packaging assays, performed in collaboration with the laboratory of Dr. Venigalla Rao (Catholic University) indicates that MotB can significantly enhance T4 DNA packaging. Our work demonstrates how a single early phage protein can improve infection in multiple ways.

Lactococcus lactis DNA-ejection adsorption process

Lactococcal phage-host interactions at a molecular level: a cheesy story

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Strains of *Lactococcus lactis* and *Lactococcus cremoris* (formerly classified as two subspecies of *L. lactis*) are routinely used as starter culture in mesophilic dairy fermentations for the production of a wide variety of cheeses, such as Gouda, Cheddar and Edam. Their extensive and wide-spread use in large-scale facilities makes such cultures particularly vulnerable to bacteriophage infections. Due to their industrial importance many lactococcal phages have been isolated and characterized, and substantial scientific detail has been gathered regarding lactococcal phage-host interactions. This presentation will cover some of the recent achievements and insights regarding the molecular players that are involved in host recognition, adsorption and DNA release of a prototypical bacteriophage that infects a lactococcal host.

structural biology | Bacteriophage | tailspike protein

Salmonella virus Epsilon15 tailspike has multiple binding sites and two catalytic activities.

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Bacteriophages are the most numerous organisms on Earth. They infect bacteria, including pathogenic ones, and have a huge influence on ecological processes. In the future, they might help to treat diseases caused by antibiotic-resistant bacteria. Phages use their receptor-binding proteins to adsorb to their receptors in bacteria. Consequently, receptor-binding proteins (RBP) control the phage host range.

The bacteriophage *Salmonella virus Epsilon15* has one RBP, gp20. It binds to a receptor present in bacteria of *Salmonella enterica* serovar Anatum. This genus of bacteria is causing many of cases of food-borne diseases to humans in Europe. The receptor of gp20 in this serovar is the O-antigen of the lipopolysaccharide (LPS). It consists of repetitions of the trisaccharide D-Galp[6Ac]-α-1 – 6-D-Manp-β-1 – 4-L-Rhap-α-1 – ROH linked by α-1 – 3 bonds.

In this project, we determined the structure of an N-terminally truncated gp20, gp20ΔN, with and without multiple fragments of its receptor. Gp20ΔN is a homotrimer composed of three domains, from the N- to the C-termini: the β-helix domain, the β-sandwich domain and the petal domain. The petal domain forms three independent petal-like appendages and has no structural homologous proteins. Only part of it resembles proteins of the SGNH esterase family.

Four oligosaccharides of Anatum O-antigen oligosaccharides bind to the three domains of gp20. Two oligosaccharides are located next to the endorhamnosidase site in the β-helix domain. Another oligosaccharide is placed in the petal domain esterase binding site. The endorhamnosidase activity hydrolyses the O-antigen chain while the esterase activity cuts the ester bonds between the galactose and the acetyl group. Together, both activities clear space to allow the virus to approach the bacterial membrane and initiate the infection.

Receptor binding protein | bacteriophage diversity | Host specificity

Diversity and evolution of tail spike proteins of *Ackermannviridae* phages

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Phages belonging to the *Kuttervirus*, *Agtrevirus*, *Limestonevirus* and *Taipeivirus* genera within the recently classified *Ackermannviridae* family express up to four tail spike proteins (TSPs) that each recognize a different polysaccharide receptor for infection.

To understand the diversity and evolution of TSPs of *Ackermannviridae* phages, we analysed 373 TSPs encoded by 99 *Ackermannviridae* phages. We showed that while the N-termini of the TSPs are conserved, the receptor binding modules (RBMs) are highly diverse. Of the 373 TSPs analysed, we found 96 different RBMs, suggesting 96 potential different receptors. Furthermore, our analysis identified a conserved sequence motif that may allow exchange of RBMs between TSPs. Our in-silico analysis thus suggests that *tsp* genes and RBM may be exchanged between phages within the same genus or subfamily. To further determine the boundaries of acquisition of new RBMs within the *Ackermannviridae* family, we demonstrated experimentally that entire *tsp* genes of *Kuttervirus* and *Agtrevirus* phages can be exchanged with the *tsps* of kuttervirus S117 thereby leading to new hosts. Moreover, based on in-silico and structural analysis, we exchanged the RBM of the TSP from the distantly related Lederbergvirus HK620 with the TSP1 from kuttervirus S117, suggesting the possibility of recombination between phages from distant families. Now we are currently investigating the ability of *Ackermannviridae* phages to further expand their host range by engineering phage S117 to carry a fifth TSP.

Overall, our results provide novel insight into the continuing evolutionary adaption of phages to infect new hosts by exchanging and combining conserved domains and diverse RBMs. Finally, our work also demonstrate the potential to engineer phages in the *Ackermannviridae* family to target multiple pathogenic bacteria for phage therapy or biocontrol.

RNA phage Engineering insertion tolerance region

Insertion tolerance regions for synthetic RNA phage engineering

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Bacteriophages (phages) have been regarded as valuable platforms for virus-based biopharmaceuticals. Despite the utility as biological nanoparticles, RNA phages have been underappreciated most likely due to difficulties in genetic engineering. As an attempt to properly appreciate the importance of RNA phages in both basic and applied aspects, we created the reverse genetic systems for the well-known leviphages, PP7 and MS2. These systems are based on the cDNA of the RNA phages, whose transcripts derived from bacterial RNA polymerases act not only as the primary mRNA for phage protein synthesis, but also as the template for phage RNA replicases (aka. RNA-dependent RNA polymerases). These systems were successfully exploited to introduce mutations into the RNA genomes and thus enabled us to reveal the critical amino acid residues of the maturation protein during phage-bacteria interaction. To further harness the RNA phage nanoparticles for engineering purposes, we have initially identified the insertion-tolerance regions (ITRs) on the phage genomes, by virtue of in vitro transposon mutagenesis technique using MuA transposase, which can accommodate 15-nt insertion without significant impairment in phage life cycles. As a result, 26 ITRs were identified from 4,555 transposants for PP7 and 43 ITRs were identified from 2,228 transposants for MS2. Topics discussed will include our recent data on characterization and utilization of the ITRs to generate the recombinant RNA phage virions with functional modalities and may provide an insight into synthetic RNA phage engineering platforms for various pharmaceutical purposes.

marine heterotrophic bacteria bacteriophage diversity

Using cultivations to discover new phage diversity in the marine environment

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The phage diversity in the marine environment has been estimated to encompass hundreds of thousands of species. Most often, metagenomic techniques, in which viral genomes are assembled from a pool of environmental phages, are used to access this diversity, due to their high throughput abilities. Cultivation methods, on the other hand side, are much more laborious and offer a comparatively low throughput. However, their advantages come, for example, from the ability to directly link phages with their hosts, even down to the strain level, to link genomic content with infection characteristics and phage morphology, and to offer model systems for the study of phage-host interactions. In the past years, together with collaborators, I focused on the large-scale isolation of marine phages from the North Sea, infecting environmentally relevant heterotrophic bacteria. This work led to the genomic characterization of hundreds of phages infecting marine *Rhodobacteraceae* and *Flavobacteriia*. We have found both dsDNA and ssDNA phages. While some phages were related to previously cultivated phages, many were completely new. Using recently developed, genome-based classification tools, for example, VIRIDIC and VirClust, we delineated new dsDNA and ssDNA phage families and subfamilies. Overall, this work shows how phage cultivations can still be used to shed light on new and diverse bacteriophage branches, to significantly extend the known phage diversity space.

RNA virosphere | diversity | evolution | Meta-transcriptomics

Global metatranscriptome analysis reveals vast diversity of novel RNA viruses in protists and other eukaryotes

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Mining 5,150 metatranscriptomes from diverse environments uncovered 2.5 million RNA viral contigs corresponding to a five-fold increase of RNA virus diversity. Extended RNA-dependent RNA polymerase (RdRP) phylogeny supports monophyly of the five established phyla, suggests the existence of two new phyla, and reveals numerous novel virus taxa within the phyla. In particular, 18 new classes versus the 4 previously known in *Pisuviricota*, 20 new classes versus 3 previously known in *Kitrinoviricota*, and 18 new classes versus 6 previously known in *Negarnaviricota* were identified. It was found that a significant proportion of the viruses in the former two phyla utilize alternative genetic codes typical of ciliates and other protists. The results strongly suggest that drastic host shifts, known as horizontal virus transfer, between distantly related hosts, such as unicellular and multicellular eukaryotes, is a major route of RNA virus evolution. Multiple virus groups with pronounced genomic rearrangements, such as fission or fusion of genomic segments and rearrangement of the ORFs encoding polyproteins, were also identified. We found that the swapping of catalytic motifs (domain permutation) in RdRP is a common feature of several virus lineages. Gene content analysis revealed multiple domains previously not found in RNA viruses and implicated in virus-host interactions. This vast collection of new RNA virus genomes provides insights into RNA virus biology and evolution, and should become a key resource for RNA virology.

ecology | phage life-cycle regulation | lytic bacteriophages

Marine viral infection networks revealed through proximity-ligation sequencing

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Phage infection of bacteria significantly impacts bacterial physiology, ecology, and evolution. Yet, we have only a minimal idea of the structure of phage-bacteria infection networks in in most ecosystems. Existing phage-bacteria interaction networks have relied on co-occurrence, bioinformatic host predictions, or plaque assays with few culturable species. Here, we reveal the structure of the phage-bacteria infection network of coral reefs by using the physical proximity between viral and bacterial genomes during active infections, lytic or lysogenic. This proximity-ligation approach revealed over 300 novel phage-bacteria pairs consisting of uncultivated dominant species on the water overlaying corals. Among the newly identified viruses, 51 represented complete circular genomes and carried auxiliary metabolic genes encoding enzymes for sulfur metabolism, cofactor and vitamin production, and energy metabolism. The network had a one-to-one structure at the species level, contrary to previous predictions of modular and nested network structures. Among the most abundant and ubiquitous phage-host pair across reef sites were a temperate phage infecting a member of the *Sphingomonadales*, and a temperate virus infecting Archaea. This study identified hundreds of active viral infections of uncultivated marine hosts through physical contact. These networks will enable us to quantify at the species level the rates of viral predation and gene transfers that impact the stability of coral reefs and other marine ecosystems.

bacteriophage ecology | transcriptomics | seasonal dynamics

Seasonality and transcriptomic responses of the *Rheinheimera* phage-host system from the Baltic Sea

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In aquatic environments, bacteria within the class Gammaproteobacteria are important recyclers of phytoplankton blooms. In the Baltic Sea, one example is *Rheinheimera* sp. BAL341, which also has shown metabolic plasticity and an ability to react in transplant experiments. In 2015 we isolated 54 phages infecting *Rheinheimera* sp. BAL341 from the Linnaeus Microbial Observatory (LMO) in the eastern Baltic Proper. They differed largely to previously known viruses and represented five different species of the same genus. Both the bacterium and phages showed strong seasonality, with recurring high abundances in late summer/early autumn coinciding with the demise of phytoplankton blooms, seen through both metagenome and isolation investigations. Similarly, *Rheinheimera* phages showed a strong response to a phytoplankton bloom induced by agriculture-influenced river water in a mesocosm experiment. Growth experiments showed reduced bacterial growth rate as well as reduced viral burst size and prolonged latent period in low nutrient medium (LNM) compared to high nutrient medium (HNM). However, in transcriptomic experiments, nutrient concentrations strongly affected host gene expression (both in infected and non-infected cells) whereas phage responses were limited. Particularly, a larger number of host genes were differentially expressed in phage-infected cells in LNM compared to HNM. For example, iron acquisition genes, which had increased expression in uninfected bacteria in LNM, were suppressed in phage-infected bacteria during the same nutrient regime. Also, there was a switch in host gene expression involved in phosphate metabolism in LNM depending on infection. Thus, the nutritional state of the environment appears to be an important factor to consider for the outcome of phage infections and the impact seen in high-nutrient laboratory experiments might underestimate the importance of phage transcriptional regulation in natural environments.

gut virome | bioinformatics | early life exposures

The influence of early life exposures on the infant gut virome

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Recent large cohort studies have contributed significantly to our understanding of the factors that influence the development of the bacterial component of the gut microbiome (GM) during the first years of life. However, the factors that shape the colonization of other important GM members such as the viral fraction remain more elusive. Here, we utilize the deeply phenotyped $COPSAC_{2010}$ birth cohort consisting of 645 children to investigate how social, pre-, peri- and postnatal factors may influence the gut virome composition at one year of age.

Among the different exposures studied, having older siblings and living in an urban vs. rural area had the strongest impact on gut virome composition. From a total of 16118 viral operational taxonomic units (vOTUs) (mainly bacteriophages, but also 6.1% eukaryotic viruses), differential abundance analysis identified 2105 vOTUs varying with environmental exposures, of which 5.9% were eukaryotic viruses and the reminder bacteriophages. The bacterial host of these phages were mainly predicted to be within the *Bacteroidaceae*, *Prevotellaceae*, and *Ruminococcaceae* families, as determined by CRISPR spacer matches. Spearman correlation coefficients indicated strong co-abundance trends of vOTUs and their targeted bacterial host, which underlined the predicted phage-host connections. Several vOTUs carried differently abundant genes coding for enzymes involved in carbohydrate metabolism (fructose, mannose, sucrose, starch), glycolysis-gluconeogenesis, as well as amino acid (Ala, Asp and Glu) and sphingolipid metabolism, implying that these phages could be related to the utilization and degradation of major dietary components by their host.

Given the importance of the GM in the maturation of the immune system and the maintenance of metabolic health, these findings potentially provide a valuable source of information for understanding early life factors that predispose for autoimmune and metabolic disorders.

Inflammatory Bowel Disease | gut virome | gut microbiota

Intestinal virome in Crohn's disease patients

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It is now generally recognised that the gut microbiota plays an important role in human health, and in particular in Crohn's disease (CD), a complex disease that results from a combination of genetic and environmental factors, with a key role for the gut microbiota. CD is characterised by chronic inflammation associated with an imbalance in the composition of the gut microbiota (i.e. dysbiosis). While considerable efforts are being made to understand the role of gut bacterial components, bacteriophages (phages), remain comparatively understudied. In particular, our understanding of the impact of phages on the structure of the gut microbiota in health and disease remains limited.

Our results from mouse models and from the study of human faecal samples indicate that phages can affect the populations of main bacterial members of the human gut microbiota, implied in the symbiosis with the host. In addition, we rigorously enumerated viruses in the stools of healthy individuals and CD patients, and found that free virion concentrations vary by an unprecedented range for a component of the microbiome: they are present at concentrations ranging from 1.10^9 to 1.10^12 particles per gram of stool. In addition, the virus to microbe ratio is highly increased in CD patients compared to healthy controls. We also performed quantitative viral metagenomic analysis from 15 healthy volunteers and 15 IBD patients, using spiking of both single-strand and double-strand DNA phages, and observed an increase in the prevalence of temperate phages in CD patients. Altogether, our results suggest a role of the virome in Crohn's disease, either direct or indirect via a modification of the structure of the gut microbiota.

Actinobacteria | antiphage defense system | secondary metabolites

Phage defense at the multicellular level – from small molecules to cellular development

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Bacteria of the genus *Streptomyces* are well-known for their ability to produce a large variety of bioactive compounds and for displaying a complex developmental cycle. We are interested in how cellular development and the secretion of secondary metabolites may aid these organisms in the protection against phage infection at the multicellular level.

The currently known antiphage defense arsenal relies on a wide range of inhibitory mechanisms, but these are mainly mediated by protein effectors acting primarily at the cellular level. Recently, we showed that aminoglycosides, a well-known class of antibiotics produced by *Streptomyces*, are potent inhibitors of phage infection in diverse bacterial species. We demonstrate that aminoglycosides do not prevent the injection of phage DNA into bacterial cells, but instead block an early step of the viral life cycle, prior to genome replication. Overall, our results expand the knowledge of potential aminoglycoside functions in bacterial communities suggesting that aminoglycosides are not only used by their producers as toxic molecules against their bacterial competitors, but could also provide protection against the threat of phage predation at the community level.

To unravel the impact of phage infection using *Streptomyces* strains deficient in different steps of development and a repertoire of novel *Streptomyces* phage isolates. Our results demonstrate an important role of *Streptomyces* development in the efficient containment of viral infections.

marine viruses | extracellular vesicles | cell-cell communication

Ecological significance of extracellular vesicles in modulating host-virus interactions during algal blooms

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Communication between microorganisms in the marine environment has an immense ecological impact by mediating trophic-level interactions and thus determining community structure. Extracellular vesicles (EVs) are produced by all cell types and can mediate pathogenicity or act as vectors for intracellular communication. However, little is known about the involvement of EVs in microbial interactions in the marine environment. Here we investigated the signaling role of EVs produced during the interaction between the bloom-forming alga *Emiliania huxleyi* and its specific virus, EhV, which leads to the demise of these large-scale oceanic blooms. We found that the production of EVs is elevated during viral infection or when cells are exposed to infochemicals derived from infected cells. These vesicles have a unique lipid composition that differs from that of viruses and their infected host cells. Additionally, proteome analysis revealed a unique signature among EV proteins, which were enriched in proline-rich motifs. EV cargo is composed of specific small RNAs that are predicted to target sphingolipid metabolism and cell-cycle pathways. In both lab and field experiments, we demonstrated that treatment with EVs leads to a faster viral infection dynamic. Furthermore, EVs can modulate the half-life of both isolated and natural EhV virions. We propose that extracellular vesicles are exploited by viruses to sustain efficient infectivity and propagation across *E. huxleyi* blooms. Since these blooms impact the cycling of carbon and other nutrients, this mode of cell-cell communication may influence the fate of the blooms and, therefore, the composition and flow of nutrients in marine microbial food webs.

Virus-host interactions | phage engineering | Phage T5

Genetic dissection of host takeover by phage T5: developing tools for genome engineering of large virulent phages

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The virulent bacteriophage T5, which infects *Escherichia coli*, injects its 122-kb linear dsDNA in two steps: 8% of the genome (carrying seventeen pre-early genes) are first transferred into the cell and the injection pauses. Following expression of pre-early genes, the host DNA is degraded and host defense systems inactivated. Viral DNA transfer resumes after 3-5 minutes to allow entry of the rest of the genome that encodes early and late proteins necessary for viral DNA replication, virion assembly and release. Thirteen of the 17 pre-early genes escape functional prediction and only four have been characterized so far. Thus, T5 constitutes an original model to study host takeover and uncover viral effectors targeting host cell functions.

To assess the significance of pre-early genes for T5 infection, mutagenesis of T5 was first performed using homologous recombination during infection assisted by the targeted elimination of wild-type phages by CRISPR-Cas nucleases after mutagenesis. These reverse genetics efforts were hampered by the resistance of T5 to Cas9. As an alternative method to Cas nucleases for mutant enrichment, we successfully set up a simple protocol of Dilution-Amplification-Screening (DAS) of phage pools. A second tool for mutagenesis of T5 was bacterial retroelements (retrons), used to provide a source of ssDNA during phage replication. Using these methods we generated ten mutants carrying either conditional amber mutations, single-gene or multiple-gene deletions. While only two genes (*A1* and *A2*) in the 15 analyzed are essential for T5 infection in laboratory conditions, our phenotypic analysis revealed that *dmp*, a gene encoding a 5'-deoxynucleotidase, and at least one other gene facilitate infection of *E. coli* by T5. These strategies should be of particular interest for genome engineering of phages with large genomes that are naturally resistant to Cas nucleases.

bacteriophage ecology | transcriptomics | in vivo

The gut environment regulates bacterial gene expression which modulates susceptibility to bacteriophage infection

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Bacterial and bacteriophage (phage) populations, although antagonist, coexist in the gut of mammals and remain stable over time. We hypothesised that the mechanisms underlying this coexistence may involve the regulation of bacterial gene expression required for their adaptation to the intestinal environment.

To identify genes specifically over- or under-expressed in the mammalian gut that may affect phage susceptibility, we performed a comparative genome-wide RNA sequencing study between in vitro (flasks) and in vivo (mice colon) conditions on a human enteroaggregative Escherichia coli isolate.

In vivo, the global gene expression pattern was found to be closer to in vitro exponentially growing E. coli cells, compared to E. coli cells that reached stationary phase growth. Virulence determinants (adhesins and aggregative fimbriae) encoded by a plasmid, and chromosomal genes involved in adaptation to the gut environment (iron acquisition, anaerobic respiration and sugar metabolism) were over-expressed in vivo, whereas genes involved in aerobic respiration were under-expressed. We also identified a set of genes potentially involved in the differential replication of phages in vivo acting on the flagellum, the LPS biosynthesis, the biofilm formation and the quorum sensing. We experimentally demonstrated that four of these differentially expressed genes (rfaL, fliA, lsrC and bssR) affected the relationships between E. coli and three virulent phages.

This work demonstrates that the gut environment, by modifying microbial gene expression, modulates phage-bacteria interactions and highlights the role of tripartite relationships between bacteriophages, bacteria and host in intestinal homeostasis. These results emphasize the benefit of in vivo experiments to elucidate the mechanisms affecting phages efficacy in the gut.

bacteriophage Antibiotic phage-antibiotic synergy

Phage-Antibiotic Synergy for filamentation-inducing antibiotics

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(Bacterio)phages are promising tools for combating antibiotic-resistant bacteria. Currently, phage therapy is only carried out in combination with antibiotics. Sometimes, when bacteria are incubated in their presence, the lytic activity of phages increases. This phenomenon is called Phage-Antibiotic Synergy (PAS) and results in enhanced antibacterial activity since the changes induced by the antibiotics in the bacterial physiology modify positively the dynamics of phage propagation.

Using single-cell techniques, the lytic cycle of phage HK620 was studied on a ciprofloxacin-treated culture of E. coli. One of the most notorious effects of the antibiotic is the inhibition of division. As a consequence, a subpopulation of cells produces filaments that increase the survival and mutagenesis of the bacteria.

This study seeks to characterize a novel dynamic of host-phage interaction: antibiotic-induced filaments become easy targets for phages due to their enlarged surfaces, as proven by fluorescence microscopy and flow cytometry techniques. Adsorption, infection and lysis happen twice as often in filamentous cells, compared to regular-size bacteria. Furthermore, the reduction of bacterial number caused by impaired division can account for the faster bacterial killing during PAS.

The impact of phages on the reduction of mutagenesis was also assessed using reporters for bacterial DNA repair. A significant percentage of hyper-mutagenic filamentous bacteria was efficiently killed by phages due to their increased susceptibility to infection. These results suggest a previously unidentified trade-off for bacterial survival: The activation of antibiotic-tolerance mechanisms makes bacteria more vulnerable to phage infection.

Finally, a model will be presented that documents phage propagation in the presence of filamentation-inducing antibotics, and its impact on mutagenesis. This will allow a systematic approach to test phages and antibiotics combinations for therapy.

Pseudomonas aeruginosa | Post-translational modification | Host take-over

Exploring protein lysine acetylations during phage infection in *Pseudomonas aeruginosa*

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Bacterial viruses, so-called bacteriophages, heavily depend on their host's physiology for progeny production. Therefore, they encode specialized proteins that can inhibit, activate or redirect bacterial functions to facilitate the infection process. Well-known host-hijacking mechanisms involve replication, transcription and translation interference. It was only recently that our group discovered a novel layer of phage-based regulation, by acetylation-based posttranslational modification (PTM) of proteins within the infected cell. Unlike the control of transcription and translation processes, which are relatively slow and energy-demanding, PTMs allow a swift adaptation of protein function/activity and thus of cell physiology and fitness. Therefore, studying phage-encoded acetyltransferases not only provides novel insights in phage biology, it additionally broadens our view on the bacterial acetylome under stress conditions.

We here present a Gcn5-related N-acetyltransferase encoded by the *Pseudomonas aeruginosa* infecting phage LUZ19. Using SILAC-based acetylomics, the phage protein was found to facilitate acetylation of nine distinct proteins ($\log_2$ fold change > 1) in *P. aeruginosa* strain PAO1, including two key enzymes of the cysteine and methionine biosynthetic pathway, MetE and MetK. Two specific lysine residues of the methionine synthase MetE showed highly increased acetylation levels ($\log_2$ fold change > 3), one of which is located near the active site of the enzyme. Metabolomics confirmed the observations as specific alterations in the cysteine and methionine metabolism were detected. Overall, this study unraveled a novel mechanism of host metabolic take-over, in which the phage uses post-translation modification to regulate specific bacterial processes. Moreover, it reveals the regulatory potential by acetylation of specific enzymes in key metabolic pathways of *P. aeruginosa*.

Aquaculture | Flavobacterium psychrophilum | bacteria-phage interaction | phage-bacteria co-evolution

Phages select for non-virulent variants of *Flavobacterium* pathogens: Implications of pathogen control in aquaculture

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Diseases caused by the fish pathogen *Flavobacterium psychrophilum* are a major bottleneck in trout farming and cause economic losses to the industry. Currently, antibiotics are used to treat these infections, despite serious concerns about the development of bacterial antibiotic resistance. Application of bacteriophages has been suggested as a strategy to control disease outbreaks in aquaculture, and in the current study, we aimed at developing novel phage-based strategies for disease prophylaxis and treatment of outbreaks with *F. psychrophilum*. Successful application of phages requires detailed knowledge on delivery efficiency to infected organs, and on the development of phage-resistant populations. The observed significant increase in fish survival upon intraperitoneal administration of phages demonstrated that the delivery of phages directly at the infection site can reduce fish mortality, even when present in relatively low multiplicity of infection in the peritoneal cavity. Further, exposure to phages selected for phage-resistant variants of *F. psychrophilum* with mutations in the Type IX secretion system gene complex, which resulted in impaired virulence properties such as gliding motility, biofilm formation and protease activity. As these mutations turned out to be reversible, the study identified a flexible phage defense mechanism in *F. psychrophilum*, based on genomic mutations that turned the virulent pathogens into phage-resistant bacteria that had become non-virulent against rainbow trout. This documentation that phage exposure is selecting for non-virulent, phage-resistant *F. psychrophilum* further emphasizes the potential of phage applications for controlling the mortality caused by this important pathogen.

Salmonella | inhibition | ultrasonic spray coating | biopolymers

Ultrasonic spray deposition of a bio-based coating loaded with the *Salmonella* phage Felix 01 on food contact materials

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The quality of foods is highly dependent on their microbiological content, which can cause changes in varied parameters, including colour, texture, pH, and total acidity. *Salmonella* remains one of the major zoonotic diseases in Europe after campylobacteriosis and is responsible for several foodborne disease outbreaks (>91,000 in EU alone). According to the European Food Safety Authority, the estimated economic burden of human salmonellosis is as high as €3 billion a year. The use of antimicrobial coatings on food packaging can have valuable benefits in promoting food safety and even prolonging product shelf life. One of the challenges of developing antimicrobial food contact materials is the functionalization process. Incorporating biological antimicrobial compounds can be challenging using conventional methods (i.e., extrusion); thus, ultrasonic spray coating can be an alternative.

In this work, we evaluated the adhesion of *Salmonella* Enteritidis to different food contact materials. Furthermore, we used an ultrasonic spray coating to apply layers of a bio-based coating loaded with *Salmonella* phage Felix 01 on these materials and assessed their inhibition efficacy at varying storage temperatures and contact periods. We further characterized the materials by SEM, FTIR-ATR, contact angle, phage release, phage stability, and moisture, among others.

The ultrasonic spray coating had an insignificant effect on the phage's viability, which remained stable stored at 4°C in vacuum-sealed bags for up to 1 month. Moreover, the phages were released from the different coated materials within 10-15 minutes, and the inhibition levels varied according to the treatment temperature. Nonetheless, the phage-loaded coatings caused an inhibition that ranged between 99% and 99.999% of viable bacteria on the surfaces.

Ultrasonic spray deposition of phages demonstrates great potential for use in food packaging materials to inhibit and minimize the growth of foodborne pathogens.

Human Gut Microbiota | bacteriophage biocontrol | metagenomics | Safety | STEC

Unveiling the impact of STEC infecting phages on the colon microbiota using an *in vitro* fermentation model

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(Bacterio)phages are considered safe for humans' consumption, being regard as excellent biocontrol tools to prevent foodborne pathogens spread. Phages' major advantage is their inherent specificity towards a bacterial species, yet some reports have shown phages ability to evolve to infect different hosts when transiting the gastrointestinal tract (GIT). And so, it is of extreme importance to understand the safety outcome of using phages as biocontrol agents in food, with particular interest in the ones that target species from *Enterobacteriaceae* family, commonly found in the human GIT microbiota. In this study, the impact of a phage infecting Shiga toxin-producing *Escherichia coli* (STEC), named *E. coli* phage vB_EcoS_Ace (Ace), towards the colon microbiota was investigated. An *in vitro* batch fermentation model was used, and the inoculum was the fecal material of three healthy donors. Fermentations' metabolome was analyzed through GC and HPLC, and the concentration of both phage Ace and STEC strain were monitored along time (up to 24h). The interference with the gut microbiota composition and functional potential was assessed by shot gun metagenomics.

We observed an increase in phage titre only when the host was present, suggesting that there was no other suitable host within the different microbiotas used. Also, the microbiotas' composition did not alter when phage Ace was added. Nevertheless, the attenuated version of STEC strain did indeed create some perturbation in the microbiota, which led to different functional potential. This was corroborated by the differences observed for both gas and short chain fatty acid acids dynamics. The microbiotas' individuality was an important factor for the observed perturbations. Moreover, phage Ace revealed to be a safe phage when intended to be used as a biocontrol agent for food products. Also, we concluded that the *in vitro* fermentation model is a reliable, easy, and non-expensive safety screening methodology for phages.

bacteriophages Salmonella veterinary

Multi-aspect safety assessment of bacteriophage products dedicated for animal health

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Developing modern phage commercial product requires selection of appropriate cocktail components and format of the product that supports effective delivery of active phages to targeted site. Equally important is precise definition of product characteristics directly related to bacteriophages' biology as well as information on the product in the final form delivered to the market. Proteon Pharmaceuticals created the technology platform that allows to successfully develop phage-based products and implement new strategies of control of pathogenic bacteria affecting animal production industry. The platform developed by Proteon Pharmaceuticals allows to design highly effective products consisting of a mixture of carefully selected and genotypically characterized virulent phages that eliminate pathogenic bacteria, without causing side effects, while supporting better performance outcomes. Proteon's flagship product BAFASAL is a feed additive for use in industrial poultry sector protecting birds against *Salmonella*. The preparation was examined in accordance with the EFSA guidelines proving its high safety for human, birds, and environment, and high effectiveness in reducing the number of *Salmonella* in birds and in environment of poultry production sites in multiple studies. While phages have excellent safety profile employing phage products on large scale in food production sector is perceived as novel technological approach. Broad acceptance of phage technology might require additional effort to better understand potential effects of their introduction into food chain or work environment. Proteon, together with MedBiome Inc., applied *in vitro* assay called RapidAIM to investigate the effect of BAFASAL on human gut microbiome. Multi-omics analyses, including 16S rRNA gene sequencing and metaproteomic, revealed that *ex vivo* human gut microbiota composition and function were unaffected by BAFASAL treatment, providing an additional measure for its safety.

bacteriophage biocontrol | Salmonella | Phage Therapy | Bacteriophage | S. Typhimurium | swine

Evaluation of spray-dried microencapsulated phage cocktail to control *Salmonella* carriage and shedding in weaned pigs

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Asymptomatic *Salmonella*-infected pigs act as major sources of infection to humans. Reduction in *Salmonella*-related infections at the farm level can mitigate *Salmonella* transmission from pigs to humans. Microencapsulated lytic bacteriophages have the ability to control *Salmonella* in pigs and potentially be used as antibiotic alternatives. Wide acceptance at the farm level would require the encapsulated phages to be economical, mass-producible, shelf-stable, easily mixable with conventional feed, and most importantly control the pathogen. We have conducted this animal trial to evaluate the ability of a pH-responsive microencapsulated spray-dried anti-*Salmonella* phage cocktail to control *Salmonella* in pigs. Newly weaned pigs (n=18) were randomly selected and designated as Negative, *Salmonella* and Phage groups. Pigs in Phage groups received microencapsulated phage orally for 12 days while Negative group received placebo. On days 6 and 7, pigs in Salmonella group and Phage group were challenged with 2 mL of $5.5x10^6$ CFU/mL *Salmonella Typhimurium* DT104Nal^R. Fecal samples were collected on 5^{th}, 7^{th}, 8^{th} and 10^{th} days of the experiment. *Salmonella* was detected in 83% pigs in Salmonella group, whereas only 66.6% pigs received Phage treatment were *Salmonella* positive. *Salmonella* shedding was significantly reduced in Phage group (3.37 log cfu/g; ($P<0.05$)) on 10^{th} day compared to Salmonella group (5.57 log cfu/g). All pigs were euthanized on the 12^{th} day, and intestinal contents from jejunum, ileum, caeca and colon were collected for analysis. *Salmonella* count (2.22 log cfu/g) in the jejunum of the Phage group was significantly lower than that in Salmonella group (4.08 log cfu/g; $P<0.05$). *Salmonella* count in the colon in Phage group was significantly lower (3.75 log cfu/g) than the pigs in the Salmonella group (4.11 log cfu/g; $p<0.05$). In conclusion, spray-dried microencapsulated bacteriophage cocktail is an economical antibiotic alternative to reduce Salmonella in weaned pigs.

Haloarchaea | Archaeal viruses | Entry Mechanism

Infection mechanism of haloarchaeal viruses

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Archaea are ubiquitous microorganisms that are found in numerous surroundings ranging from extreme environments such as hot springs, to mesophilic ones such as the ocean and the human gut. Archaea are infected by unusual viruses that are structurally very diverse and include those with unique capsid shapes such as a bottle, and viruses with cosmopolitan morphologies, such as tailed dsDNA viruses.

As the archaeal cell envelope is fundamentally different from that of bacteria and archaea, archaeal viruses face different challenges when traversing the cell envelope on their way in and out of the cell. We use haloarchaea and their viruses to study viral infection mechanisms, such as viral entry and egress. In contrast to other Haloferax strains, *Haloferax gibbonsii* LR2-5 is very susceptible to viral infection and serves as a host for a dozen of viruses. In my talk I will present infection mechanisms of viruses infecting this strain that serves as a model to study virus-host interaction in haloarchaea.

biofilm Lipopolysaccharide Salmonella

A sweet key to bacteriophage infection: Polysaccharide interactions at Gram-negative bacterial surfaces and in extracellular glycan matrices

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The encounter with a cellular boundary is a central step in a viral life cycle to start transfer of genetic material into a host cell. Bacteriophages meet a plethora of bacterial surface structures, most of them highly diverse envelope glycans, but also glycan-based biofilms. Phage-host co-existence in these glycan matrices is tightly balanced, as they play dual roles both as protective shields and as phage receptors. This talk will give an overview on how bacteriophages interact with polysaccharides as part of the bacterial envelope or of biofilms. We have analyzed bacteriophage mobility in polysaccharide-based biofilms by defining microviscosity parameters of the glycan matrix. We show which strategies phages have developed to remain mobile in highly viscous biofilms. Tailed bacteriophages infecting Gram-negative bacteria can use the outer O-antigen polysaccharide layer for infection. The interaction with this major outer membrane component has high regulatory impact on bacteriophages. In intact cells, the O-antigen serves as infection control point, whereas in outer membrane vesicles, the O-antigen mediates extracellular phage concentrations. In addition, isolated lipopolysaccharide (LPS) fragments form membrane-like assemblies exposing O-antigen to inactivate phages. Using a set of *Salmonella* model phages of different tail architectures we show that phage particles work as molecular machines that can be triggered to release their DNA by O-antigen containing membranes. We show how the interplay of bacteriophage enzymatic activity, OM properties and phage tail architecture leads to opening of the phage particle, illustrating the high regulatory power of the Gram-negative OM glycan environment on bacteriophage infection.

Cell wall | phage resistance | defence systems

Reversible bacteriophage resistance by shedding the bacterial cell wall

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The cell wall plays a central role in protecting bacteria from some environmental stresses, but not against all. In fact, an elaborate cell envelope may even render the cell more vulnerable, since it contains molecules and structures that bacteriophages recognize as the first step of host invasion. Bacteriophages are highly abundant in the environment and a major threat for bacteria. Therefore, bacteria have evolved sophisticated defense systems to withstand phage attacks, like CRISPR/Cas, restriction-modification or abortive infection. However, some bacteria are known to be able to shed the bacterial cell wall in response to several environmental stressors. We hypothesized that wall-deficient bacteria may be temporarily protected against phages, since they lack the essential entities that are necessary for phage binding and infection. To test this hypothesis, three model organisms (Streptomyces, E. coli and B. subtilis) were inoculated in osmoprotective medium and infected with bacteriophages at MOI=1. Cryo-electron tomography was to give a detailed overview of the interaction between phages and wall-deficient bacteria. We have uncovered a previously unknown mechanism by which mono- and diderm bacteria survive infection with diverse lytic phages. Phage exposure leads to a rapid and near complete conversion of walled cells to a cell wall-deficient state, which remain viable in osmoprotective conditions and can revert to the walled state. While shedding the cell wall dramatically reduces the number of progeny phages produced by the host, it does not always preclude phage infection. Altogether, these results show that the formation of cell wall-deficient cells prevents complete eradication of the bacterial population and suggest that cell wall-deficiency may limit the efficacy of phage therapy, especially in highly osmotic environments or when used together with antibiotics that target the cell wall.

Giant Viruses | anti-viral | defence systems | host-defence

Abortive infection and defensive symbiosis - novel defense strategies against giant viruses

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The discovery of giant viruses in amoeba changed our perception of the viral world. With genome and particle sizes comparable to those of bacteria and a number of cellular features, giant viruses sparked a controversy about their evolutionary origin. Frequent host-switches, and competition with other viruses and bacterial symbionts were proposed as drivers of giant virus evolution. While virophages have been shown to facilitate amoeba host survival during giant virus infection, very little is known about other defense mechanisms. Here, we report on two new defense strategies observed with two different giant viruses. First, we studied the role of bacterial symbionts of free-living amoebae in the establishment of giant virus infections. To investigate these interactions in a system that would be relevant in nature, we isolated a giant virus (Marseilleviridae) and potential *Acanthamoeba* hosts infected with a bacterial symbiont, identified as *Parachlamydia acanthamoebae*, from the same environmental sample. Systematic co-infection experiments showed that the bacterial symbiont represses the replication of the sympatric giant virus as well as other giant viruses (Mimiviridae) in both environmental isolates as well as *Acanthamoeba* lab strains. Second, we studied a new giant virus isolate (Mimiviridae) infecting members of the amoeboflagellate genus *Naegleria*. We describe how infection of *Naegleria* may lead to abortive infection, in which viral replication and dissemination is blocked by premature host cell death, ensuring the survival of the amoeba host population. Together, we show that amoebae employ diverse and as yet underexplored strategies to cope with omnipresent giant viruses.

Endolysin | Cell wall | Membrane Potential | Antibiotic resistance

Unraveling bacterial determinants of tolerance to endolysin lytic action

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WHO recognizes antibiotic resistance as one of the major current threats to global health and economy. To tackle the decreasing therapeutic efficiency of conventional antibiotics, alternative antimicrobials are needed, preferentially with new modes of action that minimize resistance development. Among such promising alternatives are bacteriophage lytic enzymes, such as endolysins [1].

Endolysins are enzymes (enzybiotics) that cleave the bacterial cell wall (CW) peptidoglycan [1]. Phages employ them to lyse host bacteria at the end of infection for virion progeny release. Endolysins' ability to cause cell lysis when added exogenously to bacteria set the basis for their exploration as antimicrobials.

It is usually considered that Gram-positive (G+) bacteria are "easy" targets to endolysins, since these bacteria lack an outer membrane protecting the CW. However, it has been observed that in nutrient rich environments, G+ bacteria can present some tolerance to endolysins. Little is known about the mechanisms of tolerance, except that they depend on the presence of certain CW secondary polymers and that they are abolished by agents that dissipate the membrane proton-motive force (PMF) [2,3].

We have used the endolysin Lys11 that targets the high priority pathogen *Staphylococcus aureus* to gain insight on the determinants of tolerance. By employing selective membrane drugs, *S. aureus* mutants affected in CW polymers, and different endolysin constructs, we show that: i) the PMF component most preponderant in tolerance is the pH gradient across the membrane; ii) selective membrane drugs affect endolysin binding to cells; iii) wall teichoic acids are the key CW-associated polymers contributing to tolerance; iv) the two catalytic domains of the endolysin respond differently to PMF dissipation.

[1] Dams and Briers, 2019. doi: 10.1007/978-981-13-7709-9_11

[2] Fernandes and São-José, 2016. doi: 10.1111/mmi.13448

[3] Gouveia et al., 2022. doi: 10.1038/s41598-022-05361-1

phage annotation | phage genomics | phage classification

Workshop on Viral Genomics and Taxonomy - from isolate to GenBank and beyond

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How many of us have isolated and sequenced a phage, gotten reads back from the sequencing facility and thought “What now?” How can I assemble the data, and should I use an automated pipeline (DFAST, RASTtk, PHANOTATE) to annotate the genome, or shall I do what my scientific ancestors in the laboratory did before me and manually scan for ORFs, Shine-Dalgarno sequences, promoters and terminators? What if there is no reference phage closely related to mine? What if there is? What is a phage species and what about all those new families?

In this workshop, we will try to guide you through the whole process, from deciding on the sequencing technology, to assembly, annotation and classification. For the most part, we will use Internet resources and freely available software that can be run on a laptop computer. We’ll teach you how to hunt for missing coding sequences and false start codons, show best practices in functional annotation and drill down the mantra “if you cannot convince your grandmother (Avó) of a protein’s function, call it hypothetical protein”.

In the second part of the workshop, we’ll introduce the participants into the wonderful – if somewhat baffling – world of official virus taxonomy. We’ll highlight some of the new tools that are available and guide you through the decision-making process.

At the end of this workshop, you will have all the knowledge and tools to go from a sequence file to a finished annotated phage genome that can be submitted to an INSDC database and can be officialised through a Taxonomy Proposal to ICTV (the International Committee on Taxonomy of Viruses).

The workshop coordinators are Chair, Vice Chair and former Chair of ICTV’s Bacterial Viruses Subcommittee and members of NCBI’s Viral Advisory Committee. In these capacities they are committed to assure that phage genomes submitted to NCBI are complete, accurate, properly annotated, and accurately classified.

Mycobacteriophage | Mycobacterium abscessus | Therapeutic use

Discovery, dynamics, engineering, and the therapeutic opportunities for phage treatment of Mycobacterium infections

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Mycobacteriophages are phages infecting *Mycobacterium* hosts. A large collection of over 10,000 individual phages have been isolated on a single host strain – *Mycobacterium smegmatis* mc^2155 – and over 2,000 have been sequenced and annotated. These provide a high-resolution view of the genomes, their overall diversity, the ways they differ from each other, the evolutionary mechanisms that give rise to pervasive genomic mosaicism, and their origins. Many if not most of these phages are temperate and use a variety of intriguing mechanisms for establishing lysogeny and maintaining their prophages during bacterial growth. These phage genomes have provided a rich source of tools for developing genetic systems for the mycobacteria – including *Mycobacterium tuberculosis* – such as integration-proficient plasmids, recombineering systems, non-antibiotic selectable markers, and phage-mediated delivery systems. We have also explored the potential therapeutic use of mycobacteriophages for the treatment of *Mycobacterium* infections, including those caused by *Mycobacterium abscessus*, *Mycobacterium chelonae*, *Mycobacterium avium*, and BCG. Through extensive screening, forward genetics, and genome engineering, we have identified, prepared, and provided phage preparations for treatment of 20 patients with antibiotic resistant and refractory infections, for which all extant treatments had been exhausted. Most of these received intravenous administrations, twice daily, for at least three months and often substantially longer. No serious adverse reactions were observed, and favorable microbiological or clinical outcomes were reported in many but not all patients. Interestingly, phage resistance was not observed and did not contribute to treatment failure, even for the eleven patients for which only a single phage was administered. Phage neutralizing antibodies were observed in several patients but did not consistently prevent favorable outcomes.

phagetherapy phage receptors Formulation of smart phage cocktails

Fundamentally applied phage biology

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In this talk, I will discuss the necessity of fundamental phage research within clinically-relevant bacterial pathogens. Specifically, I will detail our recent work on phage receptors, the emergence of phage resistance, the development of phage cocktails and pre-clinical translation in two emerging bacterial pathogens. Building this knowledge base within each relevant bacterial pathogen is critical if we are to successfully and consistently apply this for therapeutic interventions.

phage therapy | clinical application | treatment

Phage therapy: A Belgian experience

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Antimicrobial resistance (AMR) is a growing threat for human health. Phage therapy (PT) could be part of the solution.In 2008, the Queen Astrid Military Hospital (QAMH) reinitiated PT in selective cases.

We retrospectively analyzed the PT requests and treatments facilitated by the QAMH between 01/01/2008 and 31/12/2021 to get an idea of their safety and efficacy.

In the above-mentioned time period, 989 PT requests and 100 phage treatments were registered, and retrospectively analyzed. These treatments took place in 12 countries: Belgium (n=68), UK, France, The Netherlands, Germany, Spain, Portugal, Switzerland, Austria, Italy, Tunisia and Latvia (n=32). In total, 15 bacterial species were targeted for which we used 17 individual phages and 6 phage cocktails. The clinical indications were: low respiratory tract infections (LRTI, 33%), bone and prosthetic joint infections (BPJI, 23%), skin and soft tissue infections (SSTI, 22%), ear nose throat infections (ENTI, 11%), abdominal infections (AbdI, 5%), blood stream infections (BSI, 5%), and others (5%). 78 patients were treated in hospitals, 22 ambulatory. No allergic reactions or major adverse events were observed. However, 6 patients reported mild adverse reactions, possibly linked to the phage treatment. The principal modes of phage administration were local administration, for BPJI and SSTI infections, and nebulisation for LRTIs. In 18 patients, phages were administered intravenously, without any adverse reaction. The overall clinical outcome was positive in 72% of the cases with microbiological eradication observed in 52% of the patients. Phages were used complementary to antibiotic treatment in 62 patients. 2 patients were treated with phages alone intravenously.

The interest and use of PT have increased considerably. Even though no conclusion can be drawn on the therapeutic efficacy of phage therapy, our results indicate that it is more than likely safe, and possibly efficient.

Bacteriophages Microbial consortia gastrointestinal microbiome

Targeted design and manipulation of defined microbial consortia by bacteriophages

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The human gut is a complex ecosystem, consisting of eukaryotic cells, bacteria and viruses and alteration of this intestinal microbiota is associated with an increasing number of human diseases. Bacteriophages and viruses of Archea are important effectors and indicators of human health and disease by managing specific bacterial population structures and by interacting with the mucosal immune system.

To obtain functional insights into the gastrointestinal microbiome and its function in health and disease, we aim to establish a model to investigate the interaction of bacteriophages and cognate host bacteria in the mammalian gut. Therefore, we isolated specific phages from environmental samples for members of a minimal bacterial consortium, the Oligo-MM[14], which consists of 14 well-characterized bacterial strains that colonize gnotobiotic mice in a stable and reproducible manner and provides colonization resistance against *Salmonella*. These phages are used to analyze their effect on the stable community in the murine gut with respect to compositional and functional alterations as well as phage-host bacterial interaction over time.

We show, that phages lead to initial depletion of the target population and thereafter coexist with the bacteria over long periods of time. Furthermore, the addition of phages led to a significant decrease of the colonization resistance against *Salmonella*, indicating that community functions can be targeted by phages.

In summary, our work yields insights into phage-bacterial interactions in the gut and the effect of phages on fundamental microbiome functions, which will be important for evaluating the future use of phages for targeted microbiome manipulation.

m13 phage cancer PDT

Selective photokilling of colon cancer cells using a spheroid-penetrating phage retargeted to the fibronectin receptor.

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Photodynamic therapy (PDT) represents a promising therapeutic modality for cancer. Here we used an orthogonal approach (genetic/chemical) to engineer M13 bacteriophages as targeted vectors for efficient photodynamic killing of cancer cells. M13 was genetically refactored to display on the phage tip a peptide ligand (CPDIERPMC) recognizing the a5b1 integrin fibronectin receptor, a therapeutic target expressed by tumoral neovessels and tumoral cells whose overexpression is associated with a poor prognosis for patients. The refactored M13a5b1 phages demonstrated targeted tropism to HT29 and DLD1 colon cancer cells, which overexpress a5b1. Using an orthogonal approach to the genetic display, M13a5b1 phages were then chemically modified, conjugating hundreds of Rose Bengal (RB) photosensitizers on the capsid surface, without affecting the selective targeting provided by the CPDIERPMC peptide. Upon internalization, the M13a5b1-RB derivatives generated intracellular reactive oxygen species, activated by an ultralow intensity white light irradiation. The retargeted phages were then tested in PDT on both 2D and 3D (spheroids) cell cultures. Promisingly, a photodynamic killing activity on colon cancer cell lines was observed at picomolar concentrations of the phage vector. Moreover, the M13a5b1-RB derivatives were able to penetrate into the spheroid core and promote selective cytotoxicity and disaggregation of the spheroid upon light irradiation.

macrophage | phage-immune interaction | Pseudomonas aeruginosa | inflammation

Phages promote polarization of M1 macrophages

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Though medically important as therapeutic agents and ubiquitous in human and animal microbiomes, little is known about how phages interact with the mammalian immune system. The consensus is that phages are harmless; however, as viruses, we hypothesized that the host immune system can detect and respond to phages. Here, we investigate phage-immune interactions by determining their influence on macrophages, heterogeneous and highly plastic cells that are specialized in sensing and responding to microorganisms, including viruses. We show that macrophages exposed to phages of *Pseudomonas aeruginosa* transcriptionally upregulate 279 genes and downregulate 100 genes. Gene Ontology and KEGG pathway analyses suggest that this response was driven by Toll-like receptor 2 (TLR2) and TLR9 signalling pathways that induced the transcription factor NF-κB, promoting expression of predominantly proinflammatory and antiviral genes. Moreover, we demonstrate that the response was independent of TLR4 stimulation. Transcriptomic changes correlated with increased secretion of proinflammatory cytokines, including tumor necrosis factor (TNF), interleukin 1β (IL-1β), IL-6, CC chemokine ligand 5 (CCL5/RANTES), monocyte-chemotactic protein 3 (MCP3/CCL7), and interferon-β (IFNβ). These data suggest that phages promote macrophage polarization towards inflammatory M1 phenotypes via mechanisms similar to those of eukaryotic viruses. Thus, phages may play a role in host defense and cell-mediated inflammatory diseases.

anti-phage defence systems | Bacteriophages | viral evasion mechanisms

Bacteriophages mobilise pathogenicity islands encoding immune systems as weapons to eradicate competitors

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Bacteria encode sophisticated anti-phage systems carried on defence islands, which are diverse, versatile and display high genetic mobility. How this variability and mobility occurs remains unknown. Here we demonstrate that a widespread family of pathogenicity islands, the phage-inducible chromosomal islands (PICIs), carry an impressive arsenal of defence mechanisms, which are disseminated intra- and inter-generically by helper phages. These defence systems provide broad immunity, blocking not only phage reproduction, but also plasmid and non-cognate PICI transfer. Remarkably, our results demonstrate that phages mobilise PICI-encoded immunity systems to use against other mobile genetic elements, which compete with the phages for the same bacterial hosts. Therefore, the cost of mobilising PICIs is essential for phage (and bacterial) survival in nature. Our results highlight PICIs as key players controlling horizontal gene transfer in nature and demonstrate that PICIs and phages establish mutually beneficial interactions which drive bacterial ecology and evolution.

Lysis/Lysogeny | Phage-Antibiotic Synergy | Temperate Phage

Biasing Bacteriophage Behaviours

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Temperate phages can enter a lytic or lysogenic cycle, the latter akin to dormancy. These phages are responsible for over 10^{23} infections per second, and most bacteria already contain within their genomes at least one dormant phage awaiting the right signal to wake and lyse its host. The decision between lysis and lysogeny is arguably the most important in all of biology. We investigate how to bias this decision to better manipulate bacterial populations. Using the model *E. coli* phage HK97, we demonstrated that ciprofloxacin, a DNA-damaging antibiotic that induces the bacterial SOS response, canonically an inducer of temperate phages, results in a potent 450 000-fold synergy dependent on the lysis/lysogeny decision. We termed this effect temperate Phage-Antibiotic Synergy (tPAS). We found that this synergy holds true across many SOS-inducing antibiotics but, curiously, observed a comparable synergy with protein synthesis inhibitors completely independently of the SOS response. Notably, synergy here was not due to induction of the phage as it was with SOS-inducers, but rather due to the antibiotics blocking entry into lysogeny. This is the first report of a means of chemically blocking entry into lysogeny, providing a new means for manipulating the key lysis/lysogeny decision point in temperate phages. Armed with these appropriate controls for tools to block entry to, or force exit from, lysogeny, we are now embarking on high-throughput screens to identify new compounds capable of biasing this behaviour.

Lysogeny arbitrium communication

Regulation of prophage induction and lysogenization by phage communication systems and the SOS response

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Many phages cause both lytic infections, where they release viral particles, and dormant infections, where they await future opportunities to reactivate. The benefits of each transmission mode depend on the density of susceptible hosts in the environment. Many phages that infect Bacillus species use molecular signalling to respond plastically to changes in host availability. These phages produce a signal during lytic infection and regulate, based on the signal concentration in the environment, the probability with which they switch to causing dormant infections. I will discuss the adaptive significance of plasticity in phage life-history traits in fluctuating environments. Using a combination of theory and experiments, we show that host density fluctuations selects for plasticity in entering lysogeny as well as virus reactivation once signal concentrations decline. We also find that conflict over the responses of lysogenization and reactivation to signal is resolved through the evolution of different response thresholds for each trait. Finally, we demonstrate that lysis/lysogeny decisions are further modified by intracellular cues that indicate the viability of the host. Specifically, activation of the host SOS stress response alters the responsiveness of phage to its signals, enabling the phage to integrate information about the intracellular and extracellular environment to optimize its transmission. Collectively, these findings deepen our understanding of the ways phage regulate their infection strategies, which can be leveraged to manipulate host and phage population dynamics in natural environments.

Viral factories | virions warehouses | membrane-less compartments | DNA replication | particles assembly | video-microscopy

Compartmentalization of bacteriophage SPP1 replication and assembly in the Gram-positive bacterium *Bacillus subtilis*.

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Viral infection affects host cell homeostasis and draws extensive cellular biosynthetic resources. Cell machineries are also hijacked for optimal viruses multiplication. Here, we investigated the impact of these processes in the spatial organization of the bacterial cell. We show that infection by bacteriophage SPP1 leads to the formation of two types of membrane-less compartments in the cytoplasm of the Gram-positive bacterium *Bacillus subtilis*.

Phage DNA localizes in a single DNA compartment in mono-infected cells. More than 300 copies of the SPP1 viral genome are synthesized in the first 25 minutes of infection, doubling the cell DNA content. This process requires fast recruitment of the bacterial replisome proteins orchestrated by the SPP1 helicase gp40 that binds to the DnaG primase and to DnaX, a subunit of DNA polymerase III. Hybrid phage-bacterial replisomes accumulate in discrete genome replication factories within the phage DNA compartment. Collectively, our data reveal that the host replisome machinery is massively redirected and dedicated for optimal SPP1 DNA replication.

Later in infection, procapsids localize at the periphery of the DNA compartment for genome packaging whereas DNA-filled capsids fully segregate to spatially distinct warehouse compartments where viral particles accumulate. Warehouses are found mostly side by side from the viral DNA replication *foci*.

The dynamics of the SPP1 replication factory and virions warehouses were visualized during the complete SPP1 infection cycle using microfluidics. The spatial and temporal distribution in the bacterial cytoplasm highlights a sequential program of molecular interactions. It leads to an extensive re-organization of the crowded cytoplasm to achieve assembly of about 150 infective particles within 25 minutes of infection. Structuration of viral factories to confine phage DNA enzymatic reactions appear as a very efficient strategy for SPP1 to exploit the bacterial resources for its own profit.

Horizontal gene transfer host range Host specificity

Diversity Generating Retroelements: From phage host-range evolution to targeted mutagenesis tools

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Diversity Generating Retroelements (DGRs) are fascinating genetic systems used by some bacteriophages to diversify protein domains involved in host cell recognition. These systems perform a localized and controlled mutagenesis that preserves the overall protein structure while generating up to 10^{20} variants, allowing bacteriophages to reach out to infect new strains. By refactoring the DGR components onto an E. coli plasmid system, we have for the first time harnessed a bacteriophage DGR turning it into a flexible and innovative tool for generating targeted mutagenesis.

I will present our ongoing work on redirecting this system towards engineering the phage λ host range by mutagenizing the phage tail fibers and its bacterial receptor conjointly. We were able to generate bacterial receptor escape mutant resistant to the phage attachment, and then phage tail fiber mutant able to re-infect these escape mutants, essentially re-creating an accelerated version of the co-evolutionary dynamics happening between phages and bacteria at the cell surface. This dataset informs us on the structural details of the phage/host binding interface.

lateral transduction | Pathogenicity island | Staphylococcus aureus

Dual Pathogenicity Island Transfer by a Novel Form of Lateral Transduction

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The Staphylococcus aureus pathogenicity islands (SaPIs) are highly mobile genetic elements that carry genes for superantigens and toxins. They are prototypical members of the phage-inducible chromosomal islands that parasitize bacteriophages (phages) for their reproduction and dissemination. Here we have discovered that the SaPIs follow an atypical life cycle after induction, which allows them to engage in lateral transduction (LT) to mobilize large sections of the bacterial chromosome at high frequencies, and a second more sophisticated form of LT that produces transducing particles capable of delivering an intact SaPI element with host DNA. As a result of this second mechanism, that we term lateral cotransduction (LcT), SaPIbov1 can piggyback its own LT of staphylococcal pathogenicity island vSa so that both islands can be mobilised in a single infective particle and transferred to the same host cell, all in parallel to the normal SaPI life cycle. Moreover, unlike phage-mediated LT that is limited to prophage induction, we found that SaPI-mediated LT and LcT occur during lysogenic induction, infection of SaPI-containing strains, and even infection of strains that do not carry a SaPI. Therefore, our results show SaPIs mediate dynamic forms of gene transfer and they are one of the most important drivers of pathogen evolution.

anti-phage defence systems | E. coli | Phage Satellites

Phages and their satellites encode hotspots of antiviral systems

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Bacteria carry diverse genetic systems to defend against viral infection, some of which are found within prophages where they inhibit competing viruses. Phage satellites pose additional pressures on phages by hijacking key viral elements to their own benefit. Here, we show that E. coli P2-like phages and their parasitic P4-like satellites carry hotspots of genetic variation containing reservoirs of anti-phage systems. We validate the activity of diverse systems and describe PARIS, an abortive infection system triggered by a phage-encoded anti-restriction protein. Antiviral hotspots participate in inter-viral competition and shape dynamics between the bacterial host, P2-like phages, and P4-like satellites. Notably, the anti-phage activity of satellites can benefit the helper phage during competition with virulent phages, turning a parasitic relationship into a mutualistic one. Anti-phage hotspots are present across distant species and constitute a substantial source of systems that participate in the competition between mobile genetic elements.

bacteria-phage interaction | antiphage defense system | CRISPR-Cas

Type III CRISPR-Cas provides resistance against nucleus-forming jumbo phages via abortive infection

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Many prokaryotes encode CRISPR-Cas systems as immune protection against bacteriophages. During infection, jumbo phages evade DNA-targeting CRISPR–Cas immunity by physically protecting their DNA inside a viral nucleus-like structure. However, phage mRNA remains vulnerable to RNA-targeting CRISPR–Cas systems (e.g. type III) when exiting the nucleus for translation. In *Serratia* the type III-A accessory nuclease is required for defence against the jumbo phage PCH45; however, the mechanism of protection is unclear. Here we solved the structure of the accessory nuclease, which revealed it is a member of the NucC family. We show that NucC, upon binding cA_3, becomes an active endonuclease that promotes dsDNA cleavage. We demonstrate that NucC is activated upon the targeting of jumbo phage RNA, which results in destruction of the bacterial chromosome. Consequently, type III immunity against the jumbo phage results in cell death, thus providing bacterial population protection by suppressing the phage epidemic. Overall, we show that type III CRISPR-Cas systems overcome the inaccessible nature of jumbo phage DNA via abortive infection. Our bioinformatic and experimental data showed that type III jumbo phage immunity is widespread and can be elicited by accessory DNases as well as RNases.

Endolysin | Cell wall | Bacteriolysis | Heteromeric lytic enzyme | Endolysin complex

On the occurrence and multimerization of two-polypeptide phage endolysins encoded in single genes

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Bacteriophages employ endolysins to promote host cell lysis for release of the virion progeny. Endolysins are enzymes that cleave peptidoglycan, the major constituent of the bacterial cell wall barrier. Because endolysins can cause bacteriolysis when added exogenously to bacteria, they are currently viewed as promising alternative antimicrobials to fight antibiotic resistant bacteria.

Most endolysins are thought to correspond to single polypeptide, monomeric enzymes. However, a few endolysins were shown to act as two-polypeptide isoforms [1,2], with some forming heteromeric complexes [3-5]. The two isoforms are produced thanks to the presence of an in frame, internal translation start site within the endolysin gene (iTSS). One isoform corresponds to the full-length polypeptide (FLP) deduced from the gene sequence, whereas the other corresponds to a C-terminal product (CTP) initiated at the iTSS.

Surprisingly, in a recent bioinformatics study we found evidence that two-polypeptide endolysins may be rather common in phages infecting Gram-positive bacteria. We have experimentally validated the production of two protein isoforms for four distinct endolysins. In addition, by studying in detail one of the endolysins, we observed that the two isolated isoforms were inactive, and only when they assembled as a heteromultimer the active endolysin was generated. Moreover, by employing biophysical methods (chromatography, native MS and SAXS), we have uncovered a 1FLP:5CTP stoichiometry for the complex, which has never been described before for endolysins. These results challenge the established view of endolysins being mostly formed by single, monomeric polypeptide chains.

1. Catalão et al. 2011, doi: 10.1371/journal.pone.0020515

2. Abaev et al. 2013, doi: 10.1007/s00253-012-4252-4

3. Proença et al. 2015, doi: 10.1111/mmi.12857

4. Dunne et al. 2016, doi: 10.1074/jbc.M115.671172

5. Zhou et al. 202, doi: 10.1371/journal.ppat.1008394

urinary tract infection | phage engineering | Bacteriocins | E.coli | Klebsiella | Enterococcus faecalis

Enhancing bacteriophage therapeutics through *in situ* production and release of heterologous antimicrobial effectors

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Bacteriophages operate via pathogen-specific mechanisms of action distinct from conventional, broad-spectrum antibiotics and are emerging as promising alternatives. However, phage-mediated killing is often limited by bacterial resistance development. Here, we engineer phages for target-specific effector gene delivery and host-dependent production of colicin-like bacteriocins and cell wall hydrolases. Using urinary tract infection (UTI) as a model, we show how heterologous effector phage therapeutics (HEPTs) suppress resistance and improve uropathogen killing by dual phage- and effector-mediated targeting. Moreover, we designed HEPTs to control polymicrobial uropathogen communities through production of effectors with cross-genus activity. Using a phage-based companion diagnostic, we identified potential HEPT responder patients and treated their urine *ex vivo*. Compared to wildtype phage, a colicin E7-producing HEPT demonstrated superior control of patient *E. coli* bacteriuria. Arming phages with heterologous effectors paves the way for successful UTI treatment and represents a versatile tool to enhance and adapt phage-based precision antimicrobials.

RNA modifications | T4 phage | infection mechanism

How to connect RNAs with proteins? -RNAylation of proteins

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The mechanisms by which viruses hijack their host's genetic machinery are of enormous current interest. One mechanism is adenosine diphosphate (ADP) ribosylation, where ADP-ribosyltransferases (ARTs) transfer an ADP-ribose fragment from the ubiquitous coenzyme nicotinamide adenine dinucleotide (NAD) to acceptor proteins. Here, we report that bacteriophage T4 ARTs surprisingly accept not only NAD, but also NAD-capped-RNA (1) as substrate, thereby covalently linking entire RNA chains to acceptor proteins *in vitro* and *in vivo* (2). We term this reaction an "RNAylation".

This new post-translational protein modification (PTM) changes the properties of the target protein such as charge and size. In *in vitro* experiments we were able to show that the RNAylation of the ribosomal protein S1 by ModB using radiolabeled NAD-capped RNA dramatically increases the protein size. Western blot experiments showed that the RNAylation also occurs *in vivo* – in *E. coli* during T4 phage infection. Removal of ADP-ribosylation and RNAylation, during T4 phage infection dramatically decelerates lysis thereby underlining the significance of these PTMs for T4 phage infection.

Our findings challenge the established views of phage infection and exemplify that the structural and functional boundaries between different classes of biopolymers become increasingly blurry. We suggest that RNAylation by ARTs could play so far undetected roles in the interaction of phages and bacteria or even in higher organisms.

References

1. Schauerte, M., Pozhydaieva, N., Höfer, K. Shaping the Bacterial Epitranscriptome—5'-Terminal and Internal RNA Modifications. *Adv. Biology.* 2021, 2100834.

2. Höfer, K.*+, Schauerte, M.*, Grawenhoff, J., Wulf, A., Welp, L. M., Billau, F. A., Urlaub, H., Jäschke, A.+ Viral ADP-ribosyltransferases attach RNA chains to host proteins. *bioRxiv* 2021 2021.06.04.446905. *+ corresponding author,* *These authors contributed equally to this work.

algal viruses | infection process | assembly | structure | cryo-electron microscopy

Structural Insight into Giant Virus Infection of Marine Picoplankton

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Marine viruses are key drivers of nutrient and biogeochemical cycling in oceans by terminating algal blooms. The marine picoalga *Ostreococcus tauri* is among the smallest free-living eukaryotes known to date. *O. tauri* is frequently infected by giant, species-specific viruses belonging to the nucleocytoplasmic large double-stranded DNA virus (NCLDV) family. To visualize and characterize the virus infection of this highly simplified unicellular green alga, we employ an integrative approach by combining biochemical and biophysical techniques with state-of-the-art cryo-electron microscopy and -tomography (cryo-EM and -ET). We found that Ostreococcus tauri virus 5 (OtV-5) possesses a quasi-icosahedral capsid with a diameter of 150 nm and triangulation number 127 formed by major capsid proteins with the double jelly roll fold. Applying cryo-EM and -ET on late stage-infected *O. tauri* cells, we observed that the number of virions produced by an infected cell is limited by its small size to about 25, which is consistent with the previously reported burst size [1]. During the early stage of infection, OtV-5 attaches to the algal cell via one of its capsid vertices. The virus possesses an inner lipid bilayer, which fuses with the algal cell membrane and forms a tunnel through which the viral genome is transferred into the host cell. We furthermore identified various virus assembly intermediates inside the infected cell, ranging from inner membrane precursors serving as nucleation sites for partially assembled angular capsid segments, over complete empty capsids, to genome-filled virions. Our results provide further understanding of the diversity of virus structure and function, and provide insight into the processes by which viruses shape the marine community structure. Controlling biomass and primary production of phytoplankton communities might be a relevant measure to mitigate the effects of global climate change.

[1] Derelle E. *et al.*, PLoS ONE 3(5), e2250 (2008)

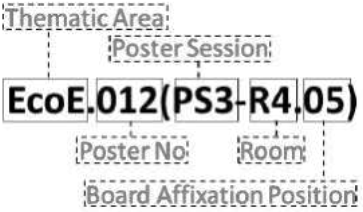
POSTER COMMUNICATIONS

ABSTRACTS

5. POSTER COMMUNICATIONS - ABSTRACTS

Poster code and distribution

Posters will be displayed in different locations of the venue, and their presentation will be distributed by 3 poster sessions (3 different days) through the following scheme:



Room	July 19 Poster Session 1	July 20 Poster Session 2	July 21 Poster Session 3
R1	EcoE.001-022	EcoE.023-044	EcoE.045-065
R2	VirS.087-106	OverB.107-127	EcoE.066-086
R3	HostD.128-149	HostD.150-171	HostD.172-194
R4	MolM.195-216	MolM.217-238	MolM.239-260
R5	AgroV.261-282	AgroV.283-305	AgroV.306-328
	BioH.329-359	BioH.360-391	BioH.392-423

Thematic areas
EcoE: Ecology and evolution of microbial viruses
VirS: Virus structures and function
OverB: Overcoming cell barriers
HostD: Host defence and viral evasion mechanisms
MolM: Molecular mechanisms
AgroV: Agro-food, veterinary and environmental biotechnology applications
BioH: Biotechnology applications in health care

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Ecology and evolution of microbial viruses

Hot spring | Bacteriophage isolation | Lysogeny | Mitomycin-C

Genomic characterization of a lysogenic bacteriophage of *Pseudomonas* sp. isolated from geothermal spring of India

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The Geological Society of India (GSI) has reported seven geothermal provinces covering nearly 400 thermal springs based on the tectonic elements and geothermal gradients across India. Despite the widespread distribution of geothermal springs in India, Indian hot springs are least explored in terms of viruses compared to their host bacteria. Vashisht hot spring is located in the Himalayan geothermal province, in the Manali region of Kullu district, Himachal Pradesh. The hot spring is situated on the other side of the Beas River. The healing benefits of the hot spring make it one of Manali's most renowned tourist attractions. Here we report, the isolation and genomic characterization of a lysogenic bacteriophage of the host *Pseudomonas* sp. strain VB3 from Vashisht hot spring, Himachal Pradesh, India. A lysogenic bacteriophage, vB_Psp_VB3 was isolated by mitomycin-C induction from *Pseudomonas* sp. strain VB3. Host range analysis with other strains of *Pseudomonas* species appeared to be limited. Genomic DNA of the phage was isolated after mitomycin-C treatment and subsequently sequenced on the Illumina HiSeq platform. In these extreme environments, the phage enters the lysogeny state, facilitating long-term co-evolution with the bacterial host, and thereby establishing a mutually beneficial interaction for their survival. The study suggested that lysogeny may be a viable lifestyle in hot springs.

bacteriophages CRISPR marine metagenomics sea-surface microlayer

Brackish sea surface slicks harbour distinctive communities of bacteriophages with metabolic benefits

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Sea-surface slicks dampen small-scale waves at the water surface, can cover up to 30% of surface area in coastal regions, and harbor a distinctive bacterial community composition. So far nothing is known about the viral communities of brackish sea-surface slicks. Here, we sampled the sea-surface microlayer (SML), the <1 mm thin skin of the water column within a surface slick and compared its virome to non-slick SML, and to corresponding subsurface water from 1 m depth (SSW) in the Kalmar Sound, Sweden. Samples underwent size-fractionated filtration to sequence metagenomes of the >5 µm, 5-0.2 µm and <0.2 µm (virome) fractions. The slick SML was highly enriched in surfactants and dissolved organic carbon compared to non-slick SML and SSW. Metagenomic analysis recovered 429 viral scaffolds >10 kb, and the viral community in the slick SML was distinct compared to the other samples across all filtered fractions. Three viruses of the same genus that were present in all samples, reached very high abundances (read coverage=14403) in the slick SML compared to non-slick SML and SSW, and shared protein similarities with *Cellulophaga* and *Flavobacteria* phages as well as k-mer frequency patterns with metagenome-assembled genomes (MAGs) from these hosts. Based on read mapping, eight viral genomes between 27 and 106 kb length were unique to the slick SML. These phages carried metabolic genes, related to e.g., folate, cobalamin, and nucleotide synthesis. Among 82 dereplicated MAGs, a complete genome of the polysaccharide-degrader *Paraglaciecola* sp. (family *Alteromonadaceae*) carried adaptive immunity in form of a CRISPR system. One CRISPR spacer targeted a 106-kb viral scaffold unique to the slick SML and other known *Gammaproteobacteria* phage isolates from Baltic Sea. Our data show that brackish surface slicks harbor distinctive bacteriophage communities providing auxiliary metabolic genes to their hosts. Abundant lytic phages likely influence carbon turnover at the air-sea boundary.

archaeal DNA viruses | major capsid protein | marine Thaumarchaeota

Diverse viruses of marine archaea discovered using metagenomics

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During the past decade, metagenomics became a method of choice for discovery of novel viruses. However, host assignment for uncultured viruses remains challenging, especially for archaeal viruses, which are grossly undersampled compared to viruses of bacteria and eukaryotes. Here, we assessed the utility of CRISPR spacer targeting, tRNA gene matching and homology searches for viral signature proteins, namely, major capsid proteins, for assignment of archaeal hosts and validate these approaches on a metavirome from Yangshan Harbor (YSH). We report 35 new genomes of viruses which could be confidently assigned to hosts representing diverse lineages of marine archaea. We show that the archaeal YSH virome is highly diverse, with some viruses enriching the previously described virus groups, including Magroviruses of Marine Group II Archaea, and others representing novel groups of marine archaeal viruses. We propose four new families within the class Caudoviricetes for classification of viruses predicted to infect Nitrososphaeria (formerly Thaumarchaeota), a class of environmentally important and widespread ammonia-oxidizing archaea, and highlight the relationship between the soil and marine thaumarchaeal viruses. Collectively, our results illustrate the utility of viral metagenomics in exploring the archaeal virome and provide new insights into the diversity and evolution of marine archaeal viruses.

Giant Viruses | algal viruses | endogenous viruses

Genomics of Endogenous Giant Viruses

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Recent discoveries have shown that endogenous giant viruses are widespread in protists, especially green algae, and that they may play important roles in host genome evolution (Moniruzzaman et al., Nature, 2020). Here we examined genomes of *Chlamydomonas reinhardtii* to see if endogenous giant viruses could be detected in field isolates of this model green alga. In six strains we discovered sequences belonging to endogenous giant viruses that reach up to several hundred kilobases in length. In addition, we have also discovered the entire genome of a closely related giant virus that is endogenized within the genome of *Chlamydomonas* incerta, the closest sequenced phylogenetic relatives of *C. reinhardtii*. Most endogenous giant viruses belonged to the Imitervirales, but one belongs to the Algavirales, demonstrating that multiple distinct lineages of viruses endogenize into this host. Endogenous giant viruses add hundreds of new gene families to the host strains, highlighting their contribution to the pangenome dynamics and inter-strain genomic variability of *C. reinhardtii*. The presence of endogenous giant viruses in *C. reinhardtii* opens up important avenues of future work that can leverage this model system to determine the molecular underpinnings of these intriguing elements. Moreover, although viruses of *C. reinhardtii* have not been detected in the past, our results indicate that they are common in nature.

metagenomics marine viromics

Long-reads improve the recovery of viral diversity from marine samples

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The recovery of DNA from viromes is a major obstacle in the use of long-read sequencing to study their genomes. In this sense, the use of cellular metagenomes (>0.2) emerges as an interesting complementary tool since they contain large amounts of naturally amplified viral genomes from pre-lytic replication. We have applied second (Illumina Nextseq; short-reads) and third (PacBio Sequel II; long-reads) generation sequencing to compare the diversity and features of the viral community in a marine sample obtained from offshore waters of the western Mediterranean. We have found a major wedge of the expected marine viral diversity directly recovered by the raw PacBio CCS reads. More than 30,000 sequences were detected only in this dataset with no homologous in the long- and short-read assembly and ca. 26,000 in the comparison to the large dataset of the Global Ocean Virome 2 (GOV2), highlighting the information gap created by the assembly bias. At the level of complete viral genomes, the performance was similar in both approaches. However, the longest average length of the sequences was provided by the hybrid long-and short-read assembly which also improved the host assignment. Although no novel major clades of viruses were found, there was a significant increase of the intra-clade genomic diversity recovered by long-reads that produced an enriched assessment of the real diversity and allowed the discovery of novel genes with biotechnological potential (e. g. endolysins).

Spaceflight | Surrogate bacteriophages | Phage infection

Stability of Bacteriophage MS2 in Spaceflight Conditions

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Recent years have demonstrated the dangers that viral diseases pose to public health. If an outbreak happens in a space habitat, the consequences would be more devastating for astronaut health than on Earth. Additionally, bacteriophages pose a real threat in spaceflight biotechnology. Considering that biotechnology plays and will play an important role in deep space exploration like oxygen/food production by cyanobacteria and synthesis of drugs/proteins by genetically engineered bacteria. Therefore, bacteriophages have the capacity to destroy bacterial cultures, halting biotechnological processes on space stations. Since astronauts will depend on those processes, bacteriophage contamination will be a significant threat. It is relevant to understand the bacteriophage infectivity and distribution in spaceflight environments, which differ significantly from the environments on Earth.

In this research, virions of bacteriophage MS2 have been exposed to some of the physical conditions of deep space: temperatures of -80 °C, and up to 80 °C, lunar dust simulant, near-vacuum pressure, high X-ray doses, and dispersion in cold droplets under presence of UV. Results show that most tested conditions of space rapidly inactivate bacteriophage MS2. However, to some of those conditions like extremely low temperatures, near-vacuum, or X-rays, viruses showed a higher degree of resistance than expected. Also, lunar dust simulant showed some degree of protection for viruses at high temperatures but aids their inactivation at low temperatures. Additionally, MS2 has been shown to be transmissible in air droplets and aerosols, even under UV light, aiding the idea of viral stability in water plumes of solar system's icy moons like Europa or Enceladus.

Taken together, this study shows that physical conditions of deep space inactivate bacteriophage MS2 to some extent. This paves the way for the future research of spaceflight virology.

phage-bacteria co-evolution | plant pathogens | bacteriophage biocontrol | host range

Jack of all strains, master of none? Host range and virulence on bacteriophages of a phytopathogenic bacterium

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Bacteriophages (phages) hold the promise of being the perfect hunters of pathogenic bacteria in a wide range of applications, from medicine to industry or agriculture. As a consequence of the extreme specificity of most phages, a broad host range is the main criterion of phage selection in therapeutic cocktails. Considering evolutionary trade-offs among phage characteristics such as phage efficacy and host range, could refine the selection of candidate phages and will help the durability of many phage applications.

Bacterial wilt caused by the *Ralstonia solanacearum* species complex (RSSC) is among the most important plant diseases worldwide, severely affecting a high number of crops. Our aim was to isolate and study new phages capable of infecting the RSSC, to be used as biocontrol tools in the South West Indian Ocean. A high genetic diversity was found in 23 phages infecting RSSC isolated in Mauritius and Reunion islands, with 7 new genera and 13 new species. In an innovative approach to assess the host range of phages, phylogenetic data of the targeted bacteria was integrated in the analysis, creating a phage phylogenetic host range index (PHI). We demonstrate that all phages preferentially attack the most abundant RSSC variant in both islands, but harbour a high host range variability. The 23 phages also show differences in their virulence, i.e. their efficiency at decreasing bacterial growth in vitro. We demonstrate a positive correlation among the phage PHI and their virulence for Mauritian phages, but no pattern for Reunion ones. Our results point out that phages with a wide host range are optimal candidates for biocontrol in complex epidemiological situations, such as in Mauritius island, but that this pattern is not extensive to every phage-bacteria interaction. We also show that different phages from the same species are able to target very different RSSC strains, suggesting that phage genomes and host range can rapidly evolve.

Auxiliary Metabolic Genes | Horizontal gene transfer | Lambdoid phages

Horizontal gene transfer from temperate and virulent phage genomes impacts bacterial genome evolution

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Phages evolve rapidly due to high mutation rates and frequent horizontal gene transfer. Gene transfer can occur among phage genomes, from bacteria to phages, or vice versa. Both the infection with temperate or virulent phages might lead to gene transfer with different regions of the bacterial genome and here we investigated both routes.

Temperate lambdoid phages have highly mosaic genomes and here we analyzed genes that are transferred together between distantly related phages and integrated prophages. We found that the genomes of all lambdoid phages could be grouped based on gene content. Nevertheless, some groups shared highly similar proteins, indicating the transfer of those genes across groups. We observed that gene transfer affects all regions to a similar extent; however, co-transfer of genes occurs more frequently in the immunity and late operon. This suggests that conditional co-transfer, where the functioning of one protein depends on the presence of another, may lead to genome mosaicism.

Next, we studied gene transfer in virulent phages by analyzing the evolution of auxiliary metabolic genes (AMGs), which have a function within the host's metabolism. We investigated whether AMGs evolve solely in phages after their transfer from bacteria or whether they might be transferred back into bacterial genomes. Due to restrictions on phage genome length, AMGs evolving solely in phages are expected to evolve into shorter versions compared to bacterial homologs. However, by analyzing 1886 virulent phage genomes with AMGs, we did not find a consistent trend towards gene shortening with only 34% of the AMGs showing a decrease in gene length. Additionally, phylogenetic reconstructions of some AMG families support multiple transfers between phages and bacteria. We conclude that AMGs might be transferred into bacterial genomes after accelerated evolution in phage genomes, contributing to bacterial diversification.

Anaerobic digestion | viral ecology | molecular microbial ecology

Characterization of virus-host dynamics in anaerobic digesters under abiotic stress

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Viruses of microbes are major players in various ecosystems, typically affecting the structuration and dynamics of microbial communities. They nevertheless remain poorly explored in anaerobic digestion (AD) plants, where biowaste is valorised into methane-rich biogas. The AD process ecosystems are very sensitive to disturbance, leading to inhibition and loss of methane production. We were therefore interested in better understanding the interplay between abiotic disturbance, microbial community composition, including the viromes, and process performance.

The variations of both viral and prokaryotic populations were followed in batch AD microcosms under abiotic stress. Four types of abiotic disturbances were tested in triplicates. During incubation, either NaCl, NH4Cl or phenol was injected into the reactors, as inhibitors previously reported in full-scale plants. Mitomycin C was also tested, since it can activate the lytic cycle of proviruses.

We first confirmed a significant impact of the tested stresses on biogas production. We performed 16S rDNA metabarcoding targeting archaea and bacteria, and shotgun metagenomic sequencing of 30 selected metaviromes.

Metavirome coassembly showed a N50 of 3,886 bases, and yielded more than 10^5 contigs longer than 1000 bp. Among them, 34,947 were predicted as putative viral contigs using VIBRANT (including 823 of high quality). There was a good agreement between the prokarotyic community composition and the predicted hosts of the viral contigs, with the dominance of Clostridiales, in both cases. We also detected auxiliary metabolic genes related to carbohydrate metabolism and the sulphur relay system, which seems relevant to this specific ecosystem. In the near future, we will identify the viral contigs that are differentially abundant according to the applied disturbance, and interpret the observed community dynamics. We also plan to apply the epicPCR technique to confirm specific virus-host pairs.

Recombination transduction Horizontal gene transfer Herd immunity adaptation CRISPR Antibiotic resistance

CRISPR, herd immunity and transduction - an unexpected way of how bacteria have 'sex'

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Unlike most eukaryotes, bacteria reproduce by binary fission, which is devoid of recombination. Recombination is known to be able to speed up adaptation by bringing beneficial alleles arising in different individuals together. In a purely clonal bacterial population, however, the beneficial alleles compete with each other in a process called clonal interference, which slows down adaptation. Although bacteria lack recombination as it occurs in eukaryotes, they can occasionally exchange genetic information directly between individuals in a process called horizontal gene transfer (HGT). How much HGT affects evolutionary dynamics within bacterial populations, however, remains largely unclear. We experimentally determined that in bacterial populations of Eschierichia coli a combination of CRISPR-Cas9-based herd immunity and HGT mediated by a general transducing phage P1 can lead to coexistence of CRISPR+ and CRISPR– strains and the phage in the population. As a result, it allows for a continuous gene flow from the susceptible fraction of the population to the resistant one. This gene flow then leads to bacterial adaptation by recombination, which exceeds adaptation by de novo mutations by one to two orders of magnitude and circumvents clonal interference. We show that the combination of CRISPR, herd immunity and transduction can lead to population-wide recombination, which is frequent enough to substitute the evolutionary advantage of sexual (i.e., meiotic) recombination described by a so-called Hill-Robertson effect.

Anaerobic digestion | Antibiotic resistance gene | metagenomics | phage

Limited role of virome on the spread of antibiotic resistance genes in anaerobic digestion

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Batch anaerobic digestion experiments were established covering different substrate types (swine manure, chicken manure and sewage sludge). The role of substrate types on the fate of antibiotic resistance genes (ARGs) were compared through the qPCR and metagenomics analysis. The effects of substrate microbial community were also revealed though the comparison between with and without autoclave sterilizer. The virome was identified from 59 metagenomic samples in the anaerobic digestion system, and 13,895 virus clusters were collected, where Siphoviridae and Podoviridae were the dominant phages. The virus-host prediction indicated that most of the virus cannot infect across the genus. Only 22 of 13,895 virus clusters were found to carry the ARGs, 3 of 22 virus clusters carried high risk rank ARGs (rank I and II), and all of them have limited host range. It was generally considered that the horizontal gene transfer (HGT) of ARGs identified between two distantly related genomes (less than 97% of 16S rRNA sequence similarity, genus level) could make more sense. The limited ARGs carrying virus clusters and corresponding limited host range indicated the limited role of virome on the spread of ARGs in the anaerobic digestion system through the transduction. The host range of ARGs was also deciphered at the contigs level, and the ARGs located together with integrative and conjugative elements (ICEs) accounted for above 60.0%, although the ratio varied from the substrate types in the anaerobic digestion. Compared to the transduction, the conjugation could have contributed more to the spread of ARGs.

Archaeal Virus microdiversity Deep Subsurface

Microdiversity of archaeal viruses from a terrestrial subsurface aquifer

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Several terrestrial subsurface aquifers are dominated by the members of the carbon fixing, biofilm forming genus *Candidatus* Altiarchaeum (*1*, *2*). Yet little is known about the microdiversity of associated viruses, although it can be important to counteract viral defense by, e.g., escape the archaeal clustered regularly interspaced short palindromic repeat (CRISPR) system. Successful countermeasures of lytic viruses could lead to increased cell lysis and thus influence the bioavailable carbon within the terrestrial subsurface. We studied the microdiversity of a published 8.9 kb circular virus (*3*) within the sulfuric spring 'Mühlbacher Schwefelquelle' (Regensburg, Germany) across assembled metagenomes spanning six years. By mapping metagenomic reads to the viral genome we identified various single nucleotide polymorphisms (SNPs) within coding sequences of uncharacterized proteins and within intergenic regions of the virus. A small set of SNPs was located within gene coding regions that are targeted by low abundant CRISPR spacers within the population. Our results demonstrate that the microdiversity of a lytic virus infecting a dominant archaeon in the subsurface results in altered protein sequences and can cause adaptation pressure within the CRISPR system of the host.

1. A. J. Probst, C. Moissl-Eichinger, *Life Basel*. **5**, 1381–95 (2015).
2. A. J. Probst *et al.*, *Nat. Commun.* **5**, 5497 (2014).
3. J. Rahlff *et al.*, *Nat. Commun.* **12**, 4642 (2021).

experimental evolution | adsorption process | adaptation | RNA virus evolution | Single stranded RNA phage | latent period | extreme environments

Evolutionary strategies for viral adaptation to low host density depending on the environment temperature

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Microbes, as well as their viruses, can be found in most environments on our planet, including some presenting very harsh conditions for life. In the same way as the formers are able to get adapted to adverse conditions, viruses have developed strategies to optimize the infection of their hosts. These strategies include improvement of the virus adsorption to the cell and of the genome translocation, an increased permanence within the host, or unusual burst sizes, just to mention some examples.

Tracking the pathways of evolution along history is a difficult task, due to the frequent interaction among selective pressures and our lack of knowledge of how they have varied over time. Experimental evolution under controlled conditions in the lab may be very useful to identify and characterize the mutations that arise under particular selective pressures, making it possible to establish interactions between genotype and phenotype.

In this work, we have used Qβ bacteriophage, a -ssRNA phage that replicates optimally at 37 °C, to carry out evolution experiments that combine two selective pressures: low host density and a non-optimal temperature (43 °C). These conditions had been previously studied separately. Qβ adaptation to low host density at optimal temperature takes place through a mutation in the minor capsid protein. The phenotypic effect of this change is displayed as an enhancement of virus entry. However, Qβ adaptation to low host density at 43 ºC takes place through a different mutation, located in the virus protein involved in the processes of virus adsorption to the bacterial receptor and viral progeny releasing. This mutation affects viral entry in the same way as the former does. However, whereas at optimal temperature the phage latent period is not modified, at 43 ºC it is significantly longer. Consequently, the virus spends less time in the external medium. This behavior is similar to that shown by viruses that infect thermophilic microorganisms in nature.

Methanogens | Archaeal viruses | human gut microbiome

Viruses of human-associated methanogenic archaea

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Methanogenic archaea are a phylogenetically ancient and diverse group of strict anaerobes that produce methane, a potent green-house gas, as a metabolic by-product. Methanogens are found in a wide range of environments such as wetlands, marine sediments, and the gastrointestinal tract of animals, including humans. Despite their broad distribution and critical ecological role, only a handful of viruses infecting methanogenic archaea have been characterized to date. Here, we present the first experimental study on the interactions between a temperate virus, Msmi-Pro1, and its host *Methanobrevibacter smithii*, the dominant methanogenic archaeon in the human gut. Our results show that Msmi-Pro1 is active and transmission electron microscopy analysis has shown that it has a siphovirus-like morphology, with an icosahedral head of ~65 nm in diameter and a long non-contractile tail of ~220 nm in length. Various forms of stress, including mitomycin C treatment, exposure to oxygen as well as nutrient limitation, were tested in an attempt to induce the replication of the provirus. Preliminary data suggests that none of the treatments tested induced the provirus replication. Instead, Msmi-Pro1 seems to be chronically produced in the liquid culture without dramatically affecting the growth of *M. smithii*, suggesting that only a small fraction of cells is lysed. These results imply that, similar to CrAssphage, the dominant phage in the human gut, viruses infecting gut methanogenic archaea co-exist with their hosts at certain equilibrium that could be advantageous for both parties.

Antarctica viral ecology metagenomics time-series

Virus diversity in marine Antarctic waters during two consecutive productive seasons

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Polar regions are undergoing global warming-induced changes expected to result in profound alterations in productivity and microbial community composition. Although viruses are known to be key modulators of their host populations, to date, there are only a few studies that targeted the viral diversity in Antarctic waters, let alone on a temporal scale. Here we present the results of a time-resolved metagenomics survey targeting the DNA virus community composition (and the potential microbial host community) in the coastal waters of the Western Antarctic Peninsula for two subsequent productive seasons. We examined the dynamics of viruses predicted to infect key phytoplankton (unicellular algae) and bacterial taxa, including their relation to environmental factors. We identified 6072 viral sequences, mostly belonging to the class Caudoviricetes (>75%), followed by NCLDVs (5%). Some of the most prolific phages were predicted to infect some of the most abundant bacterial taxa belonging to Oceanospirillales and Flavobacteriales. For example, viruses predicted to infect Oceanospirillales increased in abundance during the bacterial bloom. At the same time, the putative host was also highly abundant, suggesting significant viral-induced mortality for these taxa during this period. We also observed an increase in the abundance of temperate phages in the free virus fraction and cellular fraction during the bacterial bloom. Moreover, we detected NCLDVs predicted to infect phytoplankton, including viruses infecting *Phaeocystis*, an ecologically important phytoplankton group. These peaked during the late stage of the *Phaeocystis antarctica* bloom while virus abundances of the V4 flow cytometry group peaked. Temperature and salinity best explained the changes in viral community composition, highlighting the impact of viruses on the microbial community and the need to study these communities in the face of climate change.

viral metagenomics | virus discovery | benchmarking

Benchmarking of Bioinformatic Virus Discovery Tool across Biomes Using Real Metagenomics Data

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Viruses are globally abundant and widespread through ecosystems. Viral processes have extensive influence on the health/disease of higher organisms, evolution of cellular life and functioning of Earth's ecosystems.

As most viruses remain uncultivated, metagenomics is currently the main method for virus discovery. Detecting viruses in metagenomic data is not trivial and in the past few years, many bioinformatic tools have been developed for this task. The abundance of virus discovery tools makes it hard for researchers to choose the right tools, parameters, and cutoffs. As all these tools measure different biological signals, and use different algorithms and reference databases/training datasets, independent benchmarking is urgent to give users objective guidance.

We compared performance of eleven state-of-the-art virus discovery tools in fourteen modes using metagenomic datasets from viral and microbial size fractions of three different biomes, including marine seawater, tomato soil and human gut. We used real instead of simulated metagenomic data to benchmark the tools as the real metagenomic data is more complexed and less biased than the simulated metagenomic data.

Most tools are specific (average specificity ranges from 0.90 to 1.00) while only few tools are sensitive (average sensitivity ranges from 0.00 to 0.83). PPR-Meta is the most robust tool among all the tested tools with average specific, sensitivity, precision, and f score of 0.91, 0.83, 0.93 and 0.88, respectively. Default parameter cutoff settings were not always optimal for all tools and for all biomes, indicating that adjustment of parameter cutoffs for individual experiments should be considered.

Together, our independent benchmarking of bioinformatic virus discovery tools provides guidance on choices of virus discovery tools and suggestions on parameter adjustments for researchers who study virus metagenomic data across different biomes.

phage resistance | Phage-host interactions | bacterial community

The role of phage resistance in phage-bacteria co-existence in the mammalian gut

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Phages and their bacterial hosts are typically engaged in an arms race, which selects bacteria for resistance and phages for an increased ability to infect their hosts. Curiously, a dynamic yet stable coexistence between the two is observed in natural communities. The ecological and evolutionary mechanisms behind coexistence are underexplored, as well as the impact of the arms race in bacterial communities.

In the mammalian gut, a number of plausible mechanisms are hypothesized to contribute for these dynamics. One such mechanism regards the presence of haplotypes carrying phage resistance mutations, in which phage driven selection and the fitness cost of carrying such mutations lead to a dynamic equilibrium between phage and both resistant and sensitive bacterial populations. We intend to study this mechanism and evaluate its contributions to gut phage-bacterium stability and their impact on Oligo-Mouse-Microbiota (OMM^{12}), a widely used synthetic bacterial community model. Using the mouse native strain *Escherichia coli* Mt1B1 as a host and recently isolated, targeted *E. coli* phages P3, P10 and P17, we have performed experimental evolution *in vitro* and *in vivo* to generate and select resistant haplotypes. Through genome sequencing of phage resistant isolates, we detected putative phage resistance mutations. We have generated single gene deletion mutants, and used phage infection assays to pinpoint the role of these genes in phage resistance. Our future prospect includes *in vitro* and *in vivo* competitions in the presence of OMM^{12}, between phage resistant and phage sensitive strains, in the presence and absence of the corresponding phage, to pinpoint fitness costs and test the equilibrium hypothesis. We will also monitor the abundance of each of the OMM^{12} member and investigate phenotypic changes at the community level. This project will contribute to enlighten the role of resistance mutations in phage-host stability and to evaluate its impact in a community setting.

Bacteriophages microdiversity in vitro

Phage interactions at the single cell level - A microdiversity Tale

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In most environments phages outnumber their bacterial hosts by approximately 10 fold. Considering their high abundance and their dependence on host invasion to multiply, Phages are statistically pressured to share their host and to coexist in the virocell. The simultaneous coinfection of the host gives the rise to the “hybrid virocell” concept, in which the invading phages are interacting with each other in the cytoplasmic milieu. Investigating such phenomenon is of great importance, as it impacts the genetic diversity of both phages and bacteria, and thereby maintaining a continuous state of coevolution.

However, in the recent years only couple studies were successful to identify and describe coinfection modules, in which hybrid virocells are formed using experimental methods. In this study I described the simultaneous coinfection of a *Sulfitobacter sp.* Strain by two wild phage isolates (*Sulfiviruses*) at the single cell level. By combining innovative and classical techniques such as Phage targeting direct-geneFISH and epifluorescence microscopy, I gave visual evidence of the existence of hybrid virocells carrying the progenies of two active lytic sulfiviruses. Furthermore, I investigated the impact of the active coinfection on the replication cycles of the individual phages by monitoring the change in their genome copies in both single infection and coinfection conditions. I also tracked the evolution of the host total cell counts under the different infection conditions with Flow cytometry analysis. Surprisingly, despite the higher viral charge in the coinfection setup, the host total cell counts did not differ from the single infection counts. Moreover, the quantification of the infected cell fraction has shown an increase of infected cells for both phages at different stages of infection in the coinfection condition. Which provided evidence of a cooperative coinfection of the host by two *Sulfiviruses* related at the species level.

bacteriophage ecology | marine bacteriophages | viral metagenomics

Patterns of phage diversity reflect contrasting ecosystems along Panama's coasts

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Outnumbering their hosts by ten-fold, bacteriophages (phages) are critical components of marine ecosystems, shaping microbial community composition and modulating their bacterial hosts that drive biogeochemical cycles. Despite their significance, the evolution and drivers of phage diversity remain open questions. The contrasting tropical ecosystems on the coasts of Panama present a unique opportunity to unravel both global and local factors shaping phage communities. The Isthmus of Panama gradually formed and closed just millions of years ago, drastically altering marine species migration patterns by disconnecting two oceans. Additionally, the Western Atlantic ocean became nutrient-poor, while the Eastern Pacific ocean remained nutrient-rich. Though the impacts of the isthmus formation has been explored for macroorganisms, like shrimp, the impacts on marine viruses have yet to be investigated. In this study, we examine patterns in phage diversity between parallel, yet contrasting ecosystems on the Eastern Pacific (EP) and Western Atlantic (WA) coasts of Panama. We use metagenomes of seawater collected in mangrove and coral reef habitats on both coasts, enabling the additional comparison of local communities. Our analyses have revealed striking differences between the oceans, with less than 2.3% of phage contigs detected in both oceans. Despite these differences, phylogenies of two phage hallmark genes have revealed close relationships between phages found in different oceans, implicating potential shared evolutionary histories. Locally, phage communities were more similar between mangroves and reefs in the WA than in the EP, which potentially reflects their close proximity in the WA versus their distance in the EP and salinity differences in EP mangroves. Taken together, our work provides a foundational account of phage communities along Panama's coasts, revealing candidate factors underlying these differences to test directly in future studies.

Phage Satellites | Comparative genomics | Horizontal gene transfer | Bacteriophages

Getting to know the parasites of parasites: discovery and characterization of phage satellites from bacterial genomes

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Bacteriophages (phages) shape the composition and genetic diversity of microbial communities, and it is thus important to understand the ecological and evolutionary factors that modulate the co-existence between phage and host. One of these factors are parasites of phages, which are themselves parasites of bacteria. These mobile elements, called phage satellites, are small elements that lack components of the viral particle for autonomous transfer. Instead, they encode diverse and sophisticated mechanisms to hijack the particles of helper phages and transfer between cells. Phage satellites can have an impact in their bacterial hosts at different levels: by transducing chromosomal DNA or by encoding virulence factors or anti-phage defense systems. Some phage satellites are also costly for their helper phages, and thus impact their propagation and stability in a bacterial population. Three main types of phage satellites have been described: P4 in Enterobacterales, phage inducible chromosomal islands (PICIs) in Enterobacterales and Firmicutes, and phage-inducible chromosomal island like-elements in Vibrio spp. However, the diversity of these families of phage satellites is mostly unknown, since only a few elements of each family are described. We developed an approach to discover and characterize phage satellites integrated in bacterial genomes, by designing prototypical models that are suited to identify the major families of phage satellites. Searching for phage satellites in bacterial genomes, we unearthed and characterized the abundance and diversity of these elements across bacterial phyla. Our approach allowed us to identify thousands of putative phage satellites, including novel ones, and we characterized the phage satellites' hosts, genomic diversity and genetic relatedness within and between families. These results sheds light in the evolution of phage satellites, as well as their role as an important member in the cosmos of mobile genetic elements.

Giant Viruses | virophage | experimental evolution

Chemical-driven changes in host-virus-virophage eco-evolutionary dynamics

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Microbial community assemblages and their diversity are dominated by species interactions and co-evolution, which respond to changes in the interplay of ecological and evolutionary processes. Eco-evolutionary processes can be altered by chemical exposure, consequently changing the diversity and species coexistence of microbial communities. Here, we use a marine microbial community consisting of a heterotrophic flagellate, a giant virus and a virophage to test if and how Predicted No Effect Concentrations (PNEC) of antiviral exposures impact the microbial community at both ecological and evolutionary levels. Communities were established in chemostats for 50 days (~100 host generations) under three antiviral treatments: control, no exposure; pulse, a single pulse of 0.1 mg/L; and, disturbed, one pulse of 0.1 mg/L every day. We monitored population dynamics and assessed host and virus trait changes. Host, virus and virophage population dynamics and ratios changed depending on antiviral exposure. Host population traits diversified from ancestor populations used to start the experiments, suggesting that they evolved during the experiment. Specifically, clonal populations of the host isolated from the end of the experiment survived virus infections. This was most pronounced for those isolated from the disturbed treatments where virus pressure was expected to be lower due to antiviral exposure despite of the virus selection pressure being similar as in the pulse treatment based on virus densities from both communities. Virus and virophage trait changes are still under study.

Giant Viruses Isolation nanopore sequencing

Rapid characterization of novel giant viruses using nanopore sequencing

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Giant viruses have extraordinarily large genomes reaching up to over 2 Mbp and virions up to 1.5 µm. Majority of the viruses are thought to be pathogens of protists, such as algae and amoebae. Since the first discovery of Mimivirus in 2003, diverse giant viruses have been isolated from various environments. Large-scale metagenomic studies also support their vast diversity and ubiquity. Currently, isolation of giant viruses is largely dependent on co-culture method using several free-living amoebae, such as *Acanthamoeba polyphaga*, *A. castellanii*, and *Vermamoeba vermiformis*. This method recovered a large number of giant viruses. In contrast, their genomic diversity is not fully characterized due to the time and cost of whole-genome assembly. Here, we present a rapid whole-genome analysis of giant viruses using nanopore sequencing, which enables fast and low-cost genome sequencing. Accuracy of the sequencing method was assessed using a prototype of marseillevirus, *Marseillevirus marseillevirus*. In total, 2.3 Gb reads were obtained from a run, which were subsampled with different proportion and assembled by four assemblers, Flye, Raven, Wtdbg2, and Miniasm. We found that Flye assembler provides high-quality genomes with over 99.995% of identity to the reference genome without short-read correction at ×100 coverage. As proof-of-concept, the method was applied to newly isolated viruses from lake sediment and water. Two marseilleviruses, two mimiviruses, and a pithovirus were recovered. The marseillviruses and pithovirus were assembled into a single circular contig. Annotation of predicted proteins suggested chimeric features of these viruses. Particularly, in pithovirus, putative horizontal gene transfer events between other giant viruses were detected. Taken together, the present results demonstrated that our method is useful to rapidly investigate the genomic diversity of isolated viruses with lower cost.

Lysogeny | Antibiotic resistance gene | Phage-Plasmid

Phage-plasmids are widespread and produce antibiotic resistant lysogens.

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Phages and plasmids are major drivers of bacteria evolution and are clearly distinguished in the literature. Temperate phages tend to integrate the chromosome whereas plasmids are episomal, autonomously replicating DNA elements. Phage-plasmids infect bacterial cell as phages and replicate in host cells as plasmids, challenging the classical distinctions between these types of elements.

In our study, we set up a pipeline to detect phage-plasmids in prokaryotic genomes and found them to be numerous and widespread (5%/7% of all phages/plasmids). We used their gene repertoire relatedness to group them into different types. Whereas some are related to each other, most groups show very little similarity. The fact that these large, unrelated groups have phage and plasmid functions in common suggests that phage-plasmids are ancient. Some encode interesting traits such as virulence factors, which are often found in both plasmids and phages. In contrast, antibiotic resistance genes (ARGs) are often found in plasmids, but are very rare in phages. We thus wondered if phage-plasmids can spread ARGs as phages. We conducted experiments on phage-plasmids which we isolated from resistant strains coming from the hospital, and confirmed that bacteria can become resistant lysogens by acquiring ARG-encoding phage-plasmids.

Overall these results show that phage-plasmids are abundant and may transfer accessory genes typical of phages and of plasmids. They may thus have a remarkable impact on bacterial evolution.

Bacteriophage evolution | DNA recombination | phage engineering

Analysis of Spontaneous DNA Deletion in Bacteriophage MK1

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Deletion or mobilization of various genes during DNA replication is a major force in evolution. We isolated an *Escherichia coli* phage MK1 that forms a turbid plaque with a halo and observed that about 0.01% of the MK1 plaques became clear even after several rounds of a single plaque purification step. The phage forming a clear plaque (MK1C) was isolated and its genome was compared with that of MK1. The whole genome sequencing of MK1 and MK1C revealed that MK1C lost 4.5 kb DNA including 3' part of *MK1_020* (annotated as a phage tail fiber), *MK1_021* (annotated as a phage tail spike), *MK1_022* (annotated as a hypothetical protein), and 5' part of *MK1_023* (annotated as a phage tail fiber). The remaining regions of *MK1_020* (130-bps) and *MK1_023* (144-bps) encode a new open reading frame (ORF) homologous to a phage tail fiber protein in MK1C, however, the receptor, the host ranges, the adsorption rates, and the burst sizes were same for both MK1 and MK1C. Deletion of the 4.5 kb DNA from MK1 by utilizing CRISPR Cas9 system made plaques clear, indicating that the genes in the 4.5 kb DNA region affect plaque morphology. The 14-bps direct repeat DNA sequences were found at both ends of the deleted 4.5 kb DNA, suggesting that MK1C may be derived from MK1 by spontaneous deletion of the 4.5 kb DNA from MK1 through homologous recombination. The MK1C appeared even when MK1 infected *recA* mutant strain of *E. coli*, indicating that the deletion is *recA*-independent. The phage-resistant *E. coli* appeared faster upon MK1C infection compared with MK1 although the bactericidal effect was not much different. The reason for differences in phage-resistance development and host inhibition between MK1 and MK1C should be studied further.

bacterial community | Phage-host interactions | bacteriophage diversity

Successful propagation of the lytic *Shewanella* phage Phonos in planktonic but not in biofilm communities

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Phages regulate bacterial communities in various ecosystems through the phage-mediated predation or through effects mediated by lysogeny. To study the influence of phages on different forms of bacterial communities we isolated phages infecting our model organism *Shewanella oneidensis* MR-1. By this, we identified and characterized a novel phage, *Shewanella* phage Phonos. Phonos is a member of the family *Myoviridae* and a lytic phage, which reproduces successfully in a chronic way without a phage-dependent decrease of the bacterial population. Phonos slows bacterial growth only slightly, which we could explain by the used adsorption receptor, the major pilin subunit of the mannose-sensitive hemagglutinine (MSHA) type 4 pilus. The pilus activity depends on the second messenger c-di-GMP, which varies in amount between different growth phases and bacterial lifestyles. Accordingly, by insertion of targeted mutations into the pilus extension/retraction ATPases we could demonstrate that the activity of the pilus affects phage infectivity. Notably, the activity of the MSHA pilus is crucial for the initial cell attachment on surfaces during biofilm formation. We observe enhanced biofilm formation upon early exposure of cells to Phonos, likely through release of biofilm-promoting factors, such as extracellular DNA. However, mature biofilms were resistant to phage predation through Phonos, likely through inactivity of the pili leading to limited access to the host. Taken together, we show how a lytic phage can stablish itself successfully in a community with only small interference with planktonic culture growth by using an attachment structure, which is heterologous within the population.

Helicobacter pylori | Comparative genomics | prophages

A catalogue of the prophages of the genus *Helicobacter*

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The genus *Helicobacter* comprises species that colonize either the gastric mucosa (gastric *Helicobacter*), or the liver/intestinal tracts (enterohepatic *Helicobacter*) of distinct classes of the subphylum *Vertebrata*, including mammals, birds and reptiles. *Helicobacter pylori* is the type species and was the first described species in the early eighties of last century. *H. pylori* colonizes the stomach of the human host, causing gastritis, peptic ulcer and gastric cancer. Previously, we have described the first complete *H. pylori* prophage and showed that prophage genes tend to belong to the same phylogeographic group as *H. pylori*, reinforcing co-evolution and phage-host interactions persistence. Prophage sequences found in *H. acynonichis* and *H. felis* share homology with *H. pylori* prophages, but the co-evolutionary scenario between prophages and their *Helicobacter* hosts remains undetermined. To address this question we have retrieved 343 *Helicobacter* genomes from the Pathosystems Resource Integration Center, comprising 43 different species. These genomes were mined for the presence of prophages using the software PHASTER, and BLAST search using reference prophages as query. PHASTER identified 483 incomplete, 29 questionable and 6 intact prophages, resulting in 1.5 (± 1.0) prophages present per genome, with a minimum of zero and maximum of five; while BLAST search identified 40 prophages. This result shows that most prophages of *Helicobacter* genus are in a massive decay process, which usually involve loss of prophage DNA segments, genome rearrangement, among other inactivation mechanisms. Currently we are analyzing the prophage sequences of *Helicobacter* genus and we intend to present a congruence analysis between the phylogenetic trees of the host genome and prophage genome to evaluate the dependency and agreement between the phylogenies, as well as searching for specific events, such as duplication, host jumps, tropism of prophage integration, and integration site.

filamentous phage | Swiss-type cheese | Gram positive bacteria

Isolation and characterization of new filamentous phages active on Gram-positive bacteria in Swiss-type cheese

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In 1998, our team isolated one of the rare filamentous phages active and culturable on Gram-positive Bacteria. The phage infected *Propionibacterium freudenreichii* and was isolated from Swiss-type cheese. It is a single-stranded DNA phage, that was named B5. Until now, no related phage was found and little is known about B5-like phages diversity.

The aim of this research is to isolate new filamentous phages from Swiss-type cheese to study their diversity and discuss, by comparison with phage B5, a possible evolution in recent years.

This study was carried out using 30 pasteurized and raw milk cheese samples from different manufactures. After enrichment, each cheese sample was inoculated with mid-log phase culture of *P.freudenreichii*. Then, the phages plaques were detected using the soft agar layer method. The phages were subsequently isolated and studied using host range assay, transmission electron microscopy, S1 Nuclease hydrolysis, SDS page, and NGS sequencing.

Seven filamentous phages that infect *P.freudenreichii* were isolated from raw milk cheese. Genome sequencing analysis confirmed that these phages were distinct but related. They indeed displayed a similar genome structure, and sequence identity ranged from a hundred mutations to a 9% identity difference. Did all the newly isolated phages evolve from B5? At which step of cheese making do they occur? The diversity of these filamentous phages isolated from cheeses of different geographical origins shows that B5-related phages have been able to evolve over the last few years. More, the fact that nearly ten filamentous phages were found in relatively few cheeses suggests a large diversity.

To our knowledge, these 7 phages are the first filamentous phages active on Gram-positive bacteria found since the first in 1998. It would be interesting to continue this study by more precisely the newly isolated phages, by scanning a larger number of cheeses, and by investigating the origin of these filamentous phages.

Lysogeny | Shiga toxin | Escherichia coli | Temperate bacteriophage

Phage-mediated Shiga-toxin gene transduction from O80:H2 STEC to non-STEC strains and *in vivo* virulence assessment

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Shiga toxin-producing Escherichia coli (STEC) are major foodborne pathogens causing human diseases ranging from diarrhea to life-threatening complications such as hemolytic–uremic syndrome (HUS). Virulence of STEC strains and their ability to cause severe diseases are linked to the activity of prophage-encoded Shiga toxins (Stxs). Stx phage acquisition and stability studies are crucial in terms of public health. The first objective of this work was to isolate and characterize the Stx2d phage from STEC O80:H2, an emerging serotype in humans and calves, and then study the transfer of this phage in non-STEC strains. The second objective was to assess the survival of Galleria mellonella larvae inoculated with these convertant strains. Three temperate phages were induced and isolated from a bovine STEC E. coli O80:H2 under UV radiation. One of them was used to infect five non-STEC strains and its genome was analyzed. The three successfully converted strains (K12-MG1655, K12-DH5a and O80:H26) were confirmed by stx2d PCR and whole genome sequencing. A stability study was performed and showed that this phage was stable in the new STEC strains after three successive subculturing steps. This phage presents resistance to high temperature (60°C) and low pH (2). Galleria mellonella experiments showed that convertant strains caused significantly higher mortality rates than the corresponding non-STEC strains. In conclusion, this study showed that Stx2d phage can be transferred to non-STEC strains raising the question: “do O80 EPEC strains derive from O80 STEC strains after loss of Stx phages or are they precursors of STEC that will acquire Stx phages”? The comparison of the genome of all Stx2d phages will allow a better understanding of their epidemiology in the different serotypes of STEC strains.

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co-existence | algal viruses | viral ecology | viral evolution

Friend or enemy: The story of a frenemy relationship between a virus and its host

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Haptophytes are a dominant and diverse group of microalgae that play fundamental roles in both marine ecosystems and in global biogeochemical processes[1-3]. Despite being primarily known for bloom formation, most members of this group are present in low densities throughout the year, forming diverse communities together with other microalgae[4]. The knowledge of host–virus dynamics for these non bloom-formers is scarce. Our data indicate that the infection patterns are diverse and that this diversity is essential to ensure a stable and long-term co-existence between these viruses and their low-density hosts[5]. We will present experimental data from the infection cycles of a selection of host–virus pairs that exemplify unique infection strategies and virus traits. For instance, all tested cultures quickly developed resistance between 5-10 days post-infection, but these resistant strains presented differences in their host-virus co-existence dynamics. We also observed a high production of viruses, which did not seem to affect either growth or density of the host cultures.

References

1. Not F, et al. *Adv Bot Res. 2012;64. doi:10.1016/B978-0-12-391499-6.00001-3*
2. Liu H, et al. *Proc Natl Acad Sci*. 2009;106(31):12803. doi:10.1073/pnas.0905841106
3. Eikrem W, et al. *Springer Int Pub*; 2017:893-953. doi:10.1007/978-3-319-28149-0_38
4. Egge ES, et al. *Mol Ecol*. 2015;24(12):3026-3042. doi:10.1111/mec.13160
5. Sandaa RA, et al. *Biol Rev*. 2022;97(1):179-194. doi:10.1111/brv.12795

Mycobacteriophage endolysins NTMs

Isolation & characterization of F2 sub-cluster mycobacteriophage *RitSun* & derived endolysins as antimycobacterial agents

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Tuberculosis (TB), is one of the leading causes of death due to infectious diseases worldwide. COVID19 has overturned many years of progress in reducing the TB burden. Hence, there is an urgent need for exploring bacteriophages and encoded lysins as non-antibiotic strategies to treat drug-resistant TB & NTM infections, which could potentially shorten the treatment regimen of TB when given in combination with drugs.

Endolysins are enzymes used by bacteriophages to release their progeny from the host. Mycobacteriophages encode Lysins (A&B), which target the peptidoglycan & ester bonds in the cell wall, respectively.

We isolate mycobacteriophages using *M. smegmatis* mc^2155 as the host and derive endolysin gene sequences from the novel phages & from prophages by PCR amplification or chemical synthesis followed by their purification as recombinant proteins. In this study functional characterization of a F cluster mycobacteriophage *RitSun* and the encoded lysins (A&B) are presented. The F2 sub cluster has only six phages so far.

The analysis of domain organization of *RitSun* endolysins shows multiple modules in lysinA: chitinase domain embedded in lysozyme-like domain & amidase domain embedded in PGRP & in lysinB, a C-terminal linker domain besides the characteristic a/b hydrolase fold. The mycobacterial growth inhibition studies with endolysins were done using plate lysis method, microtitre assay & colony count method. The biochemical activity was estimated using *in vitro* assays: lysozyme & amidase for LysinA & esterase assay for LysinB.

There are limited studies on LysinA from mycobacteriophages. *RitSun* LysinA data in this study is encouraging, especially its lysozyme activity & 'lysis from without' effect on *M. smegmatis.* Further exploring the effectiveness of *RitSun* lysins on the pathogenic mycobacterial spp. can expand their scope as therapeutically relevant protein biopharmaceuticals, alone or in combination with antibiotics.

Methanogens archaeal DNA viruses CRISPR

In silico characterization of viruses of archaeal methanogens

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Methanogens are a phylogenetically diverse group of archaea that produce methane as a metabolic by-product. Methanogens are found in anaerobic environments, including fresh and hypersaline water and sediments, hydrothermal vents, and intestinal microbiomes of animals and humans. Despite the ecological importance and diversity of methanogens, their viruses are virtually unknown. Using sequence-based approaches, we aimed at identifying and characterizing viruses of methanogens including host-associated lineages.

We found that all major lineages of methanogenic archaea are associated with viruses or proviruses. In total ~250 complete viral genomes (3 – 85 kbp in length) were identified. Most viral genomes encode proteins for head-tailed virions except for tailless icosahedral viruses of Methanococcus and Methanomicrobiales, pleolipoviruses infecting Methanohalarcheum, and ssDNA smacoviruses assigned to Methanomassiliicoccus. Head-tailed viruses of methanogens are unique and not related to other archaeal viruses and follow the taxonomy of their hosts. In addition to viral sequences, we also found that methanogens are associated with diverse integrated elements, conjugative and cryptic plasmids.

The analysis of gene content revealed the presence of auxiliary metabolic genes (PAPS reductases, glutamate synthases, flavin reductase, etc.), which may be involved in the modulation of host metabolism.

Antibiotic resistance gene | viromes of pigs'gut | Bacteriophages

Viromes and microbiomes from pig fecal samples: phages and prophages are not vectors of antibiotic resistance genes

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Understanding the transmission of antibiotic resistance genes (ARGs) is essential as they have important human health implications. It is necessary to identify the animal biotopes where ARGs are present, and if they might be transmitted via mobile genetic elements and then spread into human pathogens. Previous research suggests that ARGs, which can be found in abundance in pig feces, may be encoded by bacteriophages. However, definitive proof of the presence of phage-encoded ARGs in pig viromes is still lacking, because viral sequences are difficult to distinguish from those of contaminating bacterial DNA. Using improved methods, we collected 14 pig fecal samples from various farms and performed shotgun-based deep sequencing on both total microbiota and highly purified viral fractions in order to investigate phage-encoded ARGs. We showed that ARGs were absent from the genomes of active, virion-forming phages (below 0.02% of viral contigs from viromes), but present in three prophages, representing 0.02% of the viral contigs identified in the microbial dataset. However, the genomes of these prophages were not detected in the viromes, and their genome map suggests they might be defective. Furthermore, our comprehensive virome assemblies allowed for the discovery of two new and prevailing viral taxa infecting mostly *Clostridiales*: filamentous *Tubulavirales* phages (272 species) and phages related to the *Faecalibacterium* phage Oengus (94 species). Interestingly, our analysis also suggests a way to study both phages and bacteria within total microbiota samples.

Bacteriophage isolation | bacteriophage diversity | Antarctica

Isolation and genomic characterization of novel bacteriophages from Antarctic ice-free soil samples

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Discovered in 1820, Antarctica, one of the most desolated and roughest places on planet Earth, was thought to be a relatively sterile environment for decades. However, continuous multidisciplinary research up to this date has unveiled that the continent is far from sterile, with the majority of Antarctic lifeform variety hidden in the micro-world. First microbes in Antarctica were found circa 1900, but not until several decades later attempts to not only isolate but also characterize them have been successfully made. Even though most of the existing bacteria cannot be cultured using traditional microbiological techniques, recent advancements in culture-independent microbial research approaches have allowed to partly overcome this existing limitation and revealed even greater microbial diversity in various extreme environments, including Antarctic soils.

Bacteria presence in any given environment a priori indicates the presence of phages, and genomes of phages have already proven themselves to be a “treasury” of novel genes. Needless to say that many of these genes encode products for which various practical applications, most notably in the field of molecular biology, have been found. Whereas the metaviromics approach might be the fastest way to mine such genes from the environmental samples, it substantially limits study opportunities on the individual phage characteristics, which is of utter importance to expand the global phage diversity knowledge.

Herein we would like to report our progress on the process of culture-based isolation and genomic characterization of novel bacteriophages isolated from samples of ice-free soils collected near Rasmussen Hut, Antarctica. These phages, to our knowledge, are the first phages capable of infecting *Psychrobacillus* sp. and *Sporosarcina* sp. that had their complete annotated genomes made publicly available (*Psychrobacillus* phages Perkons and Spoks and *Sporosarcina* phage Lietuvens).

Virus-host interactions | CRISPR | stable isotope probing | metagenomics

Identifying active host-virus interactions in soil by following transfer of assimilated carbon

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While we have substantial knowledge of the complexity of prokaryotic communities and their contribution to functional processes in soil, we are currently ignorant on the role of viruses in influencing their ecology. In particular, difficulties remain in identifying the frequency of active interactions between host and virus populations in situ, largely due to a lack of tools to study interactions within this highly diverse and heterogeneous environment. To examine discrete, active interactions between individual host and virus populations in situ, this study focused on the transfer of assimilated carbon from autotrophic hosts to viruses. Microcosms containing pH 4.5 or 7.5 soil were amended with ^{13}C methane or carbon dioxide and subjected to short-term incubations. Using DNA stable-isotope probing combined with metagenomic analyses, microbial networks were characterised, with primary and secondary utilisers of carbon identified, together with the transfer of host-fixed carbon to viruses. In methane incubated soils, 63% of 13C-enriched viral contigs were associated to methylotrophic bacteria with non-methanotrophic methylotroph viruses and predator bacteria also identified. Active interactions and history of virus interactions with individual hosts were also characterised via analysis of 13C-enriched virus protospacer sequences in host CRISPR arrays. In soils incubated with carbon dioxide under nitrifying conditions, active viruses of ammonia oxidising archaea and nitrite oxidising bacteria were identified. Putative viruses of both methane oxidisers and ammonia oxidising archaea contained auxiliary metabolic genes involved in central metabolic pathways, and analysis of viral genes (e.g. integrases, tail and capsid proteins) revealed that they were distinct from previously cultivated viruses. These results demonstrate that following carbon flow facilitates identification of discrete host-virus interactions within the complex soil environment.

gut virome | bacteriophage ecology | health and disease

Bacteriophages enhance resolution of microbial community analyses in preterm neonates

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Necrotising enterocolitis is a major cause of death in preterm neonates. As such, gut microbial communities have been widely studied in this population. Links between microbial community structure and disease however remain poorly understood.

We collected stool samples from very preterm infants admitted to the neonatal intensive care unit over the first 60 days of life. Using DNA sequencing technologies we profiled bacterial, free and chemically induced viral populations in preterm infant stool. Community structures were compared, along with clinical metadata to interrogate links between features of the microbiota health or disease states.

Large degrees of overlap were observed in communities of free and chemically induced viruses. Viruses with host specificity for previously observed bacterial community members were also found. Individual patients harboured distinct community structures although twin pairs shared more similar communities than non-related infants. We found little discriminative power between health & disease states, however. Future work will explore functional gene features of bacterial and viral communities in these cohorts.

We here demonstrate the potential importance of combining bacterial and bacteriophage community analysis when monitoring polymicrobial infection and community structure.

gut virome | 3C | OMM12

In vitro and *In vivo* characterisation of bacterial and prophage 3D organisations

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The gut microbiota houses a complex and diverse microbial community composed mainly of bacteria and their viruses, bacteriophages (phages). It has been shown that some human diseases or disorders are associated to variations of bacteria and or phage populations, calling for combined studies.

Here we apply Chromosome Conformation Capture (3C) and virome (ssDNA and dsDNA viruses) techniques to characterise the bacterial and viral community of a consortium of 12 bacteria colonizing the gut of mice (OMM12 mice). Similar analysis performed on in vitro samples of isolated cultures allowed the comparison between the gut and in vitro conditions. This innovative approach revealed information on both bacterial and viral populations as well as their interactions through DNA collisions.

The 3C data, beyond improving the assembly of the 12 bacterial genomes, revealed specific 3D structures of bacterial chromosomes providing key information on the architecture of non-model bacterial chromosomes as well as the metabolic activities of these bacteria in the gut compared to in vitro conditions. We also uncovered particular 3D signatures on some of the chromosomal regions annotated as prophages unravelling prophages activity. This information was cross validated with virome sequences from mice fecal pellets in order to identify the viral fraction of the OMM12 microbiome. Virome data confirmed the induction of 10 and 11 prophages among in vivo and in vitro conditions, respectively. Furthermore, this data revealed the absence of other viruses than induced prophages demonstrating that these mice are eukaryotic viruses-free.

This study shows that the dynamic interactions between phages and bacteria can be assessed in situ by associating 3C with virome data. Applied to the OMM12 mice this approach revealed that some prophages are exclusively induced in the gut, pointing towards specific induction signals possibly originating from the intestinal cells.

Bacteriophage evolution | bacterial community | coevolution

Back into the future: phage-bacterium coevolution in the pear tree phyllosphere

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Microbial communities play key roles in plant development, growth, productivity and health. They are indispensable in nutrient acquisition, metabolism, stress-relief and disease development. In recent years, we are starting to understand the role of bacteriophages, viruses infecting and actively killing members of these communities, as puppet masters in these essential and intricate interactions. Because of their nature, phages exert high evolutionary pressure on members of the bacterial community, resulting in co-evolution dynamics between the phage and its bacterial host. A large body of theory and experimental work discuss phage-host co-evolution dynamics *in vitro*, outside of the natural context. This raises the critical question how coevolution dynamics are shaping communities in a natural context. To this end, we use the pear phyllosphere as a study system and collected samples monthly over a time span of three years to isolate bacteriophages infecting different members of the microbial community, with the emphasis on *Pseudomonas* and *Erwinia*. This sampling allows us to perform time shift assays to quantify coevolutionary change. By combining wet-lab phage infection characterizations and comparative genomics, we track viral evolution dynamics and the associated trade-offs. As such, we assess trade-offs in evolution dynamics in natural environments.

gut virome | human gut microbiome | human performance

Gut microbial stability is associated with improved endurance performance

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Environmental stress can promote temperate phages to switch from lysogenic to lytic life-cycles. Highly trained athletes participate in dietary periodisation and high intensity endurance exercise to improve endurance performance. These interventions can cause substantial stress in the gut mucosa.

We investigated the impact of high protein and high carbohydrate dietary interventions on endurance performance and associated gut microbiota in a cohort of well-matched, highly trained endurance athletes. In a double-blind, repeated measures RCT we measured performance outcomes along with free virus, inducible virus and bacterial microbial communities in stool.

High carbohydrate diets significantly improved performance outcomes by 6.5% from baseline ($p=0.03$) and were associated with expansions in Ruminococcus and Colinsella bacterial spp. Improved performance was also associated with greater proportions of genes associated with energy utilisation. Conversely, endurance performance was reduced by 23.3% from baseline ($p=0.001$), during high protein diets. These changes were associated with lower inducible virus diversity ($p = 0.04$) and altered free and inducible viral community composition ($p = 0.02$). Participants who experienced the greatest shifts in community composition performed worse than those who did not.

In this well-matched, highly controlled RCT we identified greatest endurance performance was associated with gut microbial stability in a cohort of highly trained athletes. We also observed bacteriophage populations to be a greater marker of gut stress than bacterial populations. Athletes and those supporting them during dietary periodisation and training would benefit from being mindful of these results.

phage resistance | phage-bacteria co-evolution | Phage resistant mutants | Resistance mechanisms

An evolutionary arms race between nitrogen fixing cyanobacteria and their viruses

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Cyanobacteria perform a substantial part of photosynthesis and nitrogen fixation in nature, and thus their role in various ecosystems is central. Despite their ecological benefits, under certain conditions, some of the strains proliferate and form blooms that constitute an environmental hazard. One of the main factors that control the population dynamics of cyanobacteria in nature, are viruses that infect cyanobacteria (cyanophages). Cyanobacteria and their phages keep long-term coexistence in nature. To gain insight into this coexistence, we isolated and characterized (phenotypically and genotypically) cyanobacteria substrains resistant to these phages, as well as phage substrains that coevolved with these resistant strains.

Resistance conferring mutations were found in various genes related to cell surface molecules, which may serve as receptors for phage attachment. Indeed, resistance was due to a reduced ability of the phage to adsorb to its host. Moreover, mutations that might affect gene regulation have also been identified. Resistance came with an adaptive cost, manifested as lower ability to fix atmospheric nitrogen, which may reduce the ability of resistant strains to survive and form blooms under nitrogen starvation conditions. Some of the coevolved phage strains displayed mutations in genes related to the phage tail structure that are crucial for the recognition of the host and for the attachment to its cell surface. These modifications in tail structure may compensate for the modifications in the host receptors. The evolved phages had a greater ability to suppress the host populations.

Our results suggest that cyanophages can potentially affect the cyanobacterial populations both by killing them and by selecting for resistant strains with a substantial reduced ability to survive under conditions in which the susceptible strains proliferate and bloom, which may dramatically affect the cyanobacterial population dynamics and diversity.

Bacteriophage ecology | Pseudomonas syringae | Agriculture

Do phages of *Pseudomonas syringae* communities reflect the ecology and diversity of bacteria in apricot trees?

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The distribution and diversity of phages in the biosphere places them in key roles within their ecosystems. Phages influence their bacterial host populations in many ways, from their population size to driving their genetic diversity and pathogenic potential. Unlike the aquatic or intestinal environments, the dynamics governing the interactions between phages and bacteria in agriculture are poorly investigated. *Pseudomonas syringae* is a species complex harboring a great genetic diversity of strains that are widely distributed in many environments. In the context of an apricot orchard, a multitude of genetically distinct strains of *P. syringae* can be present depending on the substrate and the season, with some strains inducing the devastating apricot bacterial blight disease. This study aims to identify the phage populations of *P. syringae* strains associated with the different apricot trees substrates (soil, branches, buds, leaves and water) and to test their relationship with the bacterial diversity and the disease incidence. In a first step of the study, we collected more than 200 *P. syringae* phages from soil samples in apricot orchards in the south of France, suggesting a prevalence of phages in this substrate. We are currently identifying them by sequencing their genomes and we plan to analyze some phenotypic traits such as host range. Using this phage genomic reference data base, we will then perform metagenomic analysis of phages and bacteria in the different ecological niches. This approach will allow the identification of the prevalence and diversity of phages of *P. syringae* and how it interacts with its bacterial host population in different microhabitats.

latent period | Dynamics | simulation

Latent period variability impacts microbe-virus dynamics

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Microbial populations can be shaped by viral infection. Our understanding of virus-microbe dynamics largely depends on the study of viral traits, e.g., the latent period. The latent period in lytic virus-host systems is the time from viral adsorption to viral progeny release. The latent period of a virus-microbe pair is often estimated using one-step experiments in which free virus is measured over time in a population of infected cells. These measurements do not consider latent period variability, which can affect virus-microbe dynamics. At the cellular level, the latent period is a single value for a virus-cell pair. In a population, there is cell-to-cell variability in the latent period; hence, a better description of the trait is a distribution of times.

Here, we use nonlinear dynamical models with latent period variability to understand how population variation of this trait impacts virus-host dynamics. Our modeling approach showed that latent period variability in a population can have a profound impact on virus-microbes dynamics. Moreover, latent period estimates typically used in one-step experiments can deviate substantially from the mean latent period in a population and are highly sensitive to latent period variability. Hence, we developed a computational framework to estimate latent period variability from free-virus one-step experiments data. We tested the framework by producing *in silico* data generating virus and host dynamics. Our estimates of the mean latent period and its variance were able to recover those of the underlying *in silico* datasets. Although some studies have characterized the mean latent period across microbial realms, there may still be considerable uncertainty in these estimates, since variance is not considered. Even less is known about the variance of the latent period across diverse taxa. Reframing the latent period as a distribution will allow us to better study the trait itself and its role in shaping microbial populations.

Bacteriophages breast milk preterm infants

Characterising the core bacteriophage community in low volume breast milk using an optimised phage isolation protocol

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Bacteriophages (or phages) can be found alongside beneficial bacteria, growth factors and immune proteins in human breast milk (BM). In premise in this BM matrix that the core phage community could be transmitted to infants during feeding and may prevent overgrowth of pathobionts in the gastrointestinal tract (GIT) of infants. We propose that the core community of phages in BM of preterm mothers may play a beneficial role in GIT and development of sequalae such as Necrotising enterocolitis (NEC) and late onset sepsis (LOS) that can be fatal in extremely low birth weight preterm infants. We have designed a robust phage isolation protocol that has successfully identified a diverse community of phages in as little as 0.1 mL donor milk. We then applied this optimised protocol in a larger cohort study of 74 preterm mothers, illustrating a core phage community in the BM identified across the colostrum, transitional and mature stages of lactation. Our optimised protocol also enables isolation of phages from maternally expressed milk that can be used to infect bacterial community structure in paired- or unpaired preterm infant stool samples. The obtained results will serve as baseline information for our on-going and future research on the transmission of phages in both stored and fresh human milk and their interactions with the GIT microbiota of preterm infants.

Alteromonas | podovirus A5 | receptor-binding protein

Homologous host recognition modules in distant families of *Alteromonas* phages

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The abundance and diversity of phages in nature is an indication of the evolutionary success of these microorganisms. Random illegitimate recombination generates novel phage genomes over time and HGT plays a key role in phage evolution. Here we have identified the new *Alteromonas* podovirus A5, with unexpected similarity in its host recognition module with others found in *Alteromonas* phages with different morphologies and belonging to distant viral families. The genomic comparison among these phages revealed a conserved module including a homologous receptor binding protein (RBP) and tail fiber chaperone. In contrast, the rest of the genome was completely different. Binding assays with A5 demonstrated that gp8 is the RBP in this podovirus and is required for host recognition, but gp9, identified as a tail fiber chaperone, is not essential for host binding. In contrast, in the case of the *Alteromonas* myovirus V22, which contains the same recognition module, the tail fiber chaperone is required for RBP maturation and thus for proper host recognition. The results obtained here suggest that recombination of host recognition modules among unrelated phages may be more frequent among the entire community of tailed phages than previously thought and a major mechanism of adaptation to host evasion by target import by horizontal gene transfer. To our knowledge, this is the first time that this level of conservation in phage-host recognition proteins has been detected at the same time in marine phages belonging to the *Myoviridae*, *Podoviridae,* and *Siphoviridae* families, with no other closely related gene in the rest of the genome.

translocation antibody phageome

Transfer between ecological niches within human body: how gut phageome expands to serum phageome

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Phages are constantly present in human bodies; body regions are ecological niches- some expected for phage propagation and presence like gut, some normally free of microbes, like blood. Bacteriophages may translocate from one niche to another.

We have investigated translocation from gut to blood in humans (whole phageome) and in mouse model (defined phages). In 12 human patients, bioptates of large intestine mucosa collected by endoscopy and serum samples were processed to isolate VLP fractions and sequenced (Illumina systems). In mice, defined phages A3R (Kayvirus) or T4 (T4virus) were administered orally and detected by microbial cultures of gut content, mucus, and serum.

Most represented bacteriophages in human gut were Kayviruses (12 patients), T4viruses (11 patients), and Pbunaviruses (7 patients). It the serum samples of the same patients' they were detected in 11, 4, and 5 patients, respectively. Other types of phages were less abundant. Thus, viruses commonly present in gut were also detected in serum, but the translocation rates for specific group of viruses differed: 92% for Kayvirus, 71% for Pbunavirus, and only 36% for T4virus. In mice, translocation of active phages from gut to blood was poor, but similar for T4 and A3R phage.

Differences between phages in their frequency of effective translocation from gut to blood correlates to the frequency of phage-specific antibodies that we observed in human population in previous studies. The most frequent (above 80%) were antibodies specific to T4 phage, with lower frequency of those specific to A3R phage (30%) or DP1 phage (40%, Pbunavirus). We propose that phages can translocate from gut to blood, but with low efficiency, and depending on gut concentrations of phage. Further, specific immune response to phage, when present, may decrease the amount of phage that reach circulation.

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microbiome phage phagetherapy

Microbiota and phages

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Microbiota comprises bacteria, archaea, microkaryotes, and viruses/phages in the body of humans and other species. These microorganisms function in a commensal, symbiotic or pathogenic relationship. The bacterial microbiome is based on 16S rRNA genes, while the virome is more difficult to characterize. The human microbiome comprises 160 species per individual and 1100 prevalent species, with a total of 1014 microbial cells. It varies with diet, age, the location within the GI tract and depth, infections and disease. However, we and others evidenced that in spite of the diversity there is a conserved core microbiome involved in essential metabolism. It has been demonstrated in a "Zurich Patient" who recovered from *Clostridium difficile* after fecal microbiota transfer (FMT). Corona and other viruses can have strong effects, also Intestinal Bowel Disease (IB), neurological disorders, obesity, bacteria, and cancer. The response to immunotherapy against cancer is extremely effective in 25% of cancer patients. This is multifactorial, yet the gut microbiota of responders versus non-responders and a potential FMT as therapy is under intense investigation and will be discussed.

Success of FMT is associated with Bacteroides, Firmicutes, Bifido- and Lactobacteria and low levels of Proteobacteria. The role of phages in diversity and richness of the gut microbiome is not fully elucidated. Phages can replace FMT. Characterization of the virome is still challenging and will be discussed. Especially phages as therapy of multidrug-resistant bacteria has gained momentum recently - after almost 100 years of application. Novel approaches with gene-modified phages with AI support by AlphaFold or RoseTTAFold may help to counteract an antibiotic crisis and will be reviewed. A German Governmental approach will be discussed.

Ref: Moelling K: Viruses to kill Bacteria - a way out of the antibiotic crisis with phages. Dr. Friedrich Pfeil Press, Munich (2022).

Bacteriophages Database PhageDive

PhageDive: the new data resource on prokaryotic viruses

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Prokaryotic viruses are the most diverse and abundant biological entities on Earth. The research on bacteriophages (virus infecting bacteria) and their mechanisms has led to major advances in multiple domains especially in molecular biology and biotechnology. The recent increase in antibiotic resistances highlights the need to intensify phage research. Within the DFG priority programme SPP 2330 which focuses on “New concepts in prokaryotic virus-host interactions – from single cells to microbial communities”, our project provides essential service functions and access to state-of-the-art methodologies for studying all relevant aspects of the biology of prokaryotic viruses and is in charge of the long-term conservation, quality control and distribution of the isolated bioresources and their data. While the amount of data on viruses is currently growing exponentially, mainly due to the increasing application of metagenomics techniques, the data generated with different experimental approaches and molecular techniques are insufficiently linked. As a result, the full set of existing data set for a given bacteriophage is not accessible so far. This observation led us to develop a specific database for bacteriophages and archaeal viruses named PhageDive. Using the results generated in the SPP, data will be linked to existing information distributed across scientific publications, specific databases and other sources which will generate and guarantee an easy and comprehensive access for the scientific community. PhageDive provides fields for various experimental data (lifestyle, lysis kinetics, adsorption kinetics, host range, genomic data, etc.) and the available metadata (e.g., geographical origin, isolation source). Data are standardized employing controlled vocabulary and ontologies. An important feature is to link experimental data to the culture collection number and the repository of the corresponding physical bioresource (virus and prokaryotic host strains).

metalworking fluid | Pseudomonas | bacteriophage biocontrol | emulsion | population dynamic | kill the winner

Bacteriophages modulate bacterial populations in metalworking fluids

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Water-miscible metalworking fluids (MWFs) have three main functions: reducing friction, cooling work piece and tool, as well as removing metal chips in various metalworking operations; and they are easily populated by bacteria.

MWFs are either solutions or oil in water emulsions with a typical water content of around 95%, typically designed to work at an alkaline pH of 8.5 or higher. This creates a suitable environment for some bacteria that reach densities of up to 10^8 CFU/mL. It is thus important for developers and end users to manage these bacterial populations in order to avoid degradation of the MWF, loss of function, integrity and performance, as well as adverse health effects. To achieve this, biocides are often applied with ambiguous success, whereas “bio-concept” MWFs exert, through their constituents, a selection pressure mainly allowing the growth of *Pseudomonas oleovorans*.

As bacteriophages are the most abundant organisms on earth and are assumed to exist in every habitat that is populated by bacteria, we endeavored to establish the prevalence of phages in MWFs, elucidate their influence on bacterial population dynamics at different pH ranges and to test whether they might be a useful tool for active control.

Results indicate that phages can have a strong decreasing effect on bacterial populations of up to 6 logs at pH 8 but no decrease at pH 9.5. Besides we encountered only short-term effects presumably due to growth of resistant bacteria. We suppose that a drop of the pH in a MWF can lead to a phage attack against the dominant bacterial species (“kill the winner”) which results in a dramatic change of the bacterial species composition.

bioinformatics protein mosaicism

Evolutionarily conserved fragments: an attempt to better understand domain composition and mosaicism of phage proteins

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Some phage proteins are known to be complex and multi-domain. Since these proteins may be underrepresented in public domain databases like Pfam, we designed the following bioinformatic pipeline: First, we collected a representative subset of phage genomes from those deposited in the NCBI RefSeq database and clustered all protein sequences using mmseqs2. Then, from each cluster, a representative protein was selected and used to build an HMM profile with HHsuite using the Uniclust database. Finally, we performed all-vs-all profile-profile searches to identify evolutionarily conserved fragments (ECFs), i.e., alignable sequence regions shared between distantly related clusters. Analyses of the obtained ECFs showed that they frequently overlap with known domains defined in Pfam. However, we also found cases where ECFs were localized in regions that were not annotated by any Pfam domain, for example in receptor-binding proteins, suggesting that these could represent novel, previously unidentified protein domains. Overall, our approach highlights the need for a more systematic characterization of phage protein domains, particularly in more complex and multi-domain functional classes.

Bacteriophages | Bacteriophage ecology | Phage inactivation | environmental

The Presence of Microplastic Decreases the Number of Active Bacteriophages in the Aqueous Environments

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We demonstrate for the first time the possible link between microplastic pollution and the number of active bacteriophages, i.e., viruses of bacteria, in aqueous environments. Bacteriophages (phages) are essential in homeostasis, as it is estimated they cause the death of up to 40% of all bacteria every day. Any factor affecting phage activity is vital for the whole food chain and the ecology of numerous niches. We show that microplastic can decrease the number of active phages by releasing leachables (up to around 70% decrease) or because of adsorption of virions on solid particles (up to about 90% decrease) depending on the phage (MS2, M13, and T4 were studied) and polymer type (12 polymers were tested).

Soil virus ecology | Distribution | Host-virus interactions

Soil virome structure and virus-host interactions across pH gradients

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While viruses have ability to infect a range of hosts in highly diverse prokaryotic soil communities, co-evolutionary processes may tightly control the susceptibility of hosts through virus-bacterial interactions and local adaptation within distinct ecological niches. This work tested the hypotheses that i) soil virus community structure changes as a function of pH at local and global scales as observed for prokaryote community structure, and ii) soil pH restricts distribution of viruses capable of infecting individual populations of bacteria. Prokaryotic and viral communities were characterized in soils at a local scale using a long-term pH-manipulated soil gradient (pH 4.5 to 7.5), and viral communities subsequently compared at global scale. Viral communities were influenced by pH at local scale with 99% of viral operational taxonomic units restricted to pH 4.5 or 7.5 soil only. Analysis of viromes from other soil systems demonstrated that a selection of viral clusters from acidic and neutral pH soils were more associated with those from the local gradient pH 4.5 or 7.5 soils, respectively. To examine virus-host interactions, *Bacillus* strains were isolated from pH 7.5 soil, and virus enrichments obtained from gradient pH soil were applied to the host bacteria with infectivity quantified using a plaque assay approach coupled with metagenomic analysis. Susceptibility of individual hosts to infection changed across the pH gradient. Intriguingly, the composition and diversity of viruses infecting individual hosts varied, being greater in pH 4.5 compared to those in pH 7.5 soil that were co-localised. While direct pH effects on virion integrity and indirect selection via host composition were not distinguished, results reveal that soil pH is a factor in structuring viral communities. However, at a local scale, differences in virus community structure across a gradient does not necessarily reflect variation in ability of virus populations to infect an individual host.

prophages | bacteriophage ecology | Next generation sequencing | plant pathogenic bacteria

Hot and cold: prophage warfare in the wheat phyllosphere

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Living in the shadow of their lytic cousins, the sheer ubiquity of prophages is sometimes overlooked. Often outnumbering bacteria in their own genomes, many prophages encode auxiliary metabolic genes, virulence factors, and mediate protection from similar phages. Prophages can also act as potent bioweapons when induced, capable of infecting and killing a susceptible rival bacterial population. Although many prophages are well-characterized in the context of pathogenicity, the ecological importance of prophages is understudied in many environments.

Here, we investigate the prophages of 200 bacterial strains isolated from the wheat phyllosphere with pseudomonas-specific agar. Using hybrid genome assemblies generated using Illumina and Oxford Nanopore sequencing, we predicted active prophages *in silico*. These predicted prophages were annotated and analyzed with comparative genomics. Next, by spot-testing bacterial culture supernatants on overlay agar, we investigated the host range of prophages induced under different treatments and relate this to the distribution of predicted prophages in host bacteria.

bacteriophage ecogenomics | Virus-host interactions | Auxiliary Metabolic Genes

Potential roles of viruses in carbon and nitrogen cycling during benzene degradation under nitrate-reducing conditions

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Nitrate-dependent benzene mineralization was monitored by the addition of ^{13}C-labelled benzene and subsequent analysis of generated $^{13}CO_2$. We collected solid (sand) and liquid samples at five-time points (3 replicates per time point) for DNA extraction and whole-genome sequencing. A total of 24 metagenomes (2x125 bp, 25 Mio reads) were analyzed.

Using MetaWrap we recovered almost 2000 prokaryotic metagenome-assembled genomes (MAGs). These MAGs were separated into 193 prokaryotic Operational Taxonomic Units (pOTUs) based on average nucleotide identity with a 0.95 cutoff (a proxy for species level). Taxonomic assignment by GTDB-tk revealed Gammaproteobacteria, Ignavibacteria, and an unclassified class of Zixibacteria as the most dominant phyla.

VirSorter, VirFinder, and VIBRANT were used to recover a total of 605 complete and 365 high-quality Uncultivated Viral Genomes (UViGs) as defined by CheckV. After dereplication (Stampede-ClusterGenomes), we defined a total of 222 viral Operational Taxonomic Units (vOTUs). Taxonomic analysis with vConTACT2 suggested that all vOTUs belong to novel viral groups. Analysis using WisH indicated that 72 pOTUs (37.35%) were infected with phages ($p < 0.05$).

Functional annotation of the 222 vOTUs identified 91 genes encoding 13 proteins relevant to benzene mineralization coupled to nitrate reduction. Regarding carbon cycling, we found genes related to anaerobic degradation of benzoyl-CoA, and CO2 fixation using the Wood-Ljungdahl pathway in our vOTUs. Similarly, genes related to nitrate reduction to nitrite, nitrite reduction to N_2, and nitrite reduction to ammonium indicate the viruses' potential contribution to the nitrogen cycle. Preliminary analysis suggested that 160 vOTUs (72.1%) have a lytic life cycle.

Our data demonstrate the potential relevance of viruses in anaerobic benzene degradation and open new doors for studying viruses in anaerobic ecosystems contaminated with hydrocarbons.

Thermophilic bacteriophages | Geobacillus | siphovirus

Characterization of thermophilic bacteriophages from compost heaps

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Bacteriophages are the most abundant entities on Earth. Despite this, there is still a lack of information about extremophilic phages including high-temperature adapted bacterial viruses which are attractive objects for thermophilicity determinants and for practical aspects in industrial biotechnology and other areas employing high temperatures. Thermophilic bacteriophages have been isolated from a variety of sources including environments where temperatures are increased by either natural processes or human activity.

Here, we present characterization of twelve thermophilic bacteriophages which have been isolated from soil samples collected from compost heaps at Vilnius University Botanical Garden, Vilnius, Lithuania. Also, 40 thermophilic bacteria strains, which were used for phage propagation, have been isolated from the same environmental samples. Phage host range determination assay revealed that all phages were active against *Geobacillus thermodenitrificans* strains. In addition, five phages also infected bacteria from genus *Parageobacillus*. Efficiency of plating experiments revealed that phages were able to form plagues at 55–80^{o}C temperatures tested. The vast majority of plaques were clear, ranging in diameter from 1 to 6 mm. Furthermore, most of the bacteriophages (8 out of 12) formed plagues surrounded by halo zone indicating the presence of phage-encoded bacterial exopolysaccharide (EPS)-degrading depolymerases. TEM analysis revealed that all phages were siphoviruses characterized by an isometric head (from ~53 nm to ~71 nm in diameter) and apparently non-contractile flexible tail (from ~122 nm to ~218 nm in length).

The results of this study not only improve our knowledge about poorly explored thermophilic bacteriophages but also give new insights for further investigation of potential thermoactive and/or thermostable enzymes of bacterial viruses.

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adaptation biocontrol Bacteriophage evolution coevolution
experimental evolution host range Phage Therapy Salmonella enterica

Evolutionary training: how bacteriophages adapt to multiple strains of *Salmonella enterica*

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The efficacy of phage therapy depends not only on the ability of bacteriophages to infect pathogenic bacteria but also to limit the emergence of new resistant bacterial genotypes nor to select rare resident resistant genotypes. We performed *in vitro* evolutionary training of one isolated and purified bacteriophages (four independent lineages) by performing 6 to 7 consecutive passages against 8 not co-evolving bacterial genotypes of *Salmonella enterica* serotype Tenessee. While the ancestral bacteriophage was able to infect 3 out of 8 bacterial genotypes, evolved populations expanded their host range (8/8 infected bacterial genotypes). Moreover, bacterial growth inhibition of adapted bacteriophage populations was maintained without appearance of resistant bacteria for more than 20 hours despite a 3-4 log dilution of the bacteriophages. We will present comparison of the population sequencing of both ancestral & adapted bacteriophages. For the sake of successful phage therapy, our results demonstrate the importance of *in vitro* evolutionary training taking into account the diversity of bacteria isolated *in situ* prior to the use of therapeutical bacteriophages.

modelling | Lotka-Volterra | fitness trade-offs | Host-virus interactions

Understanding complex phage-host communities using Lotka-Volterra modelling

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Phages play a vital role in shaping microbial communities by influencing their abundance, diversity and composition via 'kill the winner' or 'arms race' strategies. Bacterial immune systems and the sophisticated viral counter-measures are a testament to this interplay. In our work, we use Lotka-Volterra equations to model the ecology and co-evolution of complex host-phage systems. Such idealized models can explain when bacterial strains evolve phage resistance at a fitness cost, when phages become more generalistic and whether diversity can be maintained. For example, the bistable regime of either domination or co-existence of susceptible and resistant bacteria emerges as a bifurcation mainly determined by bacterial carrying capacity, phage virulence and fitness cost associated with resistance. We explore co-evolution by considering fitness trade-offs in both the bacteria (e.g., growth rate vs resistance) and the phages (e.g., infectivity vs burst size). We explore how motility and spatial structure influence bacteria-phage relations by creating connected niches, modelled using graphs. Such models can shed light on the fundamental aspects of host-phage ecosystems, e.g., why bacteria evolve to be more susceptible to phages in natural systems. In addition, such models are directly valuable for guiding microbiome engineering and implementing effective phage therapy strategies.

Dynamics | Lotka-Volterra | Bacteriophage ecology

Transient Dynamics of phage-bacterial ecology

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Phage-bacterial ecosystems often display transient dynamics: sudden shifts in their dynamics caused by rapid extinctions, migrations, or explosive blooms. Here, we present a computational method to predict shifts in dynamical systems using per capita rates. We apply this method to a phage-bacterial ecosystem using a predator-prey model with four terms: bacterial growth, phage predation, phage growth rate, and phage decay. We also show how this method can be used to simplify the model by removing negligible terms.

Per capita rates show how the system changes on an individual scale. In our case, this means how bacterial and phage concentrations change per unit of time and per unit of bacteria and phage. Formally, per capita rates are obtained by normalizing the predator-prey equations by the total concentration of bacteria and phage. This individualistic approach is insightful because the rates are referenced to the total populations, and they reflect how changes in the dynamics impact the populations themselves. More specifically, we look at the per capita contributions of the four terms to the overall dynamics and their effect on the populations. That is, how per capita bacterial and phage growth, predation and decay can predict shifts in the dynamics. In particular, we set a threshold ϵ for the per capita terms. This threshold implies that there should be an impact of 100% per unit of bacteria or phage. Any per capita term below this threshold will be inactivated from the model, because it will be effectively negligible.

Our results show that large shifts in the dynamics correlate with a single term being significantly inactive than all other three. In addition, we observe that the simplified model without inactive terms gives similar results as the original model if $\epsilon<1$

Bacteriophages | environmental microbiology | gypsum karst lake | ecosystem

Bacteriophages from sulfate-type gypsum karst lake ecosystem – representatives of new viral genera

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Active gypsum karst lake phenomenon occurs as a result of the leaching of gypsum and dolomite rocks leading to the formation of gap holes, which are then transformed into small water bodies dominated by Ca^{2+} and SO_4^{2-} ions. However, the microbial diversity of gypsum karst lakes remains underinvestigated, especially little is known about bacteriophages of such ecosystems.

The aim of this study was to investigate the diversity of bacteria and bacteriophages from sulfate-type gypsum karst lake Kirkilai located near Biržai (Northern Lithuania). In total, 82 bacterial strains and 8 bacteriophages were isolated from water samples taken from different depths of Lake Kirkilai. Host range determination assay demonstrated that phages were active against bacteria from genera *Aeromonas*, *Bacillus*, *Paracoccus* and *Pseudomonas*, as well as *Pseudaeromonas* and *Pararheinheimera* for which no viruses have been reported to date. TEM analysis showed all three morphotypes of tailed phages. Efficiency of platting tests revealed ambient temperature-adapted viruses, which, with an exception of *Bacillus* phages, were able to form plaques even at 4°C. In addition, with an exception of *Bacillus* phage KLEB30-3S, all phages formed plagues surrounded by halo zone indicating the presence of phage-encoded bacterial EPS-degrading depolymerases. Genome sequencing resulted in genomes sized from ~37 to ~216 kb. Bioinformatics and phylogenetic analysis revealed that most phages have no or only few similarities to other bacterial viruses. With an exception of KLEB30-3S, none of newly isolated phages can be assigned to any genera currently recognized by ICTV, and may represent seven new ones within the order *Caudovirales*. Thus, the data presented here not only expand our knowledge on bacteriophages from unexplored environments, but also offer novel insights into the diversity and evolution of bacterial viruses.

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Host specificity | tail fibre | Caudovirales

Exploring tail fiber diversity and compatibility among Caudovirales using comparative genomics

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Although bacteriophages are promising alternatives to antibiotics, their narrow activity spectra greatly hinders their therapeutic applicability. One potential solution to this issue is to rationally direct the host specificity of a bacteriophage against a specific target bacterium by exchanging its tail fiber genes. However, our knowledge on how to find a tail fiber with (i) the desired compatibility with the phage body and (ii) specificity toward the target cell is very limited. Our project aim is to systematically map phage tail compatibilities and specificities by comparing thousands of bacteriophage genomes. Our method first identifies the N-terminal adaptor regions of the phage tail fiber genes that connect the tail fiber to the phage body. Opening the possibility of altering host specificity requires defining tails with likely interchangeable adaptor regions. To achieve this, we used positional conservation data between fibers, as well as metrics of identity and length as clustering inputs. Next, we annotate the depolymerase regions which largely define the range of bacteria that the phage can infect. Predicting the enzymatic domains of tail fibers is another important aspect of our work. We employ a variety of bioinformatics tools, calibrated by analyzing sequences of annotated fibers, available structure predictions and experimentally verified activity. Finally, we experimentally test our predictions by generating hybrid bacteriophages with altered host specificity. Overall, our global map of phage tail compatibility and specificity guides the rational exchange of tails between phage particles which may lead to intelligent design of therapeutic bacteriophages.

Phage-host interactions | DMSP | Hypersaline environments | Coral reefs

Effect of DMSP on viral infections in aquatic environments

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Dimethylsulfoniopropionate (DMSP) is a key molecule in the global sulfur cycle. This compound can be degraded and catabolized biologically to produce dimethylsulfide (DMS), a gas that may affect global climate. DMSP has been proposed to serve as osmoprotectant, antioxidant, signal molecule or sulfur (and carbon) source for marine microorganisms. Several studies have shown that DMSP and DMS can mediate on prey-predator interactions in algae. However, although there are a few studies on how DMS/DMSP may influence viral infection in *Emiliania huxelyi*, the possible role in phage-bacteria interactions remains unexplored.

The current study presents several effects of DMSP in two model bacteria and their phages; *Salinibacter ruber*, a hyperhalophile, and the coral pathogen *Vibrio mediterranei*. Both microbes live in systems where DMSP can reach a very high concentration. In both cases we detected a change in the burst size when the phage-bacteria pairs were incubated with DMSP. We also assessed the infectivity of several viral stocks that had been exposed to different concentrations of DMSP. Furthermore, we explored whether DMSP could affect to viral aggregation or viral adsorption to the cells. For this purpose, we incubated *S. ruber* and their viruses with/without DMSP with a MOI of 0.1. After 30 minutes, we detected a lower number of PFUs in the supernatant of the DMSP treatment, supporting the idea that DMSP increased the adsorption rate. Similar results were obtained with *V. mediterranei*. Finally, we tested if there is a change in protein expression when *V. mediterranei* is incubated with DMSP, finding that several membrane proteins are overexpressed in the presence of DMSP. We hypothesized that these proteins could act as viral receptors, enhancing the viral adsorption. Therefore, DMSP production in heat stressed corals, which is one of the major drivers of the *V. mediterranei* pathogenic behavior, could also play a key role in *Vibrio*-phage regulatory interactions.

prophage | bioinformatics | phage integration and excision

Precise prophage mapping

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There are far more phage genome sequences available as prophages within bacterial genomes than have been submitted to public databases as free-standing phage genomes. To exploit these and other mobilome elements, our published software TIGER precisely maps the coordinates of mobile DNAs within archaeal and bacterial genome sequences. We have now improved TIGER's yield, through cross-scaffold search, and execution speed, through careful selection of reference genome databases. Our "Phage Factory" platform identifies an appropriate prophage set against any bacterial target and develops the set into a phage cocktail, for use in therapy or microbial genome editing. Each precisely defined genomic island also maps an integrase to its integration site (*att*) sequences, with biotechnological and evolutionary implications. Precise prophage mapping has yielded discoveries of site-specific and site-promiscuous integrase clades, regulation of bacterial gene integrity, and a new class of satellites that integrate within their helper prophage genomes.

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Human oral microbiome | biofilm | bacteriophage diversity | Meta-transcriptomics | Comparative genomics | Microbiome engineering

The human oral phageome

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The human mouth harbors complex and diverse microbial biofilms. Oral microorganisms cause the most prevalent polymicrobial pathologies that affect mankind, such as dental caries and periodontal disease, as well as contribute to pathologies outside oral cavity, e.g., in gastrointestinal tract or in air ways. Consequently, oral bacteriophages constitute a growing area of interest, but research on these phages is still in its infancy.

Here, we discuss the diversity of the selected oral phages across clades and niches. Metagenomic profiling has yielded many new phage genomic sequences that still remain to be characterized. Profiling of the conserved protein domains, offer a rational level at which the phage phylogeny and functionality can be studied and used to complement other approaches. Understanding of phage roles in oral ecology is contingent upon availability of well-characterized oral phages. Only a few oral phage isolates have been thoroughly studied to date, because the isolation of oral phages poses a significant research challenge. Detection of phage nucleic acids in clinical samples using PCR or RNAseq, and extensive isolation of propagation strains using culturomics facilitate phage isolation. Phages have a considerable impact on the ecology and dynamics of dental biofilms. We highlight the utility of phages in sensitizing oral biofilms towards antibiotic therapy by specific removal of key pathogens.

To sum up, dentistry is at the very beginning of understanding oral phages. Phage isolations and insights into the phage-microbiome molecular interaction seem essential to decipher the role of oral phages in health and disease and for producing therapeutic phages.

phage-host interactions | Comparative genomics | extreme environments

Novel phage-host systems originating from an extreme environment contaminated with heavy metals

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The aim of this study was to isolate and functionally investigate novel phage-host systems originating from the same sampling location i.e. unusual ecological niche – the ancient gold and arsenic mine Zloty Stok (Poland). This ecosystem is inhabited by unique groups of microorganisms physiologically adapted to its harsh conditions (e.g. contamination with heavy metals, stable low temperature), which form structurally organized communities – microbial mats in the bottom sediments and biofilms on the mine walls. We obtained several phage-bacterium pairs. In each case the host range of phages was very narrow and restricted to individual bacterial strains. These were *Serratia* sp. OS31 – temperate myovirus vB_SspM_BZS1, *Shewanella* sp. M16 – virulent siphovirus vB_SspS_KASIA, *Aeromonas* strains MR7, MR19, MR16 – virulent autographiviruses vB_AspA_Bolek, vB_AspA_Lolek, and vB_AspA_Tola, respectively. The common features of these novel host-parasite systems were cold-activity of phages and cold tolerance of bacteria, although we demonstrated that studied viruses were able to propagate in a narrow range of external factors (e.g. temperature), as compared to the greater flexibility of their host. All newly discovered phages were thoroughly characterized in terms of morphology, infection kinetics and also genomics. The analysis of their genomes allowed among others, the identification of proteins presumably associated with host recognition and DNA modification enzymes which mimic the specificity of DNA methyltransferases of their hosts. Comparative genomic analyses of studied phages showed little or no similarity to other viruses in public databases suggesting to considered them the first of novel species.

Moreover, protein-based similarity networks received in the course of the comparative analysis, provided an updated view of the viruses infecting *Serratia*, *Shewanella* and *Aeromonas* species, providing insights into their diversity.

metagenomics marine Auxiliary Metabolic Genes

New viral biogeochemical roles revealed through metagenomics

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Viruses are the most under-explored fraction of the global biosphere. Metagenomics has brought major advancements to our understanding of the genomic diversity of viruses, but these findings have mostly focused on two ecosystems: the human gut and the photic tropical and subtropical oceans. An enormous diversity of viruses in other ecosystems remains to be discovered. For example, little is known regarding viruses from deep and dark aquatic environments such as the mesopelagic and bathypelagic zones of oceans or viruses in porlar regions. Through the analysis of metagenomes derived from polar and deep marine ecosystems we discovered thousands of viral genomic sequences, the majority of which are novel. Taxonomic classification assigned most of these viral sequences to families of tailed viruses from the order Caudovirales, namely Myoviridae, Siphoviridae, Podoviridae. Using state-of-the-art in silico host prediction approaches these viruses could be linked to their putative hosts, which led to the discovery that many of these viruses infect abundant and ecologically important taxa of aquatic ecosystems, including Proteobacteria, Cyanobacteria, Bacteroidetes, Actinobacteria and Crenarchaeota. Analysis of the gene content of these viral genomes allowed us to describe novel auxiliary metabolic genes. Among these, were included one of the first bacteriophage genomes with the potential to modulate host vitamin and porphyrin metabolisms. Together, these findings demonstrate that the viral potential to alter hosts metabolism during infection is much more diverse than previously described and covers ecological processes in which the role of viruses has historically been neglected. Thus demonstrating that the contribution of viruses to biogeochemical cycles is greater than previously anticipated.

Transposable phages | viromics | Comparative genomics

An expanded diversity of transposable phages

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Transposable phages (proposed family *Saltoviridae*) are dsDNA phages capable of generalized transduction, the transfer of bacterial DNA by phages from an infected bacterium to another bacterium. Examples include the Mu and B3 phages, whose mode of action has been described in detail at the molecular level. Despite their potential role in mediating horizontal gene transfer between microbiota, their known genomic diversity is limited to a small number of phages infecting a few members of Proteobacteria and Firmicutes.

Here we expanded the known diversity of transposable phages. First, we identified transposable phage genomes in the NCBI nucleotide database. Next, we dereplicated the genomes at the species level and searched for prevalent orthologous groups among their proteins. We identified 16 orthologs related to 10 proteins. Using HMM profiles of those orthologs, we searched for transposable phages in the database of uncultivated viral genomes IMG/VR and in the Gut Phage Database. After dereplication at the species level, we established a set of 635 putative transposable phages, explored their genomic diversity, reconstructed their phylogeny based on the portal protein, and explored the pangenome of particular genera associated with the human gut microbiome.

The established set of putative transposable phages greatly expands the proposed *Saltoviridae* family from four to seven phylogenetic clades. Furthermore, the expanded diversity includes phages infecting bacteria belonging to at least four phyla (Proteobacteria, Firmicutes, Deinococcus-Thermus, and Spirochaetes) and 47 different genera. Transposable phages were assembled from viromes derived from various environments, such as water, soil, and living hosts. This work significantly the known diversity and host range of transposable phages and sets the stage for further functional characterization of this enigmatic group of phages.

Pseudomonas Lysogeny transmission

Evolution of spontaneous induction in *Pseudomonas aeruginosa* lysogens in response to lack of susceptible cells

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The fitness of viruses is largely determined by their transmission mode. Temperate phages, bacterial viruses which can upon infection either lyse the cell or integrate into the genome of the cell, are a clinically and ecologically important component of microbial population dynamics. However, the evolutionary and ecological pressures that shape transmission mode in temperate viruses are poorly understood and have potential consequences in the context of human chronic infections, which contain co-evolving phage-host pairs. Chronic infections have a high degree of spatial structure and low strain diversity, which may mean low local numbers of cells susceptible to bacteriophage infection. In this study, we sought to explore the impact of absence of susceptible cells on the evolution of temperate viruses. To investigate this, we passaged a *Pseudomonas aeruginosa* lysogen PA14(DMS3) without susceptible cells for 12 days and measured different viral transmission parameters. We predicted that traits associated with horizontal transmission (burst size and latent period) would decrease and traits associated with vertical transmission (spontaneous induction) would be favored by the evolved viruses. Instead, we found horizontal transmission parameters to be unchanged in the evolved free viruses, while spontaneous induction is significantly decreased in the evolved viruses. We propose that spontaneous induction is an evolvable part of transmission mode that increases lysogen stability when no susceptible cells are present.

holobionts coral reefs Bacteriophage ecology

Prophages in host-associated microbes: the most abundant symbiosis on earth and its relevance in marine holobionts

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Bacteriophages are the most abundant biological entity on the planet, the most numerous members of any given food web, and key players in carbon and nutrient cycling. Temperate bacteriophages can incorporate into the genome of their bacterial host and remain there in a mutualistic fashion. These prophages typically encode functions that determine the ecology of their bacterial host, often in beneficial ways such as protection against protist predation, nutrient acquisition, carbohydrate utilization, and superinfection immunity from other phages. Here we surveyed prophages and their functional genomic content in a large database of host-associated bacteria from marine environments around the globe. Strikingly, we observed that host-associated bacteria characterized as pathogens contained significantly higher abundances of prophages in their assembled genomes than non-pathogenic host-associated marine bacteria. This specific trend has been observed in human systems, in the context of human bacterial pathogens and non-pathogenic human associated bacteria. Our data suggests that this trend is prevalent in marine environments across a variety of ecosystems and animals. We are now extending these bioinformatic approaches to understand the abundance and functional profiles of prophages in metagenomic profiles of marine holobionts to understand their role in coral reef benthic communities.

Salmonella signatures phage selection

Clouds of phages; the pursuit of properties that allow phages to best target pathogens

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Bacteriophages are currently developed for therapeutic purposes 'independently' for each disease type and geographical location. For example, if phages are needed to remove *Salmonella* found in UK chicken strains, large phage sets are isolated and tested on relevant host bacteria. Fairly lengthy processes are then followed where phages are characterised according to their phenotypes to select phages with the broadest host ranges within that species, maximum virulence and other desired required downstream properties. Genomes are checked to ensure phages don't encode integrases, toxins or other undesirable properties and phages are tested as pairs, or larger combinations.

Currently each time phages are developed for a specific bacterial species; this entire discovery and formulation process is be repeated as there is no established way to apply knowledge between phages that target different bacterial species. To capitalise on many discrete observations, we are developing ecological approaches to allow the identification of features common to therapeutically useful phages regardless of their host or geographical region.

We have been identifying signatures of good clinical efficacy in phage genomes, transcriptomes, proteomes and metabolomes and determining how these signatures map to bacteriophage ecological strategies that are suited to therapy. To better understand these features, we are establishing a 'cloud-based' framework to integrate all known phages for a given bacterial pathogen. We then compare the phages in a graph-based fashion to each other in order identify how key phenotypes map to genotypes within specific groups, and how those properties that make the phages successful. Thus, future phages could be more easily selected or eliminated by searching within key 'clouds' or groups. We share how we have successfully used this approach to delve into the genomes of specific host systems, in particular with *Salmonella*.

T4 phage | head proteins | gastrointestinal tract | Phage evolution

Evolution of the T4 phage virion is driven by selection pressure from the gastrointestinal tract environment

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Objectives: As important components of the gut microbiota of animals and humans, bacteriophages are constantly exposed to the gastrointestinal (GI) tract environment. Therefore, the selection pressure exerted by the specific conditions in GI tract may have directed the evolution of phage capsids to improve their survival in this particular niche. The study aims to elucidate how individual T4 phage head proteins contribute to its survival.

Methods: Site-directed mutagenesis of T4 phage and T4ΔHoc was applied to construct gp24 bypass mutants and Soc-deficient phages. The panel of T4 phage variants lacking one or more head proteins was then tested for their susceptibility to the factors specific for mammalian GI tract environment: body temperature, acidic and alkaline pH, bile and proteolytic digestive enzymes.

Results: Gp24-deficient phages showed impaired survival in acidic pH (pH 3) and at 37 °C, particularly when lacking also Soc protein. While none of the phages were susceptible to bile extract or trypsin, all were sensitive to a-chymotrypsin, with gp24-deficient variants being significantly more affected. T4Δ24byp24 mutants were also more prone to pepsin digestion, even more so when Soc protein was also missing from their capsids.

Conclusions: Presented data demonstrate the key role of T4-like phage capsid composition in phage resistance to environmental conditions, particularly those characterizing GI tracts of animals and humans. Gp24 appears to play the key role in resisting the GI tract environment as gp24-deficient mutants, representing more 'primitive' capsid structure, are less stable at warm body temperature and low pH, and more sensitive to inactivation by digestive enzymes. Therefore, we postulate that specific conditions inside the GI tract are the major source of evolutionary pressure on T4-like phages, shaping the structure of phage virion.

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CRISPR viral metagenomics ecology

High viral abundance and low diversity are associated with increased CRISPR-Cas prevalence across microbial ecosystems

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CRISPR-Cas are adaptive immune systems that protect their hosts against viruses and other parasitic mobile genetic elements. Consequently, selection from viruses and other genetic parasites is often assumed to drive the acquisition and maintenance of these immune systems in nature, but this remains untested. Here, we analyse the abundance of CRISPR arrays in natural environments using metagenomic datasets from 332 terrestrial, aquatic and host-associated ecosystems. For each metagenome we quantified viral abundance and levels of viral community diversity to test whether these variables can explain variation in CRISPR-Cas abundance across ecosystems. We find a strong positive correlation between CRISPR-Cas abundance and viral abundance. In addition, when controlling for differences in viral abundance, we found that the CRISPR-Cas systems are more abundant when viral diversity is low. We also found differences in relative CRISPR-Cas abundance among environments, with environmental classification explaining ~24% of variation in CRISPR-Cas abundance. However, the correlations with viral abundance and diversity are broadly consistent across diverse natural environments. These results indicate that viral abundance and diversity are major ecological factors that drive the selection and maintenance of CRISPR-Cas in microbial ecosystems.

Single-cell genomics | Host-virus interactions | Eukaryote-infecting virus

Unveiling virus-host interactions of marine protists through Single-Cell Genomics

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Viruses play fundamental roles in ocean biogeochemical cycles, particularly in the transfer of carbon and nutrients between microorganisms through the viral shunt. Most studies of marine viruses have focused on those that infect Bacteria and Archaea. Thus, viruses that infect microeukaryotes have often been neglected, despite the fact that their hosts are important primary producers and grazers. Understanding the underlying mechanisms and the specificity of the interactions between eukaryotic viruses and their hosts, and the ecological impact of these dynamics, is fundamental to obtain accurate projections of ocean ecosystems under climate change scenarios. This can only be achieved through a comprehensive understanding of marine viral diversity and associated information on their infected hosts. Mining viral genomes in eukaryotic single amplified genomes (SAG) obtained by single-cell genomics has proven to be an efficient method to unravel novel viral diversity. We applied this approach to obtain a total of 81 SAGs derived from single eukaryotic cells isolated by flow cytometry from a surface marine community sampled at the Blanes Bay Microbial Observatory (Spain). SAGs were classified in different taxonomic supergroups based on the V4 hypervariable region of the 18S ribosomal gene, such as the SAR group (Stramenopiles, 40.7%; Alveolates, 11.1%; Rhizaria, 7.4%), Archaeplastida (12.3%), Hacrobia (16.1%), Opisthokonta (6.2%), and unclassified eukaryotes (6.2%). Within these SAGs, 98 genomic fragments of eukaryotic viruses of the NCLDV supergroup were identified, as well as 159 genomic fragments of virophages of the Lavidaviridae family. These results expand our understanding of marine viral diversity, which is of special relevance for groups such as Phycodnaviridiae for which little to no viruses are currently known.

Horizontal gene transfer | gut virome | transduction | human gut microbiome

Looking for evidence of phage-mediated horizontal gene transfer in human gut viromes

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Bacteriophages are likely to play a role in horizontal gene transfer (HGT) in the human gut microbiota, potentially boosting the functional capacity of the microbiome and facilitating transfer of important genetic traits (antibiotic resistance, virulence, metabolic functions) within and between microbial species. As of now, we have no clear understanding of the extent or importance of any such transduction events. In support of widespread HGT within the gut, we have observed the consistent presence of bacterial DNA within metagenomics reads generated from purified gut virus-like particles (VLP). This could result from bacterial DNA packaged within phage particles (HGT) or could be due to contamination as a result of imperfect VLP extraction. We set out to examine this phenomenon.

We first compared the relative abundances of non-viral contigs in faecal VLP metagenomes and compared these to matched total community metagenomes. We identified contigs with highly skewed representation in the VLP fraction (elevated ratio of relative abundance in VLP versus total community) and analysed their taxonomic origin. We also performed long read sequencing on faecal samples to identify the length of bacterial reads in the VLP fraction. We looked for read lengths corresponding to sizes associated with packaged phage genomes that could represent fragments of host DNA subjected to phage-specific packaging restrictions on fragment length.

Together these results provide a perspective on the source and nature of bacterial DNA in viral metagenomes.

anti-phage defence systems | microbial genomics | bacteriophage ecology

Systematic and quantitative view of the antiviral arsenal of prokaryotes

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Bacteria and archaea have developed multiple antiviral mechanisms, and genomic evidence indicates that several of these antiviral systems co-occur in the same strain. Here, we introduce DefenseFinder, a tool that automatically detects known antiviral systems in prokaryotic genomes. We use DefenseFinder to analyse 21000 fully sequenced prokaryotic genomes, and find that antiviral strategies vary drastically between phyla, species and strains. Variations in composition of antiviral systems correlate with genome size, viral threat, and lifestyle traits. DefenseFinder will facilitate large-scale genomic analysis of antiviral defense systems and the study of host-virus interactions in prokaryotes.

gut microbiota coevolution oxidative stress

The consequences of stress on the relationship between one phage and three bacteria of the gut microbiota

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Reactive oxygen species (ROS) play a central role in the pathogenesis of inflammatory bowel diseases (IBD). These diseases are characterized by altered abundance and diversity of both bacteria and bacteriophages (phages) of the intestinal microbiota but the mechanisms underlying these changes in composition during chronic inflammation remain largely unknown. This study aims to identify the impact of ROS-induced oxidative stress on the interactions between intestinal phages and bacteria.

We set up a reductionist *in vitro* model using a defined microbial community of three *Escherichia coli* strains and a virulent phage growing in continuous culture in bioreactors. We compared the evolution of this community in the presence and absence of oxidative stress, by exposing the culture to continuous addition of hydrogen peroxide.

We studied population dynamics and the profiles of resistance and infectivity and linked these profiles to the frequencies of genomic mutations of the four populations isolated at different time points over 10 days. While hydrogen peroxide did not significantly alter bacterial viability and phage infectivity, phage concentrations were significantly lower during co-culture under oxidative stress, supporting observations of decreased virulent phages in IBD. Also, our phage populations evolved to be more specialist to their isolation strain in the presence of oxidative stress compared to the control condition. Finally, genomic analysis highlighted high-frequency mutations, signatures of phage-bacteria arms-race and a comparative analysis is currently ongoing.

These data contribute to the fundamental understanding of how environmental variations may affect bacteria-phage interactions and hence the equilibrium of the gut microbiota. Determining the impact of inflammation-driven abiotic factors in altering microbial diversity is a step towards understanding the pathophysiology of IBD.

Antimicrobial Resistance | Metabolism | Lysogeny

Temperate phages of cystic fibrosis and bronchiectasis drive physiological changes in PAO1 relating to chronic infection

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Pseudomonas aeruginosa (Pa) is an opportunistic respiratory pathogen of Bronchiectasis (BR) and similar symptomatically to autosomal recessive genetic disorder Cystic Fibrosis (CF). Epithelial dysregulation associated with inflammation and scarring (CF,BR) and altered ion transfer through mutation of the CF conductance regulator gene (*cftr*) leads to a thick mucus environment linked to lowered lung function and poor clinical outcomes. Temperate bacteriophages that integrate into the genomes of Pa carry genes that aid bacterial selection in the lung and drive evolution. Our aim is to characterise the genomes of phages that can mobilise from PAO1 to disseminate genetic traits and function.

In this study we created 20 lysogens of PAO1 using phages induced from early and late CF and BR Pa isolates. Lysogeny was confirmed by sequencing and aligning to the naïve PAO1 host.

We show that temperate phages can alter PAO1 susceptibility to sub inhibitory concentrations of ceftazidime, colistin, meropenem and piperacillin. The lysogens were also shown to alter *Galleria mellonella* survival in an infection model reducing virulence, seen by the increase lifespan of the larvae. We finally show that the lysogens of PAO1 have an influence on the metabolomic profiles when compared to the naïve host. Comparing over 3K metabolites, 146 metabolites and 26 pathways were shown to be significantly different (p value <0.05) compared to the PAO1 naïve host. Similarity was seen between aetiology of infection driving metabolomic change. Six pathways were shown to be impacted the greatest novobiocin and arginine biosynthesis and metabolism of folate, arginine/proline, glycerophospholipid and biotin.

This study shows how phages have adapted in the lung and can subvert their host, by using PAO1 as a host we can infer the effects of temperate phage and begin to look for the gene responsible for change in bacterial fitness, virulence, and AMR susceptibility.

viromics databases bacteriophage phage lifestyle

Environmental distribution of public virus genomics data and virus lifestyle preferences

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Viruses are the most abundant biological entities on earth and play a major role in ecosystem functioning. The ever-expanding databases have allowed researchers to exploit metagenomic data and to achieve major advances in viral genome discovery. Virus genomics is a powerful tool to investigate virus-host interactions, lifestyle, auxiliary metabolic genes, and evolution. Despite viral ubiquity, global ecosystems have not been evenly studied, and understanding the current status of data availability is key to any meta-study. In this study, we aimed to get an overview of the publicly available data that can be resolved into virus genomes and investigate viral lifestyle. To survey what data is available, we collected the metadata associated with all uncultivated virus genomes (UViGs) from JGI's IMG-VR and with all metagenomes from EBI's MGnify platform. We also compared the global distribution of soil and freshwater samples using geospatial data. Finally, we predicted the lifestyle of all viral contigs from IMG-VR per environment using the DeePhage tool. The IMG-VR database is the largest UViGs resource and contains ~1,9 million viral contigs. Among the environmental libraries, 2,766 (32,5%) are marine, 2,159 (25,4%) are from soil and 1,892 (22,2%) are from freshwater. Among the environmental metagenomes in MGnify, 4,295 are marine (64,5%), 1,726 are from soil (25,9%) and 417 (6,3%) are from freshwater. Furthermore, our preliminary results showed that in aquatic environments the ratio between virulent and temperate viruses is the highest (median >50%), while in other environments the median is below 50%. In summary, by number, environments are more evenly represented in the IMG-VR than in the MGnify database. Furthermore, by linking sample geolocation with geospatial data, we uncovered the potential blind spots in global viral genome research. Further analyses are needed to validate the results on viral lifestyles predominating in different environments.

phage genomics | taxonomy | Klebsiella

Genomic characterisation of *Klebsiella michiganensis* phage Kmi2C reveals a new genus

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Klebsiella michiganensis is an emerging pathogen of both humans and animals. Recent reports describe increasing antibiotic resistance within the species, including isolates encoding extended spectrum B-lactamases and carbapenemases. Therefore, it is important that alternative treatment modalities are investigated. Bacteriophages (phages) are one such option.

Phage Kmi2C (vB_KmiS_Kmi2C) was isolated on a multidrug-resistant clinical isolate of *K. michiganensis* (PS_Koxy2). Host range analysis against 79 clinical and veterinary isolates belonging to the genus *Klebsiella* revealed a narrow host range (5/47 *K. michiganensis* lysed, 1/24 *K. oxytoca*, 0/7 *K. grimontii,* 0/1 *K. pneumoniae*). Electron microscopy identified a *Siphoviridae*-like morphology. Genome sequencing revealed a genome size of 42,234 bp encoding 55 predicted genes. Interesting genomic features include a Sak4-like ssDNA annealing protein with single-strand DNA binding protein; a Cas4-domain exonuclease; a holin and a Rz-like spanin. Further gene annotation refinement via BLASTp identified a possible receptor binding protein (RBP) sharing similarity to RBPs from *Acinetobacter baumannii* prophage.

Initial BLASTn genomic and ViPTree proteomic tree analysis showed Kmi2C shares little similarity with other deposited phage genomes whilst PhageClouds was unable to identify any related phage genomes. Network-based analysis using vConTACT suggests Kmi2C shares a viral cluster with 38 other phages from multiple genera and shares a low level of sequence identity (VIRIDIC 18.8%) with phage vB_Pae-Kakheti25 identified as its closest sequence relative. These results suggest Kmi2C is novel and constitutes a new genus or possibly family of phage.

Studies are ongoing to categorically determine the RBP of this novel phage and its lytic potential against isolates outside the genus *Klebsiella*.

Giant Viruses | jumbo phage | Virus Life History | Entry Mechanism | Capsid stability | Allometric Exponent | Packaging density | Energetic cost

Scaling relation between genome length and particle size of viruses provides insights into viral life history

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In terms of genome and particle sizes, viruses exhibit great diversity. The interplay between particle and genome sizes of viruses and their life histories is poorly understood. However, with the discovery of several large eukaryotic DNA viruses and jumbo phages, size has emerged as an important criterion for understanding virus evolution. We use allometric scaling of capsid volume with the genome length of different groups of viruses to shed light on its relationship with virus life history. We find that the allometric exponent between genome length and capsid volume of icosahedral dsDNA bacteriophages and the nucleocytoplasmic large DNA viruses (NCLDVs) infecting unicellular eukaryotes such as protozoan and algae are approximately 1 and 2, respectively. This finding indicates that with increasing capsid size, DNA packaging density statistically remains the same in bacteriophages but decreases for NCLDVs. We argue that the different exponents of these two groups of viruses are largely shaped by their entry mechanism and capsid mechanical stability. Thus, in NCLDVs, the potential to add more genome and generate higher autonomy from the host appears to be a consequence of the larger capsid and higher allometric exponent of 2. We further show that these allometric size parameters are also intricately linked to the relative energy costs of translation and replication in viruses and can have further implications on viral life history.

jumbophage freshwater genomes

Jumbophages and a megaphage among boreal freshwater phage isolates

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In the age of increasing sequence data, the demand to understand the biology behind the data is growing. Thus, we isolated and characterized phage-host pairs from Finnish lakes and rivers to study the phage diversity and to connect the phage genome sequence to its morphology and host specificity. We characterized 44 phage-host pairs, of which ten phages had a genome size over 200 kbp. Seven of these infected *Flavobacterium* species, and *Pseudomonas*, *Janthinobacterium* and *Herbaspirillum* all were infected by one jumbophage isolate. Interestingly, one of the Flavobacterium phages had a genome size of ~646 kbp. To our knowledge this is the first cultivated isolate of a megaphage (genome size >600 kbp). Virions of the megaphage displayed a myovirus morphology with a capsid of ~165 nm in diameter and ~180 nm long tail. From the whole genome sequence, 1001 open reading frames and 68 tRNA genes were predicted. BlastP searches yielded hits to an uncultured marine phage with a similar size genome, however many of the ORFs received no results. We speculate that freshwater bacteria harbor a great diversity of phages that are conveniently cultivated in the laboratory and thus serve as an excellent source for studies on phage diversity.

bacteriophage diversity | flavobacterium | Host-virus interactions

Unique Life Cycle of a Novel *Flavobacterium* Infecting Virus Type

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Flavobacterium infecting, lipid-containing phage (FLiP) is icosahedral bacteriophage with a circular ssDNA genome and an internal lipid membrane. This combination of characteristics makes it unique among published bacteriophages, and for time being, the only species in virus family *Finnlakeviridae*. So far, phage-host interactions of FLiP have remained poorly understood. Here, we addressed this issue by characterizing the life cycle of FLiP under different environmental conditions, e.g. in host cells in different growth phages, oxygen depletion, different temperatures and under competition. According to our results, FLiP shows some peculiar life cycle characteristics. FLiP prefers to adsorb to and infect cells in stationary phase compared to logarithmic growth phase. In logarithmic phase cultures FLiP only adsorbs onto isolation host while adsorption to two other known hosts is negligible. However, when stationary phase cells are used, FLiP adsorbs more efficiently to all hosts. FLiP only moderately propagates in liquid culture of isolation host. It does not propagate in liquid cultures of other known hosts until time scale of several days is reached, i.e. there is enough time for biofilm formation. FLiP shows delayed lysis when host experiences stressful conditions like anaerobic, cold or competitive environment in a mixed culture. In such conditions FLiP plaque size is substantially larger and surrounding plaques around clear drop area are common. To conclude, FLiP life cycle shows plasticity determined by environmental conditions. Weakness of infection in liquid-suspended cells, especially during logarithmic growth, could be explained by expression of phage receptor and / or other infection allowing host factors only after host cell is old enough or attached to a surface like agar on plates or biofilm in liquid cultures. FLiP might in challenging conditions rely on lysogeny or pseudolysogeny leading to delayed infection and lysis.

Bacteriophage Bioinformatics metagenomics

The Hidden Bacteriophage of Chronic Obstructive Pulmonary Disease (COPD)

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Chronic Obstructive Pulmonary Disease (COPD) is currently the third major cause of mortality in the United States and the United Kingdom, and encompasses progressive lung conditions, including emphysema and chronic bronchitis. Until recently, the microbiome of the lungs was quite novel for healthy individuals. As COPD includes several conditions and fluctuating bacterial infections, little has been studied regarding the bacterial or viral microbiome of these lungs. Using bioinformatic techniques on human sputum and DNA extractions from bacterial isolates of COPD patients, we can begin to map more of the microbiome. From this data, bacteriophages are predicted and identified. Learning this brief portion of the viral biome, we can compare viral and bacterial microbiomes within the lungs of COPD individuals.

metaviromics orthologous groups Phage Cocktails

The database of Virus Orthologous Groups and its application in phage cocktail genomics

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The sequencing of genomes of bacteriophages from cultures and from the environment is a key method to understand the function, diversity, abundance and host relations of phages in the biosphere. Current metaviromic experiments are producing lots of metagenome assembled viral genomes or viral fragments out of which most are so far hard to cultivate or study experimentally. However, the rapid evolution of virus genomes limits the computational analysis of virus genomes, as often no homologs of identified genes can be found in the sequence databases. Methods for detecting remote homology, e.g. grouping of genes by orthology, increase the sensitivity of virus annotation workflows substantially. Therefore, we developed VOGDB, the database of Virus Orthologous Groups (VOGs). VOGDB groups viral proteins by their with distant evolutionary relationships. The database is based on all completely annotated viral genomes (both phages and non-phages) from NCBI RefSeq. Clustering of viral protein-coding genes is a two-step process where in the first step phages and non-phages are separated and clusters are calculated based on the pair-wise sequence similarity and in the second step, Hidden Markov Models of the previously created clusters are compared to each other and grouped into VOGs in order to capture remote evolutionary relationships, now combining phages and non-phages. VOGs are functionally annotated based on the annotation transfer from UniProt/Swiss-Prot database. VOGDB was used in many projects already. We recently analyzed and compared the composition of metagenomes of five phage cocktails from Eastern Europe. Cocktails come from different producers and are designed to treat different types of infection. Phage genomes from cocktails were assembled and putative genes were predicted and mapped to VOGs. Similarity of the cocktails was assessed from the overlaps of the VOG contents and the annotations of the most common VOGs in the cocktails.

Average nucleotide identity Bacteriophage mmseq2

ANImm : A fast and accurate tool for calculating average nucleotide identity between pairs of viral genomes

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One of the most common metrics for the assessment of a relationship between pairs of microbial genomes is Average Nucleotide Identity (ANI). While ANI was originally developed with bacterial genomes in mind, in recent years it has become commonly used to assess relationships between viral genomes, including bacteriophages. There are several methods currently available for systematic calculation of ANI between pairs of genomes such as PyANI, fastANI and Mash. However, these tools have not been systematically assessed on viral samples and do not take into account a high degree of genetic mosaicism between some viruses. Furthermore, it is unclear how well the current tools scale to large datasets. Here we present a new approach called ANImm that uses mmseqs2 to systematically calculate ANI and alignment fraction (AF) values for pairs of genomes by applying the same approach used by Goris and colleagues. We assessed the accuracy and speed of ANImm using 200 NCBI RefSeq phage sequences and found that it reproduces the accuracy of PyANI (R2=0.999), a BLAST-based tool, at about 1/100 of running time. We then further assessed the performance of ANImm by generating an in-silico dataset of 126 simulated pairs of DNA sequences with a known ANI and AF values. Our results showed that ANImm, with the right parameterization, was able to accurately predict ANI values even below the PyANI prediction threshold of 60% ANI. We were able to process the entire NCBI RefSeq (4500 genomes) in 2hours 56minutes, making ANImm applicable to intermediate-to-large datasets. Altogether, ANImm is a fast, accurate and scalable tool to investigate genetic relationships between pairs of viral genomes.

Tectivirus Plasmid bacteriophage diversity

Systematic discovery of plasmid-dependent bacteriophages

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Plasmid-dependent bacteriophages are an under-explored group of bacterial viruses that use plasmid-encoded receptors to infect bacteria. Despite their interesting biology, there has not been a systematic search for them or an efficient strategy for their isolation. We developed a method that allows us to efficiently screen for phages that infect bacteria carrying specific conjugative plasmids. With this method, we were able to isolate an unprecedented collection of bacteriophages of the Tectiviridae family, which are broad host range viruses that depend on IncP-type conjugative plasmids. We discovered that these bacteriophages are more common and abundant than previously thought, and our collection expands the known diversity of these phages by an order of magnitude. We are exploring how this special group of phages is able to infect diverse bacterial hosts containing IncP plasmids, and how plasmid-dependent phages shape the evolution of broad-host plasmids.

Prophage Antibiotic Induction

Evaluating the effect of antibiotics on prophage induction in soil bacteria

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Terrestrial systems are hotspots for unexplored viral diversity and virus-bacteria interactions. Phages (viruses of bacteria) infect bacteria via the lytic or lysogenic cycle. The lysogenic cycle is characterized by the integration of the phage genome into bacterial chromosome as a prophage. Prophages are induced by environmental triggers or disturbances, resulting in the lysis of bacterial hosts and the release of new phages into the environment. In this work, we studied if antibiotics applied to livestock farming or agriculture, and which persist in the environment, induce prophage. Specifically, five veterinary antibiotics, i.e. ciprofloxacin, trimethoprim, tetracycline, amoxicillin and kanamycin, as well as mitomycin C were tested for induction of soil bacterial isolates. Induction with MIC and half-MIC concentrations showed that ciproflocaxin and tetracycline induced most soil isolates. In addition, we compared prophage induction in soil microbial communities after the exposure of soil microcosms to concentrations of ciprofloxacin and tetracycline usually found in manure (5 and 50 µgkg-1), and to mitomycin C. Phage numbers after induction were quantified by an improved flow cytometry protocol, and the microbial community was assessed by 16S rRNA gene amplicon sequencing. Both tetracycline concentrations resulted in the strongest changes in community composition, while only 5 µg kg-1 tetracycline resulted in significantly higher numbers of released phages, indicating induction of prophages.

Prophages Toxins Vibrio

Network distribution of prophages carrying *zot* toxins on different *Vibrio* genomes

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Vibrios are one of the most abundant bacterial groups in marine environments. Many vibrios carry prophages on their genomes, and some of them are involved in the virulence of the bacteria, carrying toxins such as the *zonula occludens toxin* (*zot*). Therefore, it is important to explore the distribution of those prophages among the different vibrio species and to evaluate if they are more related to individual species.

We evaluated 4,619 genomes of vibrios, which include 127 species, for the presence of prophages carrying the *zot* toxin using the PHASTER tool. In total, 2,030 potential prophages of 13 different types were detected in 43 species of vibrio. The prophages have ranging sizes from 3.6 kb to 158 kb, with an average size of 9.9 kb. As expected, most of the prophages were found in *V. cholerae* and *V. parahaemolyticus* genomes; however, if the number of genomes sequenced is considered, then the prophages were found preferentially on species such as *Vibrio antiquaries*, *Vibrio diabolicus* or *Vibrio alginolyticus*. Some prophages, such as CTX or Vf33 were found in two or three species. In contrast, phiVCY or VfO3K6 were found on 28 and 20 *Vibrio* species, respectively.

Seven different prophages were found in *V. cholerae*, being the CTX the most frequent (645; 73%), and Vfo3k6 the less frequent (2; 0.2%). While in *V. parahemolyticus*, nine different prophage types were found, with VfO3K6 (417; 49%) as the most frequent and fs1 found only in one genome (1; 0.1%).

Finally, the different *zot* sequences found in the genomes were classified using a sequence similarity network analysis, obtaining 6 clusters. Cluster 3 was found in nine types of prophages and 17 vibrio species, while cluster 1B was found uniquely in one genome of *V. cholerae*, showing different distribution patterns of the different *zot* types.

We expect this analysis to contribute to understanding the distribution patterns of *zot*-containing prophages across the vibrio genomes.

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prophage | extreme environments | Network Analysis

In silico identification and characterization of *Psychrobacter* prophages

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Psychrobacter spp. have been isolated from a variety of environments, of which most are low-temperature geographical locations. *Psychrobacter* spp. due to their capability of growing at extremely low temperatures are considered psychrotolerant bacteria. They belong to *Moraxellaceae* family which also accommodates two other genera: *Moraxella* and *Acinetobacter*, for which the majority of representatives were recognized as pathogens. Until now, only one *Psychrobacter* phage has been described with an indication of a potential drift of phages between *Psychrobacter* spp. and *A. baumannii*. However, this assumption requires further investigations and insights into the *Moraxellaceae* virome.

In this study we screened 266 genomes of *Psychrobacter* spp. for the presence of prophages. Using PhiSpy algorithm and manual annotation, we identified 122 previously unindicated complete prophages within 83 *Psychrobacter* genomes. Among them 28 revealed to be polilysogens carrying up to 4 complete prophages Then, using the protein-based similarity networking we revealed the diversity of available *Moraxellaceae* (pro)phage genomes where *Acinetobacter* (pro)phages showed the highest diversity, forming four separate clusters. The majority of *Psychrobacter* prophages which shared similar proteins with all known *M. catarrhalis* prophages and several *A. baumannii* viruses were grouped together in one compact cluster. Moreover, the analyses showed that a range of *Psychrobacter* prophages are similar to viruses infecting other *Gamma-, Alpha-* and *Betaproteobacteria*. What is more, detailed analyses of *Psychrobacter* phages revealed their mosaicism.

Based on our results we assume that induced prophages of *Psychrobacter* and *Moraxella* could be able to cross-infect their hosts, rather than to infect *Acinetobacter* strains.

Virus structures and function

protein structure | receptor-binding protein | mosaicism | functional annotation

Domain architecture shows extensive mosaicism of phage receptor binding proteins

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During millions of years of bacteria-phage coevolution, bacteriophages have been forced to keep the pace with evolution and diversification of bacterial receptors. We hypothesized such coevolution has shaped the domain architecture of phage proteins engaged in host tropism, like receptor-binding-proteins (RBPs). To address it, we downloaded representative genomes of phages from NCBI RefSeq and created deep HMM profiles for all proteins therein using UniClust30. We used remote homology detection (HHblits) for an all-by-all profile-profile comparison, protein domain detection using Pfam and putative function assignment to each profile using homology detection with three complementary approaches (PHROGs, GOs, PhANN). Our results show that the function of a structural protein often cannot be uniquely determined and, when using low coverage threshold, some functions (eg tail fibres and spikes) cannot be distinguished using homology-based approaches. We found that such functional ambiguity is related to domain sharing between proteins from different functional categories. We then systematically compared domain architectures of structural proteins from various functional categories. Domains found within these proteins were shared between otherwise dissimilar proteins and co-occurred in multiple combinations. Such understood mosaicism was more frequent within RBPs than other structural components like capsids or major tails. To account for under- or overrepresentation of a domain database like Pfam, we carried out an all-by-all comparison between all phage structural proteins and found that sharing fragments between otherwise dissimilar proteins is much more frequent within RBPs than any other structural protein class.

Altogether these results highlight the importance of the underlying protein domain architecture in RBPs in helping phages to adapt to novel bacterial surface receptors, for example via horizontal gene transfer of protein domains into new structural types.

Pseudomonas aeruginosa bacteriophage cryo-electron microscopy structure replication strategy

A hunting strategy and virion structure of *P. aeruginosa* bacteriophage JBD30 revealed by cryo-electron microscopy

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Pseudomonas aeruginosa is a human pathogen, whose treatment is complicated by its frequent antibiotic-resistance. *Siphoviridae* bacteriophage JBD30 infects and kills bacterium *P. aeruginosa*, which makes it a potential agent for phage therapy. Here we present the structure of bacteriophage JBD30 virion and its replication strategy, revealed by the combination of cryo-electron microscopy analysis techniques and cryo-electron tomography.

The virion of bacteriophage JBD30 is composed of non-enveloped icosahedral capsid, long flexible non-contractile tail and baseplate decorated with fibers. The capsid with a diameter of 60 nm is built from major capsid protein organised in T = 7 icosahedral lattice and decorated with trimers of minor capsid protein. In one vertex of the capsid, the penton of major capsid protein is replaced by dodecameric portal. The portal complex forms an interface between the capsid and 180 nm long tail. The tail is built from 44 hexameric discs of major tail protein. Distal tail protein trimer follows-up the last tail disc and forms an attachment site for the long tail fibers. The baseplate is terminated with a tripod complex of receptor binding protein trimers.

Using cryo-electron tomography we followed the infection process of *P. aeruginosa* by JBD30 phage from attachment to bacterial cell, to the production of new phage progeny and host cell lysis. Bacteriophage JBD30 uses its long tail fibres for binding to *P. aeruginosa* pili type IV. After attachment to pili, the virion is pulled towards the cellular surface, where it irreversibly binds by its receptor binding proteins. Afterwards, the phage punctures the outer cellular membrane, degrades the peptidoglycan layer and injects its DNA into host cell. New phage progeny is released approximately after 80 minutes post infection.

The combination of cryo-electron microscopy methods allowed us, to propose the mechanism of key stages of phage infection and describe it at molecular level.

GBS bacteriophage Bacteriophage isolation

vB_GBSs_1 temperate bacteriophage of *Streptococcus agalactiae*

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GBS-Group B streptococci (GBS) or *Streptococcus agalactiae* is found in 20% of healthy women as part of normal gastrointestinal and genital tract flora. They are the leading bacterial cause of meningitis and bacterial sepsis in newborns. GBS case treatment mainly performed with empiric antibiotics. The use of broad-spectrum antibiotics has certainly played a major role against bacterial infections, but increased number of antibiotic-resistant strains requires starting new insights into alternative antibacterial. Bacteriophages are often suggested as an alternative therapeutic agent against bacterial infections. Between March 2019 and March 2020 56 *Streptococcus agalactiae* bacterial strains were provided by Clinic “Curatio”.The samples were cultured following the CDC recommendations. For phage isolation, we sub-grouped bacterial strains and used different combinations for wastewater sample enrichment procedures. We isolated bacteriophage vB_GBSs_1. Electron microscopy revealed that it belongs to the *Syphoviridae* family, Phage vB_GBS_1 had a 50 nm diameter icosahedral head and a 112 nm tail. Phage vB_GBS_1 was able to productively infect 58.1% of *GBS* strains. The approximate time for 89% of phage to attach host strain was 7 min. Phage genome was sequenced and it revealed that vB_GBSs_1 contains sdDNA with 44.875kb in length and low CG content (GC percentage: 35.98%). A total 72ORFs were identified. 65ORFs has start codon as ATG (M), for 2ORF start codon is TTG (L) and only 5 ORF has GTG (V) start codon. 11ORFs from 72 were annotated as structure proteins: tail proteins, tail tape measure protein, head-tail adapter protein, head-tail connector protein,capsid protein and portal proteins;The products of the 8 ORFs belonged to the DNA metabolism replication modules; packaging module involved 1 ORF -terminase large subunit. 2 ORFs encodes host lyses genes –lysin and holin.1 lysogeny control genes were identified as integrase. Phage vB_GBSs_1 genome does not encode tRNAs.

RNA quadruplex | Transcript slippage | Single stranded RNA phage

Ribosomal protein S1 may recognize G-quadruplex as a signal for termination of the RNA synthesis by Qβ replicase

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Bacteriophage Qβ is a positive-strand RNA virus which infects *E. coli*. Its propagation depends on recruitment of host proteins that normally take part in protein synthesis. Three of them switch to RNA replication by forming viral RNA-depended RNA polymerase (Qβ replicase) together with viral-encoded β-subunit after infection. This enzyme is remarkable because it can exponentially amplify RNA in vitro at an unprecedented rate. Synthesis of GGG, complementary to the 3'-terminal CCC of the template, drives the replicase into a closed conformation, from which the growing RNA strand cannot dissociate. Termination (release of the completed RNA strand) is promoted by the ribosomal protein S1, one of the Qβ replicase subunits, as well as by its N-terminal fragment OB_{12}. We discovered that with GTP as the only substrate, Qβ replicase produces long polyG strands, which on denaturing gel electrophoresis produce a ladder with at least three clusters of bolder bands of about 15, 25 and 35 nt. Varying the GTP concentration or incubation time changes the distribution of material among the clusters, but the positions of the clusters in the gel remain preserved. This synthesis is template directed, it only occurs in the closed replicase conformation, and is prevented by incorporation of the next template encoded nucleotide; the latter indicates that it results from transcript slippage. This is the first time that slippage is demonstrated for the replicase of a positive strand RNA phage, and the first time ever that transcript slippage is found to generate products whose amount periodically changes with their size. The most intriguing observation is that protein S1 and its fragment OB_{12} promote release of the G_{15} product suggesting that they recognize it as a termination signal. In view of the known propensity of G-rich sequences to form quadruplexes, this indicates that a G quadruplex-like structure may be formed by the replicative complex at the termination step.

Yersinia phage YerA41 | DNA polymerase | jumbo phage | Modified nucleotides

The DNA polymerase of bacteriophage YerA41 replicates its T-modified DNA in a primer-independent manner

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Yersinia phage YerA41 is morphologically similar to jumbo bacteriophages. The isolated genomic material of YerA41 could not be digested by restriction enzymes, and used as a template by conventional DNA polymerases. Nucleoside analysis of the YerA41 genomic material, carried out to find out whether this was due to modified nucleotides, revealed the presence of a ca 1 kDa substitution of thymidine with apparent oligosaccharide character. We identified and purified the phage DNA polymerase (DNAP) that could replicate the YerA41 genomic DNA even without added primers. Cryo-electron microscopy (EM) was used to characterize structural details of the phage particle. The storage capacity of the 131 nm diameter head was calculated to accommodate a significantly longer genome than that of the 145,577 bp genomic DNA of YerA41 determined here. Indeed, cryo-EM revealed, in contrast to the 25 Å in other phages, spacings of 33–36 Å between shells of the genomic material inside YerA41 heads suggesting that the heavily substituted thymidine increases significantly the spacing of the DNA packaged inside the capsid. In conclusion, YerA41 appears to be an unconventional phage that packages thymidine-modified genomic DNA into its capsids along with its own DNAP that has the ability to replicate the genome.

DNA polymerase | lytic bacteriophages | Protein X-Ray Crystallography

Biochemical and structural studies of newly discovered phage DNA polymerase EfaS-271

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DNA replication is one of the most important processes occurring in cells. Although many different proteins may be involved in the regulation of DNA replication, the most important enzyme is DNA polymerase responsible for adding nucleotides to the 3'-end of a DNA strand. The literature provides information on the characteristic hand-shaped structure or the typical activity of specific polymerase families, however, with the development of technologies that enable more precise studies, rare features of these enzymes are still being discovered. During the characterization of bacteriophage vB_EfaS-271, which was selected to target *Enterococcus faecalis* infections, it was discovered that its genome contains a gene encoding a DNA polymerase, EfaS-271. EfaS-271 belongs B-family of DNA polymerases but has low sequence homology compared to other enzymes within the B-family. The gene was cloned, expressed in *E. coli*, and purified to high purity and homogeneity as judged by SDS-PAGE and dynamic light scattering measurements. Based on size exclusive chromatography the polymerase is a monomer and exhibits canonical 5'-3' polymerase and 3'-5' exonuclease activity.EfaS-271 apoenzyme was crystalized. The crystals diffracted to 2.17Å, however, solving the structure by molecular replacement (MR) was not possible. It may be due to low homology with MR models or unusual conformation of EfaS-271 polymerase.Further biochemical and structural characterization of EfaS-271 polymerase is currently under way. A better understanding of the diversity of molecular mechanisms involved in DNA synthesis may further our knowledge of bacteriophage physiology. This is crucial for introduction of phage therapy as an alternative to antibiotics in the era of antibiotic resistance.

bacteriophage UV_protection Dye

A molecular, selective sunscreen protecting bacteriophages from UV exposure.

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Bacteriophages are viruses that infect bacteria. To completion of the replication cycle usually results in the death of the host cell. Thus phages are considered natural antibacterial agents. They are specific, easy and cheap to produce, and safe to operate. Phages are used in various branches of medicine, industry, and science. However, bacteriophages are inactivated by UV light – one of the most commonly used disinfection agents. Thus combined utilization of UV and bacteriophages against bacteria was only hardly explored, because of its self-limiting character. Here, we found the solution for this issue that allows for more efficient simultaneous application of UV and bacteriophages to fight bacteria. The method allows for the combined action of viruses and ultraviolet radiation thanks to the specific azo dye that selectively shields bacteriophages from the harmful radiation, simultaneously providing antimicrobial protection by at least 99.9% of bacteria or yeasts. Finally, we show the application of the developed method to reduce the biofouling of membranes. Biofouling is a significant problem in wastewater treatment.

analysis bacteriophage gut human gut microbiome Next generation sequencing Phage infection phage sequence

The impact of storage buffer and conditions on fecal samples for bacteriophage infectivity and metavirome analysis

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Background: It has become increasingly clear that the complex bacterial community inhabiting the human gut plays a pivotal role in health and disease. More recently the viruses particularly phages were found to influence not only the gut bacterial community but also the trajectory towards health or disease. There is thus an increasing interest in investigating the human gut virome. However, relatively little is known about how conventional storage buffers and storage conditions influence the infectivity of bacteriophages and down-stream metavirome sequencing. Here, we longitudinally evaluated 5 storage buffers on the infectivity of phages T4, c2 and Phi X174 spiked into fecal samples stored at different temperatures and storage times. Further, the effect of these factors on down-stream metavirome sequencing was also evaluated.

Results: Our results showed that the infectivity and genome recovery rate of the different bacteriophages is variable and highly dependent on storage buffers. Regardless of storage temperature and timespan, all the tested phages lost their infectivities immediately in DNA/RNA Shield and >90% of the tested phages are inactivated in both StayRNA and RNAlater. Generally, SM buffer at 4°C have a good maintenance of phage infectivity up to 30 days and phage genomes up to 100 days. The metavirome sequencing indicated that preservation buffers can induce more bacterial contaminations and higher alpha-diversity compared to SM buffer, longer time storage (500 days) at – 80°C influences the viral diversity differently in the different buffers. Although the samples stored in CANVAX or DNA/RNA Shield buffer has the least shifts in metavirome composition after long time storage, they increase more contigs that cannot be assigned to a taxonomic unit (“viral dark matter”). In addition, compared to SM buffer, the preservation buffers induce more “sneaker contigs” that should be assigned to bacterial chromosome rather than viral category.

Conclusion: Fecal sample storage conditions (buffer, time, temperature) strongly influences bacteriophage infectivity and viral composition as determined by metavirome sequencing. Generally, the choice of buffer had stronger influence compared to storage temperature and time and can induce bias for viral sequencing and analysis. Based on these results, we recommend for the fecal virome study, SM buffer is suitable to study infectivity, but it is better to do virome extraction with SM buffer to increase the accuracy of viral analysis. SM buffer at 4°C for fecal virome samples that can be proceeded within 2 weeks, and CANVAX or DNA/RNA Shield for longer storage at – 80°C for metavirome studies.

bioinformatics major capsid protein bacteriophage

Predicting viral capsid architectures from metagenomes

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Most viruses protect their genome in capsids made of multiple copies of the same protein. These shells span two orders of magnitude in size and thousands of different architectures. However, the landscape and evolution of capsids across ecosystems remain poorly understood. The main challenge is obtaining empirical evidence of both the genomic and associated molecular structure of viral capsids in the environment. This talk will outline the Luque lab's approach to tackling this problem for tailed phages, the most abundant viruses on the planet. First, I will describe how to use the generalized geometrical theory of icosahedral capsids as a framework to quantify distinctive physical features in viruses from different structural lineages. Second, I will show how we combined biophysical models, bioinformatics, and statistical learning to predict the capsid architecture of tailed phages (HK97-fold structural lineage) from metagenomically assembled circular genomes and the major capsid protein sequence. Third, I will share our findings analyzing tailed phages in gut metagenomes. Our two main observations were the existence of small putative phage capsids that have never been isolated and a relatively high presence of putative jumbo phages. Finally, I will discuss how our open-source biophysical-computational approach opens a route of monitoring the ongoing evolution and selection of viral capsids across ecosystems.

virus-host protein-protein interactions receptor-binding protein

integrated pest management

Invertebrate Iridescent Virus 6 (IIV6) Open Reading Frame 118L Encodes a Functional Matrix Protein

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Invertebrate iridescent virus 6 (IIV6) is an insect virus belonging to the Iridoviridae. IIV6 is of considerable scientific interest due to its utility as a model for dsDNA viruses, and its ability to induce a type I immune response in mammalian cells and several innate immune signaling pathways in D. melanogaster cells. The genome of IIV6 contains an open reading frame (ORF 118L) that encodes a structural protein including transmembrane domain and myristylation sites. Studies with its homologues have shown that 118L protein may be a member of the cell entry-fusion complex and may have an important role in initiating virus replication. In this study, we aimed to identify the function of 118L by deleting it from the viral genome, silencing its expression with dsRNA and neutralizing virus using 118L specific antibodies. 118L ORF was replaced with the green fluorescent protein gene (gfp) in IIV-6 genome by homolog recombination. Plaque assays were performed to purify the recombinant virus. Silencing of the 118L was performed by RNA interference (RNAi technique. Progeny virus production was determined by EPDA. The target gene was expressed in baculovirus vector system and used for antibody production. The 118L specific antibodies was used for virus neutralization. However, after several repetitive plaque assays, recombinant IIV6 could not be purified from wild-type virus. This indicated that 118L encodes an essential protein for virus replication. This result was also confirmed by silencing 118L ORF using gene specific dsRNA. After silencing, the progeny virus in dsRNA transfected cells was decreased significantly (99%). Antibodies produced against 118L protein were able to neutralize the virus and inhibit virus infection. These results showed that 118L protein has an important role in virus replication and virus could not reproduce in the absence of it.

bacteriophage | biotechnology | phage display | thermophilic phage | geobacillus stearothermophilus

Construction of a novel phage display system based on thermophilic *Geobacillus stearothermophilus* phage TP84

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Phage display technology, which is based on the presentation of peptide sequences on the surface of bacteriophage virions, was developed over 30 years ago and has been already applied in the numerous fields of biotechnology and medicine. However, this method still encounters several limitations. For example, problems with the production and presentation of hydrophobic peptides on phage surfaces or the formation of aggregates by recombinant proteins remain unsolved. The possible reason is that the mesophilic bacteriophages used in the phage display systems known so far cannot deal with more challenging peptides or proteins that are difficult to fold properly. It might be assumed that the construction of a phage display system in thermophilic bacteriophages could provide a solution. Here we show the basics for the construction of phage display system based on the thermophilic *Geobacillus stearothermophilus* phage TP84 from *Siphoviridae* family. With 81 identified coding sequences in its 47.7 kb double-stranded DNA genome and quite complex, multiprotein capsid, TP84 constitutes a powerful platform for presenting peptides on virion's surface. We chose several structural proteins as candidates for display, made in-frame fusions with different tags and showed that at least some of the phage particles containing fusion proteins are viable and infective. Besides, we presented the method of *in vitro* packaging of recombinant phage genome into capsids. These results should lead us to the construction of the first phage display system using thermophilic bacteriophage, that could significantly improve the range of biotechnological and biomedical applications of this technology.

crAssphage major capsid protein Comparative genomics

Exploring the diversity of Crassvirales' structural proteins in the human gut phageome

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The human gut microbiome comprises a large diversity of microorganisms and viruses that engage in intricate relationships with each other and the human host. The majority of viral diversity in the human gut is associated with bacteria viruses (bacteriophages, or phages), collectively known as the human gut phageome. These complex communities of bacteriophages play essential roles in regulating the microbiome by killing bacteria or assisting bacteria in horizontal gene transfer between strains. The major obstacle in phageome research is that most gut phages remain uncharacterized, unclassified, and not linked to particular bacterial hosts. Recently a highly prevalent order of bacterial viruses – Crassvirales, was identified in the human gut phageome. Crassvirales (also known as crAss-like phages) were predicted to infect bacteria of the phylum Bacteroidetes and comprise tens of novel phage species, with only a few of them ever isolated in pure culture. Their exact host range, mode of interaction with host cells, and significance for gut health remain largely unknown.

Taking advantage of a recently reconstructed cryoEM atomic-resolution model of a Crassvirales virion (crAss001), we first investigated the diversity of structural proteins, particularly those responsible for host recognition, encoded by hundreds of metagenomically assembled genomes of uncultured Crassvirales. Next, we created a comprehensive network of sequence similarities across orthologous and paralogous structural proteins encoded by Crassvirales. Finally, Investigate correlations between various predicted tail architectures and predicted host range and taxonomy of individual Crassvirales clades.

siphophages' structures AlphaFold2 lactic acid bacteria

Structural diversity of lactic acid bacteria siphophages' host-binding devices revealed by AlphaFold2

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Lactic acid bacteria (LAB)-infecting siphophages use diverse host-binding devices, yet the overall picture of the interactions between LAB phages and their host remains incomplete. Unraveling the molecular details of phage-LAB interactions is essential not only for decoding phage biology, but also for industrial purposes since LAB are important micro-organisms in food fermentations. While phages infecting *Lactococcus lactis*, one of the main LAB used in dairy fermentations, have enjoyed extensive scrutiny over the last decade, there is a significant knowledge gap on the interactions between other LAB species, from various ecosystems, and their phages. How diverse LAB phages' host-binding devices are?

To address this question, we explored the structural variety of host-binding devices from seven representative phages infecting either *Streptococcus thermophilus*, the other LAB widely used in dairy plants, or *Oenococcus oeni*, the winemaking LAB. To this end, we used the powerful protein structure prediction program AlphaFold2 (AF2), which is revolutionizing structural biology. Notably, AF2 allows high-confidence structural analyses of large and flexible assemblies reluctant to experimental methods.

Our structural models show different and unprecedented architectures of host-binding devices. Interestingly, these devices are LEGO®-like assemblies built from common structural and functional domains. Particularly, carbohydrate-binding modules (CBM) are found in all phages, indicating their potential role in mediating contacts with LAB cell surface. Also, diverse combinations of CBMs highlight the likely advantages of these domains for LAB phages to bind to specific hosts. Moreover, enzymatic domains that may disrupt components of the host cell wall can be identified. Based on these models, we propose host-binding mechanisms. AF2 is likely to become a valuable tool in the field, for the structural biology of phages as well as for the annotation of their genomes.

bacteriophage | receptor-binding protein | tail fibre | tailspike protein | Endolysin

Crystallographic structures of bacteriophage receptor-binding proteins and endolysins

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Most bacteriophages recognize their host cells via specialized spike or fibre proteins. The overall goal of our group is to determine their structures in complex with their natural receptors, which may lead to different biotechnological applications, such as elimination of specific bacteria. We also study the high-resolution structures of endolysin proteins, involved in bacterial wall degradation. This research aims to understand their mechanism of action and specificity, which, in turn, may lead to produce information that could lead to recombinant lytic enzymes with improved properties.

Bacteriophages | Receptor-binding Proteins | Diagnosis

Viral proteins to improve the detection of secondary bacterial infections (SBI) associated to COVID-19

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Bacteriophages (phages) are characterized for their high specificity being able to discriminate their host up to the strain level. This feature is largely dependent on specific structural proteins encoded on the phage's genomes. These proteins recognize specific receptors on the bacterial cell surface and are known as phage receptor binding proteins (RBP). The ability to specifically recognize and bind to certain bacteria make RBP valuable biorecognition elements with high potential for the development of new diagnosis methods.

Considering the slow turnover of the conventional culture methods and the limitations of the immune and molecular assays, it is crucial and urgent the development of new diagnostic methodologies able to rapidly and accurately detect and identify the etiological agent of important bacterial infections. This is exacerbated in COVID-19 patients for which a high rate of deaths was attributed to secondary bacterial infections (SBI).

Through bioinformatics and functional analysis we identified RBP encoded in the genome of two lytic phages. These two RBP were able to specifically recognize and bind to 2 of the most important bacteria responsible for SBI associated with COVID-19: *Pseudomonas aeruginosa* and *Staphylococcus aureus*. By fusing the RBP to different fluorescent proteins we developed a method to detect and identify these bacteria in multiplex through epifluorescent microscopy and spectrofluorimetry. Fusion of the RBP to the NanoLuc luciferase improved the limit of detection 100 times when compared with the fluorescent proteins.

This new methodology was tested against more than 200 bacteria isolated from COVID-19 patients with a specificity of 100% and 90%, and a sensitivity of 44% and 96%, against *P. aeruginosa* and *S. aureus* respectively.

In conclusion, we developed here a new methodology based on viral proteins able to fast and accurately detect *P. aeruginosa* and *S. aureus* that will improve diagnosis of SBI associated with COVID-19.

Acinetobacter baumannii phage | Modified nucleotides | 1228 Da nucleoside

vB_AbaM_ISTD carries 5-HmdU and a novel “jumbo” nucleoside instead of thymidine

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Carbapenem-resistant *Acinetobacter baumannii* is designated as an urgent threat. In our previous work, we characterized its novel phage vB_AbaM_ISTD in detail. The phage DNA resists restriction enzyme digestion, and its sequencing was unsuccessful albeit several different second- and third-generation sequencing platforms, including Illumina HiSeq/MiSeq, PacBio's SMRT and Nanopore sequencing were used.

To analyze the possible modifications in the phage DNA, it was digested to single nucleosides and subjected to LC-MS analysis using XSelect column, performed on an Agilent 1290 Infinity II UHPLC system equipped with LC/MSD XT Single Quadrupole Mass Detector. With the goal of preparing the sample for chemical structure analyses, sample was also run on Phenomenex Develosil Column as its preparative scale version is available.

The chromatogram obtained with XSelect column demonstrated retention times specific for all canonical nucleosides except 2’-deoxythymidine. Instead, two peaks were obtained: one corresponding to 5-HmdU, a known small-size modification of deoxyuridine; and the other not corresponding to any known nucleoside. Mass analysis showed that this peak contained 1228 Da molecule, which would make it the largest non-canonical deoxynucleoside reported so far. When the same samples were run on other column, two non-canonical peaks could be observed in addition to 5-HmdU.

DNA of the phage vB_AbaM_ISTD is totally absent of canonical deoxythymidine, which is replaced with at least two different modified nucleosides: 5-HmdU and 1228 Da unknown nucleoside. We hypothesize that this “jumbo” nucleoside is derived by the post-replicative modification of 5-HmdU, possibly by the addition of a pentasaccharide. The emergence of second peak suggests that this peak and the 1228 Da peak may differ by a single repeating unit of moiety adducted to the nucleobase. Mass analyses of obtained peaks, as well as their purification and preparation for structural analyses are ongoing.

Bacteriophage | Klebsiella species | Phage Therapy

Characterization of bacteriophages targeting clinical isolates of *Klebsiella spp.*

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The uncontrolled use of antibiotics worldwide has led to the development and spread of drug-resistant bacteria. Nowadays, the problem is the most prominent for Gram-negative microorganisms. Bacteriophage treatment is considered to be a promising option to fight bacterial infections. A substantial number of individualized phage preparations are prepared against multiple pathogens at the Eliava Institute. Over the last years, it has become prominent, that the highest demand for individualized phage preparations is for phages against *Klebsiella* strains.

A prerequisite for fast and easy selection of the correct phage(s) for the treatment is a well-systematized collection of characterized phages suitable for therapy. For the purpose of further development and enrichment of such a collection (present at Eliava Institute), *Klebsiella* phages previously used for treatment were studied in detail. Selected phages were classified morphologically into three major groups *Myoviridae*, *Siphoviridae*, and *Podoviridae*. It is noteworthy that studied bacteriophages successfully lyse beta-lactamase-producing, genetically diverse clinical strains of *Klebsiella* species. Phages grow effectively on non-host bacteria with high EOP, which is one of the most important characteristic feature of virulent phages suitable for therapy. Phages are characterized by rapid adsorption. The latent period varies within 30-40 min for different phages.

The rate of formation of phage-resistant mutants is quite high; however, the resistance was found not to be stable as it disappears with the first cultivation of these mutants.

Based on our research, we can conclude that studied phages are virulent and that they represent good candidates for treatment options either as alternatives to antibiotics or in combination with other antibacterials.

Bacteriophage cryo-EM structural biology

Structural Elucidation and Characterization of The Bacteriophage N4

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Bacteriophages (phages) are viruses which infect bacteria and are highly host-specific. The order *Caudovirales* comprises the tailed phages which encapsidate dsDNA and represents one of the most abundant and ubiquitous entities in the biosphere. Despite their crucial role in biology, they remain incompletely understood, making them a biological dark matter. Furthermore, the rise of antibiotic resistant bacteria has caused a recent surge of interest into bacteriophage research and bacteriophage therapy. The phage N4 is characterised by an icosahedral head and a short, non-contractile tail. N4 is unique in its ability to package and inject the large ~382 kDa viral RNA polymerase (vRNAP), which renders it independent of the host's machinery for the transcription of the early phage genes. We aim to understand how does N4 inject this massive protein into the host. Here, we use cryo-electron microscopy to elucidate the complete structure of the phage capsid in its different forms during phage maturation, as well as the multi-protein tail complex necessary for the ejection of DNA in the infection process. These results will pave the way towards exchanging the recognition target of the phage, so that it can specifically bind to a bacterium of choice. Additionally, it will also help us with the modification and eventual replacement of the vRNAP with a different complex.

Bioinformatics | Function Prediction | Network Analysis

Shedding light on viral dark matter with synteny-based functional annotations

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With advancements in high-throughput DNA sequencing technology, metagenomic studies and specialised bioinformatics tools, the amount of viral genomic sequencing data is ever-expanding. As a result, almost 5,000 bacteriophage (phage) genomes and half a million phage proteins have been discovered, constituting our existing knowledge of phage biology. Functional labels can be assigned to these protein sequences using computational pipelines which compare the similarity of novel sequences to sequences with known functions. However, as two-thirds of phage proteins have no known homologs, wide gaps persist between the number of known phage sequences and known phage functions. Despite this, phages have a mosaic genome architecture, where genes are arranged according to the order which proteins must be produced to support viral replication. It has been shown that this 'gene-order' is highly predictive and conserved across phage genomes.

Here, we address some of the challenges associated with annotating viral genomes by leveraging viral genome synteny to assign functions to viral proteins. We use network analysis and machine learning techniques to predict gene functions, irrespective of sequence homology. Our predictive framework can subsequently be used to generate more robust annotations of viral genomes, predict protein functions and identify viral genomic regions where further experimental characterisation is required to inform unknown viral functions.

bacteriophage | Pseudomonas aeruginosa | Antibiotic resilience

Susceptibility of Pseudomonas aeruginosa clinical strains to newly isolated phages and its comparison with antibiotics

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P. aeruginosa is among the 12 critical pathogens in the list of bacterial infections of the multidrug resistant pathogens established by the World Health Organization (WHO). Consequently, since P.aeruginosa has a "pan-drug-resistanne" (PDR), it is a global priority to develop the alternative therapy methods and means of treatment of the associated diseases.

Bacteriophages (phages) are promising alternative antimicrobial agents. Pathogen-specific phages are perceived as effective treatment against multidrug resistant bacteria (Phage Therapy). Due to unavoidable antibiotic resistance crisis, interest and popularity towards phage therapy is gradually increasing worldwide.

The study aimed comparative analysis of antibiotic- and phage- susceptibility patterns of the *P.aeruginosa* clinical strains from Eliava IBM R&D collection.

Susceptibility of 30 *P.aeruginosa* clinical strains was studied against 13 antibiotics presenting the following groups: Penicillins, Cephalosporins, Fluoroquinolones, Aminoglycosides, and Carbapenems. The Kirby-Bauer disk diffusion method was used. Results were classified as susceptible or resistant according to the EUCAST and CLSI criteria.

Bacteriophages susceptibility screening was performed using the spot test. Fifteen *P. aeruginosa* phage clones (titer 10^7 cfu/ml, MOI=0.1) from the Eliava IBMV R&D collection have been used. Susceptibility was assessed by the degree of transparency of the lysed culture.

Results showed that all strains with excluding three cultures (PS1123; 1215354; PS119) appeared to be sensitive to at least one antibiotic. In case of phages, five strains (including PS1123; 1215354; PS119) showed resistance to all phages used, one strain (#638) showed a very weak sensitivity to one single phage, and the other 7 strains out of 10 appeared to be sensitive to at minimum 5 and at maximum 15 phages.

Bacteriophage bioinformatics Caudovirales

Characterization of *Lactococcus laudensis* prophages by whole genome sequencing

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Lactococcus laudensis is a newly described *Lactococcus* species, that has been associated with Italian raw cow's milk (Meucci et al. 2015). Profiling of a traditional Danish dairy starter (DL-starter) allowed isolation of 61 isolates dominated by *Lactococcus* and *Leuconostoc* members, amongst which 9 isolates could be identified as *Lactococcus laudensis* (ANI = 0.97), as determined by whole genome sequencing based on Oxford Nanopore GridIon technology. Prediction of prophage regions (VIBRANT) on complete bacterial chromosomes, indicated the likely presence of prophage regions in 5 isolates. Clustering analysis of the viral-associated proteins (open reading frames - ORF) revealed low similarity (50% cut-off identity) to those found in the P335 quasi species. The genomes of the 5 putative prophages were approx. 35 kbp. Alignment of the 5 prophage genomes indicated that they belonged to the same phage species.

The 5 isolates with likely prophage regions were then subjected to prophage induction with mitomycin C. Using epifluorescence microscopy, we quantified $>10^8$ viral-like particles per ml of induced lysate (VLP/ml) in isolates with positive induction. Electron microscopy images, sequencing data from induced prophages, and susceptible host-range will be presented at VoM2022.

Ref.: Meucci et al. (2015) IJSEM 65, 2091– 2096

Virus-host interaction: overcoming cell barriers

Antibiotic resilience | Stress response | phage-antibiotic synergy | Dormant Bacteria

Phage Paride exploits bacterial stress responses to kill dormant, antibiotic-tolerant cells

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Bacteriophages, the natural predators of bacteria, constitute a large reservoir of unknown biology. In their habitat, bacteria and phages mostly interact under poor nutrient conditions and are constantly exposed to a variety of stressors. Therefore, most bacteria on our planet are thought to spend most of their existence in a non-growing, dormant state. Non-growing bacteria are also found within infections and have been linked with high levels of antibiotic tolerance, the evolution of antibiotic resistance and the insurgence of chronic, relapsing infections. Given the near-infinite abundance and diversity of phages, we therefore wondered whether any phage developed the capacity to directly predate on dormant, antibiotic-tolerant bacteria. We initially confirmed that most phages fail to replicate on dormant hosts and instead enter a state of hibernation or pseudolysogeny. By developing a tailored isolation process, we obtained a new Pseudomonas aeruginosa phage named Paride with the exciting ability to directly kill dormant, antibiotic-tolerant hosts by lytic replication, causing sterilization of deep-dormant cultures in synergy with the β-lactam meropenem. Intriguingly, efficient replication of Paride on dormant hosts depends on the same bacterial stress responses that also drive antibiotic tolerance. We therefore, suggest that Paride hijacks weak spots in the dormant physiology of antibiotic-tolerant bacteria that could be exploited as Achilles' heels for the development of new treatments to prevent and combat the emergence of treatment-resistant bacterial infections.

Lactococcus Endolysin peptidoglycan modification

Cell envelope stress responses and phage infection in *Lactococcus lactis*

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Bacteriophages infecting the dairy starter *Lactococcus lactis* impose a serious burden to milk fermentations worldwide. In this work, we asked the question if the cell envelope stress response (i.e. the CES response) orchestrated by the two-component system CesSR and modifications in the peptidoglycan of *L. lactis* may impede phage predation. The cell wall is a barrier that a phage encounters twice. First, at the host recognition step and, subsequently, at the end of the lytic cycle when phage endolysins must degrade the peptidoglycan (PG) to release the phage progeny. Three endolysins with different catalytic domains (CHAP, amidase and lysozyme) from the lactococcal phages 1358, p2 and c2, respectively, were purified. Unfortunately, we were unable to observe the lytic activity of the amidase LysP2. The other two were tested in turbidity reduction assays against a bank of *L. lactis* mutants featuring an altered CES response, either lacking or overexpressing members of the CesR regulon and PG modifying enzymes. The results showed that the exolytic activity of Lys1358 with a CHAP catalytic domain and LysC2 with a lysozyme domain was compromised when tested against *L. lactis* overexpressing *spxB*, a main effector of the CesR regulon that increases the degree of PG *O*-acetylation, and against *L. lactis* Δ*ftsH*, lacking the CES responsive membrane protease FtsH. Lys1358 was also sensitive to the overexpression of *cesSR*, while LysC2 lysed quicker cells with a lower degree of PG *O*-acetylation (*oatA*) and those with a lower degree of deacetylated N-acetylglucosamine (*pgdA*). The consequences for phage infection are currently under evaluation using the phage c2, able to propagate on our *L. lactis* mutants. In this way, we hope to decipher to which extent a thwarted endolysin activity may or may not restrict phage propagation and help us to select robust dairy starters able to thrive in the presence of phages.

Antibiotic resistance | host range | Phage receptor

Repurposing bacteriophages P1 and P2 for antimicrobial delivery

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Antimicrobial resistance (AMR) is one of the world's major health concerns that need addressing. The use of bacteriophages (phages) has been investigated as an alternative to antibiotics to combat AMR. Bacteriophages are highly specific in respect to their host, which make them an attractive antibiotic alternative with minimal disruption to other commensal bacteria. We have developed a transduction system based on bacteriophages P1 and P2. To expand the application of these phage-based transduction systems, we have modified the host range of both phages P1 and P2. The host range of P1 has been expanded to a novel host such as *Salmonella enterica* serovar Typhimurium via two main routes: 1) the use of O-antigen degradation enzyme (phage P22 tailspike) as an adjuvant and 2) engineering of chimeric tail fusion. The host range of phage P2 has also been modified to recognise a novel host such as *Escherichia coli* O157:H7 via tail fibre engineering. The tail fibre of P2 has been fused with a tailspike from phage phiV10, which makes the chimeric P2 highly specific to *E. coli* O157:H7. Currently, our lab is developing both P1 and P2 phages as a CRISPR-Cas9 antimicrobial delivery vehicle to kill important gut pathogens such as *S.* Typhimurium and *E. coli* O157:H7 in a sequence-specific manner. This system allows precise elimination of pathogens and offers a promising solution to fight AMR.

depolymerase | Klebsiella pneumoniae | capsular polysaccharides

Insight into key structural points of capsule-targeting phage depolymerases specific to Klebsiella pneumoniae K63

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Phage depolymerases are enzymes capable of degrading bacterial capsules, making cells accessible for antimicrobials and the immune system. Depolymerases are a highly interesting anti-virulence tool against encapsulated K. pneumoniae (WHO priority 1 pathogen - CRITICAL). Apart from reports regarding the biological activity of depolymerases there is not much data available on structural characteristics with particular emphasis on the catalytic centre. Those are generally large proteins (>50kDa), forming trimers characterized by a modular organization with various stability features. We intended to prepare ”mini-enzymes” (truncated versions with a catalytically active domain but lower MW and more stable). Here we present two depolymerases encoded by Klebsiella-specific phages KP34 (KP34p57), and KP36 (KP36p50), both active against capsular type K63. The structural prediction done using Artificial Intelligence (AlphaFold software) allowed us to identify the crucial amino acid residues of the catalytic pocket of both enzymes, a finding which we verified experimentally by site-directed mutagenesis. Also, it allowed us to design a spectrum of truncated forms, with different stabilities and catalytic properties. We believe that our results will facilitate the identification of catalytic sites of other depolymerases in the future and allow for the selection of the appropriate truncation positions for such enzymes. These could be applied for the preparation of active mini-enzymes with preserved enzymatic functions. Project supported by National Science Center grants 2017/26/M/NZ1/00233 and 2020/38/E/NZ8/00432.

prophages | capsular polysaccharides | Klebsiella pneumoniae | Comparative genomics | depolymerase

Characterisation of prophages and their depolymerases within *Klebsiella pneumoniae*

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Bacteria of *Klebsiella pneumoniae* are known to harbour prophages in their genomes. Like in lytic phages, entry of a temperate phage into the cell often relies on a successful binding between the bacterial surface polysaccharide, like the capsule, and the phage receptor-binding protein (RBP), such as tail fibre/spike. We know from previous studies that *K. pneumoniae* exhibits a large repertoire of capsule diversity, and that the specificity of the capsule-RBP interaction is a strong determinant of the phage host range in lytic and temperate phages. However, little attention has been paid to an interplay between capsule diversity in *K. pneumoniae* and the diversity of structural proteins and RBPs in their prophages. To investigate this interplay, we analysed 391 Illumina-sequenced genomes (99 of which were hybrid-sequenced) from a collection of *K. pneumoniae* strains sampled from patients in Melbourne, Australia. K-loci were detected in genomes using Kaptive, prophage regions by a combination of VirSorter and PhiSpy, quality assessed using CheckV, and annotated with Phanotate and PHROGs. Prophage variants (PV) were defined as clusters of highly similar genomes (>90% in coverage). As expected, we found that clonal groups with a greater K-locus diversity harboured a greater number of phage variants. We detected RBPs in 64% of all prophages, with 12% of them carrying two RBPs. HHpred analysis revealed carbohydrate depolymerases in 9% of all prophages, with 68% of those hits within proteins annotated as RBPs or other tail proteins. A comparison of prophages found in distant bacterial isolates showed many dissimilar prophages sharing putative host-range determinants like tail proteins, RBP and lysis genes, with interesting examples of highly similar RBPs shared between distant prophages found in isolates with the same K-locus. Altogether, this analysis suggests that the capsule diversity might play an important role for the evolution and diversity of Klebsiella prophages.

bacteriophage | tail fiber | tailspike protein | phage tail-like bacteriocins | R2-pyocin | Shiga toxin | E. coli | Receptor binding protein | O-antigen

Synthetic phage tail-like bacteriocins targeting Shiga toxin-producing *Escherichia coli*

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Next to pandemics and wars, antibiotic resistance is a drastically increasing problem the world must prepare for. Shiga-toxin producing *Escherichia coli* (STEC), belonging to the critical priority pathogen list defined by the World Health Organization, is a severe foodborne disease with an estimated 2.2 reported cases per 100,000 population in 2019. The use of broad-spectrum antibiotics does not only disrupt the microbiome and contribute to the spread of antibiotic resistance, but in the case of this specific pathogen also induces the release of the Shiga toxin, which causes bloody diarrhea and can even lead to the life-threatening haemolytic uraemic syndrome (HUS). Phage tail-like bacteriocins (PTLBs), more specifically R2-pyocins, are antimicrobial compounds that are morphologically similar to head-less myoviruses. R2-pyocins are produced by Pseudomonas aeruginosa to protect themselves from their competitors. Here we show that the natural receptor-binding proteins of R2-pyocins, necessary to initiate contact with their host, can successfully be swapped with those of bacteriophages targeting STEC serovars, resulting in highly specific, antimicrobial compounds to combat STEC pathogens. Synthetic PTLBs rely on a simple and efficient killing mechanism, conserve the high specificity of bacteriophages, and may have a regulatory advantage since they do not multiply or carry DNA and thus, do not evolve. Therefore, synthetic PTLB cocktails may provide a valuable alternative for antibiotics, inspired by phages.

biofilm formation | Bacteriophages | E. coli | T4 phage | Continuous Bioprocesses

Bacteriophage T4 interaction with E.coli biofilms in a continuous system

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Bacterial biofilms are dynamic structures of bacteria often involved in many chronic infections due to their low susceptibility to host immune defenses and antibiotic treatments. Bacteriophages as anti-biofilm agents have already been in use for decades and are regaining interest today. Biofilms present sub-optimal environment for bacterial growth and consequently affect bacteriophage infection and growth since bacteriophage production depends on physiological state of their host. To characterize biofilm formation and to study interaction between phages and biofilm, a lytic bacteriophage T4 and bacteria *E.coli* as host were used in a custom built continuous system consisting of a mixed bioreactor and tubular bioreactor connected in series. Biofilms were formed under controlled physiological state of bacteria and exposed to several conditions including nutrient limitation where bacteriophage efficacy towards biofilm was studied. Bacteriophages successfully infected biofilm bacteria whereas bacterial lysis and biofilm eradication did not occur until fresh nutrients became available.

Phagogram phage susceptibility Phage Therapy

Disambiguating our understanding of phage sensitivity to develop a more rapid and credible tool for routine therapy

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The personalized phage therapy model has the potential to sustainably serve a large and growing population of patients suffering from untreatable microbial disease. However, for this approach to be implemented, it will be necessary for the phage community to build both a common conceptual framework for understanding phage sensitivity and a toolset with which clinical microbiologists can routinely assess it for patients.

Here we present a reference assay for understanding phage sensitivity that disambiguates the relationship between a phage and clinical isolate as reflecting either a lack of meaningful utility, a lethal activity without meaningful propagation, or an ability of the phage to substantially propagate at the expense of the isolate. This assay, which is constructed from traditional phage methods, can serve as a foundation for how we as a community understand the Ecopharmacology of phage therapy and explain it to clinicians. However, traditional phage methods cannot meet the needs of patients who require rapid, on-site, and reliable diagnosis to be credibly served at scale. Indeed, traditional methods cannot be implemented outside of distant core facilities or at required lead times and costs.

To disrupt these challenges, we present a semi-automated low-footprint multiplexed device, which can be implemented in any clinical laboratory that currently provides an antibiogram, and which provides the same disambiguated diagnostic answer within 3.5 hours. We do this with a simple kit that allows for a semi-quantitative direct detection of the phage-mediated lysis of the target bacteria in a liquid culture using an optical measurement of the ATP released using Luciferin/Luciferase. By returning scores reflecting the percentage of the culture that a low-MOI of each phage can lyse, the Inteliphage Phagogram both faithfully predicts the relationship between phage and host while allowing for the most effective phage(s) to be chosen for an individual patient.

T4 phage | Phage T7 | m13 phage

Viral transport mechanisms of DNA across the bacterial membrane

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Bacteriophages employ a wide variety of mechanisms to transport their DNA into the host cell. For Gram-negative host bacteria, two membranes as well as the periplasmic space have to be traversed by the phage genome. During this process, a damage of the plasma membrane has be avoided to assure the maintenance of the membrane potential and the host metabolic energetics during the propagation of the phage.

Myophage T4 has developed a mechanical machinery to traverse the outer membrane by perforating it using the force of its tail contraction. In the periplasm, the lysozyme activity of the gp5 tail protein locally removes the murein network such that the tail tip can reach the outer surface of the plasma membrane. Still unknown is the mechanism how the phage DNA is transported through the inner membrane of *Escherichia coli*.

Podophage T7 with its very short non-contractile tail first injects internal head (core) proteins into the periplasm that assemble into a DNA-transporting device. For this to happen, the internal head proteins have to pass through the narrow portal structure of the phage and therefore have to unfold transiently. Reaching the periplasm the core proteins have to refold and assemble into an ordered structure. Still unknown is how this reordered complex is used to transport the DNA across the inner membrane.

Filamentous phage M13 transports its single-stranded DNA when it enters the host cell but also when the progeny particles leave the host by a secretory process. The DNA movement is achieved by a phage assembly motor localized in the inner membrane using ATP to drive the secretion of the entire phage. The assembly motor simultaneously binds coat proteins to shingle the DNA as it leaves the inner membrane. In the outer membrane a huge pore structure is formed to allow the passage of the new phage particles.

To resolve the molecular details of these events is currently the focus of our research.

Klebsiella pneumoniae | receptor-binding protein engineering | synthetic phages

Development of a synthetic, VersaTile strategy for receptor-binding protein engineering of *Klebsiella pneumoniae* phages

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Klebsiella pneumoniae has been labeled as an 'ESKAPE' organism by the WHO, due to its growing multidrug resistance and virulence, urgently requiring antibiotic alternatives. *Klebsiella* phages are highly specific against their host bacterium, recognizing the capsule serotype with their receptor-binding proteins (RBPs). To overcome limited host specificity in traditional phage therapy, we elaborate a synthetic phage framework in which a diversity of receptor-binding proteins (RBPs) can be plugged in. The bivalent *Klebsiella* phage KP32 is used as a scaffold and possesses a dual RBP system with an anchor-branched structure. This branched RBP system can be divided into the following modular building blocks: a N-terminal anchor for attachment of the first RBP to the phage tail, including a T4gp10-like branching domain; a different C-terminal specificity domain with depolymerase activity for the first and second RBP, respectively, and a conserved peptide to attach the C-terminal specificity domain of the second RBP to the T4gp10-like branching domain of the first RBP. Using the VersaTile technique as a modular DNA assembly technique, a collection of modular building blocks derived from different *Klebsiella* phages (KP32, K11, KP34 and KP36) were assembled, resulting in chimeric RBP gene clusters, that were subsequently integrated into the well-characterized phage scaffold of KP32. In the future, this strategy may allow us to create customized phages, consisting of a safe and well-known phage scaffold in which specificity to the bacterial host can be reprogrammed by only changing the RBPs targeting the patient-specific bacterial pathogen.

Endolysin | Antimicrobial peptides | Gram-negative pathogens

Enzymatic activity of *Klebsiella* phage KP27 endolysin does not seem to be indispensable for its antimicrobial activity

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Introduction: Endolysins are phage-encoded peptidoglycan-degrading enzymes. Derived from phages targeting Gram-negatives are usually active against a broad range of species in the presence of outer membrane permeabilizers. Endolysin KP27gp166 derived from *Klebsiella* phage KP27, possesses peptidase activity and undergoes thermal inactivation at 110^{O}C for 30 min. Since KP27gp166 is characterized by positively charged N-terminal part with the net charge at pH 7 = 5, the goal of this project was to characterize the antibacterial potency of this enzyme in both, native and heat-inactivated (HI) form against intact and permeabilized Gram-negative pathogens including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Acinetobacter baumanii*.

Results: In kinetic experiments, the KP27gp166 in both native and HI forms, showed lytic activity against all chloroform-permeabilized bacteria at a concentration of at least 20 µg/ml. In general HI endolysin was from 10-fold to 50-fold less active than the native enzyme, depending on bacterial strains tested. Moreover, using the spot test, we demonstrated the bactericidal activity of native and HI endolysin against all four pathogens tested without any exogenous permeabilizers, wherein non-fermenting, intact representatives of Pseudomonadales are more sensitive to KP27gp166 than representatives of Enterobacteriales. The bactericidal efficiency of both forms of endolysin was accompanied by perturbation of bacterial membrane.

Conclusions: The results suggest that KP27gp166 retains its lytic potency after heat-inactivation and the antimicrobial activity of this enzyme consists of both: the hydrolytic activity and the primary structure interacting with cell surface structures.

bioinformatics machine learning receptor-binding protein

Identifying phage receptor-binding protein sequences based on protein domains and machine learning

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Phage engineering allows for the precise adaptation of phage host specificity by modifying or swapping receptor-binding proteins (RBPs) between phages. Additionally, the ever-increasing amount of publicly available data enables to study RBP sequences in new, data-driven ways. However, non-standard or missing annotation often complicates these analyses. As much as 90% of phage genes in publicly available genomes may have no annotation. Recent tools such as PhANNs partly alleviate this problem but have not focused specifically on RBPs and show that these are among the most difficult sequences to annotate. We have developed two parallel approaches to overcome the complex identification of RBP sequences. The first approach consists of a collection of RBP-related hidden Markov models (HMMs), collected from the Pfam database as well as custom HMMs developed by us. The second approach entails a machine learning model to accurately discriminate phage RBPs from other phage proteins. We show that our approaches are complementary to one another and that our machine learning approach surpasses both our HMM-based approach and PhANNs. Finally, we hypothesize that many phage RBPs remain to be discovered in complex metagenomics data, and that both our approaches could be valuable tools to discover RBPs in such settings.

Archaeoviruses Haloarchaea Haloferax

Interaction between haloarchaeal virus HFTV1 and its *Haloferax* host

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Archaeal viruses face different challenges during viral entry and egress, compared to their bacterial counterparts, as the archaeal cell envelopes and their appendages fundamentally differ from those of bacteria. For only a few archaeal viruses, the entry and egress mechanisms have been studied, so far. To investigate the main principles underlying infection strategies of euryarchaeal viruses, we use the recently isolated Haloferax tailed virus 1 (HFTV1) and its host *Haloferax sp.* LR2-5 as a model system.

To study the interaction between *Hfx. gibbonsii* LR2-5 and HFTV1, we sequenced and analysed the host genome and determined optimal conditions for host growth, motility and virus production. We further investigated host cell characteristics like the surface layer, filamentous surface structures, and cell shape that might play a role in its virus susceptibility (Tittes *et al.*, 2021; Tittes, Schwarzer and Quax, 2021).

Notably, HFTV1 is one of the first known viruses infecting a *Haloferax* strain and was isolated together with its host from saline Lake Retba (Senegal) in 2011. HFTV1 has a double-stranded DNA genome and an icosahedral capsid with a long non-contractile tail (siphovirus morphotype) (Mizuno *et al.*, 2019). The characterization of the halophilic virus isolate HFTV1 showed a lytic life cycle hampering the hosts cell growth. Analysis of its reproduction showed that the release of progeny virions resulting from cell lysis occurs after ~6 hours. Remarkably, HFTV1 virions adsorb to the host cell surface much faster than other haloarchaeal viruses. We characterized the details of the adsorption process and examined the impact of viral infection on the host cell with a combination of light and electron microscopy. This experimental analysis of host behaviour and viral life cycle contributes to the identification of infection strategies of the relatively unexplored haloarchaeal viruses.

mycobacteriophage Holin recombineering

Deciphering the physiological relevance and molecular properties of Mycobacteriophage D29 Holin

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Phage therapy is gaining attention for the treatment of pathogenic organisms such as *Mycobacterium tuberculosis* due to the emergence of drug-resistant strains. Mycobacteriophage D29 is a virulent phage that has the ability to infect and kill most mycobacterial species including *M. tuberculosis*. To ensure timely and efficient host cell lysis, D29 lytic cassette encodes three proteins *viz*. peptidoglycan hydrolase (LysA), mycolylarabinogalactan esterase (LysB), and Holin. Holin is a small membrane perforating protein, which forms non-specific holes into the cell membrane at a genetically-programmed time. Here we dissected the importance of Holin in D29 physiology by knocking it out from the phage genome (D29Δ*gp11*). We show that D29D*gp11* is viable but forms smaller plaques, and the host cell lysis is significantly delayed as compared to the wildtype D29. D29D*gp11* further shows longer latent period and reduced burst size. We thus show that while holin is dispensable for phage viability, it is essential for the optimal phage-mediated host cell lysis and phage propagation. We also show that coiled-coil (CC) domain present in the protein's C-terminal region is indispensable for an efficient Holin-mediated host cell lysis. We demonstrate that the positively charged residues present in C-terminal region are crucial for Holin-mediated toxicity. These data allowed us to engineer a highly toxic version of Holin, HolHC, by duplicating its C-terminal region. HolHC protein shows toxicity in both *Escherichia coli* and *M. smegmatis*, and causes rapid killing of both bacteria upon expression, as compared to the wild-type. A similar oligomerization property of HolHC as the wildtype Holin allows us to suggest that D29 Holin C-terminal region determines the timing, and not the extent, of oligomerization and, thereby, hole formation. Such knowledge-based engineering of mycobacteriophage Holin will help us in developing engineered phages to kill pathogenic mycobacteria.

bacteriophage bacteriophage diversity bacterial-phage inactivation

In vitro analysis of phage-bacteria interactions as a guide for the therapeutic phage cocktails formulation and dosage

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The worldwide increase of antimicrobial resistance has spurred scientists to find alternatives to antibiotics. The specificity of phages and their capability to evolve to overcome bacterial phage resistance have been identified as very attractive characteristics with respect to their use as additional tools in the fight against infections. There is an ample supply of phages in nature, where they were identified as major dynamic components of micro-biocenosis, controlling bacterial populations' growth and adaptation to changing environmental conditions. In vitro studies of the interplay between bacteria and phages will provide insights that are important for the development of more robust therapeutic phage cocktails and application protocols. In this study, 12 Pseudomonas aeruginosa and 15 Klebsiella pneumoniae new phages were isolated from five different natural and industrial sewage environments. All new phage lysates were plaque and culture purified following Good Microbiological Practices, prior to their use in the present study. The lytic activity of phages was analyzed using an OmniLog™ system, which uses redox chemistry to automatically measure cell respiration. Phages were tested separately and in different combinations against clinically relevant bacterial strains with different genetic backgrounds. The phage inoculum mixes were designed based on the combinations that were initially identified in the processed environmental samples. We observed that therapeutic phage cocktails designed based on phage compositions observed in the environment best prevent the development of immediate bacterial phage resistance and lead to a maximal bacterial control in liquid cultures. The most promising new phages and the most interesting bacterial strains were selected for whole genome sequencing, to broaden our understanding of the molecular basis of the observed phage-bacteria interactions, and to improve the formulation and dosage of therapeutic phage preparations.

Phage Therapy | Antibiotic resistance | Host specificity

To target or not the capsule – the dilemma of *Klebsiella* phages

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Background: Phage efficiency relies on their ability to recognize and bind on the bacterial surface, which depends on high specificity towards surface structures. This makes Klebsiella phages highly specific towards its most external component, the polysaccharidic capsule. However, resistance to capsule-targeting phages have been shown to evolve rapidly, leading to the exposure of other membrane structures. In this work, we devised a strategy to isolate phages that target capsular mutants. These phages may be useful in phage cocktails as they would target resistant mutants emerging during treatment with first-line phages. We also aim to characterize the phage-Klebsiella host interactions.

Methods: Based on a collection of 7,388 Klebsiella genomes, we selected six wild type Klebsiella and seven capsular mutant strains for their surface structures prevalence and diversity. Phages were isolated and genomically characterized. Phenotypic characterization including host-range, infection curves and efficiency of plating was also performed. Phage resistant Klebsiella clones were isolated and sequenced. Phage efficiency experiments in vivo were performed using OMM12 mice model.

Results: 67 phages were isolated belonging to 4 different taxonomic families. Phages that do not target the capsule have a broader host-range and resistance against them took longer to emerge in vitro. We also discovered one broad host range phage that infected virtually all different O-antigen types of Klebsiella. Resistance to these phages evolved through mutations in O-antigen or LPS synthesis genes. We could observe in vivo replication of both phage-types, targeting or not the capsule.

Conclusion: This study demonstrates the feasibility of a new strategy to isolate phages against non-capsular structures and that have a broad host-range within Klebsiella. Such phages may represent interesting additions for phage therapy cocktails.

endocytosis macrophage scavenger endothelial cells

The role of scavenger endothelial cells in bacteriophage pharmacokinetics

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The application of phages is widely considered useful for topical and localized usage, and intravenous (i.v.) administration of phages is primarily used in critically ill patients suffering widespread infections. Studies of phage pharmacokinetics and dynamics in individuals receiving phage therapy are limited, and few reports exist about the cells and mechanisms involved in phage blood clearance. Endocytosis of phages is postulated as a major process determining pharmacokinetic but has been studied mostly in cell lines, which are often very different from normal cells in the body. According to current literature, phages accumulate in liver and spleen after systemic administration. In the liver, Kupffer cells and liver sinusoidal endothelial cells (LSECs) are important scavenger cells actively engaged in the clearance of foreign antigens, including viruses. Particularly, LSECs are pivotal in the elimination of many soluble macromolecules, nanoparticles, and colloids (≤200nm) by receptor-mediated endocytosis and play a central role in liver innate immunity. Our leading hypothesis is that due to their very high endocytic capacity, many phages reaching the liver will be endocytosed by LSECs and degraded. This may constitute a vital role in elimination of phages from blood during phage treatment. We recently reported that T4 phages are efficiently endocytosed and degraded by primary rat LSECs *in vitro*. Hence, updated information about the liver interaction with bacteriophages is warranted. To this end, we aim to investigate how various i.v. administered phages interact with scavenger cells in a complete physiological system, using the mouse model. Preliminary results from our current work with bacteriophage K1F (T7-like lytic phage against *E. coli*) show elimination of 80% of circulating particles as quickly as 20 minutes after inoculation, with a concurrent high uptake of phage in liver (4x that of spleen).

Giant Viruses | Virus-host interactions | algal viruses

Genomes of two giant viruses and their capacity in the infection cycles

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Here we report the genome analysis of two giant virus isolates Haptolina ericina virus RF02 (HeV_RF02) and Prymnesium kappa virus RF02 (PkV_RF02) belong to the group of Imitervirales, the members of which share 32 core genes functions for infection initiation, virion architecture, DNA replication and transcription. The function distribution of the ORFs predicted from the two genomes shows the potential capacity in each step of the infection cycle, which is supported by transmission electron microscope images. Virions attach to the cell surface and DNA fibres are injected into the host cell by fusion of capsid inner membrane and host cell membrane. The host cell experiences membrane reorganization and compartmentation to organize the viral factory. The empty capsids are constructed followed by viral assemblage. The predicted genes related to the above process are identified from both viral genomes. The predicted proteins involved in DNA replication including helicase, primase, PCNA and RFC also suggest similarities to eukaryotic DNA replication. A series of ORFs coding for the transcriptional apparatus indicate similarities to eukaryotic transcription with elaborate regulation occurring in the cytoplasm. Only limited number of ORFs are involved in the translation indicating that the viral translation is greatly dependent on the host apparatus. Apart from the ORFs for virus proliferation, there are considerable number of ORFs participating in biomacromolecule modification, metabolism, energy support and signal transduction, which indicates a well-organized process of infection cycle. By sequence comparison with proteins packed in giant virus capsids, no hits were found for DNA packaging proteins which indicates a possible novel DNA binding family in our two viruses. The gene content of the two viruses reveals their potential ability to take over host life activities. Our results provide new insight to the different steps in the giant virus infection of algal hosts.

bacteriophage P1 bacterial cell lysis Holin

Strategies of bacteriophage P1 to optimize cell lysis process of different hosts and at different cell growth conditions

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Lytic development of bacteriophages in bacteria ends with cell lysis to release progeny phages. Optimal lysis time depends on several factors such as e.g., host cell density and bacterial growth conditions. Premature lysis results in the release of immature phages while delayed lysis leads to failure in evolution with other phages. Double-stranded DNA phages break a barrier of cell wall at their progeny release with the help of a pair of proteins. One of them, endolysin, can digest cell wall peptidoglycan. Typically it is free in the cytoplasm and requires holin which forms large nonspecific pores in a cytoplasmic membrane (CM) ensuring endolysin the access to cell wall and lysis triggering. In certain phages endolysin is equipped with the N-terminal signal-arrest release (SAR) domain and is anchored to CM in the inactive form, which is activated and released with the help pinholins - proteins that form a multidude of nanometer scale pores in CM and cause membrane depolarization. Most often phages encode either endolysin-holin pair or SAR-endolysin-pinholin pair. Wide host range bacteriophage P1 encodes SAR-endolysin Lyz and three proteins of holin properties, LydA, LydC and LydD. Lyz was shown previously to pair in cell lysis with holin LydA encoded by an operon unlinked to the *lyz* operon. Here we show by analysis of various P1 mutants that it can also pair with LydD, which is encoded by the *lyz* operon, and that LydD is a pinholin. Whether Lyz-LydA pairing or Lyz-LydD pairing predominates in cell lysis by P1 depends on cell growth conditions and on a host of P1. Moreover, while lysis of different P1 hosts is associated with Lyz-LydD pattern of morphological transitions, in the case of mutants depleted of the *lydD* gene it is host-specific. LydC protein appears to play a function of accessory holin in cell lysis of various hosts tested in this study. However, its high lethality to cells suggest that it also has a potential to pair with Lyz in cell lysis.

biofilm microfluidics bacteriophage dose-dependency phage therapy

Are more phages better? An argument for low doses

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Pseudomonas aeruginosa is a major nosocomial multidrug-resistant pathogen that is difficult to treat because it persists as biofilm. There is renewed interest in studying bacteriophages (phages) as antimicrobial agents against *P. aeruginosa* due to their antibiofilm activity. To better understand the effect of phage dose on the physiology and destruction of biofilm, we developed a microfluidics platform to model biofilm growth under hydrodynamic conditions, where shear stress promotes wider coverage, greater cohesion, and increased biomass. 3D microscopy showed that GFP-expressing pseudomonas biofilm grew with topographical heterogeneity of ridges and canyons carved by fluid flow. We found that adding phages ranging from 10^2 to 10^8 PFU/mL caused dose-dependent sporadic clearing throughout the microchannel, where the lowest dose promoted the highest level of biofilm degradation. The negative correlation between phage concentration and clearance of biofilm suggests that higher phage infection pressure “desensitizes” biofilm to lysis. Conversely, planktonic cell lysis was positively correlated with phage concentration, where the higher phage dose (10^8 PFU/mL) caused greater reduction in bacterial density. Therefore, biofilm was resilient to phage concentrations normally effective against planktonic growth. This suggests that reductions in phage pressure avoid triggering bacterial defenses through an unknown mechanism, leaving biofilms more susceptible to lytic destruction.

E.coli | Receptor binding protein | Bacteriophage isolation

Host binding of *Tequatrovirus* phages through their receptor binding protein gp38

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Escherichia coli phages belonging to the *Tequatrovirus* genus may use gp38 as a receptor binding protein (RBP) to bind to their receptor located in the bacterial outer membrane. The gp38 RBP is located at the tip of the long tail fibers and has a modular structure containing conserved glycine-rich motives and hypervariable loops. During phage binding, these hypervariable loops interact with the outer loops of the host receptor. Here, we aim to determine how the diversity of gp38 affects binding efficiency of *Tequatrovirus* phage to diverse *E. coli* strains.

We established a *Tequatrovirus* phage collection by screening piglet feces filtrates for *Tevenvirinae* phages using a PCR protocol targeting the major capsid protein of these phages. Positive samples were plated on the *E. coli* laboratory strain MG1655 and isolated plaques were purified three times. In this way, 26 new *Tequatrovirus* phages could be specifically isolated. In addition, six *E. coli* phages from our present collection were included. All phages were sequenced and *in silico* analysis allowed identification and comparison of gp38 RBPs that were categorized into three groups according to their similarity. To identify gp38's binding partner, we spotted our phages on *E. coli* mutants lacking 14 different common phage receptors and found that most of our phages use OmpA, Tsx and TonB as a receptor.

Future work will determine the binding efficiency of diverse gp38 to the host receptor fusing gp38 to luciferase and determine luminescence indicating binding of gp38 to the host. Finally, we aim to characterize the binding mechanism further using cross-link mass spectrometry.

Virus-host interaction: host defence and viral evasion mechanisms

phage resistance | Phage-host interaction | phage therapy

Mutagenesis and lateral gene transfer are involved in bacterial resistance and interference to bacteriophages

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Bacteriophages are becoming a real alternative to antibiotics for fighting against antimicrobial-resistant bacterial strains. However, aspects such as the emergence of mechanisms involved in resistance to phages or interference with their multiplicative cycle remain unclear. This work aimed to determine the emergence of these mechanisms in *Salmonella* exposed to a cocktail composed of three bacteriophages (UAB_Phi20, UAB_Phi78, and UAB_Phi87) under three scenarios: i) laboratory cultures (LAB), ii) cooked ham (FOOD), and iii) oral phage therapy in broilers (PT). In the LAB scenario, after 24 h almost all the bacteria lost their susceptibility to the three phages, however, in the FOOD scenario, only 3.2% of isolates were not susceptible to the cocktail. From broilers contaminated with *Salmonella* and treated with phages, 3.3% of isolates presented a mechanism of resistance or interference with the multiplicative cycle of some of the phages of the cocktail. This percentage was 9.7% in the untreated group.

Eleven isolates were selected and sequenced. Mutations in *rfc* and *rfaJ* genes, involved in the synthesis of LPS (receptor of phages), were identified in bacteria resistant to phage in both LAB and FOOD scenarios. LPS profiles agreed with the presence of these mutations. Regarding PT isolates, they acquired large conjugative plasmids by lateral gene transfer, which encode determinants involved in mechanisms of interference with the multiplicative cycle or adsorption of phages. According to these results, no mutations were found in LPS synthesis encoding genes. Also, a significant decrease in both the efficiency of platting (EOP) and the efficiency of centres of infection (ECOI) was observed. These data show a role for conjugative plasmids in spreading mechanisms of interference with phages through in vivo lateral gene transfer. The impact of both LPS mutations and these mechanisms in food biocontrol and oral therapy does not impair the success of phages.

bacteriophage CRISPR-Cas defence systems

Panimmunity against phages in the marine bacterium *Marinomonas mediterranea*

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The marine bacterium *Marinomonas mediterranea* MMB-1 possesses two CRISPR-Cas systems of type I-F and III-B, respectively. Cas1 in the III-B system is fused to a reverse transcriptase (RT) allowing the acquisition of spacers from RNA. The systems I-F and III-B cooperate in the defense against some podoviruses, as spacers in the I-F array targeting some of those phages could be used by both, the I-F and the III-B system. This cooperation contributes to the survival of the bacteria when phages escape the I-F system, for example, by mutation in the Protospacer Adjacent Motif (PAM). Genomic analyses of two other *M. mediterranea* strains, MMB-2 and MMB-3, revealed a different set of CRISPR-Cas systems. While the I-F system was conserved, strain MMB-2 didn't show any III-B system, and in strain MMB-3 the III-B system did not contain Cas1 fused to RT. The spacers in the three strains showed a high diversity, with only a few spacers conserved among the different *M. mediterranea* strains.

With the aim of studying the resistance to phages in the three *M. mediterranea* strains, several novel podoviruses have been isolated. They showed a very similar genomic organization with differences mainly in the number and sequence of genes in the initial region. In the strain MMB-1 the resistance to all phages was mediated by CRISPR-Cas systems. On the contrary, strains MMB-2 and MMB-3 showed resistance to some phages which was CRISPR independent. Some phage escape mutants have been isolated which are able to infect, either the strain MMB-2 or MMB-3, but not both of them. In mutants able to infect strain MMB-2 the mutations are located in early genes. These results indicate the existence of different alternative mechanisms of phage resistance in those strains. This constitutes an example of the importance of analyzing the panimmune systems in bacterial species to understand bacterial-phage interactions.

anti-viral prophages bacteriophage

Characterization of *Streptococcus thermophilus* satellite prophages

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Streptococcus thermophilus (S.t.) is a widely used lactic acid bacterium (LAB) for the manufacture of cheeses and yogurt. However, strains of this bacterial species are also sensitive to phages. If virulent phages present in heat-treated milk infect these added industrial LAB strains, the resulting "phage attack" can lead to low quality fermented products. S.t. can effectively use CRISPR-Cas systems as natural anti-phage mechanisms but phages producing anti-CRISPR proteins have been isolated. Thus, new defense strategies are needed. Previous studies have shown that satellite prophages (SP) found in Staphylococcus aureus are able to significantly reduce the replication of specific virulent phages. We noted that structurally similar SPs are present in S.t. Thus, the aim of this project was to characterize SP in S.t. to increase our toolbox of anti-phage mechanisms.

The first objective was to characterize the diversity of SPs in S.t. and identify probable phage resistance mechanisms. Using bioinformatic tools, some SPs of S.t. were found to carry restriction-modification systems and potential abortive infection (Abi) systems. Interestingly, we showed that an Abi system found in several SPs of S.t. provided resistance against some virulent phages infecting Lactococcus lactis strains, another LAB widely used for the manufacture of cheeses.

The second objective was to characterize the SP of the reference strain S.t. SMQ-301. We observed that this SP could be excised from the bacterial genome seemingly without induction as it could be found in an overnight culture using PCR with primers specific to a circularized SP. In addition, the presence of this SP was detected by PCR in lysates of the virulent phage 73 treated with DNase, indicating that phage 73 can package this SP when infecting S.t. SMQ-301.

Taken altogether, this study provides the first glimpse of new tools that could be developed for LAB, including the development of phage-resistant strains.

antibiotics | aminoglycoside | defence systems | Streptomyces

Molecular multitasking: inhibition of phage infection by aminoglycoside antibiotics

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In response to viral predation, bacteria have evolved a wide range of defense mechanisms, which rely mostly on proteins acting at the cellular level. Here, we show that aminoglycosides, a well-known class of antibiotics, are potent inhibitors of phage infection. We observed a broad phage inhibition by aminoglycosides, as diverse phages infecting the model Gram-negative bacterium *E. coli* as well as the Gram-positive bacteria *Corynebacterium glutamicum* and *Streptomyces venezuelae* were impacted. We demonstrate that aminoglycosides do not prevent the injection of phage DNA into bacterial cells but instead block an early step of the viral life cycle, prior to genome replication. Importantly, inhibition of a *Streptomyces* phage could be reproduced using the supernatants from a natural aminoglycoside producer, hinting at a broad physiological significance of the antiviral properties of aminoglycosides. Interestingly, acetylation of the aminoglycoside antibiotic apramycin abolishes its antibacterial effect, but maintains its antiviral properties, suggesting a potential decoupling between these two key features. Altogether, this study expands the potential functions of aminoglycosides in bacterial communities. It further suggests that aminoglycosides are not only used by their producers as toxic molecules for their competitors but could also serve as a protection against the threat of phage predation at the community level.

gingipains | Porphyromonas gingivalis | Defense mechanism | anti-phage

Gingipains of Poryrphyromonas gingivalis: do they also act as a defense mechanism against phages?

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Poryrphyromonas gingivalis is a keystone pathogen in the etiology of Periodontitis. Among its variety of virulence factors, its gingipains are significantly harmful. These secreted proteases provide nutrients for the bacterium by breaking down the host's tissue, enhancing biofilm creation, and disabling innate immune system components. We hypothesize that the gingipains might have an additional role as a defense mechanism against bacteriophages.

We observed that different strains of P. gingivalis reduced the PFU/mL of the Staphylococcus aureus phages, OMS1 and OMS2, by eight orders of magnitude when cultured together. Furthermore, the supernatant of these bacteria maintained this ability. We found that the neutralizing effector is >100kD, sensitive to heat and proteases but not to DNAase or RNAase. These characterizations fit the gingipains features. Moreover, this effector was inhibited by a general inhibitor of proteases, isn't produced by gingipains-null mutants and electron microscopy shows that the bacterium's outer membrane vesicles are explicitly attached to the spikes of the phages. These results strongly support the notion that the anti-phage effector in the supernatant of P. gingivalis is the gingipains. Currently we are finalizing the characterization of the effector, testing its effect on a variety of phages.

This work adds another layer of knowledge on the growing data of anti-phage mechanisms of bacteria. We speculate that P. gingivalis is not the only bacteria utilizing secreted proteases as a defense.

anti-phage | defence systems | Escherichia coli | Thoeris

Interrogating the Thoeris novel anti-phage defense system in its native bacterial host

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Bacteria and phages have been locked in a perpetual arms race, driving the evolution of a diverse arsenal of prokaryotic immune systems and viral countermeasures. Recent advances have expanded this arsenal to include a wide variety of previously unknown and functional diverse set of bacterial anti-phage defense systems. One of those systems, called Thoeris, is characterized by an NADase domain of its effector protein ThsA. Additionally, it encompasses one or more ThsB proteins containing Toll/interleukin-1 receptor (TIR) or TIR-like domains, which are also associated with eukaryotic innate immunity. It has recently been hypothesized to function as an abortive infection system, inducing NAD depletion leading to cell death. We investigate a Theoris system encoded in *Escherichia coli* purported to belong to a distinct subtype based on domain architecture. This *E. coli* strain carries six different defence systems in total, allowing us to investigate the role of Thoeris as part of the whole anti-phage arsenal. We identified Thoeris-mediated protection against different phages and found differences between Thoeris in its native context and when heterologously expressed in a different *E. coli* strain. By applying a cell-free transcription-translation (TXTL) system, we investigated the effector domain architecture and infection induced NADase activity. The observed NADase kinetics do not support abortive infection through NAD depletion, further promoting the hypothesis of a distinct subtype. Our goal is to investigate the role of Thoeris as part of the anti-viral repertoire in its native host, the interplay with other existing defense mechanisms, as well as modes of escape deployed by phages to circumvent the system. By investigating the system in its native host, we hope to broaden the understanding of antiviral immunity beyond any single defense system.

phage biofilm formation Antibiotic resistance

Imaging process of phage infection of *Staphylococcus aureus* biofilm by light sheet fluorescence microscopy

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Staphylococcus aureus is a major human pathogen causing wide-range of diseases including infections acquired in hospitals. The persistence of *S. aureus* infections is related to its ability to form biofilms. Bacteria in biofilm are more resistant to antibiotics and to the host immune system. Extracellular matrix components play an important role in unique lifestyle and virulence of biofilms. Phage therapy is alternative approach to treat infections caused by antibiotic-resistant bacteria.

We used light-sheet fluorescent microscope with an integrated microfluidic system to study the formation of *S. aureus* biofilm and its infection by phages. To visualize the biofilm-forming cells, we modified *S. aureus* to stably express red fluorescent protein mCherry. The main components of biofilm matrix, such as extracellular DNA and polysaccharide intercellular adhesins, were labelled by specific fluorescent dyes. We introduced different phages into the mature biofilm and used time-lapse monitoring to detect their effect on biofilm disintegration.

Light-sheet fluorescent microscope with microfluidic system and time-lapse monitoring enabled us to detect the distinct stages of biofilm formation and the impact of phage infection on the biofilm development.

Pseudomonas aeruginosa | phage-host ineractions | phage resistance

Phenotypic consequences of large genomic deletions induced by infection with lytic phages in *Pseudomonas aeruginosa*

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The common outcome of phage invasion is bacterial mutations in genes encoding phage adhesion receptors, located on the surface of bacterial cells. However, the extent of the mutational changes also implicates genes not directly involved in the expression of surface receptors. Our previous work, based on whole-genome sequencing analysis of phage-resistant *P. aeruginosa* PAO1 mutants, revealed that phage infection led to mutational changes both in genes related to external receptors such as lipopolysaccharide (LPS), Type-4 pili (T4P), exopolysaccharide (EPS), and flagella, as well as in global regulatory genes and other genes of unknown function. The appearance of large genomic deletions (20 to 500 kbp) was also relatively frequent especially when bacteria were contacted with two (or more) phages simultaneously.

Here, we present the analyses of selected phage-resistant PAO1 mutants with large genomic deletions, which affected several phenotypes including pathogenicity. The common feature of these deletions is the removal of three important genes: *galU*, *mexXY* and *hmgA*. Lack of *galU* and *mexXY* resulted in impaired production of LPS O-antigen and increased susceptibility to several antibiotics, respectively. The loss of *hmgA* is responsible for the accumulation of homogentisic acid (intermediate product of the pheomelanin synthesis process) resulting in a characteristic brown phenotype. This may be beneficial to the bacterial population, increasing its resistance to pyocins and oxidative stress. The presence of deletion was next accompanied by the acquisition of complement-sensitive phenotype and induction of proinflammatory cytokines. The formation of such large deletions is associated with the MutL protein, which functions in DNA mismatch repair processes. Complementing the previously published results of basic phenotypic parameters, we decided to thoroughly analyse the metabolic capabilities of strains with large deletions, using OmniLog Biolog technology.

anti-crispr protein | Antibiotic | phage-host ineractions | CRISPR-Cas

Translation inhibitor antibiotics favour anti-CRISPR phage infection despite decreasing anti-CRISPR proteins efficiency

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Pathogenic bacterial infections can be treated by bacteriophage (phage) therapy, sometimes in combination with antibiotics. However, bacteria have a wide range of defence mechanisms against phage, among which is CRISPR-Cas. CRISPR-Cas system relies on storage of phage genetic material from previous failed infection which is then used to guide sequence-specific cleavage of phage genetic material in subsequent infections. On the other hand, phages have evolved counter-defence mechanisms, such as anti-CRISPR (Acr) proteins. These proteins are expressed in the early stages of the infection and can inhibit one or several steps of the CRISPR-Cas mechanisms to allow the phage to replicate.

Previous results in *Pseudomonas aeruginosa* have shown that sub-inhibitory doses of bacteriostatic antibiotics (which inhibit cell growth without killing) slow down DMS3 phage development. This leaves more time for bacteria to acquire CRISPR-Cas based immunity against DMS3, overall favouring bacterial in their competition against phages.

Since Acr-phage infection success depends on expression of Acr proteins, we hypothesized that translation inhibitor antibiotics could disadvantage Acr-positive phages when infecting CRISPR immune bacteria. Here, we show that sub-inhibitory doses of bacteriostatic translation inhibitor antibiotics decrease both Acr and CRISPR-Cas efficiency. However, on a population level, both bacteriostatic and non-bacteriostatic translation inhibitors antibiotics favour phage amplification in *P. aeruginosa* carrying a functional CRISPR system. This suggests that inhibition of CRISPR-Cas system has more impact than inhibition of Acr, thus shifting the phage-host relationship in favour of the phage. These results highlight a potential role for translation inhibitors when selecting antibiotics for combined phage-antibiotics therapy.

DNA modification | Deazaguanines | defense system

Deazaguanine and how to protect your DNA from restriction enzymes

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In the continuous war for survival between bacteriophages and their bacterial hosts, both sides engineer new weapons; one to degrade the invader nucleic acids, the other to resist such aggression. To escape restriction enzymes, one of the most common bacterial defense systems, double-stranded DNA phages modify their genomic DNA with complex modifications. Recently, we showed that phages insert 7-deazaguanine derivatives in their DNA. These modifications were previously thought to occur only in RNA. We have identified so far fourteen families of phage encoded proteins that take part in the synthesis of eight unique deazaguanine DNA modifications. Modification level varies from 0.1 % to 100 % of the guanines. Each of these modifications confers resistance to restriction enzymes that have at least one guanine in their recognition site, with levels of resistance ranging from partial to total. A transglycosidase that we named DpdA (for deazapurine in DNA), is the signature enzyme responsible for the insertion of the deazaguanine derivatives in phage DNA. A combination of experimental and bioinformatic analyses show that the DpdA can be split in five subgroups (DpdA1 to DpdA5), that seem to have specific substrates and rates of insertion. We showed that the expression of DpdA proteins is sufficient to insert deazaguanine derivatives in DNA when the correct deazaguanine substrate is present. After accurately predicting and validating the pathways for four of these modifications, we are now characterizing the synthesis pathway for the last four. So far, we successfully validated the function of a new methylase that further modifies $preQ_1$ inserted in DNA. We also identified two other enzymes with novel chemistries that remain to be validated: $preQ_1$ formylase and 7-carboxy-7-deazaguanine decarboxylase.

Giant Viruses | host-defence | virophage

Giant-virus induced reactivation of endogenous virophages from wild flagellate population

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Recent studies have uncovered various endogenous viral elements (EVEs) in eukaryotic genomes. Most of these EVEs represent viral fossils; however, some of them are still active. The marine heterotrophic flagellate Cafeteria burkhardae contains several EVE types related to the virophage mavirus, a dsDNA virus that parasitizes the lytic giant virus CroV. Here we show that mavirus-like EVEs, which are silent under normal conditions, produce infectious virus particles in response to CroV infection. Interestingly, only one type of mavirus-like EVEs responded to CroV infection, implying that other EVE types may be specific for different strains of giant viruses.

We characterized ten different reactivated virophages by electron microscopy, comparative genomics, and infection experiments. All these virophages replicated and inhibited the production of CroV during co-infection, thereby preventing lysis of the infected host cultures in a dose-dependent manner.

This is the first report showing reactivation of naturally occurring virophage EVEs after giant virus infection, and providing a striking example of EVEs that become active under specific conditions. Moreover, our results support the hypothesis that virophages can act as an adaptive antiviral defense system in marine protists.

S. aureus | Effectiveness of commercial phage cocktails | human serum

The efficacy of bacteriophages against clinical *Staphylococcus aureus* strains under physiological relevant conditions

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Antibiotic resistance is often referred to as a silent pandemic and has been predicted that in 2050 drug-resistant bacteria will cause 10 million deaths annually. Due to high prevalence of antibiotic resistance of *Staphylococcus aureus* (*S. aureus*), the World Health Organization marked it as a worldwide threat in 2014. An alternative for antibiotics could be the use of bacteriophages (or phages). However, before these can be widely used in the clinic there is the need to evaluate their efficacy on clinically relevant strains and conditions. Here, we analyze the efficacy of three commercial phage cocktails on ten genetically diverse and antibiotic resistant *S. aureus* strains. Three different assays to evaluate phage efficacy are compared; the standard spot test and optical density (OD) assay in bacterial media, which are often used for this purpose. In addition, by using microcalorimetry, we determined bacterial lysis in 80% human serum after exposure to the three phage cocktails, as well as single *S. aureus* specific phages obtained from one of the cocktails. In both the spot test and the OD assay all three cocktails were able to lyse several clinical strains. However, whereas the spot test didn't show a major difference in phage susceptibility between methicillin susceptible *S. aureus* (MSSA) and resistant *S. aureus* (MRSA) strains, the OD assay indicated less MRSA strains to be susceptible to the cocktails. In addition, microcalorimetry showed significantly reduced phage efficacy in high concentrations of human serum in comparison to the other applied assays. Discrepancies between these assays, especially the declined efficacy of phages in human serum, emphasizes the need to assess phage susceptibility under physiological relevant conditions to prevent overestimation of efficiency of phages screened for phage therapy.

RNA virosphere | Phage Lytic Proteins | host range | Meta-transcriptomics

Metatranscriptome mining uncovers multiple clades of RNA bacteriophages

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High-throughput RNA sequencing offers unprecedented opportunities to explore the Earth RNA virome. By mining over 5,000 metatranscriptomic assemblies, spanning diverse habitats (soil, fresh water, oceanic, sediment etc), we were able to recover >2.5 million RNA viral contigs. Phylogenetic analysis of the only conserved protein in all RNA viruses, the RNA-dependent RNA polymerases (RdRP), revealed that this expansion corresponds to a five-fold increase in diversity, including two putative novel phyla, and numerous novel classes and orders. Identification of CRISPR spacer matches and genes encoding bacteriolytic proteins, along with conservation of Shine Dalgarno like sequences, suggests that subsets of picobirnaviruses and partitiviruses, as well as one of the novel phyla, likely infect bacterial hosts. Coupled with the vast expansion of the Leviviricetes class (consisting of bona fide bacteriophages), phages now roughly account for a fifth of the global RNA virome diversity. These findings represents a shift in the understanding of host adaptations, suggesting the domain barrier may be more surmountable than previously believed.

biofilm formation | experimental evolution | Bacteriophage isolation

The importance of interspecies interactions and spatial organization in multispecies biofilms during phage predation

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The eco-evolutionary advancement of bacteria is greatly influenced by the presence of their natural predators, bacteriophages. Although bacteria mainly reside in biofilms, most bacteria-phage interactions are studied in planktonic cultures. Thus, there is a gap in how well we understand the dynamics of bacteria and phages in several natural settings. Further, the biofilm-phage studies that have been conducted have mainly been done so in mono-species biofilms.

In this project we have modified the genome of different robust biofilm formers, so they express different fluorescent proteins. Further we have isolated and characterized novel phages, targeting these biofilm hosts. This catalogue of bacteria and phages enables us to study the importance of temporospatial arrangements of bacteria in different combinations during phage exposure. We hypothesize that non-susceptible bacteria can partly shield susceptible cells, and that spatial organization is of great importance for the success of the viral infection. Currently, we characterize how cells distribute and affect one another in a biofilm depending on priority effects, and how this impact phages ability to penetrate the matrix.

With this unique model system, we will hopefully increase our knowledge on phage-bacteria dynamics in nature and perhaps impact how phages are applied in respect to bacterial biofilms.

Streptomyces | Development | Microscopy | Phage Tolerance

Streptomyces venezuelae development is crucial for efficient containment of viral infections

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Bacteria of the genus *Streptomyces* undergo a complex life cycle starting from single spores developing into a branched mycelium that is followed by the formation of aerial hyphae and maturation of spores under unfavorable conditions. As all free-living bacteria, *Streptomyces* are under the constant threat of infection by bacteriophages. In this study, we focused on investigating the impact of phage infection on cellular development using the model species *Streptomyces venezuelae*. Genome analysis showed that WhiB-like proteins represent the most abundant transcriptional regulator in phages infecting Actinobacteria (actinobacteriophages). In addition to 31 % of phages infecting *Streptomyces* harboring *whiB*, further developmentally relevant genes like *parB*, *ftsK* and *ssgA* were found to be encoded. These findings suggest that development is playing an important role during phage infection. Stereomicroscopic investigation and fluorescence microscopy of *S. venezuelae* infection by phage Alderaan revealed enhanced mycelial differentiation at the infection interface. During plaque formation, we first observed an enlargement of the lysis zone on wild type lawns, which was followed by a significant decrease upon progressing infection. This shrinkage is based on the development of a phage-tolerant mycelium enabling re-growth into the plaque area. In contrast, for a *bldN* mutant strain, which is defective in cellular development and features vegetative growth only, we did not observe resumed growth in the lysis zone. The comparative analysis of *S. venezuelae* WT and mutant strains deficient in key developmental genes *bldD*, *bldN* and *whiB* revealed the importance of cellular differentiation for the establishment of a phage tolerance phenotype in *Streptomyces*.

Pseudomonas aeruginosa | bacteriophage | phage susceptibility

Screening 505 clinical strains of *Pseudomonas aeruginosa* to Bacteriophages and antibiotics sensitivity

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Introduction*: P.aeruginosa* (PA) is one of the top bacteria in the CDC's 2019 list of antibiotic-resistant bacteria. Often *PA* becomes highly resistant to antibiotics, defined as Multi Drug Resistant (MDR) or Extensively drug-resistant (XDR). Currently the treatment options of MDR and XDR PA strains are very limited.

One of the promising solutions to achieve successful treatment of such resistant bacteria is the use of bacteriophages as part of the therapy.

The aim of this study was to assess the phage, antibiotic susceptibility and phenotypic and genotypic profile of a collection of 505 MDR and XDR *PA* clinical strains collected for decades in the Hadassah Medical Center.

Methods: Bacterial susceptibility to antibiotics was performed using either disc diffusion method or Vitek2. Phages susceptibility was tested using 28 phages from the Israeli Phage Bank (IPB), using agar plates, and kinetics measurements in liquid cultures.

Results: Out of We succeeded to achieve a coverage of 70% the 505 XDR and MDR PA strains with a minimum of 10 phages out of the 28 tested. We found significant differences in the efficacy of the phages depending on the susceptibility testing method (solid agar vs liquid conditions). Currently we are sequencing the al PA isolates aiming to find putative correlation between genomic markers and the phage susceptibility. In addition, we are screening additional phages that will be able to cover the remaining 30% PA isolates.

Conclusions: In this work we demonstrate that large proportion of MDR and XDR PA isolates can be susceptible to limited number of phages.

Phage-host interaction Eggerthella phase variation

The existence of a phase variation mechanism of *Eggerthella lenta* affecting susceptibility to phage infection

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The human gastrointestinal tract hosts a wide variety of microorganisms (bacteria, archaea, yeasts, protists and viruses), with the number of bacteria residing in the human gut estimated to be 10^{11} CFU/g of feces. These bacteria are understood to play an essential role in the metabolism of nutrients and xenobiotics. *Eggerthella lenta* has been found in 81.6% of tested individuals and is implicated in the metabolism of a vast array of substances, both medicinal and dietary. Bacteriophages (phages) are viruses that specifically infect bacteria. Their numbers are estimated to be equal to or less than that of their bacterial hosts in the human gut and are understood to influence the gut metabolome due to phage predation. We have been investigating the interaction between phages and *E. lenta* to determine the role phage infection may play in the colonisation of this bacterium in the human gut. Phase variation of human gut bacteria is increasingly understood to be an important mechanism by which host bacteria evade their phages, often by causing reversible changes to phage receptors on the host cell surface. We have identified the existence of a phase variation mechanism that potentially enables *E. lenta* DSM2243 to switch between phenotypes of sensitivity and insensitivity to infection by *Eggerthella* phage LE1-1. The examination of phase variants shows the presence of large genomic inversions with junction points situated within gene clusters implicated in exopolysaccharide biosynthesis (or possibly capsular polysaccharides). One of these gene clusters possesses a transposase with its target site located within sugar transferase genes situated in both genes clusters and implicated in the observed genomic structural changes. We are currently investigating these genomic inversions and their influence on phage LE1-1 infection.

Bacterial immunity | Phage-encoded defence systems | Phage-host interactions | Temperate bacteriophage | Toxin-antitoxin system

"It's a trap!": three novel prophage-encoded immunity systems defend *Escherichia coli* against phage attack

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Ubiquitous predation by bacteriophages forces bacteria to continuously update their repertoire of antiviral defences. While most phages are virulent and always kill their host for viral replication, temperate phages integrate into the host genome as prophages to form a transient mutualistic symbiosis known as lysogeny. Temperate phages often encode powerful bacterial immunity systems since their spread and survival is bound to the fate of their lysogenic host. Despite the abundance of prophages in pathogenic and ecologically important bacteria, we are just beginning to understand the diversity of anti-phage immunity systems and their roles as obstacles to phage therapy or as a largely untapped reservoir of biotechnological potential.

We, therefore, studied bacterial immunity provided by a newly isolated P2-like temperate phage named Likho that infects *Escherichia coli*. Intriguingly, Likho encodes three novel bacterial immunity systems each comprising one or more proteins with domains of unknown function. Using the BASEL phage collection (a set of ca. 80 phages representing *E. coli* phage diversity, we found that each immunity system defends bacteria against a different range of phages by inducing abortive infection. In each case, viral escape mutants enabled us to identify specific and distinct phage factors that trigger the immunity system and, consequently, bacterial cell death. Interestingly, one of the three defence systems is a new three-component toxin-antitoxin system whose toxin is liberated upon phage infection and inhibits bacterial translation to impair phage replication. We are currently unravelling the molecular mechanisms underlying antiviral immunity provided by these phage-encoded bacterial immunity systems. Our work highlights the abundance, diversity, and potency of prophage-encoded bacterial immunity systems as obstacles for phage therapy as well as their unusual molecular biology that might inspire applications in biotechnology.

phage resistance | Phage resistant mutants | E. coli | phage-pathogen interactions | phage sequence

Isolation and characterization of *Escherichia coli* phage resistant mutants for successful therapeutic phage selection

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Escherichia coli, a typical pathogen, is mostly associated with urinary tract and gastrointestinal infections. Because recurrence rate of E. coli infections is getting higher due to antibiotic resistance, phage therapy is considered as potential treatment. E. coli phages belonging to the genus Kagunavirus, subfamily Guernseyvirinae, are still less characterized than many other phage groups, yet they are considered suitable for phage therapy. To learn more about the suitability of Kagunaviruses to phage therapy, we wanted to study the mechanisms of bacterial resistance to these phages and the characteristics of Kagunavirus-resistant bacterial mutants. E.coli strains #5506 and #5521 isolated from human blood were used as hosts to isolate six phages belonging to Kagunaviruses, vB_EcoS_fKuEco01, vB_EcoS_fTaEco01, vB_EcoS_fTaEco03, vB_EcoS_fFiEco02, vB_EcoS_fFiEco03, and vB_EcoS_fPoEco01. Altogether 13 E. coli mutants resistant to these phages were isolated, their genomes were sequenced, and the growth kinetics and serum resistance of the mutants were analyzed. Two of the mutants grew clearly slower than the parental strains #5506 and #5521 even in rich culture medium, and all the mutants were more sensitive to serum than the parental strains. The phage DNA had not been inserted into the bacterial chromosome in any of the phage-resistant mutants, indicating a lytic life cycle of the phages. To conclude, the Kagunavirus -resistant bacterial mutants showed clearly attenuated growth in the presence of human serum, which indicates weaker pathogenicity. We are currently analyzing the sequence data of the mutants to understand the phage resistance mechanisms. We hope that this study will provide us insights to the phage-pathogen interactions, which later impacts to the selection of the phages for personalized phage cocktails for the patients.

bacteriophage | defence systems | host-defence | Phage Satellites

P2-like phages and their P4 satellites encode hotspots of antiviral systems

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Bacteria carry diverse genetic systems to defend against viral infection, some of which are found within prophages where they inhibit competing viruses. Phage satellites pose additional pressures on phages by hijacking key viral elements to their own benefit. Here, we show that E. coli P2-like phages and their parasitic P4-like satellites carry hotspots of genetic variation containing reservoirs of anti-phage systems. We validate the activity of diverse systems and describe PARIS, an abortive infection system triggered by a phage-encoded anti-restriction protein. Antiviral hotspots participate in inter-viral competition and shape dynamics between the bacterial host, P2-like phages and P4-like satellites. Notably, the anti-phage activity of satellites can benefit the helper phage during competition with virulent phages, turning a parasitic relationship into a mutualistic one. Anti-phage hotspots are present across distant species and constitute a substantial source of systems that participate in competition between mobile genetic elements.

prophage macrophage lateral transduction polylysogeny active lysogeny

Prophage taming by the adherent-invasive *Escherichia coli* LF82 upon macrophage infection

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Adherent-invasive *Escherichia coli* (AIEC) strains are frequently recovered from stools of patients with dysbiotic microbiota. They have remarkable properties of adherence to the intestinal epithelium, and survive better than other *E. coli* in macrophages. The best studied of these AIEC is probably strain LF82, which was isolated from a Crohn's disease patient. This strain contains five complete prophages, which have not been studied until now. We undertook their analysis, both *in vitro* and inside macrophages, and show that all of them form virions. The Gally prophage is by far the most active, generating spontaneously over 10^8 viral particles per mL of culture supernatants *in vitro*, more than 100-fold higher than the other phages. Gally is over-induced after a genotoxic stress generated by ciprofloxacin and trimethoprim. However, upon macrophage infection, Gally virion production is decreased by more than 20-fold, and the transcription profile of the prophage indicates that part of the structural module is specifically repressed while the replication module is overexpressed compared to unstressed culture conditions. We conclude that strain LF82 has evolved an efficient way to "tame" its most active prophage upon macrophage infection, which may participate to its good survival in macrophages. The results are discussed in light of the active lysogeny process.

evolution | Antiviral defense | Comparative immunology

Evolution and conservation of antiviral mechanisms between Prokaryotes and Eukaryotes

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Viruses are a constant threat to both prokaryotic and eukaryotic cells. The arms race between cells and viruses has led to innovation and diversification of anti-viral mechanisms. A consequence of this evolutionary trend is that phylogenetically distant organisms across Kingdoms encode different and specific antiviral mechanisms For example restriction-modification and CRISPR-Cas systems are only present in Prokaryotes. Recent discoveries in the field of anti-phage defense challenged this view, as it was uncovered that multiple eukarytic antiviral systems have prokaryotic counterparts including TIR domains, cGAS, Viperins and gasdermins. Here, we trace the evolutionary history of the eukaryotic anti-viral systems inherited from Prokaryotes by employing bioinformatics analysis combined with experimental methods. We characterize the antiviral arsenal across eukaryotic proteomes and demonstrate that specific anti-viral systems have been transferred from Prokaryotes to Eukaryotes during or after the emergence of the Eukaryotes. Focusing on the evolutionary history of Viperins, we explore molecular transitions marking the emergence of enzymatic specificities of the eukaryotic versions of such system. Finally, we extend our observations from Viperins to other antiviral systems allowing us to propose a scenario for the evolution of prokaryotic anti-viral systems to eukaryotic ones. Our results contribute to our understanding of the emergence of the eukaryotic antiviral immune system.

prophage | antiphage defense system | Human Gut Microbiota

Prophages and antiphage defense systems in genomes of gut commensal enterobacteriaceae

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Millions of years of co-evolution gave rise to a delicately balanced yet intricately diverse and complex microbial community that inhabits the gastrointestinal tract of animals, collectively known as the gut microbiota. This collection of microorganisms is dominated by bacteria and viruses, with bacteriophages (phages) composing the lion's share of the viral component of the microbiota (the virome). Our research focuses on bacterial- phage interactions in the gut.

We isolated murine commensal Enterobacteriaceae strains from faeces of healthy adult animals, and analysed them using 16s rRNA sequencing. We found these strains to belong to four major genuses of Enterobacteriaceae commonly found in the large intestine: Escherichia coli, Klebsiella, Enterobacter, and Proteus. We then conducted whole genome sequencing on selected isolates and analysed the genomes for the presence of prophage. We found each of the isolates to contain several prophages, partial phages and putative phages. Exposure of live cultures to a DNA-damaging agent resulted in a significant increase in the ratio of phage to bacteria DNA copies, indicating prophage induction and production of phage DNA.

Additionally, we found that the commensal gut strains encode various antiphage defense systems including restriction modification systems, CRISPR-cas and AVAST.

Our data suggest a high prevalence of active prophage and antiphage defense systems in the genomes of commensal bacteria. Thus, we propose that phages and antiphage defense systems contribute to colonisation of commensal gut bacteria ergo possibly contributing to shaping the composition of the endogenous gut-associated microbial community.

defence systems | restriction-modification systems | epigenetics

A mobile restriction–modification phage defence system resolves an epigenetic conflict with an antagonistic endonuclease

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Epigenetic DNA methylation plays an important role in bacteria by influencing gene expression and allowing discrimination between self-DNA and intruders such as phages and plasmids. Restriction–modification (RM) systems use a methyltransferase (MTase) to modify a specific sequence motif, thus protecting host DNA from cleavage by a cognate restriction endonuclease (REase) while leaving invading DNA vulnerable. Other REases occur solitarily and cleave methylated DNA. REases and RM systems are frequently mobile, influencing horizontal gene transfer by altering the compatibility of the host for foreign DNA uptake. However, whether mobile defence systems affect pre-existing host defences remains obscure. Here, we reveal an epigenetic conflict between an RM system (PcaRCI) and a methylation-dependent REase (PcaRCII) in the plant pathogen *Pectobacterium carotovorum* RC5297. The PcaRCI RM system provides potent protection against unmethylated plasmids and phages, but its methylation motif is targeted by the methylation-dependent PcaRCII. This potentially lethal co-existence is enabled through epigenetic silencing of the PcaRCII-encoding gene via promoter methylation by the PcaRCI MTase. Comparative genome analyses suggest that the PcaRCII-encoding gene was already present and was silenced upon establishment of the PcaRCI system. These findings provide a striking example for selfishness of RM systems and intracellular competition between different defences.

antiphage defense system | viral evasion mechanisms | Phage evolution

Systematic discovery of phage determinants that trigger bacterial immune systems

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To survive infection by phages, bacteria have developed various immune systems that enable them to defeat phage infection. Multiple new families of antiphage defense systems have been discovered recently. Although it has been demonstrated experimentally that these systems provide defense against phages, the mechanisms of action remain largely unknown. One of the fundamental questions regarding these systems is what is the phage component that is sensed by each of these systems, activating antiphage activity. To address this question, we set out to identify the specificity determinant for each of the defense systems in a systematic manner. For this, we designed a large-scale screen to isolate phages that escape from each of the newly discovered systems. Examining the genome sequences of phage mutants that escaped defense revealed genes whose mutation enabled the phage to circumvent bacterial immunity. We hypothesize that these genes encode the components that are sensed or targeted by the antiphage systems. Our results identify phage-encoded specificity determinants for multiple new defense systems, bringing us closer to understanding their mechanism of action.

abortive infection | TIR domain | anti-phage

Phages overcome bacterial immunity via dedicated anti-Thoeris proteins

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To overcome bacterial immunity, phages utilize proteins that directly inhibit CRISPR-Cas and restriction modification systems. It was recently shown that bacteria employ, apart from CRISPR-Cas and restriction systems, a large diversity of additional phage resistance systems, but it is unknown how phages cope with this multilayered bacterial immunity. One such anti-phage system is Thoeris, which involves Toll/interleukin-1 receptor (TIR) protein domains that have functional homology to human and plants immune proteins. By comparing genomically-similar *Bacillus* phages displaying differential sensitivity to the bacterial immune system Thoeris, we identified a family of phage proteins that inhibit this defense system. We demonstrate that the anti-Thoeris proteins are necessary and sufficient for the phage to overcome the system. Homologs of these anti-Thoeris proteins are found in hundreds of phages that infect taxonomically diverse bacterial species, and by cloning such homologs into Thoeris-containing Bacillus subtilis cells we show that they efficiently cancel the defensive phenotype. Finally, we study the molecular mechanism of the phage anti-Thoeris proteins and reveal that they inhibit the core functionalities of the defense system.

anti-phage defence systems | Escherichia coli | microbial genomics | mobile genetic elements | pan-immune system

The defence island repertoire of the *Escherichia coli* pan-genome

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In recent years it has become clear that anti-phage defence systems cluster non-randomly within bacterial genomes in so-called “defence islands”. Despite serving as a valuable tool for the discovery of novel defence systems, the nature and distribution of defence islands themselves remain poorly understood. In this study, we comprehensively mapped the repertoire of defence islands within >1000 strains of *Escherichia coli*, the most widely studied organism in terms of phage-bacteria interactions. We found that defence islands preferentially integrate at several dozens of dedicated integration hotspots in the *E. coli* genome. Defence islands are usually carried on mobile genetic elements including prophages, integrative conjugative elements and transposons, as well as on other genetic elements whose nature of mobilization is unclear. Each type of mobile genetic element has a preferred integration position but can carry a diverse variety of defensive cargo. On average, an *E. coli* genome has 4.1 genomic hotspots occupied by a defence system-containing element, with some strains possessing up to 7 defensively occupied hotspots. Studying the distribution of mobile genetic elements and their defensive cargo is crucial to understanding the wider context of the phage-bacteria arms race and the nature of defence islands themselves.

Phage-bacteria interaction | Pseudomonas | CRISPR-Cas | host range | machine learning

The paradoxical relationship between CRISPR-Cas and phage susceptibility in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an important opportunistic pathogen and our labs focus on its interactions with bacteriophages. From a genomics perspective, *P. aeruginosa* presents a tremendous amount of diversity, with pairs of strains harboring widely different accessory genetic components. As part of our research, we use machine learning approaches to explore large-scale omics data and model interactions between diverse sets of strains and (pro)phages.

We present here a story that takes us from observations gathered during such computational modeling to a concrete biological insight into the evolutionary dynamics of *P. aeruginosa*, its phages, and the CRISPR-Cas defense systems.

CRISPR-Cas systems are part of the pan-immune system of *P. aeruginosa* and were recently shown to limit horizontal gene transfers in that species. Strains equipped with these systems tend to have smaller genomes and CRISPR spacers targeting integrative conjugative elements, phages, and plasmids.

Driven by insights from our models of phage susceptibility, we explore the genomic effects and phenotypic consequences of CRISPR-Cas systems in *P. aeruginosa*. First, we describe multiple associations between the presence of these systems, their subtypes (I-C, I-E, and I-F), and the population structure. Second, we show a paradoxical, positive correlation between the presence of CRISPR-Cas and the likelihood of the strain to be susceptible to distinct virulent phages. Finally, we reconcile these observations by establishing a link between this increased phage susceptibility and a depletion of other components of the pan-immune system in the presence of CRISPR-Cas.

We conclude by 1) discussing potential issues of co-selection of antibiotic resistance and resistance to phages in *P. aeruginosa*; 2) describing other computationally relevant determinants of host-virus interactions to predict phage susceptibility; and 3) projecting how this analysis expands to other bacterial species.

adaptation algal viruses anti-phage defence systems Bacterial immunity bacteriophage bacteriophage ecology Bacteriophage evolution co-existence coevolution experimental evolution Freshwater Phage resistant mutants phage-bacteria co-evolution Resistance mechanisms

The tradeoff between resistance to phages and nitrogen fixation in bloom-forming cyanobacteria

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Cyanobacterial blooms negatively impact aquatic environments worldwide. Diazotrophic (nitrogen fixing) cyanobacteria, such as *Cylindrospermopsis*, can even form blooms under nitrogen starvation, due to their ability to fix atmospheric nitrogen (N_2)**.** Phages could potentially control cyanobacterial growth; however, cyanobacteria are able to adapt to the presence of phages quite rapidly by acquiring resistance to the phage, and thus, the role of phages in bloom dynamics is -as yet- unclear. We examined the resistance to phages of two strains of diazotrophic cyanobacteria: the invasive bloom-forming *C. raciborskii*, and the model strain *Nostoc* PCC 7120. Our results demonstrate that this resistance, comes with reduced ability to induce heterocyst cells, in which N_2 is fixed. This reduction causes reduced N_2 fixation by the mutant strains, along with reduced growth or even death under nitrogen starvation. Such cost can prevent the survival of phage-resistant cyanobacteria under nitrogen starvation, and may suggest that spontaneous resistance to phages is a transient trait in diazotrophic bloom-forming cyanobacteria. Whole genome sequence analysis of the resistant strains reviles new genes essential for heterocyst cells development and function, and suggests a possible explanation to this intriguing pleiotropy.

bacteriophage biocontrol | Acidovorax citrulli | Bacterial fruit blotch

Biocontrol of bacterial fruit blotch with a novel bacteriophages infecting *Acidovorax citrulli*

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Acidovorax citrulli is a gram-negative bacterium causing an economically important seed-borne disease in Cucurbitaceae family known as bacterial fruit blotch (BFB). Despite the importance of this bacterium, control methods against the bacterium are considerably limited. The use of copper-based antibiotics, heating, and seed treatment methods was not able to completely eradicate this disease. Therefore, new methods to control this disease are highly necessary. Bacteriophages, the viruses of bacteria, are highly suitable as an alternative control method. We aim to isolate and characterize bacteriophages infecting *A. citrulli* and to use these isolated bacteriophages to control BFB. As result, we isolated over 100 bacteriophages, and two phages with a wide host range were identified and characterized. Bacteriophage ACPWH and ACP17 were among the firstly isolated phages belonging to *Siphoviridae* and *Myovoiridae* family, respectively. Initiative results of seed coating with bacteriophage ACPWH showed up to 90% of germination and complete survival of the watermelon plants compared to 100% death of non-treated control. We also aim to investigate the possible phage resistance of *A. citrulli* and the relationship between phage resistance and pathogenicity. Phage resistant mutant named as AC-17-G1 was isolated among 3,264 *A. citrulli* mutants produced by transposon mutation system using spot assay and plaque assay, which was confirmed as avirulent in seed coating method. The mutant has the integrated Tn5 in the middle of Cupin protein gene. Furthermore, site-directed mutation of this gene from wild type by CRISPR/Cas9 system resulted in the loss of pathogenicity and phage resistance, which showed that the Cupin protein gene is the responsible gene for the pathogenicity and phage resistance. All these data suggest that the Cupin protein is a phosphomannos isomerase involved in LPS synthesis determinant of pathogenicity and phage susceptibility of A. citrulli.

Phage Satellites | Horizontal gene transfer | phage defense

Bacteriophages mobilise genetic islands encoding immune systems to spread virus resistance

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Bacteria encode diverse anti-phage immune systems which display high variability between related strains, indicating high mobility. How this mobility is achieved, however, remains poorly understood. Phage-inducible chromosomal islands (PICIs) represent a widespread family of pathogenicity islands which hijack the life cycle of their cognate helper phages to spread. Here, we demonstrate that PICIs carry an impressive arsenal of anti-phage systems which can be mobilised intra-and intergenerically. In *Staphylococcus aureus*, the PICI pT1028 carries a novel defence system that inhibits phages not normally hijacked through the canonical PICI life cycle, broadening the anti-phage activities of pT1028. We also show that mobilising pT1028 helps spread anti-phage immunity to naïve bacteria, which can be beneficial for the helper phage since pT1028 can later protect the helper phage against different phages. The relationship between phages and PICIs is therefore not strictly parasitic, but can also be mutualistic. Bioinformatic searches in Gram-positive and Gram-negative bacteria identify multiple known and hypothetical anti-phage systems within PICIs. Overall, our results demonstrate a new facet of PICI biology, highlighting that PICIs play a complex role in the dynamics between bacterial hosts and phages, and between phages and phages, driving bacterial ecology and evolution.

anti-CRISPR | molecular mechanisms | phage defense

AcrVA2 inhibits Cas12a biogenesis via translation-dependent mRNA degradation

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Bacterial CRISPR-Cas systems protect their host from bacteriophages and other mobile genetic elements. Many temperate phages, in turn, encode various anti-CRISPR (Acr) proteins to inhibit the immune function of CRISPR-Cas and enable lysogeny. Effective inactivation of these nucleases is important for phage, prophage, and lysogen fitness. We previously reported the discovery of three Cas12a anti-CRISPR proteins (AcrVA1, AcrVA2, and AcrVA3) encoded in the same operon of a *Moraxella bovoculi* prophage. Whereas AcrVA1 inactivates Cas12a by cleaving the CRISPR RNA, here we show that AcrVA2 inhibits Cas12a biogenesis by specifically downregulating Cas12a mRNA and protein levels. AcrVA2 downregulates mRNA of Cas12a orthologs that are highly diverged, codon-modified, and expressed off artificial promoters, suggesting that AcrVA2 does not affect Cas12a transcription initiation. Interestingly, disruption of the Cas12a start codon abrogates AcrVA2-mediated Cas12a mRNA destruction, indicating that Cas12a mRNA must be translated to trigger downregulation. Consistent with this, an inactive mutant of AcrVA2 co-precipitates with Cas12a protein, confirming a non-inhibitory protein-protein interaction. Altogether, these data suggest that AcrVA2 recognizes the Cas12a polypeptide and triggers the destruction of its mRNA before translation is complete. The strategies employed by these anti-CRISPRs to inhibit pre-existing Cas12a complexes (e.g. AcrVA1) as well as CRISPR-Cas biogenesis (e.g. AcrVA2) likely allow phages to survive during infection as well as maintain a stable co-existence with their bacterial hosts.

Antiviral defense | abortive infection | structural biology | virus-host protein-protein interactions

Investigation of Bacterial Abortive Infection AbiV Antiviral System

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The abortive infection (Abi) system is abundant in bacterial genomes. It is one of the innate defense strategies that prevent phage infection from spreading to uninfected cells. The Abi strategy is considered as an altruistic trait as the infected cell commits suicide by preventing the phage lytic cycle to benefit its community. More than twenty different Abi systems, designated AbiA to AbiZ, have been reported. Literately, the mode of action of Abi can function through toxin-antitoxin (e.g. AbiE, AbiQ) or CRISPR-associated systems. However, detailed mechanism of most Abi systems has not been well elucidated.

AbiV is a member of the higher eukaryotes and prokaryotes nucleotide-binding (HEPN) superfamily that may serve as an endoribonuclease. It can be found in many bacteria including *L. lactis*, *L. paracamosus, S. pneumoniae, S. orlis,* and others. In our study, we used the non-pathogenic *L. lactis* as the model organism, which provides resistance against virulent lactococcal phage of the 936 and c2 species. Our previous study had shown that phage p2 gene encoded a protein SaV that activates the AbiV system. However, neither AbiV nor SaV has any structural homologs in PDB and the molecular mechanism of anti-phage remains unclear. To better understand the mode of action of AbiV, we investigated the AbiV system from the basis of gene architecture, transcription regulation, biochemical and structural aspects.

Bacterial immunity | Pseudomonas aeruginosa | Innate immunity

Complementary and overlapping phage defense specificity in clinical *Pseudomonas aeruginosa* strains

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Bacteriophages impose a strong evolutionary pressure for the development of mechanisms of survival. These mechanisms include receptor adaptations that result in decreased phage adsorption, as well as molecular pathways that have specifically evolved in microbes to suppress phage infections and which can be grouped in adaptive and innate immunity. The CRISPR-Cas system is currently the only adaptive immune system known in microbes, while innate immune systems are much more diverse with dozens of new systems discovered in recent years. Identification of these new defense systems was aided by the observation that multiple systems coexist and cluster in defense islands. However, the purpose of this multi-layered defense and its implication for phage host range remains unclear.

Using a unique collection of antibiotic-resistant clinical strains of *Pseudomonas aeruginosa* that encode up to 16 defense systems, and their infecting phages, we demonstrate that the accumulation of multiple layers of defense results in improved protection from phage attack. In addition, we reveal that non-receptor-associated defense systems are key determinants of phage host range in *P. aeruginosa*.

Overall, our results have broad implications for understanding the multi-layered phage immunity evolved in microbes, as well as for the development of antibacterial therapeutics, as the widespread use of phages may select for naturally occurring multi-phage resistant bacterial phenotypes that will limit the efficacy of phage therapy.

bacterial defence systems | anti-phage | bacteriophage

A bacterial defence system provides broad and robust anti-phage protection

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To prevent phage infection, bacteria harbour numerous defence systems. Recently, many bacterial defence systems have been computationally identified, and while new studies have begun to elucidate these anti-phage mechanisms, many await to be uncovered. Here, we collected *Pseudomonas* clinical strains encoding multiple defence systems, among which the uncharacterized gene cluster *qatABCD* was found. This four-gene cassette is comprised of an ATPase (*qatA*), a hypothetical protein of unknown function (*qatB*), a 7-cyano-7-deazaguanine synthase-like gene (*qatC*) and a TatD-like DNA nuclease (*qatD*). Here we show that QatABCD provides strong resistance against multiple phages when expressed heterologously in *P. aeruginosa* PAO1. Interestingly, we isolated a *Pseudomonas* phage that evades QatABCD protection through an unknown mechanism. Our results provide new insight into the vast and fast-growing field of bacterial defence systems. From a translational perspective, our findings could lead to development of new technologies and advancement of phage-based therapeutics.

bacterial immunity | CRISPR-Cas | jumbo phage | phage therapy

Type III CRISPR–Cas provides resistance against nucleus-forming jumbo phages via abortive infection

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Bacteria have diverse defences against phages. In response, jumbo phages evade multiple DNA-targeting defences by protecting their DNA inside a nucleus-like structure. We previously demonstrated that RNA-targeting type III CRISPR–Cas systems provide jumbo phage immunity by recognising viral mRNA exported to the nucleus for translation. Here, we demonstrate that recognition of phage mRNA by the type III system results in cA_3 production that activates an accessory nuclease, NucC. Although it cannot access phage DNA in the nucleus, NucC degrades the bacterial chromosome, triggering cell death, and disrupting phage replication and maturation. Hence, type III-mediated jumbo phage immunity occurs via abortive infection with suppression of the viral epidemic protecting the population. Type III systems that target jumbo phages have diverse accessory nucleases and an RNase (Csm6) also provides immunity. Our study demonstrates how type III CRISPR–Cas systems overcome the inaccessible nature of jumbo phage DNA to provide robust immunity.

Antiviral defense | Archaeal viruses | Haloferax

A virus of the archaeal model organism Hfx. volcanii reveals dramatic differences between chronic and lytic life cycles

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Viruses are important ecological, biogeochemical and evolutionary drivers in the environment. Upon infection, viruses often cause the lysis of the host cell. However, some viruses exhibit alternative life cycles, such as chronic infections without cell lysis. The nature and the impact of chronic infections in prokaryotic host organisms remains largely unknown. We isolated and characterized a novel haloarchaeal virus, Haloferax volcanii pleomorphic virus 1 (HFPV-1), the only isolated virus infecting the model haloarchaeon Haloferax volcanii DS2. HFPV-1 is a pleomorphic virus that causes a chronic infection with continuous release of virus particles during which host and virus coexist without lysis or emergence of resistant host cells. Even though the chronic infection with HFPV-1 only has a minor impact on the reproduction of the host, it causes extensive remodeling of the host transcriptome, including interactions between HFPV-1 and a pre-existing provirus, revealing a new virus defense mechanism. We further present a comparison of the characteristics of HFPV-1 with those of a lytic virus we isolated on Halorubrum lacusprofundi, also a host for HFPV-1. We highlight fundamental differences between the two viruses, including host range, host attachment and interactions with the host defense machinery. Our work demonstrates that HFPV-1 and H. volcanii are a great model system for studying virus-host interactions in archaea.

tRNAs | anti-phage defence systems | host range | Bacteriophages | Codon Usage Bias Hypothesis | T4 phage | Bacteriophage evolution | Bacterial immunity | phage resistance

Why do bacteriophage encode for tRNAs? Codon Usage Bias or host-range?

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Bacteriophages are an integral component of the evolution of bacteria through population control, spreading of genetic material by horizontal gene transfer, and introducing genetic novelty through the selection for resistance. Bacteriophage-host interactions have been the basis on which many biotechnological tools were discovered, such as restriction enzymes and CRISPR-Cas9. It has been known for several decades that phage T4 encodes tRNAs, but more recently, we have found that about 20% of fully sequenced phage genomes also encode tRNAs. Yet why this is the case remains unknown. The main hypothesis, the Codon Usage Bias Hypothesis (CUBH), suggests that differences between bacteriophage and host in codon choice necessitates that bacteriophage encode their own tRNAs to complement host tRNAs. Here we show that CUBH does not explain the presence of tRNAs in phage genomes. Instead, through experiments in T4 and *E. coli*, we show preliminary evidence that tRNAs are involved in host-range by reducing the rate at which spontaneous phage resistance mutants arise. We are currently investigating the mechanistic basis for the control of host-range by tRNAs in this model system.

Mycobacteriophage | Lysis/Lysogeny | Virus-host interactions | tRNAs | Pseudolysogen

Pseudolysogeny in *Mycobacterium smegmatis*: What genetic factors contribute to the change in host resistance?

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Many clinical isolates of the human pathogen *Mycobacterium* are now multi-drug resistant. Using viruses that kill Mycobacteria (phage therapy), could be an effective alternative treatment to antibiotics. Two major phage life-history strategies exist lytic, where phage kills their host, and lysogenic, where the phage integrates into the host's genome and is spread throughout the population. Phages suitable for phage therapy should be lytic and should cause low rates of spontaneous resistance. During the investigation of four lytic phages of *Mycobacterium smegmatis*, we observed a high frequency of phage-resistant mutants (PRMs). We noticed that lawns of PRMs could produce spontaneous plaques (SPs) at different rates. SPs derive from an intracellular agent, given they cannot readily be washed off. SPs can infect isolates that were previously resistant to the ancestral phage and some SPs are positive for marker genes of the ancestral phage, whilst others remain negative. SPs are rapidly lost after several generations of growth in the absence of exogenous phage. We are currently investigating the nature of these 'pseudolysogens' using long-read sequencing and pulsed-field gel electrophoresis. Our data raise subtle exceptions to traditional boundaries of lytic and lysogenic modes that should be considered when selecting phage for phage therapy.

mycobacteriophage | Ms6 | phage resistance | superinfection exclusion

Mycobacteriophage Ms6 Pin protein is involved in a superinfection exclusion mechanism

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Bacteriophages live in a close relationship with their hosts, playing an important role in microbial environments. For their own survival phages have developed resistance mechanisms to prevent a second viral attack. These may act at different levels: adsorption, DNA injection, replication or phage maturation. Several resistance strategies have been described for phages infecting Gram-positive or Gram-negative bacteria, but knowledge on mycobacteriophage resistance is scarce. We have previously observed that synthesis of mycobacteriophage Ms6 Pin protein from a recombinant *Mycobacterium smegmatis* (Msm_13B) confers resistance to Ms6. In this study, we sought to elucidate the resistance mechanism associated with Pin, a small protein that localizes in the inner membrane. We hypothesize that Pin could be associated with a superinfection exclusion mechanism. To confirm this, we performed adsorption and transfection assays on the wild-type (wt) and Msm_13B and assessed Ms6 fluorescently-labelled DNA entry.

Our results revealed that: i) Ms6 successfully adsorbed to wt and Msm_13B strains, indicating that Pin does not interfere with phage adsorption; ii) transfection of phage DNA into both strains resulted in plaque formation, excluding resistance mechanisms triggered after DNA injection; iii) infection of Msm_13B with SYBR Gold-labelled Ms6 showed that fluorescence of these cells increased slowly along time and that their signal was significantly reduced when compared to the wt; iv) infection by other mycobacteriophages was only prevented for those closely related to Ms6.

These observations support that Pin inhibits Ms6 infection by compromising the DNA injection step. Thus, we suggest that Ms6 Pin participates in a mycobacteriophage-encoded superinfection exclusion system. Since current options of selectable markers for use in mycobacteria are limited, these systems may be subsequently exploited as useful alternatives to selection by antibiotic resistance in this genus.

PI phage | BREX | Dar anti-restriction system

Phage P1 anti-restriction protein DarA inhibits BREX defence *in trans*

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P1 is a temperate bacteriophage with a broad host specificity, which is widely used as a tool for generalized transduction. Any DNA has a chance to be packaged in P1 capsid and is thought to be protected from the degradation by the host Type I Restriction-Modification (R-M) system due to activity of the anti-restriction Dar (defence against restriction) system. It has been proposed that Dar system acts only *in cis* and is unable to protect co-infecting phages. P1 is also capable of infecting bacteria carrying the defence system BREX, and we found that Dar system contributes to the BREX inhibition. It was suggested that Dar is a multi-component system, however, the expression of DarA protein turned out to be sufficient to inhibit BREX. The DarA protein lacks predicted catalytic motifs, but has a central coiled-coil region splitting two domains of unknown function, and all 3 domains are required for anti-BREX activity. BREX system resembles R-M in a way that it exploits methylation to discriminate between self and invading DNA, however, DarA expression did not interfere with BREX methylation. To determine a target of DarA inhibition we performed *in vivo* pull-down experiments with strep-tagged DarA and found that it co-purifies with one of the BREX system proteins – BrxC. BrxC has an ATPase domain and is one of the core components required for both: BREX defence and methylation, although the exact role of this protein is not determined. We suggest that anti-BREX activity of phage P1 is associated with the ability of DarA to inhibit BrxC and further study of this interaction should elucidate the role of BrxC in BREX defence.

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mobile genetic elements | phage defense | Staphylococcus aureus | prophages | Pathogenicity island

A search for potential phage defense encoding loci in the mobilome of *Staphylococcus aureus* strains

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Staphylococcus aureus is among the most dangerous pathogens, being both morbid and mortal. What makes the species a successful pathogen is the genetic shuffle of its genome, possible due to a variety of genetic elements interacting with each other and 'wandering' between isolates. Such accessory components together can comprise one fifth of the *S. aureus* core genome and include prophages, plasmids, *S. aureus* pathogenicity islands (*Sa*PIs) and others, like insertion sequences or transposons. Certain of them may encode phage defense systems protecting their hosts from infection with new invading phages. In this study, 47 *S. aureus* isolates used for phage sensitivity testing and representing 12 clonal complexes (CC) were sequenced using NGS Illumina and MinION hybrid technologies, providing gap-free genomic sequences. Various approaches were used to identify and analyse sequences of their prophages, plasmids, as well as mobile and defective *Sa*PIs. Prophages were most abundant, as they were missing in 3 isolates only. Prophage classification was performed with primer pairs developed by Kahánková et al., and targeting the integrase, tail appendices and cell lysis encoding loci, while phylogenetic relationships were resolved with support of ICTV-refined virus database. Two new type of integrases were identified, for which specific primers were designed. Among 48 plasmids found in isolates of 10 CCs, most were either of size below 5 kb (22 plasmids) or in the range of 20-30 kb (20). One larger, conjugative plasmid was also identified. Potentially mobile genomic islands were less numerous than the defective ones. Further analysis is under way to find out if and which of the mobilome elements can potentially provide to their hosts a protection from invading phages.

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algal viruses | Resistance mechanisms | Host-virus interactions | phytoplankton | single molecule fluorescence

Studying the unknown mechanisms of resistance to viral infection in marine algae

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Marine phytoplankton are responsible for approximately half of the photosynthesis on the planet and are at the base of marine food webs. Viral infection of marine phytoplankton is a significant cause of mortality; thus, viruses are key regulators of phytoplankton populations and thereby influence global carbon cycles. Algae from diverse lineages developed resistance to viruses, whereby after virus-induced lysis and population decline, virus-resistant cells regrow. Despite their ecological importance, little to nothing is known about the molecular basis of virus resistance in marine algae.

This work focuses on two model organisms and their giant DNA viruses: *Emiliania huxleyi* - bloom-forming coccolithophore, and *Ostreococcus tauri* - green alga and the smallest known eukaryote organism. Using Single molecule fluorescence in situ hybridization (smFISH), we can distinguish actively infected cells in the population in high throughput method, and follow the dynamic of viral infection and recovery into resistance in a single cell resolution. We also break apart the phrase “resistance producers” which is meant to describe a population that recovered from infection and gained the ability to co-exist with the virus. We show that this population is phenotypically heterogenetic and consists of both resistant and susceptible cells, and that the balance between them changes with the physiological state of the culture.

Also presented is the ongoing effort to find genes that have a role in algae resistance mechanisms. We use comparative transcriptomic from multiple experiments, narrowing down a shortlist of candidate R genes. We then validate these potential R genes' correlation to resistance and aspire to phenotypically characterize them by creating overexpression mutants. This work presents a strategy to unravel viral resistance mechanisms in algae, and show major footsteps in our ability to follow infection dynamics by targeting virus and host genes transcription.

defense system | abortive infection | bacteriophage

Zorya - a novel abortive infection system widespread in bacteria

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Bacteria use various strategies to avoid viral infection. These include adaptive immune systems such as CRISPR and innate immune systems, such as restriction-modification systems (RM) and abortive infection systems (Abi). Recently a novel Abi system called Zorya had been described. Its components contain homologs of a proton channel used in the motors of bacterial flagella. These domains had never been identified before as being involved in defense.

In our studies, Zorya showed strong protection against a broad range of phages, including several morphological families of dsDNA and ssDNA phages, pointing to a high degree of flexible molecular specificity. We first measured the culture dynamics of multiple phage infections using a robot-based system. We showed that Zorya is an abortive infection system, i.e., infected bacterial cells commit suicide, preventing lysis of the entire cell population. We then followed phage infection under the microscope using a combination of fluorescently labeled proteins and membrane and nucleoids staining. We confirmed that only 3-8% of infected bacterial cells lysed due to phage replication, while the rest of the infected bacteria lost membrane permeability without completing phage infection. Next, using several experimental setups with fluorescently labeled proteins, we microscopically examined the successful completion of each step of phage infection. We showed that although the phage successfully injects its DNA, no protein synthesis of the phage occurs. We then calculated the ratio of phage DNA to bacterial DNA during infection and showed that Zorya system abolishes phage DNA replication. To gain a deeper understanding of the mechanism of action of Zorya system, we used super-resolution microscopy to localize components of the system during phage infection. The details of the defense mechanism of Zorya system will be further discussed.

Bacteriophages | Phage resistant mutants | Host specificity

Large-scale Mutagenesis of Phage Tail Fibers to alter Host Specificity

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The rapid spread of antibiotic resistant pathogens represents a dire healthcare threat. Due to the alarmingly quick decrease of the efficiency of both novel and current antibiotics, there is an urgent need for effective alternatives. One of the most promising avenues is the therapeutic application of bacterial viruses (phages). Clinical usefulness of phages is gravely constrained by their innate narrow-host specificity and the rapid emergence of the resistance developed by bacteria against them. The most common way of acquiring resistance is to change the receptors that serve as attachment sites for the phages. Here, we developed a genome engineering technique to efficiently mutagenise phage tail fiber genes to alter host specificity. First, we optimised a DivERGE technique to mutagenise plasmid encoded version of the T7 fiber gene gp17. Then, we generated transducing phage particles with mutagenised phage tails and selected for improved host recognition on *E. coli* Δ*WaaR* strain. This strain is engineered to become resistant against the wild type T7 phage infection by truncating its lipopolysaccharide motif. We isolated several mutant phage tails that transduced the Δ*WaaR* strain in a highly efficient manner comparable with the wild-type counterpart. Next, we successfully demonstrated the same experiment with hybrid T7 phage particles that display mutagenized phage tail fiber genes derived from phages that infect clinically relevant *Klebsiella pneumoniae* strains. Our next aim is to expand this methodology to propagating phages. To this end, we mutagenized the tail fiber encoding regions during the intracellular phase of phage infection. While we observed similar mutations during this process compared to those we identified before with the transducing phage particles, the diversity of generated library was very low. Our current goal is to overcome this limitation in a way that the method is applicable not only to model but to phages with therapeutic potential as well.

Staphylococcus epidermidis bacteriophage wall teichoic acid

Identification of a novel bacteriophage receptor in *Staphylococcus epidermidis*

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Bacteriophages are the most abundant biological entities on earth and considered important for bacterial population dynamics and horizontal gene transfer. Phages infecting the genus Staphylococcus are most thoroughly investigated for their prominent member Staphylococcus aureus. For attachment and infection, bacteriophages need to bind a specific surface structure via their receptor binding proteins (RBPs). In case of S. aureus, these receptors are glycosyl moieties of the cell wall bound glycopolymer wall teichoic acid (WTA). Up to now, other species of Staphylococci have been neglected in this research, but might function as hubs for antibiotic resistance genes, which are spread by phages via horizontal gene transfer. Understanding phage mediated horizontal gene transfer is therefore essential to understand transfer of antibiotic resistance between bacteria. Since coagulase-negative Staphylococci (CoNS) share a similar WTA backbone consisting of polyglycerol-phosphate (GroP), we used the well-known coagulase-negative species Staphylococcus epidermidis for identification of the bacteriophage receptor binding site. We therefore created a transposon mutant library of S. epidermidis strain 1457 and challenged it with siphovirus ΦE72. Survivors were screened for a resistance phenotype, which was subsequently verified via clean knockouts, resulting in a partial resistance of S. epidermidis in the presence of ΦE72. The identified genes encode enzymes involved in the synthesis and transfer of sugar precursors, which are probably involved in wall teichoic acid synthesis or glycosylation. This resistance mechanism is mediated via a decreased adsorption capacity of the phage to the cell envelope and is universal for other S. epidermidis phages. We therefore assume that the identified enzymes are responsible for the synthesis of a phage receptor, most likely connected to the glycosylation of GroP-WTA present in most CoNS.

CRISPR-Cas Bacteriophage regulation viral evasion mechanisms

The phage-bacterium arms race of CRISPR-Cas and evasion strategies

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Bacteria have evolved ‘immune systems’ as a result of their constant exposure to foreign mobile genetic elements, including bacteriophages and plasmids. To thwart these invaders, bacteria have many resistance strategies, including innate immunity, such as restriction-modification and abortive infection systems, and adaptive immunity provided by the CRISPR-Cas systems. Recently, there have been major advances in our understanding of these systems and the different strategies that phages have to evade these immune mechanisms. In this talk I will present aspects of our recent research into bacterial CRISPR-Cas systems, how they are regulated and the evasion strategies used by phages to avoid these adaptive immune systems.

spatially structured environments | swarmers | bacteria-phage interaction | liquid crystals

Modeling spatially structured environments with lyotropic liquid crystals for studying bacteria-phage interaction

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Both intercellular and bacteria-phage interactions in spatially structured environments have critically different features from those in isotropic media. Partially this is due to the transition between different bacterial morphotypes from swimmers to swarmers, and eventually to biofilm-forming cells. Such bacterial forms differ in their antibiotic sensitivity and the dynamics of interaction with phages. Thus, obtaining spatially structured anisotropic media as experimental model systems for studying the dynamics of bacterial populations and their sensitivity to phages and antimicrobials is essential. Our idea is based on using a new class of materials combining properties of both the liquid and the solid phase, lyotropic chromonic liquid crystals (LCLCs), for that purpose.

Our pilot study was aimed to examine the behavior of the population of *Proteus vulgaris* — the causative agent of serious diseases such as urinary tract infections, etc. The study design included examining the bacterial growth, motility, and morphology under the transition of pre-grown population from different isotropic nutrient media to anisotropic microcosms based on LCLCs. Growth kinetics, motility pattern as well as morphotype conversion from swimmers to swarmers changed significantly after the transition of the bacterial population to different microcosms based on LCLCs as compared to those in isotropic conditions.

A search for a candidate phage to be added to the microcosms with LCLCs and bacteria was performed. A broad-host-range siphophage with 50 nm head and up to 270 nm tail, able to infect both *P. vulgaris* and *P. mirabilis* species was isolated and selected for further research.

Our findings indicate the attractiveness of artificial spatially structured microcosms based on LCLCs for the study of the phenomena of swimmer-swarmer transition. We expect our further studies to shed light on the significance of swarmer morphotype in *Proteus* interaction with phages.

Transposon anti-phage defense island

Transposon-encoded CRISPR-Cas systems facilitate mobilization of anti-phage defense genes

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Canonical CRISPR–Cas systems maintain genomic integrity by leveraging guide RNAs for the nuclease-dependent degradation of mobile genetic elements, including plasmids and viruses. We describe a notable inversion of this paradigm, in which bacterial Tn*7*-like transposons co-opted nuclease-deficient CRISPR–Cas systems to catalyze RNA-guided integration of mobile genetic elements into the genome. Mobilization of these CRISPR-associated transposons (CRISPR-Tn) in Escherichia coli requires both CRISPR- and transposon-associated molecular machineries, and is fully programmable through manipulation of the guide RNA. Large customized genetic payloads of up to 10 kilobases were successfully integrated, and deep-sequencing experiments reveal highly specific, genome-wide DNA insertion across dozens of unique target sites. We next established a bioinformatic and experimental pipeline to comprehensively explore the natural diversity of Type I-F CRISPR-Tn, leading to the characterization of 18 additional systems and the identification of a highly active subset that exhibit complete orthogonality in transposon DNA mobilization. Finally, we analyzed transposon-encoded cargo genes and found the striking presence of anti-phage defense systems, suggesting a role in transmitting innate immunity between bacteria. This discovery of a fully programmable, RNA-guided integrase lays the foundation for genomic manipulations that obviate the requirements for double-strand breaks and homology-directed repair.

Bacillus subtilis | Plaque | SPO1 | SPP1 | Phi29 | Protective Mechanism

Bacterial Factors that Limit Plaque Size

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Plaque occurrence on a bacterial lawn manifests successive rounds of bacteriophage infection. Yet, mechanisms evolved by bacteria to limit plaque spread have been hardly explored. Here we investigated the dynamics of plaque development by lytic phages infecting the bacterium *Bacillus subtilis*. We report that plaque expansion is followed by a constriction phase owing to bacterial growth into the plaque zone. This phenomenon exposed an adaptive process, herein termed "phage tolerance response", elicited by non-infected bacteria upon sensing infection of their neighbors. The temporary phage-tolerance is executed by the stress response RNA polymerase sigma factor σ^{Ξ} (SigX). Artificial expression of SigX prior to phage attack largely eliminates infection. SigX tolerance is primarily conferred by activation of the *dlt* operon, encoding enzymes that catalyze D-alanylation of cell wall teichoic acid polymers, the major attachment sites for phages infecting Gram-positive bacteria. D-alanylation impedes phage binding and hence infection, thus enabling the uninfected bacteria to form a protective shield opposing phage spread. To further identify factors that restrict plaque expansion, we conducted transposon mutagenesis, infected the mutants with lytic phages, and systematically screened for those displaying aberrant plaques. A prominent candidate that emerged from the screen was the stress response *yjbH* gene. Knockout of *yjbH* exhibited large plaques whereas over-expression showed plaques smaller than the wild type bacterium, indicating the involvement of this gene in limiting plaque size. We are now characterizing the mechanism of action of the identified factor.

cystic fibrosis | Phage Therapy | Phage-prophage interactions

Phage-host relationships relevant to phage therapy for pan-resistant *Achromobacter* infection in cystic fibrosis patients

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Microbial resistance to antibiotics is rising across the globe, especially in individuals with chronic illnesses. Cystic fibrosis (CF) patients face chronic antibiotic-resistant bacterial infections in their airways and lungs, among other organ systems. Over the lifetime of the patient, these infections become progressively more resistant. Of interest is the treatment of *Achromobacter xylosoxidans* infections. *A. xylosoxidans* is a Gram-negative, pan-resistant opportunistic pathogen, that colonizes immunocompromised individuals. Phage therapy is being tried as an experimental last resort treatment for these infections and for patients that cannot be treated with CFTR modulators and correctors (e.g., Trikafta).

Several challenges arise when preparing phages for therapy of *Achromobacter*; these include the lack of a well-characterized, plasmid-free, prophage-free, and toxin-free “reference” strain, the evolution of phage-resistant strains in the patient (even before phage therapy treatment), and an insufficient understanding of the phages for the safest, most predictable clinical use (e.g., optimal dosing, stability, and synergy among phages and between phages and antibiotics).

While following *Achromobacter* patient strains isolated over time, we found that these strains change their phage sensitivity. We are investigating the mechanisms that confer resistance to phages. One mechanism is related to prophages present in the patient strains. We are mapping the relationship between specific prophages and the lytic phages that are candidates for therapy using microbiologic and bioinformatic approaches. The results will allow us to pick the optimal strains to raise specific phage lysates. We are also using transposon mutagenesis (Himar1) to map the receptors and host factors for these phages. Together we are building a genetic system for *Achromobacter* that will increase our understanding of its role in CF infections and will facilitate optimizing phage therapy treatments.

Bacteriophage | Phage Therapy | Antibiotic resistance | Staphylococcus aureus | Vancomycin

Antibiotic exposure leads to reduced phage susceptibility in Vancomycin Intermediate *Staphylococcus aureus*

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In the time of antimicrobial resistance, phage therapy is frequently suggested as possible a solution for such difficult-to-treat infections. Vancomycin Intermediate *Staphylococcus aureus* (VISA) remains a relatively rare, yet increasing occurrence in the clinic for which phage therapy may be an option. However, the data presented herein suggests a potential cross-resistance mechanism to phage following vancomycin exposure in VISA strains. When comparing genetically similar strains differing in their susceptibility to vancomycin, those with intermediate levels of vancomycin resistance displayed decreased sensitivity to phage in solid and liquid assays. Serial passaging with vancomycin induced both reduced vancomycin susceptibility and phage sensitivity. As a consequence, the process of phage infection was shown to be interrupted after DNA ejection from adsorbed phage, but prior to phage DNA replication, as demonstrated through adsorption assays, lysostaphin sensitivity assays, electron microscopy, and qPCR. At a time when phage products are being used for experimental treatments and tested in clinical trials, it is important to understand possible interference between mechanisms underlying antibiotic and phage resistance in order to design effective therapeutic regimens.

algal viruses | Comparative genomics | virulence

Using comparative genomics to reveal genes associated with the virulence of a giant virus infecting marine algal blooms

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Emiliania huxleyi is a microscopic alga that forms massive blooms that cover vast oceanic areas. E. huxleyi blooms are routinely infected by the *Emiliania huxleyi* virus (EhV), a giant, double-stranded DNA virus, leading to their demise. Although it was shown that different strains of EhV have different infection dynamics, only one EhV strain was ever fully sequenced, and the mechanisms that determine the virulence of different EhV strains are still completely unknown. In this study, we used comparative genomic analysis to understand how genetic differences between virus strains can affect their different infection dynamics. We sequenced and assembled the genomes of four EhV strains (EhV201, EhV163, EhV-ice and EhV-M1) that were isolated from multiple oceanic sites and display different infection dynamics. We found many differences between the virus strains, most notably the unique gene set of EhV201, which contains 167 genes not shared with the other viruses we examined. Some of these genes are related to transferase activity and carbohydrate-binding and may play a role in the ability of the virus to adsorb to their host cells. We show that some of these genes are clustered together in the viral genome and are co-expressed during the early phase of viral infection. We also show that these genes are phylogenetically related to algal proteins. We suggest they this gene cassette might be associated with high virulence of EhV201. We further expanded the repertoire of genes that we predict to originate in the host, most notably lectins and transferase genes, and show that some of these genes are strain-specific. All EhV strains were shown to encode genes that stem from multiple evolutionary origins, and we suggest that these proteins may play a role in host recognition and virus replication. Overall, these findings suggest that differences in virulence are correlated with specialized genes, some of them acquired from other species, including the algal host.

bacteriophage diversity | Klebsiella pneumoniae | Antimicrobial Resistance

Increasing ciprofloxacin sensitivity in MDR *Klebsiella pneumoniae* challenged with the Drexlervirial phage LAPAZ

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The continuing rise and spread of multi-drug resistant bacteria (MDR) represents a global health concern. However, this development does not mean that traditional antibiotics will eventually wind up as being entirely useless. A number of studies have shown that the concerted activity of phages and “useless” antibiotics control the bacteria stronger than the phage alone and that phage resistance emergence can be reduced. In addition, development of phage resistance can occur at the expense of re-converting bacteria to an antibiotic sensitive genotype. Here we describe a newly isolated phage belonging to Drexlerviridae (Webervirus) with lytic activity and positive interactions with antibiotics against drug resistant clinical isolates of *Klebsiella pneumoniae*. Phage LAPAZ lysis 14% of tested strains. Its genome consists of 51,689 bp and encodes for 84 ORFs, of which 40% could be assigned to proteins of known functions. The latent period is 30 min with an average burst size of 27 PFU/cell. Upon exposure up to 50˚C the phage titer only decreased by 10%. Phage infectivity is maintained over a pH range from 4 to 10 (with pH 6 being optimal). Unlike exposure to the phage alone a complete bacterial eradication was achieved when combining LAPAZ with sub-inhibitory concentrations of meropenem. Phage resistance emergence could be markedly delayed with the co-presence of ciprofloxacin. Conversely, emerging phage resistance came along with a 16 times higher sensitivity to ciprofloxacin. Whole genome analysis revealed only one mutational aberration in the phage resistant strain affecting a membrane transport protein belonging to the Major-Facilitator-Superfamily (MFS). We speculate that this frameshift mutation compromises ciprofloxacin efflux efficiency in the bacterial host and that the non-mutated protein might be involved in phage receptor binding.

Phage-host interactions | Phage Defence | Bioinformatics

The Prokaryotic Antiviral Defence LOCator (PADLOC) for the identification and discovery of diverse novel defence systems

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Many types of antiviral defence systems have evolved in bacteria that protect against invasion by viruses and mobile genetic elements. The defence systems encoded by any given bacterium are an important determinant of their susceptibility to viral infection and capacity for exchange of genetic material. In the past four years, the number of known types of defence systems has increased more than 5–fold, and the discovery of new defence systems is quickly outpacing the development of tools that make use of these new insights. To address this, we developed the Prokaryotic Antiviral Defence LOCator (PADLOC) to provide widespread accessibility to newly discovered system types and ensure consistency between system annotations. With PADLOC, almost all currently known defence systems can be detected in microbial genomes (currently >160 types), allowing users to develop a comprehensive view of the defence arsenal of any microbe. Additionally, PADLOC has allowed us to perform large-scale defence system identification in more than 200,000 archaeal and bacterial genomes and probe the results for novel defence systems. By focusing on the subset of Doron *et al.*'s 'deity' defence systems and examining the proteins frequently encoded in their proximity, we identified six new defence system variants and a novel system comprising a helicase, methylase and ATPase (Hma). Further investigation revealed several types of known systems and other diverse genes frequently embedded in Hma systems. By cloning these embedded gene clusters into *E. coli* and screening against diverse phages from the BASEL phage collection, we have identified several additional novel defence systems encoding putative DNases, RNases, ATPases, toxins, and other proteins with unknown functions. The data presented here further expands the ever-growing spectrum of microbial defence systems against viruses, and showcases the utility of PADLOC for defence system identification and discovery.

qPCR GWAS host range Erwinia amylovora

A Novel Genome-Wide, qPCR-Based Approach to Identify Genetic Determinants of Phage Host Range

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Bacteriophage therapy has been gaining renewed interest due to its ability to eliminate antibiotic resistant microbes and facilitate targeted modifications in microbiome populations. Phage host range is a critical parameter in the selection of therapeutic phage cocktails and the establishment of phage libraries. Understanding the parameters that make a bacterial host susceptible to phage infection will significantly improve the design of high efficacy phage cocktails. Quantitative host ranges were established for four *Erwinia* phage species against a global collection of the phytopathogen *Erwinia amylovora*, and several orchard bacterial epiphytes. This was done using quantitative real time PCR (qPCR) to measure the relative titre of phage produced on each of the hosts. The host range data and host genomic sequences of 80 of the tested strains were used to perform a genome-wide association study (GWAS) to identify host genes associated with susceptibility to phage infection. This approach identified mutations in 10 host genes that likely resulted from natural phage-host co-evolution, and which affect the host ranges of three of the *Erwinia* phage species. Based on the functions of these genes, we propose two novel contact dependent, community level phage defense mechanisms which use the type V and VI secretion systems. In addition, we propose that the *Myoviridae* phage ϕEa21-4 uses the bacterial flagella as its initial binding receptor. These findings introduce several avenues for further study and will help us to deepen our understanding of the complex molecular interactions between these phages and their target hosts.

adaptation | bacteria-phage interaction | bacterial defence systems | CRISPR | modelling

A scaling law in CRISPR repertoire sizes arises from avoidance of autoimmunity

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Some prokaryotes possess CRISPR-Cas systems that use DNA segments called spacers, which are acquired from invading phages, to guide immune defense. Here, we propose that cross-reactive CRISPR targeting can, however, lead to "heterologous autoimmunity," whereby foreign spacers guide self-targeting in a spacer-length-dependent fashion. Balancing antiviral defense against autoimmunity predicts a scaling relation between spacer length and CRISPR repertoire size. We find evidence for this scaling through a comparative analysis of sequenced prokaryotic genomes, and show that this association also holds at the level of CRISPR types. By contrast, the scaling is absent in strains with nonfunctional CRISPR loci. Our results suggest that heterologous autoimmunity is a selective factor shaping the evolution of CRISPR-Cas systems, analogous to the trade-offs between immune specificity, breadth, and autoimmunity that constrain the diversity of adaptive immune systems in vertebrates.

Salmonella enterica | phenotypic resistance | in vivo | T5-like | phage resistance | gut

O-antigen phase variation promotes the co-existence of *Salmonella* and virulent phages during intestinal colonization

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Salmonella enterica Typhimurium (*S*.Tm) is an entero-pathogenic bacterium causing enteric disease in a wide range of hosts, including humans. Salmonellosis outbreaks are associated with the consumption of contaminated animal products and controlling *Salmonella* in the food supply chain has become a public health priority. In this context, bacteriophages (phages) may provide solutions to eradicate *Salmonella* at several steps of food production, from livestock to the final processed products. Here, we address the role of phenotypic resistance in the evolution of the interaction between *S*.Tm and phages, and its impact on phage-based antimicrobial strategies.

We have observed that the T5-like phage ϕ37 fails to eradicate and co-exists with *S*.Tm during multiple passages *in vitro*. We found that ϕ37 infection is prevented in *S*.Tm cells expressing GtrABC or OpvAB, two systems modifying the O-antigen moiety of *S*.Tm lipopolysaccharides. The co-existence between *S*.Tm and ϕ37 relies on the reversible phenotypic resistance generated by the bimodal expression of GtrABC and OpvAB. Using infections in mice to characterize the interaction between *S*.Tm and phages in the intestinal tract, we observed similar co-existence between *S*.Tm and phage ϕ37 *in vivo*. The double inactivation of GtrABC and OpvAB prevents stable co-existence. In this case, the loss of ϕ37 is caused by the fixation of *S*.Tm phage-resistant mutants unable to produce BtuB, the main receptor of ϕ37.

These results show that epigenetic phenotypic switches can prevent the fixation of mutations potentially costly to the bacteria. This also demonstrates that phenotypic resistance is a key parameter that must be overcome when using phages to control microbial populations.

Bacteriophage | Staphylococcus epidermidis | preterm infants

Characterising Prophage diversity and fundamenta

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Staphylococcus epidermidis is a leading opportunistic pathogen due to its ability to form biofilms on indwelling medical devices. MLST analysis of *S. epidermidis* has identified strain type 2 and strain type 5 as important in human infections and these carry a larger number of antimicrobial resistant genes. *S. epidermidis* is one of the most predominant bacteria found in human breast milk and has emerged as a leading pathogen in late onset sepsis in preterm infants, which leaves survivors with poor developmental outcomes. Previously, it has been shown that intestinal bacteriophage can be identified in the first few hours following birth with *Siphroviridae* being most abundant in breast-fed infants. It has been proposed that phage predation within microbial communities from lysogenic phages can alter the composition of consortia and drive bacterial evolution. Therefore, phage prediction from previously deposited bacterial genome sequences can be key resource for investigating the genetic potential of prophages within these environments. The aim of this study was to identify prophage-like regions from human-associated *S. epidermidis genomes* and investigate the prophage diversity within the species. There were 93 complete, human-associated *S. epidermidis* genomes present in the NCBI database included in the study. A total of 251 prophage-like regions were identified through analysis in the prophage prediction tool ProphET. The average number of prophages per genome was 2.70. Further analysis of *S. epidermidis* genomes by MLST revealed strain type 2 to be the most common and have the highest number of phage-like regions. Additionally, of the identified prophages, it was predicted that the majority of *S. epidermidis* phages belong to the family *Siphoviridae*. The role of prophage induction in *S. epidermidis* by antimicrobial agents given to preterm infants may be the key driver of bacterial community development and warrants further investigation.

Staphylococcus aureus | Pseudomonas aeruginosa | Phage-antiseptic interaction | bacterial-phage inactivation

Interactions between phages and antiseptics against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Introduction

It is generally assumed that bacteria do not develop tolerance against antiseptics. However, latest findings indicate that some members of the ESKAPE group can built up tolerance to antiseptics after repeated contact. In this study the effect of a simultaneous challenge of *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) by the phages Sb-1 or NP-3 with polyhexanide or octenidine was investigated.

Material/Methods

The growth/kill dynamics of antiseptic-tolerant SA- and PA-strains and their parental antiseptic-sensitive strains exposed to antiseptics and phages was determined via optical density measurements every 20 minutes for 16 hours. CFU and PFU determination was done at the beginning and the end of the experiment.

Results

NP-3 alone was able to supress the growth of the parental PA-strain. A stronger suppression was achieved with co-addition of each antiseptic. In contrast, the PA-strain with tolerance against 128 mg/mL octenidine and 64 mg/mL polyhexanide, respectively, could not be suppressed by NP-3 anymore. A suppressive effect could be restored when sub-inhibitory amounts of antiseptics were co-added to the phage.

In contrast, the phage Sb-1 alone could suppress the growth of both, the parental SA-strain and the strain with tolerance against 32 mg/mL polyhexanide. Antagonistic effects between phage and antiseptic were observed for the parental strain, while a dose-dependant enhanced suppression was achieved with the phage/antiseptic combination against the antiseptic tolerant strain.

Conclusion

Antiseptics can influence the killing efficiency of the phages Sb-1 and NP-3, where negative and positive interactions were observed. Development of bacterial tolerance to antiseptics can also enable evasion of phage suppression. Nevertheless, within given ranges of antiseptic concentrations phages might represent a meaningful complementation for topical treatment of wound infections.

bacterial immunity | abortive infection | prokaryotic argonaute | short pAgo | NAD+

Short prokaryotic Argonaute systems trigger

cell death upon detection of invading DNA

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While long prokaryotic Argonaute proteins have been shown to mediate DNA-guided DNA interference, the function and mechanism of short prokaryotic Argonaute proteins is poorly understood. Our work shows that short prokaryotic Argonaute proteins and their associated TIR-APAZ proteins form heterodimeric (SPARTA) complexes. Upon RNA-guided detection of invading DNA SPARTA is activated to deplete NAD. We describe how SPARTA is activated *in vitro* and *in vivo*, thereby providing insights in its function and mechanism.

T1 phenotypic resistance population dynamic E. coli microbial genomics

Generating distinct *E.coli* responses to T1 by varying carbon source

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Bacteria are able to rapidly develop resistance to bacteriophage through numerous pathways. Here we investigate how the mutational pathway depends on environmental conditions. Escherichia coli, grown in a range of media with various carbon sources, were exposed to T1 bacteriophage. In all cases the bacteria developed a resistance to the phage at long times. However, the population dynamics of the bacteria post-infection varied with both carbon source and the number of phage added, with these phenotypic differences also visible under optical microscopy. Long-read and short-read sequencing of surviving mutant bacteria was undertaken. These results suggest a coupling between carbon source and the progression of a bacterial populations response. This may have profound implications for phage therapy, as there could be significant differences between the mechanism and rate of resistance development in vivo and in vitro due to different environmental conditions.

Propionibacterium freudenreichii | restriction-modification systems | m5C methylation | phage-encoded methylase

Lifestyle-associated activity of phage-encoded m5C methylase in Propionibacterium phage PJS22

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Propionibacterium freudenreichii is a robust, dairy-associated Actinobacterium known to be generally resistant to phage attacks. *Propionibacterium* phage PJS22 was first detected as both prophage and episome during PacBio sequencing of the carrying strain *P. freudenreichii* JS22. The phage belongs to family *Siphoviridae* and, based on its DNA sequence, can be assigned to the genus *Doucettevirus*, with the highest sequence identity to *Propionibacterium* phage B22. *Propionibacterium* phage PJS22 was isolated from the carrying strain through autoinduction during normal growth in liquid Yeast Extract-Lactate medium. It was found to form plaques on *P. freudenreichii* strains TL110 and TL29, but not on 23 other tested *P. freudenreichii* strains. The phage readily lysogenizes both sensitive strains and the lysogens are resistant to superinfection. In addition, similarly to the original carrying strain JS22, the phage spontaneously induces and accumulates during growth, reaching titers of 10^8 PFU/mL without pronounced effect on the bacterial growth.

The *Propionibacterium* phage PJS22 genome encodes a 722 AA m5C methylase belonging to Type II Restriction-Modification systems. The phage DNA was isolated from autoinduced particles from the carrying strain JS22 as well as during infection of both sensitive strains TL110 and TL29 and subjected to Illumina sequencing as well as RIMS-seq for detection of m5C methylation. Illumina sequencing revealed that the phage genome was identical to the prophage and episome sequenced previously, whereas RIMS-seq allowed for detection and assignment of CGAT methylation motif to the phage-encoded methylase. Interestingly, activity of the phage methylase was detected only during active infection of strain TL110 and TL29, but not after autoinduction from lysogenized strain JS22, pointing to a complex phage-host relationship in the lysogen.

prokaryotic argonaute | defense system | assay | Enzyme characterization

Mechanisms of Long prokaryotic Argonautes

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Long Prokaryotic Argonautes (pAgos) characterized so far are stand-alone proteins. In this poster, we describe long pAgos that are associated with other proteins, their in vitro activity, and possible interactions.

CRISPR-Cas | CRISPR acquisition | phage/antibiotic combinations | anti-phage defence systems | adaptation | bacteriophage | bacteria-phage interaction | flavobacterium

Conditions affecting CRISPR acquisition of II-C and VI-B systems in Bacteroidetes

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Clustered Regularly Interspaced Short Repeats (CRISPR) together with their *cas* (CRISPR associated) genes constitute CRISPR-Cas systems that mainly defend prokaryotes from parasitic nucleic acids. Spacing sequences (spacers) between CRISPR repeats are acquired from foreign elements, and their transcripts are used for target recognition through complementary base pairing. Bacteroidetes species *Flavobacterium columnare* codes a DNA targeting II-C system and an RNA targeting VI-B subtype. Here we set to make a comprehensive study about how different experimental conditions (shaking, temperature, nutrient concentrations, volumes of medium and container, initial cell and virus concentrations) or added compounds (salts, antimicrobials, mucin) affect spacer acquisition rate. The acquisition protocol included incubation of cells and virus in diluted medium for weeks, inoculation into fresh medium, and performing population PCR from liquid culture biomass. Different from previous studies with this organism, we found spacer acquisition in undiluted medium or even without a virus.

Initial experimental conditions that increased the chance of CRISPR spacer acquisition were incubation of cells for 1h before adding the virus and using a MOI of 0.1. Agents like Mg^{2+}, Ca^{2+}, or DNA-damaging antimicrobials facilitated the process; while others like Mn^{2+}, Fe^{2+}, Tween20, or catalase produced inhibition in CRISPR adaptation. An enhancing role of oxygen stress was also suggested. Our study helps to understand the factors and conditions that promote and hinder CRISPR adaptation. This information is essential to reduce variation in experimental results. These results may also provide clues to CRISPR adaptation under more natural settings.

bacteriophages | abortive infection | antiphage | defense system | phage defense

A new abortive infection system identified in the nitrogen-fixing bacterium *Sinorhizobium meliloti*

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We have identified a gene in the nitrogen-fixing alpha-proteobacterium *Sinorhizobium meliloti* that confers defense against bacteriophages from both the *Myoviridae* and *Podoviridae* families. The results of colony counts and live/dead staining experiments indicate that phage infection causes loss of viability for cells carrying the gene, while growth curve experiments conducted at multiplicities of infection > 1 show that culture densities plateau instead of collapsing when the gene is present, suggesting that cell lysis does not occur. We therefore conclude that the system acts through an abortive infection mechanism in which infected cells die but do not undergo lysis or produce new phages. We also show that a gene encoding a homologous protein from an environmental *Escherichia coli* isolate provides some protection against several well-studied *E. coli* phages, including T7, T4, and lambdoid phage HK97. Other homologues are found across a broad range of bacterial phyla, as well as in some archaea, and display evidence of horizontal transfer. We propose that these genes represent a new, widely-distributed group of single-component abortive infection systems.

Uropathogenic Escherichia coli | urine | phage resistance | scavenging

Exploring the phage-resistance mechanisms of uropathogenic *Escherichia coli* EC958 (ST131) in artificial urine

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Antibiotic resistant urinary tract infections (UTIs) are a cause of concern due to increased risk of recurrence and severe complications. Understanding the phage resistance mechanisms of clinical pathogens can help avoid the failure of phage therapy. We aim to study the phage-resistance mechanisms of *Escherichia coli* EC958 to the most effective phage in our library –phage LUC4—in artificial urine. For this, single colonies from the phage-resistant population were recovered, and challenged in a second phage infection. We noticed that in the early timepoints, the resistant population was mainly composed of permanently resistant variants, which were confirmed to have mutations in the phage receptor OmpC by whole genome sequencing. Of note though, at later timepoints, this fixed resistant population was outcompeted by a population with reversible resistance. To study this transient resistance, a transcriptomic analysis of phage-infected and non-infected EC958 at mid-exponential phase was carried out. 788 genes were differentially expressed with significance (FDR<0.05). The most differentially expressed genes included an operon with a putative deacetylase and an acetyltransferase. While deletion mutants of these genes did not show a difference in their response to LUC4 phage infection, they also recovered but grew more slowly after being infected by the phage (but not in a rich medium) indicating that the genes were likely involved in the scavenging capacity of the strain. Further transcriptomic studies are now being carried out to define the transient resistance mechanism but the large number of genes impacted in the original study indicates the likely importance of altered nutrient acquisition taking advantage of the lysed bacterial population.

Virus-host interaction: molecular mechanisms

T4 phage | cell-puncturing device | injection mechanism | infection process | virus-host protein-protein interactions | host cell interaction

Assembly and membrane association of the bacteriophage T4 injection complex

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The bacteriophage T4 has an efficient mechanism to infect the host *Escherichia coli* cell. During infection a phage virion undergoes large structural changes. The most obvious change of T4 is the contraction of its tail. The phage particle adsorbs onto the host cell surface by its tail fibers which triggers conformational changes in the baseplate. Subsequently, the tail sheath contracts and the tail tube penetrates the outer membrane of *Escherichia coli* with a spike-shaped protein complex (gp5-gp5.4) at its tip. After digestion of the peptidoglycan layer the tail tube can reach the inner membrane and the phage DNA is injected into the host cytoplasm.

During phage assembly the needle protein gp5 undergoes a maturational cleavage into the N-terminal gp5* which contains lysozyme activity, and the C-terminal gp5C, both remain associated with the tail. It is assumed that gp5C together with gp5.4 dissociates after the complex penetrates the outer membrane, enabling the full lysozyme activity of gp5*. Gp27 and two distal phage proteins, gp48 and gp54, play a role in connecting to the baseplate, gp5 and the tail tip. It is not known whether the tail tip directly interacts with the inner membrane surface on the periplasmic side or if one protein of the injection complex binds to the membrane.

To investigate possible interactions between host plasma membrane or periplasmic components and phage tail proteins, binding experiments with purified T4 cell-puncturing device (gp27, gp5, gp5.4) proteins and spheroplasts of osmotically treated Escherichia coli cells are performed. Gp5C-gp5.4 complex was found to be crosslinked to the periplasmic protein PpiD. This suggests that gp5C-gp5.4 remains in the periplasm and possibly gp5* contacts the inner membrane allowing the translocation of the viral DNA.

Lysis process | bacteriophage | Holin | Endolysin | Bacillus cereus

Getting outside the cell: holins and endolysins used by the siphovirus Deep-Purple targeting the Bacillus cereus group

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The phage lytic cycle often ends with the release of the virion progeny through bacterial lysis. In tailed phages, this process is mediated by at least two proteins cooperating to ensure efficient bacterial killing and encoded in a lysis cassette: transmembrane proteins, called holins, and peptidoglycan (PG)-degrading enzymes, referred to as endolysins. In the canonical lysis pathway, holins form large non-specific holes in the inner membrane allowing the transport of endolysins from the cytoplasm to the periplasmic space where they break down the PG meshwork. Here we describe the holin and endolysin partners of phage Deep-Purple, a siphovirus infecting members of the Bacillus cereus group[1]. This phage encodes PlyP32, an endolysin that has a typical modular organization with a N-terminal muramidase domain and a C-terminal SH3b binding domain[2]. PlyP32 displayed antimicrobial activity against all the tested strains from the B. cereus group as well as a few other Bacilli while the binding range was specific to B. cereus. PlyP32 activity combined with its biochemical properties makes it an interesting candidate for the control and detection of bacteria from the B. cereus group. Regarding the holin, two proteins with holin features (i.e. small size, transmembrane domain and charged C-termini) are encoded in Deep-Purple lysis cassette: HolP30 and HolP33[3]. These candidate holin were expressed in Escherichia coli and induced bacterial lysis, albeit to different extent. Fluorescent experiments confirmed the localization of both proteins at the cell periphery when produced in both E. coli and Bacillus thuringiensis. In B. thuringiensis, the co-expression of both holins was required to observe lysis, suggesting that they may interact to form functional pores.

1. Hock L., Gillis A., & Mahillon J. (2018). Arch Virol 163, 2555-2559

2. Leprince A., Nuytten M., Gillis A., & Mahillon J. (2020). Viruses 12, 1052

3. Leprince A., Nuytten M., July E., Tesseur C., & Mahillon J. Submitted

LPS | Stepping stone mutations | Overcoming resistance

Evolutionary exploration of a bacterial genotype-phenotype map of LPS structures with phages

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Predicting phenotypes for a given genotype is the holy grail in biology. While it is easy to determine the genotype of almost any given organism, determining the corresponding phenotypes is difficult. For example, efforts have been made to understand the phenotypic diversity of lipopolysaccharide (LPS) structures through deletion and complementation experiments. However, this approach likely underestimates the available phenotypic diversity. To explore LPS diversity, we have generated LPS mutants in *Escherichia coli* C by selecting for ΦX174 resistance, a bacteriophage that solely relies on binding to the core LPS to infect its host. An analysis of 32 *E. coli* C mutants resistant to ΦX174 reveals that almost all carry a mutation in genes linked to core LPS biosynthesis or assembly. Based on which genes are mutated, we predicted their core LPS structures and tested our predictions by evolving phages to recognize each evolved LPS structure. Interestingly, different phages that evolved to infect the same predicted LPS structure were not always able to cross infect each other's hosts, suggesting that core LPS structure diversity was higher than predicted. Altogether, our results show that phages are a useful tool to study LPS structures, and conversely that the study of LPS structures helps to understand phage evolution and biology.

bacteriophage biocontrol | phage cocktail | Virus-host networks

Comparison of heuristic algorithms and exhaustive design of phage cocktails from host range matrices

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The misuse and overuse of antibiotics have triggered the proliferation of multidrug-resistant (MDR) bacteria, which are considered a major public health issue for the next decades. Phage therapy has become a promising alternative in the treatment of infections caused by MDR pathogens, without many side effects of current available antimicrobials. Phage therapy is frequently based on phage cocktails, that is, combinations of phages able to lyse the target bacteria. Designing phage cocktails might become extremely cumbersome and time consuming due to the large size of phage-bacteria infection matrices, which entails complex combinatorial analysis. In this work, we present five computational methods to design phage cocktails using host range matrices. Whereas some algorithms use global properties of the phage-bacteria infection networks, others evaluate individual phage-hosts interactions. All the methods are included in two different packages, developed for the platforms R and Cytoscape. Both packages are freely available for any user and provide relevant statistics. Additionally, we have compared the methods studying the variations in computational runtime and the quality of the results, measured by considering cocktail size and expected success (fraction of infected hosts). Finally, we suggest different pipelines depending on the bacteria targeted.

Listeria bacteriophages host interactions

The *Listeria* Phage-Host System: Interspecies Dynamics of Inducible Phage Elements from *Listeria monocytogenes*

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The *Listeria monocytogenes* (Lm) phage-host system includes lytic phages, prophages and monocins. Monocins have been described as defective prophages with similar structural proteins to Lm phages, such as receptor binding proteins. Our group has previously analyzed the broad host range of lytic *Listeria* phages. Data from these studies established that there is a distinction between the host range of a sample as determined by inhibitory activity versus plaque formation. Genomic analysis of 16 Lm genomes representing different genetic lineages and serotypes, showed a prevalence of both prophage and monocin genes. The objective of this study was to test if prophage and monocin genes are actively expressed in the host and determine their function in the Lm phage-host system.

Eleven of 16 strains house one or more predicted prophage; fourteen house a monocin locus. Bacterial cultures were induced by mitomycin C, centrifuged and filtered to collect potential phage elements, then diluted and spotted onto lawns of Lm to assess host range. Plaques were only observed in samples from 10403S, J1-0049, and H7858. Zones of inhibition with varied host range were observed for all samples. Based on genomic, experimental and TEM data, we hypothesize that monocin particles are responsible for the zones of inhibition. To investigate this, the monocin locus of select *Listeria* strains was expressed in a *B. subtilis* strain and assessed for host range. Our results verify that some of the observed inhibitory activity can be attributed to monocins, but other antibacterial elements may be a contributing factor.

This study demonstrates that prophages and monocins act as a selective pressure that shapes bacterial populations through interspecies inhibitory activity. The data suggests that host range analysis of lytic phages can be influenced by the presence of phage elements (such as monocins) in Lm strains. We suggest the deletion of the monocin locus in future phage propagation hosts.

Archaeal Virus | Archaea | Virus-host networks

Divide and rule: the role of eukaryotic-like cell division apparatus in archaeal virus infections

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Viruses that infect archaea represent one of the most enigmatic parts of the virosphere. They are highly diverse and share very few structural and genomic similarities with bacterial or eukaryotic viruses, suggesting that the mechanisms of virus-host interactions might be also unique. Here we study the relationship between archaeal viruses and the archaeal cell division apparatus based on the ESCRT (Endosomal Sorting Complex Required for Transport) machinery. In Eukaryotes, ESCRT machinery plays a key role in many membrane remodelling processes, including membrane abscission during cytokinesis, formation of extracellular vesicles and budding of enveloped viruses, such as HIV-1 and Ebola virus. In archaea, ESCRT machinery has been shown to mediate membrane constriction during cell division and budding of extracellular vesicles. However, whether the importance of ESCRT proteins can be extended to the release of lipid-containing archaeal viruses remains unclear. To answer this question, we exploited a collection of strains of *Saccharolobus islandicus*, a hyperthermophilic and acidophilic archaeon, in which different components of the ESCRT machinery were either knocked out or knocked down using the CRISPR technology. The *S. islandicus* strains were infected with two different spindle-shaped viruses and their replication was monitored with the aim of identifying the components of the ESCRT machinery that are important for infection. The results of this work should provide insights into new aspects of virus-host interactions in archaea and clarify the role of the cell division machinery in virus reproduction.

Pseudomonas aeruginosa | prophage | phage integration and excision

Revisiting LES prophages genomes and determining their integration in *P. aeruginosa* PAO1 under single and co-infection

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Pseudomonas aeruginosa is an important opportunistic pathogen, causing nosocomial infections. The Liverpool Epidemic Strain (LES), a significant cause of mortality and morbidity in cystic fibrosis patients, harbours five prophages associated with increased fitness and survival in infection models. However, ~76.5% of the LES prophage genes are hypothetical, encoding predicted proteins of unknown function. Moreover, the combined influence of the co-habiting LES prophages on the success of their bacterial host is not well studied. In this study, we re-annotated the original LES prophage genomes (Φ2, Φ3, Φ4, Φ5 and Φ6) and improved the prediction of gene function using the VIGA pipeline and manual curation. Additionally, we aimed to identify the molecular mechanisms by which they affect the biology of the *P. aeruginosa* host. We re-annotated the genomes of Φ2, Φ3 and Φ4 following lysogenic infection of the well-characterised *P. aeruginosa* strain PAO1 to create single, double, and triple lysogen variants harbouring all combinations of these prophages. We also determined alternate integration sites for each LES prophage when carried alone or in combination with others in the model host PAO1 genome. The reannotation of the LES prophages increased the number of putative coding sequences 1.17-1.43 times. We identified multiple genes related to DNA recombination and host cell lysis and new ncRNA elements in these prophages, mainly tRNAs in Φ2 and Φ5 and a Hammerhead-II ribozyme in Φ4. Finally, we retrieved high-quality DNA genomic backbone sequences for the different *P. aeruginosa* PAO1 lysogens. This information will be helpful for future RNAseq experiments to map the expression profiles of each LES prophage under inducing and non-inducing conditions to characterise interactions between the prophages and their lysogen host, being fundamental for unveiling the vast dark matter of temperate phages and enhancing our understanding of how bacterial and prophage genomes co-evolve.

Bacteriophages | host interactions | Cell wall

Host- Regulatory switch phage interaction: aberrant host cell's morphology emerges as consequence of lysogeny.

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Temperate bacteriophages are known as the main source of intra-species genetic diversity and as gene transfer agents. Among this category there are phages able to follow the regulatory switch mechanism (RS), a type of the lysogenic life cycle, but characterized by integration into functional genes. The prophage integration/excision results in a switch OFF/ON of the attachment gene expression in the host. Furthermore, as a consequence of the excision, the phage does not kill the host, but it is maintained as an episome in its cytoplasm. Investigating the host- RS phage interaction, we noticed that lysogeny for a specific RS phage results in an aberrant cell morphology. This phenomenon is visible also at a spore stage of the host bacterium. The unusual phenotype is stable and heritable. We also discovered that the superinfection with a second RS phage, belonging to the same group, restores the conventional rod-shaped morphology. This second phage appears to exert a protective role in preventing the morphological change caused by infection of the first phage alone. By TEM and light microscopy analysis we aim to investigate if the cause of this morphology shifting resides in the cell wall modification. A sequencing analysis is required in order to explore the impact of the double lysogeny on the host genome, in comparison with the single lysogens. The growth assay is also crucial to observe the consequence of single and double lysogeny by these peculiar prophages on host's fitness. The outcome of this research, through the investigation of the phage impact on host morphology, physiology and ecology, will bring us closer to understanding the role of temperate phages in controlling bacterial hosts, a knowledge that is crucial to control bacteria for use in biotechnology, medicine and agriculture.

Staphylococcus aureus | Chromosomal Island | DNA replication | helicases

Staphylococcal self-loading helicases couple the staircase mechanism with inter domain high flexibility

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DNA replication is a crucial biological process. Replicative helicases unwind DNA providing the template strand to the polymerase and promoting fork progression. Helicases are multi-domain proteins which use an ATPase domain to couple ATP hydrolysis with translocation by a process called the staircase mechanism. However, the role that the other domains might have during translocation remains elusive. Here we study the unexplored self-loading helicases called Reps, present in Staphylococcus aureus pathogenicity islands (SaPIs). SaPIs replicate autonomously because they harbor their own replication module composed by a primase, the aforementioned Rep and an origin of replication located downstream the rep gene. Sometimes the pri and rep genes are fused so that they generate a single functional polypeptide. We have solved the cryoEM structures of the PriRep5 (encoded by SaPI5), the Rep1 (SaPI1) and Rep1 in complex with single stranded DNA. In all cases they form a three layered ring-shaped hexamer where the ATPase domain is sandwiched between the amino and carboxi terminal domains (NTD and CTD). The CTD is responsible for ori recognition, binding and melting, in other words, initiation activity. We show that in both PriRep5 and Rep1, the CTD undergoes two extensive and distinct movements respect the ATPase domain: rotation and tilting. Surprisingly, we experimentally show that a CTD defective mutant looses its ATPase and helicase activity indicating that not only plays a role in the initiation but also during translocation. Our Rep1-ssDNA complex cryoEm structure allowed us to structurally characterize the staircase mechanism. In vitro and in vivo single point mutants confirmed that DNA contacting amino acids are necessary for Rep to provide fully replication support. We speculate that this high flexibility between domains couples Rep´s activity as initiators and as helicases.

Phage-host interactions | Stress response | Phage T5

Phage T5 encodes a new pathway for RpoS degradation, the master regulator of the general stress response in *E. coli*

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Host takeover by bacteriophages is a prerequisite for successful infection. In phage T5, it is uncoupled from the rest of the lytic cycle, thanks to T5 unique ability to transfer its genome in 2 steps. During the First-Step Transfer (FST), pre-early genes (about 8% of T5 DNA) are injected, which are sufficient to degrade host DNA, evade cellular defenses and hijack the cellular machineries. Both the FST-encoded A1 nuclease and A2 DNA-binding proteins are involved in host takeover, and required for resuming DNA transfer. In E. coli, the sigma factor RpoS is the master regulator of the general stress response (GSR). Under optimal growth conditions, RpoS interacts with the adaptor RssB and is then degraded by the ClpXP protease: the GSR is thus silenced. Under stressful conditions, anti-adaptor proteins prevent RssB/RpoS interaction, leading to RpoS accumulation, which can then fire the GSR. Phages evolved various strategies to temper with RpoS action, for example by preventing its binding to promoters as is the case with phage T7 Gp5.7 protein directly interacting with RpoS. Here we show in T5 that RpoS degradation via ClpXP is triggered upon infection during FST, independently from A1 and A2 but surprisingly also from RssB, the only known RpoS adaptor to date. The aim of our project is to investigate the FST gene product(s) responsible for this phenotype. We show here that a FST fragment only containing gp016 and its 100 bp-upstream regulatory region is sufficient to trigger RpoS degradation, suggesting a new pathway to target RpoS to degradation by ClpXP, and independently of RssB. Our bioinformatics analyses suggest the presence of a promoter and a putative small ORF of unknown function (ORFX) upstream gp016. We present here our investigations on the genetic elements encompassed in this FST small fragment required for RpoS degradation by ClpXP independently from RssB. This work highlights a novel strategy evolved by a phage to temper with the host GSR.

Staphylococcus aureus | hlb-converting phages | phage life-cycle regulation

Molecular cross-talk between Sa3int phages and their *Staphylococcus aureus* host

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As a major opportunistic pathogen of human and animals *Staphylococcus aureus* asymptomatically colonizes the nasal cavity, but is also a leading cause of life-threatening acute and chronic infections. More than 90% of the human nasal isolates of *S. aureus* were found to carry Sa3int phages, which integrate as prophages into the bacterial *hlb* gene thus disrupting the expression of the sphingomyelinase Hlb, an important virulence factor under certain infection conditions. The virulence factor-encoding genes carried by the Sa3-phages are all highly human-specific and probably essential for bacterial survival in the human host. Thus, both insertion of the prophages into and excision from the bacterial genome have the potential to confer a fitness advantage to *S. aureus*. However, how the *S. aureus* host modulates the life cycle of its temperate phages remains largely unknown (1). Our data suggest that the bacterial factors supposedly involved in the interaction of the bacterial host with its phages are strain specific, with certain *S. aureus* strains being more prone than others to support either a lysogenic or a lytic life cycle (2). We constructed and integrated Sa3int phages into different phage-cured *S. aureus* strains and found significant differences in phage transfer rates between different strains. Based on this finding, strains were grouped into low and high transfer strains. Indicating that in low transfer strains, the phages are more directed towards lysogeny. To get a more precise picture of the regulatory circuits we constructed replication deficient mutants, performed differential RNAseq to determine the transcriptional units and analysed a set of mutant strains. By this means some bacterial and phage genes were identified which are likely to play a role in the regulation of the strain dependent phage life cycle.

1. Rohmer, C. and C. Wolz. 2021. Microbial Physiolog 31: 109-122

2. Wirtz, C., W. Witte, C. Wolz, and C. Goerke. 2009. Microbiology 155:3491-9.

Archaea | Archaeal Virus | Genetics

A Novel Euryarchaeal Virus-Host System

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Viruses can have considerable impacts on host populations, from eukaryotic viruses causing pandemics that shake society to bacterial viruses which can massively impact major ecosystems. Like all other domains of life, Archaea face viral infections. However, while viral infections of eukaryotes and bacteria have been studied in detail, archaeal viruses and their infection mechanisms are less well understood. Particularly euryarchaeal viruses are still understudied. A robust, genetically accessible virus-host model system would greatly help drive research into this field forwards (Tittes, Schwarzer, & Quax, 2021).

Hypersaline lakes can be rich sources of halophilic euryarchaea and their viruses. A novel virus-host system was recently isolated from one such lake, consisting of the siphovirus-like *Haloferuvirus* Haloferax tailed virus 1 (HFTV1) and its host, *Haloferax gibbonsii* LR2-5 (Mizuno et al., 2019; Tittes, Schwarzer, Pfeiffer, et al., 2021). The strain grows well in defined media and is well suited for light microscopy. Furthermore, the strain is highly motile. Meanwhile, HFTV1 is a very specific virus, as it cannot infect *Hfx. volcanii* H26 nor the *Hfx. gibbonsii* strain Ma2.38. While the reason for this host specificity is not yet known, early bioinformatic analysis of the host indicates that its surface differs from closely related strains (Tittes, Schwarzer, Pfeiffer, et al., 2021). This indicates that specificity may be related to early infection steps. Both virus and host are fully sequenced and annotated (Tittes, Schwarzer, Pfeiffer, et al., 2021).

Recently, transformation and gene expression protocols have been established for *Hfx. gibbonsii* LR2-5. Due to its close relation to the model organism *Hfx. volcanii,* its lack of obvious defense systems and the presence of a known lytic virus, *Hfx. gibbonsii* LR2-5 and its virus are promising candidates for a new virus-host model system.

filamentous phage | uptake | Tol Pal system | virus-host protein-protein interactions

Characterization of the molecular mechanisms involved during filamentous phage import in its host.

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Filamentous phages are subclass Inoviridae . These non-lytic viruses can participate to the fitness of their bacterial host and carry pathogenicity factors. The mechanisms involved during infection are still poorly described, even for the well-known model couple Escherichia coli - phage Fd. In this case, the steps allowing the phage to cross the envelope involve the minor capsid protein pIII. This protein is organized into three distinct domains pIII-N1, pIII-N2 and pIII-C. During an initial stage of reception, the phage Fd binds to the pilus-F thanks to its pIII-N2 domain. Then the phage particle is translocated into the periplasm where pIII-N1 interacts with the TolA protein of the Tol-Pal system. The Tol-Pal system is a macro-complex of the envelope dependent on the proton-motive force and conserved in Gram-negative bacteria. This system is known to be involved in cell division and in the homeostasis of the cell envelope. Finally, the phage injects its genetic material into the host cytoplasm, possibly through an inner membrane pore consisting of pIII-C.

The literature suggests that the TolQ and TolR proteins of the Tol-Pal system also participate in phage import, although no direct interaction has been observed so far. In order to characterize the molecular mechanisms involved, we combined different approaches of biochemistry, genetics and phenotypic analyses. Our recent results provide new information that will help to decipher the key steps occurring during phage translocation across the host envelope.

Archaeal Virus | Halovirus | Haloarchaea | Host specificity | Haloferax

Insight into the host specificity determinants of viruses infecting *Haloferax*

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Viruses can infect members of the three domains of life. Yet, many aspects of the life cycle of archaeal viruses remain unexplored. For instance, the primary and secondary receptors of most archaeal viruses remain unknown. Factors determining host specificity are also not yet fully resolved. A recent study proposed the host range determinants to be virus specific, as closely related viruses display different host ranges[1]. In this study we aimed to get a better insight into the host range determinants in haloarchaeal viruses by using several species of the halophilic euryarchaeon *Haloferax* as a model system. Our results show that *Haloferax gibbonsii* LR 2-5 is an *Haloferax* strain that is infected by most viruses. On the other hand, our genomic analysis of viruses infecting *H. gibbonsii* LR2-5, suggests that adhesins are one of the key determinant of host range specificity.

1. Liu, Y. *et al.* Diversity, taxonomy, and evolution of archaeal viruses of the class Caudoviricetes. *PLOS Biol.* **19**, e3001442 (2021).

T4 phage protein RNA-seq

Dual transcriptome and proteome of E. coli infected by bacteriophage T4

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Bacteriophage T4 is a lytic phage from Myoviridae family infecting Escherichia coli. T4 is one of the most complexly built phages, whose genome is composed of 169 kbp and encodes 273 proteins.[1] These proteins are needed to infect E. coli in a highly regulated and efficient manner. The hijacking of the host genetic machinery starts simultaneously with the injection of the DNA into the host and ends after only ~25 min with the lysis of the bacterial cell. Even though research on phage T4 has enormously contributed to understanding the fundamental biological processes, the biological function of the majority of viral proteins remains unknown. Moreover, the molecular mechanisms for the controlled takeover of E. coli by bacteriophage T4 are poorly understood. Recent developments in multi-omics approaches set the foundation to unravel the molecular basis of the bacteriophage T4 infection and to study the mutual effects on bacterial host metabolism.

Here, we present the first application of the next-generation sequencing and protein-profiling technologies to study the infection of E. coli by bacteriophage T4 on the transcriptome and proteome level. We set out to investigate the transcriptome and proteome of both E. coli and phage T4 during the infection (dual transcriptome and dual proteome) in a time-resolved manner. Our data shows the temporally resolved appearance of bacteriophage T4 transcripts and proteins, which confirms previously described subgrouping for T4 gene products into early, middle and late [2]. Moreover, we investigated the stability of E. coli originated transcripts and proteins in the course of infection. This study aims to complete the overall understanding of the phage T4 infection process on the molecular level by providing the first comprehensive insight into the early transcriptomic and proteomic takeover by the bacteriophage T4.

Salmonella enterica | Phage-host interactions | motility | flagellotropic phage | Phage Therapy

Interactions between bacteriophage χ and the flagellum of the notorious pathogen *Salmonella enterica*

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Flagellotropic (flagellum-dependent) phages are viruses that begin their infection process by attaching to the flagellar filaments of their respective host cells and using the rotation to reach the cell surface. This results in the unique requirement of host motility for infection. Since motility is a significant virulence factor for many species of pathogenic bacteria, including *Salmonella enterica*, flagellotropic phages may be particularly effective as antimicrobial agents. Flagellotropic phages force an exploitable evolutionary tradeoff: a bacterial cell which represses motility to avoid infection by a flagellotropic phage would likely also attenuate its own virulence. Bacteriophage χ (Chi) is a flagellotropic phage which infects three species of potentially pathogenic organisms: *Salmonella enterica*, *Escherichia coli*, and *Serratia marcescens*. Interactions between phage and host flagellum are complex and poorly understood. It is well known that χ requires a motile host because cells lacking functional flagella are resistant to infection. The long tail fiber of χ phage is thought to wrap around the flagellar filament, positioning itself into the filament's grooves. At this point, the rotation of the filament pulls the phage to the cell surface like a nut moving down a bolt. However, the nuances of this interaction are understudied, as are the reasons why the flagella of some serovars of *Salmonella* allow χ phage adsorption while others are entirely resistant. Via targeted and random mutagenesis, we have determined specific domains within the flagellar filament protein flagellin that are involved in χ phage attachment. These findings help to elucidate the factors determining the host range of χ. With further knowledge about interactions between flagella and the phage tail fiber, coupled with χ phage's naturally broad host range, it may be possible to genetically engineer host-range mutants, which could be tailored to infect very specific strains of motile pathogens.

transcription regulation | molecular mechanisms | Phage ORFan | N4-like phage | transcriptomics

'Drc' matters: A small ORFan with a big role in phage transcription regulation

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ABSTRACT

N4-like phages are distinguished by a specific transcription pattern which allows these phages to shift transcription from early genes towards middle and eventually late genes [1]. These different stages are each marked by a separate RNA polymerase and accompanying single-stranded DNA-binding protein (SSB). Homologs of these proteins can be found amongst many N4-like viruses. However, multiple clades of N4-related viruses lack a homolog of the *Escherichia* virus N4 gp2. In N4, this SSB is crucial for activation of middle promoters by binding their ssDNA region and recruiting the N4 RNAPII complex by direct interaction [2].

Pseudomonas virus LUZ7 is one of the members without a gp2 homolog. We recently found that one of its ORFans, a gene without homology to any known gene, encodes a protein that acts as an SSB with a role similar to that of N4 gp2 [3]. Using X-ray crystallography we demonstrate that LUZ7 gp14 (termed Drc; ssDNA-binding RNA Polymerase Cofactor) achieves DNA binding with a unique protein fold that is distinct from that of known SSBs. Based on DNA and protein interaction studies we establish a model by which Drc activates transcription from middle gene promoters. Now, a novel RNA sequencing method we developed (ONT-cappable-seq) provides us a full transcriptional blueprint of this phage with unprecedented detail, which sheds further light on this peculiar transcription pattern and the role of Drc therein.

REFERENCES

[1] Lenneman, B. R. & Rhotman-Denes, L. B. (2015). *biomolecules*, 5: 647-667.

[2] Carter, R. H., Demidenko, A. A., et al. (2003). *Genes Dev.,* 17:2334–2345.

[3] Boon, M., De Zitter, E., et al. (2019). *Nucleic Acids Res., 48:445-459*

host interactions | infection process | lamdba

The role of small open reading frames in *Shewanella oneidensis* phage LambdaSo in host takeover and phage proliferation

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Many phages contain numerous genes that encode proteins of various sizes whose function is elusive. LambdaSo is one out of four prophages in the genome of *S. oneidensis* MR-1 with a genome of about 51 kbp. The genome of LambdaSo harbors a gene cluster, cluster C, whose deletion results in a drastic decrease in the production of plaque-forming units.

The cluster consists of six genes (*lcc1-lcc6*), which encode rather small proteins in the range of 41 to 137 amino acids with no apparent homologies to known proteins. Two of the proteins (Lcc4 and Lcc6) are predicted to possess a transmembrane domain. We found that deletion of *lcc6* results in highly elongated cells, in which phage particles are produced. However, the cells fail to lyse and to release the phage progeny from the cell. On the other hand, cells lacking *lcc4* do not display the usual elongation after phage induction. Overexpression of *lcc4* leads to significant cell elongation, which can be prevented by additional expression of *lcc6*. Lcc6 is also able to silence the cell filamentation effect of the SOS-response division inhibitor protein SulA. Protein interaction studies suggest that both Lcc4 and Lcc6 interact with proteins of the cell division machinery such as FtsZ, FtsW, FtsN and FtsI.

The results strongly indicate that the *lcc* genes are involved in the take-over of major host cell functions by interfering with cell division and elongation processes through direct interaction with the underlying cellular machinery.

Pseudomonas aeruginosa | virulence | lytic bacteriophages | antivirulence | Genomic dark matter | Phage-host interactions

Lytic phages: A treasure trove for virulence attenuating proteins against *P. aeruginosa*?

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Every year, the opportunistic pathogen *Pseudomonas aeruginosa* is responsible for a significant part of all health-care associated infections. The bacterium causes both acute and chronic infections associated with high mortality rates. Chemical compounds arresting essential, cellular processes are commonly used as a strategy against *P. aeruginosa* infections. However, these antibiotics place a burden on the bacterial cell, which might result in the development of resistance against this factor. Therefore, novel approaches to combat this critical pathogen are gaining attention. Bacteriophages can share both predatory and mutualistic relationships with their bacterial host. Moreover, it is known that temperate phages drive the bacterial virulence and some of those features have already been exploited for biotechnological purposes[1].

In this research, we performed several high-throughput analyses using a library of individually expressed, phage proteins to identify potential effectors of virulence factors of *P. aeruginosa*. We discovered four different phage ORFans that specifically and significantly attenuate key virulence factors of the pathogen, including the Type IV pili, protease IV (a factor secreted by the T2SS) as well as ExoS (a T3SS product). Next, we will determine the bacterial interaction partners of these phage proteins to elucidate their biological role.

To our knowledge, we are the first to prove that lytic bacteriophages are not only of interest for phage therapy or discovery and exploitation of novel antibacterial targets[2], but also for their virulence attenuating proteins. The identified phage-encoded virulence attenuators expand the diversity of regulatory mechanisms encoded by phages to impact the bacterial physiology and may serve as a source for diverse biotechnological applications.

[1] Schroven *et al.* (2021) *FEMS Microbiology Reviews*

[2] De Smet *et al.* (2017) *Nature Rev Microbiology*

bacteriophage S. aureus Phage-host interactions

vB_SauP_EBHT, a lytic phage with propagation host-dependent host specificity

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Receptor binding proteins of phage tails, such as tail fibers or spikes, are the main determinants of phage host specificity. The host range of a phage can change due to e.g. mutations in the tail fiber encoding genes. However, the role of the production host in the determination of phage host range is not fully understood. In this work, we introduce a Staphylococcus aureus –specific phage vB_SauP_EBHT (EBHT), a lytic Rosenblumvirus having a 17 kb genome. The peculiarity of EBHT is that changing its production host changed the host range as well, while the phage genome remains unaffected. The EBHT produced in the original isolation host S. aureus #6662, denoted as ϕEBHT, infected 40 % of the 109 tested Staphylococcus strains in contrast to the host range variant, denoted mEBHT and produced in S. aureus #6433, which infected 28 % of tested strains. The host range may change if EBHT acquires host-derived proteins that influences the phage host range or if the host restriction-modification systems differ between the hosts. Therefore, we studied the role of phage proteomes in the host range variation of EBHT. The purified phage particles were subjected to proteomics analysis and cryo-electron microscopy. Here, in addition to phage structural proteins, nine and eight proteins of host origin were identified from ϕEBHT and mEBHT particles, respectively. Both particles contained deblocking aminopeptidases, with sequence identity of 99 % and M42 glutamyl aminopeptidase with 98 % sequence identity, while the other host-derived proteins had house-keeping functions. However, the role of these peptidases remains to be confirmed. EM has so far revealed no apparent structural differences between the variants; further high-resolution analysis of the tail is required in establishing the cause of the differing host ranges. To conclude, the phage production host may have more significant role in determining the host range than what has been this far understood.

phage-host interactions | E.coli | Y. enterocolitica

Phage host range alteration via *in vivo* recombination

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Phage host ranges are largely determined by the binding specificities of phage tail or spike proteins. Phages can be trained for broader host range or to infect specific bacterial strains, but this is time consuming and relies on chance for specific mutations. In T3 and T7 group of phages, the major adsorption determinants are tail fibers encoded by gene *17* (Gp17). In this work, we studied if Gp17 of T7 group of viruses is the sole host range determinant using T3 and ϕYeO3-12 as model viruses. While the two phages are highly similar with 94% genomic sequence identity, T3 infects *Escherichia coli* and ϕYeO3-12, *Yersinia enterocolitica* serotype O:3. Accordingly, the Gp17 amino acid sequences of the phages are only 51% identical. We set up an experiment to replace the T3 gene *17* with the ϕYeO3-12 homologue by *in vivo* recombination to find out if that would alter the host range of T3. To this end, plasmid pGp17T3, carrying the ϕYeO3-12 gene *17* flanked by T3 specific regions was introduced to the *E. coli* strain C600. The C600/pGp17T3 bacteria were then infected with the T3 gene *17* amber mutant 17amH26, and the obtained phage lysate was screened for *Y. enterocolitica* specific recombinant phages using strain YeO3-c as host. The isolated recombinant phage, named H3, was not stable, with its titer dropping 97% even within a week. However, the stability increased when H3 was passaged on YeO3-c bacteria for six serial rounds. The adapted variant, called H3VI, only suffered from 40% titer loss in 4 weeks and 60% in 8 weeks. There was also a clear change in the plaque morphology, the diameter increasing from the original 1 mm of H3 to 3 mm of H3VI. Both H3 and H3VI are being sequenced to get insight on the exact induced genetic changes and the molecular mechanism of host adaptation. To conclude, the tail fiber protein Gp17 was enough to change the host specificity of phage T3, but there are other factors affecting the adaptation of the phage to a new host.

Stationary phase | Bacillus subtilis | Bacteriophage ecology | SPP1 | Nutrient limitation | Irreversible adsorption | Lytic bacteriophage

Persistent lytic phage infection as a novel strategy for exploitation of nutrient-limited bacteria

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Most wild bacteria live under nutrient limitation in states of low or arrested metabolic activity. Given the high biosynthetic costs of phage production this poses a major challenge for phages, who must stage productive infections in order to persist in the environment. Temperate phages may circumvent this issue through lysogeny. Strictly lytic phages however, cannot exploit nutrient deprived hosts in this manner, and have often been assumed to require actively growing hosts for productive infection. Here, we asked if, and how, a strictly lytic phage may exploit the non-growing, nutrient limited hosts they are most likely to encounter.

We discovered that the lytic phage SPP1 successfully infects stationary phase populations of the Gram-positive bacterium Bacillus subtilis. Slow, albeit continuous production of low numbers of infective phage particles characterise the first 10 hours of infection, followed by extensive culture lysis and release of remaining intracellular phages. Ongoing infections are also observed after extensive lysis, likely fueled by lysis-mediated nutrient release. Importantly, nutrient influx during stationary phase infection also led to a significant increase in phage production.

Host cell phage receptor availability influenced infection dynamics, but not phage yield, showing that adsorption limits the rate but not productivity of stationary phase infection. Throughout infection, phage late-gene expression and procapsid scaffolding protein production, the latter a reporter for viral particle assembly, were lower than during peak phage production in exponential phase, reflecting low stationary phase infection productivity. Together, our results reveal a novel infection strategy of lytic phages, characterised by persistent infection, gradual phage release and maximal exploitation of the host population. This represents at least one way in which lytic phages have adapted to productively exploit nutrient-limited bacteria in nature.

Pseudomonas aeruginosa | transcription regulation | phage-based synthetic biology | nanopore sequencing | transcriptomics

ONT-cappable-seq: a new approach to explore the transcriptional architecture of bacterial viruses

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Classic RNA sequencing has become the method of choice to profile the transcriptional landscape of phage-infected bacteria. However, short-read RNA sequencing approaches generally lack the capacity to differentiate between the original termini of a transcript and their processed counterparts and fail to capture RNA molecules in full length. This impairs the discovery of key transcription initiation and termination events and hides information on operon organisation. Yet, the elucidation of these elements is fundamental to achieve a global understanding of gene regulation mechanisms during the infection process, which is critically important to develop alternative strategies to combat bacterial pathogens and for the development of SynBio applications inspired by phages. To this end, we developed ONT-cappable-seq, a specialized long-read RNA sequencing technique that allows end-to-end sequencing of primary prokaryotic transcripts using the Nanopore sequencing platform. We applied ONT-cappable-seq to study the transcriptional architecture of phages infecting *Pseudomonas*. ONT-cappable-seq provides a comprehensive genome-wide map of viral transcription start sites, terminators and complex operon structures that fine-regulate gene expression. Furthermore, the regulatory elements were experimentally validated using a combination of primer extension assays and *in vivo* fluorescence assays. Our new method enables the exploration of dense phage transcriptional landscapes in unprecedented detail and can provide new insights in their still cryptic biology and transcriptional regulatory features.

campylobacter | Phage-host interaction | phase variation | Receptor binding protein | Recombination | prophage | Fletchervirus | phage-host adaptation

Adaptable host recognition of *Fletchervirus* phages infecting *Campylobacter jejuni*

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Fletchervirus represent a unique group of phages infecting specifically *Campylobacter jejuni*. These phages are generally dependent on capsular polysaccharides (CPS) for infection, where the phase variable O-methyl phosporamidate (MeOPN) modification functions as a common phage receptor.

We have shown that *Fletchervirus* phages encode up to four different receptor binding proteins (RBP1 to RBP4), where RBP1 is responsible for binding to the MeOPN receptor. However, in the absence of MeOPN, *Fletchervirus* phages can bind and infect *C. jejuni* through expression of RBP2. Interestingly, both RBP2 and RBP3 contain hypermutable polymeric G (polyG) tracts promoting phase variable expression by slipped strand mispairing during DNA replication. Thus, *Fletchervirus* phages mimic their host by phase variable RBP expression creating phenotypically diverse phage populations able to infect *C. jejuni* independently of the variable MeOPN receptor. Moreover, phase variable expression of both receptor and RBPs generates population dynamics allowing *C. jejuni* and *Fletchervirus* to co-exist in their shared niche, the chicken gut.

Fletchervirus phages are only distantly related to other phage genera and phages within the genus are highly conserved, including the sequences of RBP1-RBP4 responsible for binding to the CPS. Phage F341 however, is an unusual *Fletchervirus* phage, as it infects *C. jejuni* independently of the CPS, but instead relies on motile flagella for successful infection. We discovered that phage F341 encodes a novel RBP consisting of an N-terminal with sequence similarity to RBP1 and a C-terminal identical to the tail fiber protein H found in the unrelated cryptic CJIE1 prophage present in several *C. jejuni* genomes. These findings suggest recombination events between lytic *Fletchervirus* phages and the CJIE1 prophage. Thus, cryptic prophages in *C. jejuni* genomes may represent a genetic pool from where the highly conserved lytic *Fletchervirus* phages can acquire novel traits.

competition Temperate Phage gastrointestinal microbiome

Novel bacteriophage Kapi1 modifies host O-antigen and contributes to kin-recognition and competition in *Escherichia coli*

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The gut microbiome is a vital part of human health, however, our understanding of the viral aspect of the microbiome is lagging far behind our understanding of the bacterial component. Studies have shown that most enteric bacteria are lysogens, suggesting a role for temperate phage in the gastrointestinal (GI) environment. We aim to explore the interactions between temperate phage and their bacterial hosts to understand the role(s) of temperate phage in the mammalian GI tract. In this study, we isolated a novel temperate phage Kapi1, infecting commensal *Escherichia coli*. Kapi1 interacts with its host in complex ways, including modification of the O-antigen via lysogenic conversion. Intriguingly, we also find that Kapi1 lysogens behave differently when grown in simulated intestinal fluid, favoring the lysogenic cycle more strongly than they do when grown in conventional lab media (LB). Kapi1 lysogens also strongly out-compete non-lysogens in the murine GI tract. We further explored the role of Kapi1 in interbacterial competition *in vitro* and show that Kapi1 is induced when lysogens are grown in the presence of non-lysogenic competitors. This suggests that Kapi1 lytic replication is important for interbacterial competition, and that Kapi1 lysogens can sense when they are in competition. We determined that Kapi1-mediated kin-recognition likely requires cell-cell contact, as spent supernatant from non-lysogenic cultures do not induce a lytic response in Kapi1 lysogens. We hypothesize that Kapi1-mediated kin-recognition is somehow mediated by the differing O-antigen structures between Kapi1 lysogens and non-lysogens. These findings shed light on the complex nature of phage-host interactions in physiologically relevant conditions, supporting a strong role for temperate phage in the GI environment, as contributors to bacterial competition and kin-recognition.

prophages | Pseudomonas aeruginosa | cystic fibrosis

The role of temperate bacteriophages in the polylysogenic Liverpool Epidemic Strain of *Pseudomonas aeruginosa* PAO1

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Pseudomonas aeruginosa is one of the major causes of morbidity in cystic fibrosis (CF) patients. *The* Liverpool Epidemic Strain (LES) is associated with more severe disease than other *P. aeruginosa* strains and is transmissible between hosts. Previous studies revealed five novel prophages in the LES genome, and all the phages were detected as free phages in the sputum of CF patients. Moreover, phages Φ2, Φ3 and Φ5 were shown to confer competitive advantages to their host in a rat model, but the specific mechanisms are not known. Our study aims to identify the molecular mechanisms underpinning how LES prophages impact the biology of their *P. aeruginosa* host. We used the laboratory strain of *P. aeruginosa* (PAO1) that is susceptible to lysogenic infection by three LES phages. This enabled the construction and whole-genome sequencing of constructed lysogen variants harbouring three LES prophages (specifically Φ2, Φ3 and Φ4) in all possible combinations, creating single, double, and triple lysogens. To compare the gene expression landscape of naïve PAO1 vs lysogens, we first used growth profiling to identify the conditions in which phage production was minimal and extensive.We used qRT-PCR to qualify and quantify the expression profiles of key genetic markers for lysogeny along with early and mid/late lytic replication of the LES phages to validate the stability of each prophage and the timing of the replication cycle of each phage, respectively. Further, whole transcriptome studies of these lysogens, grown under varying conditions, will help identify how the LES prophages impact the phenotype of their host cells in the CF lung.

I**mportance**: Our study is designed to unpick how the LES phages have increased the fitness of their host through specific phage host interactions. Further investigation in clinically relevant conditions could identify unique targets for tackling recalcitrant *P. aeruginosa* infections.

bacteriophage | infective endocarditis | phage-resistance

Treatment of *Staphylococcus aureus* experimental infective endocarditis with *Podoviridae* selects for *tarS* mutations.

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Background: *Staphylococcus aureus* is one of the most prevalent multi-drug resistant bacteria and a leading cause of nosocomial and health-care related infections. Despite aggressive treatments, *S. aureus* infections are still associated with substantial mortality rates reaching 50% in some instances. We have been evaluating the use of bacteriophages cocktail in combination with β-lactams for the treatment of methicillin sensitive *S. aureus* (MSSA) experimental endocarditis (EE) in rats and isolated phage-resistant mutants.

Aims: To characterize MSSA phage-resistant mutants selected during EE after 48h of intravenous therapy with a two-phage cocktail (*Podoviridae* phage 66 and *Myoviridae* phage vB_SauH_2002), combined with sub-therapeutic doses of flucloxacillin mimicking human kinetic treatment of 2g given every 12h.

Results: We screened 36 MSSA isolates recovered from cardiac vegetations after 48h therapy and identified 13 resistant mutants to the *Podoviridae* phage 66 but none to the *Myoviridae* phage vB_SauH_2002. Comparative genomics revealed in the genomes of the phage 66-resistant clones, previously undescribed mutations in *tarS* encoding for a cell wall teichoic acid β-glycosyltransferase, i.e. (i) an IS257 insertion, and (ii) a 11 nucleotides indel leading to a premature stop. These mutations likely lead to the expression of a truncated non-functional TarS protein. Complementation *in trans* of the mutated genes with the wildtype *tarS* gene fully restored *Podoviridae* phage susceptibility of both mutants.

Conclusion: Point mutations in *tarS* gene have long been recognized *in vitro* as a major *S. aureus* resistance mechanism to *Podoviridae*. Our study confirms for the very first time that mutations in *tarS* also occur *in vivo* during phage therapy and revealed two new mechanisms of mutations. As for antibiotics, a thorough understanding of resistance mechanisms to phages that occur *in vivo* during treatment is needed to avoid therapeutic failure in the future.

bacteriophage parasite predator

Are bacteriophages parasites or predators?

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Bacteriophages have played crucial roles as models in genetics and molecular biology, and as tools in genetic engineering and biotechnology, for many decades. Their essential role in natural environment and their importance in human health and disease are also commonly recognized. Despite our understanding of mechanisms of development of bacteriophages, in the literature, these viruses are described as either parasites or predators. However, in biology and ecology, there are fundamental differences between parasites and predators. Therefore, I asked whether bacteriophages should be classified as former or latter biological entities. Analysis of the literature and biological definitions led me to the conclusion that bacteriophages are parasites rather than predators, and they should be classified and described as such. On the other hand, strictly virulent bacteriophages can be included into the group of parasitoids (being at the border between parasites and predators, as they propagate in living organisms, like parasites, but eventually kill their hosts, like predators), while phages which lysogenize host cells and those which develop according to the chronic cycle, like filamentous phages, reveal features of classical parasites.

S. aureus | Phage-prophage interactions | High Frequency transfer of bacterial DNA

Phage-Prophage interactions result in Lateral Transduction-like transfer of bacterial DNA

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Bacteriophages (phages) play a major role in driving the adaptation and evolution of their hosts, owing to the accessory genes that they carry and to their ability to transfer bacterial DNA. Phages can adopt two distinct life cycles: lytic or lysogenic. The majority of *Staphylococcus aureus* (*S. aureus*) strains are lysogenic, and some isolates may contain up to 4-6 prophages. Therefore, most bacteria-phage interactions in *S. aureus* involve more than one phage, as lytic replication often takes place in lysogenic or poly-lysogenic strains. Here we propose that *pac*-type phages recognise the DNA-packaging signals of other phages with DNA-packaging modules like their own. In this work, we studied *S. aureus* phages phi11 and 80a, which are two distinct phages with highly related small terminase (TerS) proteins. To interrogate our hypothesis, we infected a phi11 lysogen with 80a and showed that a cadmium resistance (Cd) marker inserted 5Kb downstream of the phi11 *attB* site was packaged and transferred at very high frequencies compared to the rates of generalised transduction. High frequency transfer of the Cd marker was dependent on the phi11 *pac* site, showing that 80a recognised and initiated DNA packaging from the phi11 prophage. Furthermore, analysis of the DNA packaged in 80a capsids (from infection of phi11 lysogens) revealed that a relatively high percentage of the reads mapped to the phi11 genome downstream of the DNA-packaging module and continued on into the bacterial chromosome. These results show that phage-prophage interactions can result in bacterial gene transfer that is similar, in effect, to the recently identified mechanism of lateral transduction, in which large portions of bacterial chromosome are packaged and transferred at high frequencies. This phenomenon in turn can contribute to genetic exchange among bacteria, driving adaptation and promoting their fitness and survival.

Phage-host interactions | transcriptomics | viral non-coding RNA transcripts | RNA-based regulation | phage-based synthetic biology | lytic bacteriophages | Pseudomonas aeruginosa

Hunting for phage-encoded small non-coding RNA transcripts

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Resolving the mechanisms used by bacteriophages to infect and replicate within host cells has been the focus of many studies, improving our knowledge on protein-based regulation during infection. However, to fully understand the infection process on a molecular level, RNA-RNA interactions between phage and bacterium need to be studied in greater detail. To achieve this, the availability of RNA sequencing data is key, as it facilitates the discovery of small non-coding RNA transcripts that are largely missed during phage genome annotation. In this regard, the number of classical RNA sequencing studies has steadily increased, offering a first look into possible non-coding RNAs encoded by phages. Furthermore, the integration of classical and global RNA studies, will provide novel insights into potential RNA-based regulatory elements as highlighted by our gradient-sequencing approach of model giant virus ϕKZ.

To date, only a handful of potential viral small RNA transcripts have been identified, in strictly lytic phages, thought their functions remain unknown. In the present study, we aim to elucidate the function of small RNA transcripts of lytic *Pseudomonas* phages and investigate their impact on the host cell. In ongoing experiments, we observe various phenotypic changes of the bacterial cell in relation to the expression of small RNA transcripts of different phages, such as delayed growth and changes in motility. Moreover, transcriptional data from *Pseudomonas* cells pulse-expressing viral non-coding RNA transcripts will be analyzed to reconcile our phenotypic observations with the molecular signaling pathways that are impeded during expression. Through this, we hope to uncover interaction partners of these transcripts that would be subsequently validated. This study would allow us to draw conclusions pertaining to the function and impact of such viral RNA transcripts during the phage infection process and their potential in diverse fields such as synthetic biology.

Phage-host interactions | HiC | Phage genome dynamics

Choreography of phages PAK-P3 and PhiKZ genomes during *Pseudomonas aeruginosa* infection.

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If bacteria appears to be the most diverse cellular domain of life on earth, they are outnumbered by the viruses that can infect them. With an estimation of 10^{31} particles, bacteriophages (phages) are the most abundant genomic entities across all habitats and a major reservoir of genetic diversity. Up to date, the vast majority of sequenced phage genomes are dsDNA and smaller than 100kb. In recent years, several publications have expanded our understanding of phages biodiversity and demonstrate the existence of phages with large genomes, rising numerous questions concerning how these genomes folds within their capsid but also during their infection cycle. Recently, a study proves the existence of a compartment that separated viral DNA from the cytoplasm in *Pseudomonas chlororaphis* phage 201f2 and demonstrates that large phages have developed innovative mechanisms to succeed in their infection cycle. Large phages typically contain more genes implicated in genome replication, nucleotide metabolism or coding for DNA binding proteins and could, therefore, have developed new strategies concerning their 3D genome organization and the hijacking their host.

To tackle this question, we have used chromosome conformation capture (HiC) to characterize the phage-host genomes interactions during the infection of *Pseudomonas aeruginosa* by two different phages, PAK-P3 and fKZ (PAK-P3 is a 88kb virulent phage, and fKZ is a 280kb giant bacteriophage). We performed a kinetic of both infection cycles and followed, concomitantly, the variation of genomes architecture through time. Our data show a correlation between variations in phages genomes folding and its transcriptional program. In parallel, we observed a global disorganization in the host genome, with a decreasing of the signal of the observed borders in the genome. Our results demonstrate that phages are highly dynamics genomic entities when they are active and pave the way to in-depth analysis of their infection cycle.

Klebsiella phage | capsule degrading depolymerase | capsular polysaccharides | Phage resistant mutants

Resistance to phage-derived depolymerase as a tool for *Klebsiella pneumoniae* infection control and virulence analysis

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Unlike conventional antibiotics, phage-borne depolymerases designed to disarm (anti-virulence) rather than kill bacterial pathogens. Therefore, they provide promising therapeutic alternatives that will be ‘evolution-proof’ and ‘tailored-pathogen'. Hence, the question of the depolymerase-resistant bacterial phenotype is important and to whether and how depolymerase-driven resistance will be developed and spread.

In this study, we analysed the phenotypic and genotype changes in *K. pneumoniae* mutant selected by the capsule-targeting phage-derived depolymerase. Based on a comprehensive genome-wide examination combined with the surface polysaccharides structural analysis, we prove that point mutation in the *wbaP* located within the *cps* cluster confers the resistance against depolymerase and phages using the same bacterial target for adsorption. Simultaneously, resistance to capsule-targeting depolymerase sensitizes *K. pneumoniae* to phages recognizing an alternative receptor. Loss of functional WbaP abolishes capsule formation at the first possible step and interestingly, increase the production of smooth LPS. This indicates a redirection of the cellular Und-P pool to LPS synthesis, without adversely affecting bacterial fitness.

The modification of the surface polysaccharide layers did not affect antibiotic susceptibility or complement-mediated killing. However, it made bacteria more prone to phagocytosis by monocyte/macrophage cell line combined with the higher adherence and internalization to human lung epithelial cells. This probably represents an innate defence mechanism to contain the infection of some respiratory pathogens.

Summing, the phage-borne depolymerases is doubly effective in the light of their application as anti-virulence agents: first, because of the enzymatic degradation of bacterial protective shields, and second, by the selection of resistant population presenting a decreased potential for pathogenicity.

adaptation prophage transcription regulation

YbcM, a transcriptional regulator from prophage origin involved in *Escherichia coli* physiology

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Temperate phages have the particularity to perform a lysogenic cycle resulting in their insertion into the bacterial genome; they are then referred as prophages. Genes carried by prophages can be expressed and confer selective advantages to their host such as virulence factors acquisition or increased fitness or stress resistance. However, only few studies have been undertaken to elucidate the molecular mechanisms improving the bacterial physiology. We decided to investigate the role of transcriptional regulators encoded by prophages in *E. coli* physiology. The *E. coli* MG1655 strain carries 9 prophages that contain 9 transcriptional regulators unrelated to classical regulators of phage cycle. Among them, YbcM, a transcriptional regulator from the AraC/XylS family encoded by the DLP12 prophage has never been characterized. Only two global studies suggest a potential role for this regulator in motility and oxidative stress response. We first confirmed that YbcM is involved in the control of bacterial mobility. Indeed, YbcM overproduction leads to an inhibition of motility, whereas its deletion makes the cell more motile. In order to identify genes directly regulated by YbcM that can explain this defect in motility, we performed ChIP-Seq experiments. We identified 8 direct YbcM-targets involved in diverse pathways, suggesting a broader role for YbcM on bacterial physiology. In parallel, we also focused on *ybcM* regulation. By using different reporter fusions, we show that *ybcM* is regulated at the post-transcriptional level; this regulation involves RpoS, the master regulator of the general stress response, therefore including YbcM in the adaptive stress response network.

Altogether our results show that YbcM is imbedded in the bacterial regulatory network and suggest its implication in stress adaptation. This work will help to give a global vision of prophage contribution to the physiology of their bacterial host.

RNA phage | lysis protein | membrane association

Mutational analysis of RNA phage lysis protein to define the regions required for membrane association

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Single-stranded RNA phages such as PP7 achieve host bacterial lysis by expressing a single subunit lysis protein (LP). However, the lysis mechanism by LP remains elusive. In this study, we created an LP-mediated cell lysis system by using the LP gene from the Pseudomonas aeruginosa phage, PP7. The PP7 LP gene was cloned into an IPTG-inducible mini-Tn7-based vector, with the ribosome binding sequence optimized for translation in P. aeruginosa. Based on the sequence characteristics, the 55-aa LP is divided into 3 regions: the N-terminal hydrophilic region (1-20 aa), the transmembrane domain (21-43 aa), and the C-terminal hydrophobic region (44-55 aa). A total of 14 point mutants were constructed and tested for their capability of membrane association and host lysis. Those from the transmembrane domain resulted in complete loss of the killing activity, suggesting their presumable role in membrane association for host lysis. The membrane association of the wild type LP was visually verified by N-terminal mNeonGreen tagging in Escherichia coli, whereas the mutants for the transmembrane domain lacked membrane association. Moreover, the LP function was associated with reactive oxygen species and displayed synergistic effect with antibiotics. These results demonstrate that LP is associated with bacterial cytoplasmic membrane involving the transmembrane domain which results in membrane disruption to ensure the appropriate host lysis.

Lipid A acylation RNA phage phage lifecycle

Lipid A acylation is required to prevent RNA phage entry in Pseudomonas aeruginosa

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The host range of a phage is determined at the various lifecycle stages during phage infection. We previously reported the limited phage-receptor interaction between the RNA phage, PP7 and its host Pseudomonas aeruginosa strains including PAO1 and PA14. PAO1 has susceptible type IV pilus (TFP) pilin, whereas PA14 has resistant pilin. In this study, we have created a PA14 derivative (PA14P) that has the pilin gene (pilA) replaced with that from PAO1 and found that PP7 did not form discernable plaques on PA14P unlike on PAO1, suggesting that other determinants than pilin could limit the PP7 infectivity in PA14. A total of 31,474 transposon mutant clones of PA14P were screened to isolate 11 mutants, on which PP7 formed clear plaques as on PAO1. Two of those mutants had the transposon insertion in a gene (htrB2) encoding an acyltransferase in lipid A biosynthesis. The lack of this enzyme increased the entry of RNA phages, which is deemed attributed to the loosened lipopolysaccharide (LPS) structure of the htrB2 mutant. Polymyxin B treatment also increased the RNA phage entry, suggesting that the compromised lipid A structure allowed the entry of the RNA phages. These results demonstrated that the LPS structures that might differ in various strains could limit the entry of RNA phages, providing a new determinant for the host range during the phage lifecycle in diverse P. aeruginosa strains.

bacteriophage | phage-host interactions | Antimicrobial | DNA replication | Escherichia coli | Phage T5

A T5 phage protein exploits the bacterial UNG to selectively nicks dUMP containing DNA

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T5 is an obligatory lytic phage of the siphoviridae family; injects its genome in 2 stages: first step transfer (FST) and second step transfer (SST). The function of most of the pre-early genes that reside within the FST region remains to be understood. Bacteriophages have evolved for eons a myriad of mechanisms to take over the host cells. Here we identify a T5 pre early gene product, T5.015 which upon expression inhibits bacterial cell growth. The whole-genome DNA-seq based method identifies E. coli DNA repair protein uracil DNA glycosylase (Ung) as the target of T5.015. Ung works by removing deoxy-uracil from DNA which was incorporated as an error in replication or as the result of cytosine deamination. The glycosylase activity of Ung generates abasic sites which are further cleaved by cellular enzymes and thus the DNA got repaired. Pull-down experiment, as well as fluorescence microscopy, shows that Ung directly interacts with T5.015. In-vitro experiment with purified protein shows that T5.015 uses the activity of Ung to selectively nick dUMP containing DNA. The endonucleolytic activity of T5.015 leads to replication blockage followed by blocking cell division which was evident from microscopy data as the cells were getting elongated upon induction of T5.015. Thus by inhibiting replication and cell division, T5.015 may enhance the utilization of host cell resources. Previous reports indicate lower incorporation of dUMP in phage DNA due to phage-encoded strong dUTPase activity. This might be the reason why the phage DNA was protected from the endonuclease activity of T5.015. We strongly believe that this kind of self vs foreign discrimination mechanism is general to many different kinds of bacteriophages.

Phage-host interactions | quorum sensing | Staphylococcus aureus

Quorum-sensing Signal Induces Phage Stab20 Infection on *Staphylococcus aureus*

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Quorum sensing (QS) is a cell-cell communication system, whereby bacteria sense population densities and respond accordingly. Recent reports suggest that some phages have evolved to eavesdrop or utilize their hosts' QS systems to achieve their intra- or inter-species communication, revealing a new sight in bacteria-phage interactions. However, such 'communication' between the human pathogen, *S. aureus* and its phages remains to be studied.

In this study, we examined the susceptibility of a phage-free *S. aureus* strain Newman to the infection by the lytic phage, Stab20. We found that wild type cells could only be infected in late stationary by Stab20 while mutants lacking the *agr* QS system could not be infected. These results indicate that QS induction is required for Stab20 infection. In *S. aureus*, QS is mediated by auto-inducing, cyclic peptides (AIPs) and while *S. aureus* peptides induce QS controlled gene expression, AIPs from a variety of coagulase-negative staphylococci inhibit QS in *S. aureus*. Interestingly, addition of *S. aureus* AIP to wild type cells allowed Stab20 infection already during exponential growth while the addition of the inhibitory *S. hyicus* AIP prevented infection. In *S. aureus*, the cell wall is decorated by wall teichoic acids (WTAs) and they appear to be primary phage receptors. By RT-qPCR we observed a lower expression of *tarM* that are responsible for α-O-GlcNAc glycosylation in QS induced cells compared to untreated cells, indicating that such a difference could account for the increased susceptibility to Stab20. Analysis of the cell wall by Fourier Transform Infrared Spectroscopy analysis indicated that deletion of *agr* contributed to the loss of the β-O-GlcNAc modification of WTA, suggesting this type of WTA glycosylation is required for phage Stab20 infection.

Based on these findings, we propose that QS signals from *S. aureus* can help with phage infection by affecting the biosynthesis of WTA on the cell wall.

infection process | complexes | translational control

Giant phages hijack post-transcriptional regulation and translation in the host

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The Gram-negative bacterium Pseudomonas aeruginosa is a major cause of nosocomial infections due to an intrinsic antibiotic resistance, a large genome, and wealth of regulatory mechanisms. Stress drives regulation and adaptation, hence we asked how the cellular complexome changes upon application of a biological stress in form of a phage infection for which the molecular mechanisms are still poorly understood. To illuminate how the phage transcriptome and proteome merge with cellular complexes and affect RNA-based regulation, we analysed RNA and protein complexes in glycerol gradients coupled to sequencing and mass spectrometry (Gerovac et al. 2021, Grad-seq: Smirnov et al. 2016). We selected the giant bacteriophage ΦKZ that encodes for hundreds of uncharacterized factors but no non-coding RNAs (Wicke et al., 2021). Notably, we observed phage transcripts that sedimented like non-coding RNAs. In the host, non-coding RNAs shifted in sedimentation profiles towards ribosomal fractions together with a high load of phage transcripts. Translation of host transcripts was inhibited. Strikingly, we observed phage proteins in ribosomal fractions that do not match with known translation factors and could mediate new modes of post-transcriptional control. In conclusion, the translation machinery is not just overwhelmed by phage transcripts but also post-transcriptionally regulated, which makes new opportunities for biotechnological applications.

Mycobacteriophage | Endolysin | Tuberculosis

Biochemical characterization of Lysin A activity of Mycobacteriophage TM4

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Bacteriophage endolysins are crucial for progeny release at the end of the lytic cycle. In TM4, a bacteriophage that infects mycobacteria including *M. tuberculosis*, a cassette encoding putative LysA, LysB and holin (gp29-30-31) was identified.

Through bioinformatic analysis we were able to establish that LysA consists of 3 modules; a C-terminal domain that probably binds to the cell wall, a central domain with high similarity to an amidase-2 and an N-terminal domain proposed to encode for a peptidase. In the amidase domain, the catalytic Zn ion is coordinated by His226, His335, and Asp347 and we also identified the amino acid Glu290 as the catalytic residue.

Four derivatives of the protein containing a mutation on each of these key residues were constructed.

Purified fractions of LysA were incubated with MDP, a synthetic molecule that emulates the bonds on peptidoglycan. The products of the reaction were analyzed by HPLC-MS, and N-acetyl-muramic-acid and L-Ala-D-isoGlutamine dipeptide were detected, confirming that LysA has an amidase activity, as predicted *in silico.*

We also assessed the ability of LysA to lyse *E. coli* or *M. smegmatis* from within, monitoring the optical density of cultures transformed with a plasmid expressing either LysA WT or the mutants. There was a significant decrease in optical density of the cultures expressing the LysA WT version but no lysis was observed in any of the mutants. These results indicate that the four predicted residues are essential for the function of the protein *in vitro* and when expressed in a homologous or heterologous host.

We also generated phages either carrying the E290 or the H266S mutations. Interestingly, no difference in the efficiency of plating was found between the mutated versions of the phage and the WT. This could indicate that either these mutations are not sufficient to abrogate lysis *in vivo*, or that a cryptic endolysin gene is encoded in TM4 genome

bacteriophage | tail spike protein | depolymerase

Spontaneous mutations and reversions in the tail spike protein of a phage ΦKp_Pokalde_001 switch the plaque morphology

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We isolated phage FKp_Pokalde_001 (Accession number GenBank: MW590329.1), a novel podovirus that infects carbapenem resistant *Klebsiella pneumoniae* (TUKp01) isolated from a patient's urine sample. The genome of the phage composed of a linear, double-stranded DNA of 44,535 bp in length with an average G+C content of 54%. The genome of ΦKp_Pokalde_001 had 53 open reading frames (ORFs), 13 promoters, 2 Rho-independent terminator sequences and 247 bp direct terminal repeats at both ends. On protein BLASTp and PHASTER analysis, the phage ΦKp_Pokalde_001 genome found to be strictly lytic. Moreover, the genome of the phage did not encode the lysogeny module and any known harmful genes such as toxins, antibiotic resistant genes (ARGs) and virulent factors (VFs). Hence, the phage found to be an excellent candidate for therapeutical application against *K. pneumoniae* infections. During phage isolation and initial characterization, we found phenotypic variations in plaque morphology where a mixture of plaques was observed with or without a halo region surrounding the clear center in lysates originating from a single plaque. These two plaque phenotypes were reversible between phage generations. We focused our attention to understand this dynamic. Genome sequencing led us to identify independent spontaneous mutations that were all located in the tail spike gene (gp53) encoding a depolymerase enzyme responsible for modulating such phage behavior. We found the alterations in the tail spike protein had a dual effect influencing the enzymatic activity and the phage adsorption kinetics. This study revealed a gene-specific high frequency mutational switch that modulates plaque morphotype. The physiological significance of this switch remains to be investigated.

phage-resistance | quorum sensing | Vibrio | transcription regulation | Metabolism

Perturbation of quorum-sensing during the development of phage-resistance is responsible for novel traits in Vibrios

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Phage-resistant molecular mechanisms in bacteria species continuously report novel and complex gene pools that can confer phage resistance. Nevertheless, bacterial species that do not possess any of these mechanisms, could develop bacteriophage resistance by a complex metabolic adaptation strategy, instigated by mutations, which often appear in transcriptional regulators and/or in phage adsorption proteins. *Vibrio* species have been proven a valuable tool to study the orchestrated metabolic response of gram-negative marine bacteria, against abiotic and biotic challenges. Previously, we showed that *Vibrio alginolyticus* diminish the expression of specific receptors and transporters in their membrane and potentially abort infection by lytic bacteriophages. Here we study how the development of bacteriophage resistance in *Vibrio* species, disrupts the quorum sensing metabolism and subsequently affects bacterial physiology and metabolic capacity. By using an rt-QPCR platform, we studied quorum sensing receptor genes, as well as auto-inducer biosynthesis genes, and finally cell density regulatory proteins. Results showed that bacteriophage-resistant bacteria diminish the expression genes of quorum sensing permeases such as *LuxM*, *LuxN* and *LuxP*, attenuating the normal uptake of QS peptides and subsequently diminishing the expression of cell density regulatory proteins, such as *LuxU*, *AphA* and *HapR*, which transcribe allosterically. These results correlate with the diverse phenotypic traits observed, such as biofilm formation, planktonic growth, and virulence of the phage-resistant strains. The transcriptional shift was also linked with genomic mutations in transcriptional regulators *UhpA* and *MerR*, responsible for orchestrating many of membrane proteins abundances. Interestingly, this phenomenon appears to be phage specific, a fine-tuned metabolic engineering, imposed by the different phages the bacteria have interacted with, updating their role in microbial marine ecology.

D29 mycobacteriophage | Endolysin | Phage derived therapy

N-terminal catalytic domain of mycobacteriophage D29-encoded endolysin functions as a cysteine peptidase

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Antibiotic resistance establishment in *Mycobacterium tuberculosis*, the causative agent of tuberculosis, has promoted the disease to a serious global threat, generating a need for an alternate efficient solution. In this regard, mycobacteriophage, a bacterial virus that infects and kills mycobacteria including the pathogenic species, is considered as the efficient alternative to combat these infections. The peptidoglycan hydrolase of the lytic D29 mycobacteriophage consists of two enzymatically active domains viz. N-terminal domain (NTD) and a lysozyme-like middle domain (LD), and a C-terminal cell wall binding domain (CTD). We have previously shown that NTD alone is sufficient to kill *M. smegmatis* upon its expression within the cell and that the isolated protein can hydrolyze mycobacterial peptidoglycan nearly as efficiently as the full-length LysA protein. Our biochemical studies now show that the isolated NTD protein is able to kill *M smegmatis* cells externally on administrating in combination with sub-lethal dose of anti-mycobacterial drugs and also inhibits *M. smegmatis* biofilm and disrupt it in combination with TB first line drugs. Our molecular dynamics simulations coupled with biophysical and biochemical experiments carried out with both the wildtype and the mutated versions of NTD allowed us to decipher the catalytic region of the protein with C41as a nucleophile and H112 as base. Our data also provide significant insights into the residues that are important for both structure (L44 and E67) and function (Y25, C41, S42, S111, H112, and D121) of the protein. We therefore conclude that NTD functions as a cysteine peptidase. We believe that our work will help in the designing and engineering of this catalytic domain of mycobacteriophage endolysin as a potent anti-bacterial molecule that can be used as enzybiotics in alternative phage-derived therapy.

Yersinia | Temperate bacteriophage | diversity

Genotypic and phenotypic properties of 11 temperate phages isoaltes from pathogenic *Y. enterocolitica* strains

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Yersinia (*Y.*) *enterocolitica* is a heterogeneous species comprising highly pathogenic, weakly pathogenic and non-pathogenic strains. Previous data suggest that gene exchange may occur in *Yersinia*. Though, only scarce information exist about temperate phages of *Y. enterocolitica,* even though many prophage sequences are present in this species. We have examined 102 pathogenic *Y. enterocolitica* strains for the presence of inducible prophages by mitomycin C treatment. Eleven phages were isolated from ten strains belonging the bio/serotypes B2/O:5,27, B2/O:9 and 1B/O:8. All phages are myoviruses showing lytic activity only at room temperature. Whole-genome sequencing of the phage genomes revealed that they belong to three groups, which, however, are not closely related to known phages. Group 1 and group 2 are each composed of five phages (type phages vB_YenM_06.16.1 and vB_YenM_06.16.2) with genome sizes of 43.8 to 44.9 kb and 29.5 to 33.2 kb, respectively. While the attachment sites (*attP*) of group 1 phages has only a length of 7 nucleotides, *attP* of phages belonging to group 2 is much longer (50-57 nucleotides). Group 3 contains only one phage (vB_YenM_42.18) whose genome has a size of 36.5 kb, which is moderately similar to group 2. *AttP* of this phage is 11 nucleotides long. Similar to the *attP* sites, the host range of the phages differed significantly. While group 1 phages exclusively lysed strains of bio/serotype B2/O:5,27, phages of group 2 and 3 were additionally able to lyse B4/O:3, some of them even B2/O:9 and 1B/O:8 strains. Initial experiments indicated that some of the phages are able to transmit chromosomal DNA by generalized transduction.

adaptation E. coli Post-transcriptional regulation Temperate bacteriophage Stress response Genetics

Pleiotropic and adaptive effects of the prophage encoded regulator AppY in E. coli

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Bacterial genome diversity is largely due to prophages, which are viral genomes integrated into the genomes of bacteria. Most prophage genes are silent, but those expressed can provide adaptive traits to their host. We focused our work on AppY, a transcriptional regulator from the AraC/XylS family, encoded on the DLP12 prophage in *E. coli* K-12. By performing RNA-Seq experiments, we showed that AppY production modulates the expression of more than 200 genes, mainly involved in stress adaptation; among them, 13 were identified by ChIP-Seq as direct AppY targets. AppY directly and positively regulates several genes involved in the acid stress response including the master regulator gene *gadE*, but also *nhaR* and *gadY*, two genes involved in biofilm formation. Moreover, AppY indirectly and negatively impacts bacterial motility by favoring the degradation of FlhDC, the master regulator of the flagella biosynthesis. As a consequence of these regulatory effects, AppY increased acid stress resistance and biofilm formation while also causing a strong defect in motility. Across our research, the prophagic protein AppY has emerged as a pleiotropic bacterial regulator. Our work shed light on the importance to consider the genetic dialogue occurring between prophages and bacteria to fully understand bacterial physiology

transcriptome Bacteriophage P. aeruginosa

Impact of phage predation on bacterial transcriptome under simulated human airway conditions

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Bacteriophages have been proven to be efficient in the combat of bacterial multidrug-resistant infections, including those caused by *Pseudomonas aeruginosa*. Nevertheless, the interactions of phages with bacteria in the human body remains unexplained and its disclosure could lead to advance research and development in phage-based therapies.

In this work, RNA-sequencing of phage-infected *P. aeruginosa* PAO1 adhered to a human epithelial cell monolayer (Nuli-1 ATCC® CRL-4011™) was performed to assess bacterial transcriptional processes occurring in phage–bacteria–human cells, i.e., mimicking phage predation under more realistic settings. To achieve that, adhered bacteria were infected with phage LUZ19, and total RNA was extracted from the complex cell mixture. Thereafter, bacterial rRNA/human RNA was depleted and cDNA libraries were prepared to sequence. The differentially expressed genes (DEGs) were quantified using uninfected bacteria as control.

In human airway-simulated conditions, there were 21, 39, and 129 bacterial DEGs after 5, 10, and 15 min-post infection, respectively. From DEGs, some genes were identified as part of LUZ19 typical induced responses (prophage, glycerol metabolism, and spermidine synthesis genes). However, unique responses were also captured including upregulation of pyochelin syntheses, LPS modification, sulfate starvation, exopolysaccharide-related genes, and downregulation of bacterial global regulators. These changes are associated with starvation-like conditions (iron and sulfate) and bacteria adaptation to the host, but its role in phage infection progression is still unknown. The study of its impact on bacterial virulence or phage efficient infectivity under human physiology is of most importance.

This comprehensive study allows the comparison of bacterial and phage transcripts in the presence of host cells, contributing to a better understanding of phage-bacteria-host interactions, which are relevant in a phage therapy context.

virus-host protein-protein interactions | Tectivirus | Bacillus thuringiensis | high-throughput sequencing | yeast two-hybrid

A yeast two hybrid-high throughput sequencing approach for unraveling interactions between Bam35 and its *Bacillus* host

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Bacillus virus Bam35 is the model virus of the *Betatectivirus*, genus of the family *Tectiviridae,* whose members are typically tailless, icosahedral, membrane-containing bacteriophages. These phages not only prey relevant human and animal pathogens from the *Bacillus cereus* group, but also are of great interest due to their possible evolutionary relationship with diverse groups of prokaryotic and eukaryotic viruses. As temperate viruses, the intricate network of molecular interactions between the phage and *Bacillus* proteins remains largely unexplored. Here, we present the first genome-wide analysis of protein-protein interactions for a tectivirus-host system by studying the Bam35-*B. thuringiensis* model. To this end, we have developed a novel and proficient approach which integrates the traditional yeast two-hybrid (Y2H) system and high-throughput sequencing technology.

In this study, we generated and analyzed a fragment-based genomic library of Bam35's host *B. thuringiensis* HER1410 and screened interactions with each of the viral ORF using different combinations of bait-prey vectors. A total of 156 screens have resulted in the prediction of over 4,000 putative interactions. After successive filtering steps, our core virus-host predicted interactome contains 182 candidate interactions, selected in terms of enrichment and specificity as well as biological meaning. Overall, host metabolism proteins and peptidases are particularly enriched in the interactome, showing differing patterns compared to other known host-phages protein interactomes. In addition, our results allowed us to propose functions for several Bam35 proteins of unknown function. This work resulted in a better understanding of the Bam35-*B. thuringiensis* interactions at the molecular level and holds great potential for the generalization of the Y2H-HTS approach for other virus-host models.

Phage Therapy | virus-host protein-protein interactions | RNA sequencing | phage resistance

A transcriptomics-based approach to understand host-phage interactions between *Staphylococcus aureus* and bacteriophage K

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Our lab is presently investigating the genetic aspects and utility of bacteriophage therapy targeting Methicillin-Resistant *Staphylococcus aureus* using phage K as a model, with a focus on phage-host dynamics and resistance mechanisms. 18 spontaneous host mutants resistant to phage K at 30°C were isolated using the clinically relevant *S. aureus* strain NRS384. 7 of these 18 mutants had single missense mutations mapping to the host RNA polymerase β' subunit. To examine the hypothesis that these mutations affect the transcription of phage genes, we performed RNA-seq analysis on total RNA samples collected from NRS384 wild-type (WT) and *rpoC* mutant cultures infected with phage K. The percentage of reads mapping to the phage genome increased steadily over the course of infection, complimenting the decrease in the percentage of reads mapping to the WT host, suggesting a progressive takeover of the host transcription machinery by the phage. We also detected reads mapping to two ~500 bp long intergenic stretches designated as long non-coding RNA regions (lncRNA1 and lncRNA2), which were found to be highly conserved among Kayviruses. Based on gene expression patterns and predicted location of regulatory sequences, we were able to identify transcription units showing early, middle, and late expression. Compared to the WT infection, the percentage of reads mapping to the phage genome decreased significantly for samples collected from the *rpoC* beginning 20 minutes after infection. While the expression of early genes was largely unaffected, the genes transcribed at later stages of infection showed a drastic decrease in expression levels indicating that the mutant is deficient in making the switch from early to late phage gene expression. Furthermore, we have been able to isolate spontaneous phage K mutants that overcome the resistance conferred by the host *rpoC* mutations, suggesting that interaction between host and phage factors plays a role in the transcription takeover.

Bacteriophage transduction genome engineering Staphylococcus aureus Temperate bacteriophage

Easy to use experimental pipeline for the analysis and engineering of *Staphylococcus aureus* clinical isolates

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Increasing problem with curing infections with antibiotic resistant *Staphylococcus aureus* strains is a driving force in the development of easy to use molecular biology tools that could accelerate functional genomics of *S. aureus* and identification of new targets for antibacterials. A source of such tools are *S. aureus* bacteriophages that have the ability to transfer genetic material between *S. aureus* strains by transduction. Upon co-culturing *S. aureus* strains of various clonal complexes we isolated a siphovirus (vB_SauS_ASZ22RN; ASZ22RN), that appeared to represent a new *Phietavirus* genus species and could productively infect laboratory strain RN4220. It originated from a prophage of a CC7 clonal complex strain, but could infect 46 of 47 strains of 12 different clonal complexes, of which 9 were infected productively and 38 were lysed from without. When propagated in RN4220 cells containing a shuttle, low-copy number *S. aureus - E coli* plasmid replicating via the theta-mode, ASZ22RN was able to transduce this plasmid to plasmid free RN4220 with a low frequency. Transduction frequency was increased up to 4 orders of magnitude if the plasmid contained the small terminase subunit gene of ASZ22RN, *terS*. Phage transducing particles were packed with plasmid concatamers as was determined by hybrid (Illumina, MinIon) NGS sequencing of phage progeny. Transductants could be obtained with strains that were productively infected with ASZ22RN, strains that were lysed from without, and, surprisingly, a strain resistant to ASZ22RN infection. The genomic sequences of tested strains were determined to identify their markers of ASZ22RN sensitivity/resistance. Taken together our results open a possibility to develop a feasible pipeline allowing for the introduction of plasmids constructed in *E. coli* to any *S. aureus* strain to study the influence of cloned genes on a given strain physiology or to modify the genome of a given strain by recombinational replacement.

structural biology | Phage T5 | cell wall perforation

Tail tip complex of phage T5: native structure and structural rearrangement occurring upon cell wall perforation

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The vast majority of bacteriophages possess a tail that allows host recognition, cell wall perforation and safe viral DNA channelling from the capsid to the cytoplasm of the infected bacterium. The majority of tailed phages, the *Siphoviridae*, bear a long flexible tail formed of stacked tail tube proteins (TTP) that polymerise around and along the tape measure protein (TMP). At the distal end of the tail, the tail tip complex harbours the receptor binding protein·s (RBP). Interaction between the RBP with the host surface triggers cell wall perforation and DNA ejection, but little is known on these mechanisms for *Siphoviridae*. We aim at characterising the mechanism of cell wall perforation and DNA ejection for the sipho-coliphage T5. We have determined the structure of T5 TTP and tail tube, before and after interaction with its *E. coli* receptor, FhuA, and shown that host binding information is not propagated to the capsid by the tail tube [1]. We now focus on the structure of the tail tip complex: we will present the structure of T5 tail tip complex, before and after interaction with FhuA reconstituted into nanodiscs. Structures were determined by cryo-electron microscopy and single particle reconstruction on purified T5 tails. We could trace all the proteins that compose the tail tip complex [2], except the RBP: we will discuss structural homologies with other phages and phage-derived complexes and the conformational changes induced by receptor binding leading to the tail tube opening and anchoring to the membrane.

[1] Arnaud, Effantin, Vivès, Engilberge, Bacia, Boulanger, Girard, Schoehn and Breyton (2017) *Nat Com,* 8, 1953

[2] Zivanovic, Confalonieri, Ponchon, Lurz, Chami, Flayhan, Renouard, Huet, Decottignies, Davidson, Breyton and Boulanger (2014) *J. Virol.* 88:1162-74

Phage T5 | structural biology | Llp Lipoprotein | superinfection exclusion protein

Functional and structural study of T5 bacteriophage immunity protein Llp

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Phage infection is triggered by host recognition thanks to the Receptor Binding Protein binding to its receptor at the surface of the cell: this interaction allows viral DNA to be delivered into the host cytoplasm. This first step of infection is followed by viral replication and eventually liberation of the new virions. During this vulnerable time, and during the lysogenic cycle, phages protect the new viral factory/home from over-infection. In coliphage T5, protection is mediated by a periplasmic lipoprotein, Llp [1], targeted to the inner leaflet of the outer-membrane, which binds the phage receptor FhuA [2], [3]. Llp biological function is probably also to prevent the inactivation of progeny phage by active receptors present in outer-membrane debris of lysed cells, thereby increasing their chances of infecting a new host. We aim to decipher the mechanisms of T5 host inhibition by Llp at the molecular level.

We over-expressed Llp in an acylated (Ac-Llp) and soluble (Sol-Llp) form in quantities compatible with biochemical and structural studies, and solved Sol-Llp (7.5 kDa) structure by NMR. We could show that Ac-Llp protects the overexpressing strain from T5 infection and we characterized the FhuA:Ac-Llp complex by biochemical and biophysical methods. Surprisingly, we also showed that T5 was able to infect a Salmonella strain and that Llp was also able to protect this strain against T5.

[1] K. Decker, et all « Lytic conversion of Escherichia coli by bacteriophage T5: blocking of the FhuA receptor protein by a lipoprotein expressed early during infection »

[2] V. Braun, et all « Inactivation of FhuA at the cell surface of Escherichia coli K-12 by a phage T5 lipoprotein at the periplasmic face of the outer membrane. »

[3] I. Pedruzzi, et all « Inactivation in vitro of the Escherichia coli outer membrane protein FhuA by a phage T5-encoded lipoprotein »

Acenitobacter baumannii phage hunt | phage cocktail | phage resistant variant | genome sequencing | Eastern europe

Hunting phages against Acinetobacter baumannii clinical isolates spreading across Eastern Europe

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The multi-drug resistant opportunistic pathogen Acinetobacter baumannii is a serious and an ever-growing healthcare concern, as it has emerged worldwide as a dominant pathogen in a broad range of severe infections. Therapeutic application of bacteriophages (phages) is an attractive alternative to control and treat this rapidly spreading nosocomial pathogen. In this study, we have carried out a phage hunt against A. baumannii clinical isolates spreading in the hospitals of Eastern European. Specifically, we sequenced 42 A. baumannii extensively drug resistant (XDR) clinical isolates collected from hospitals. Multilocus sequence typing (MLST) demonstrated the presence of 4 dominant sequence types: ST :1, ST :2 ST :636, ST :492, belonging to different serotypes. Using this information, we selected a representative set of strains and screened wastewater samples collected from five different waste-water treatment plants for candidate phages. Next, we sequenced the genome of obtained phages via NGS. In total, eight distinct lytic, double stranded DNA phages belonging to Siphoviridae and Myoviridae family were identified. The isolated phages were then characterized for activity spectrum, growth characteristics, and stability under various conditions. We observed that prophages are highly prevalent across the investigated strains which characteristic has prevented the isolation of phages against the majority of phenotypes. As a next step, we generated phage resistant variants of each selected strain and used the evolved hosts for another round of phage hunt. This in turn will expand our understanding about how A. baumannii can evolve resistance against phages and how to overcome the resistance. Overall, the results will aid the development of a phage cocktail to effectively eradicate A. baumannii infections and contribute to the improvement of therapeutic phage applications in general.

human serum | Staphylococcus aureus | Bacteriophage | Phage Therapy

Human serum affects the lytic activity of Staphylococcal bacteriophage Sb-1

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Phage application is mostly local, even though systemic administration represents the most suitable route to treat deep seated infections. The aim of this study was to elucidate the effect of human serum on lytic activity of the Sb-1 phage versus *Staphylococcus aureus* ATCC43300 *in vitro*. The lytic activity of Sb-1 (10^8-10^6 PFU/ml) was assessed in presence of serial dilutions of heat-inactivated serum samples from different donors. Further serum processing methods included pre-treatment with PANSORBIN® to remove IgG and fractioning by using 10, 30 and 100KDa cut-off filters. Additionally, a pre-incubation (1h at 37°C) of bacteria with serum before testing phages and a phage serum-neutralization assay were performed. Finally, the effect of 30% (v/v) bovine and fetal calf serum on Sb-1 activity was assessed.

The Sb-1 phage (10^8 PFU/ml) eradicated *S. aureus* (10^6 CFU/ml), in absence of serum. In contrast, when Sb-1 was tested vs bacteria in presence of either 30% human serum or its dilutions up to 1:256, PANSORBIN-pre-treated, and bovine serum, no CFU reduction was observed. These findings indicated an inhibitory effect of serum on phage activity. Sb-1 activity against *S. aureus* was observed when the co-incubation was performed in presence of <10KDa and 10-30KDa serum fractions, resulting in cell eradication and 2-log10 CFU/ml reduction, respectively. No CFU reduction was obtained with the >30KDa fraction. Pre-incubation of Sb-1 with serum did not reduce the phage titer, suggesting that no phage neutralization occurred. Notably, when bacteria were pre-incubated with serum, Sb-1 caused a 2-log10 reduction compared to the untreated control. Interestingly, bacteria eradication was observed in presence of fetal calf serum. To conclude, a relevant inhibitory effect of serum on Sb-1 lytic activity versus *S. aureus* was observed *in vitro*. Ongoing studies will elucidate serum factors accounting for the serum-mediated inhibition and differences between human and calf serum.

RNA sequencing | Pseudomonas aeruginosa | Autographiviridae | Temperate bacteriophage

Bacteriophage LUZ100: an unusual temperate member of the *Autographiviridae* family infecting *Pseudomonas aeruginosa*

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The *Autographiviridae* are generally considered to be a diverse yet distinct clade of bacterial viruses marked by a strictly lytic lifestyle and a generally conserved genome organization. Typical hallmarks include a unidirectional transcription profile and the presence of a single subunit RNA polymerase that is responsible for the middle/late or late transcription of the phage. Moreover, this T7-like RNAP has been utilized extensively in biotechnological applications, most importantly in the recombinant pET-expression systems.

We here characterize *Pseudomonas aeruginosa* phage LUZ100 which includes the hallmarking genome organization of the *Autographiviridae* yet also encodes key genes associated with a temperate lifestyle. Therefore, we've studied its temperate behavior to further unravel these contradicting characteristics. In addition, we have elucidated its transcriptional landscape using an in house developed state-of-the-art long-read RNA sequencing method (ONT-cappable-seq). This technique brought light to some peculiar mechanisms driving LUZ100's gene regulation. Besides these paradigm shifting insights into the biology of *Autographiviridae*, this phage will also be exploited for biotechnological applications.

jumbo phage | phage nucleus | Erwinia amylovora | phylogeny | Microscopy | cryo-EM

Jumbo phage RAY phage nucleus formation during its replication cycle in *Erwinia amylovora*

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Bacteriophages shape the microbiomes of our planet. They are the most numerous biological entities, yet we know the function of less than half of the genes in any given phage's genome. Recently, jumbo phages, bacteriophages with genomes greater than about 200kb, have become more widely studied, and due to their large genome sizes, the gap in knowledge about phage-encoded genes is becoming more and more important to bridge. Certain jumbo phages have been found to use some of these previously uncharacterized phage genes to set up unique replication systems, such as the phage nucleus. The phage nucleus is a protein-enclosed compartment found within infected bacterial cells that functions analogously to a eukaryotic nucleus: It houses and protects phage DNA; enzymes needed for replication, transcription, and recombination are imported inside; and translation and metabolic processes are kept separate in the cytoplasm. Understanding the genes required for a phage to establish such a complex structure will greatly increase our knowledge of phage biology, a resource for identifying new biological mechanisms. Here we characterize the Erwinia jumbo phage vB_EamM_RAY (RAY) replication cycle and show that it proceeds through the formation of a phage nucleus with complex and dynamic subcellular organization. Unlike previously characterized Pseudomonas-infecting nucleus forming jumbo phages, RAY does not degrade the host chromosome and forms a large phage nucleus that must compete with the host nucleoids for space. By studying RAY and analyzing its genome, we have uncovered new groups of conserved proteins that are likely involved in phage replication.

Bacillus thuringiensis | RBPs | phage receptors | adsorption process

Bacillus thuringiensis receptors recognized by Vp4 tail proteins upon adsorption

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Adsorption is a key step in phage recognition of sensitive host cell implying the specific interaction between Receptor Binding Proteins (RBP) often located at the distal part of the phage tail and receptors on the surface of their bacterial host. Adsorption is generally described as a three-step process initiated by random collisions between phage and its host, followed by reversible binding of the virus to bacterial surface components and the final irreversible bound of the RBP to its cognate receptor. These crucial adsorption steps, governing phage host spectrum, have been widely studied in Gram-negative bacteria but remain so far less understood among Gram-positive bacteria. Vp4 is a lytic phage that belongs to the *Herelleviridae* family of long contractile tail phages infecting bacteria of the phylum *Firmicutes.* This study aims to bring new insights into the interactions between Vp4 and its *Bacillus cereus - Bacillus thuringiensis* hosts through the characterization of Vp4 tail proteins and the identification of the bacterial receptor(s).

The tail proteins Gp112 (putative RBP) and Gp119 (baseplate wedge protein featuring a carbohydrate binding domain) fused to a GFP confirmed their binding capacity to host cells and their likely implication in the adsorption process. In parallel, four independent spontaneous mutants of the *B. thuringiensis* BGSC4BA1 insensitive to Vp4 phage were isolated using the improved protocol of plaque assay with double-layered agar. These mutants displayed an impaired phage adsorption suggesting an alteration in the receptor. Analysis of their chromosomal sequences pinpointed a mutated collagen triple helix repeat domain protein in all four mutants. This gene was knocked-out and preliminary results showed an impaired adsorption in comparison to the wild-type suggesting a potential implication in the adsorption process. Further confirmation will be obtained by complementation experiments in which the phage susceptibility should be regained.

Haloarchaea | Archaeal viruses | Halovirus

When life gives you lemons, sequence them: isolation and characterization of novel haloarchaeal viruses

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Viruses are present in all domains of life and have been shown to play important roles in microbial communities such as nutrient cycling and horizontal gene transfer. In comparison to eukaryotes and bacteria, archaea and their viruses are substantially understudied. The characterization of archaeal viruses is hindered mainly due to the lack of cultivable hosts and hosts that can be genetically manipulated. Archaeal viruses are diverse in virion architecture and gene content and a number of archaeal viruses exhibit morphologies that are not known for viruses that infect bacteria and eukaryotes. One of these morphotypes are, spindle-shaped virus, that are unique to archaea and are omnipresent in hypersaline environmental samples (Bath et al., 2006; Krupovic et al., 2014; Oren et al., 1997; Sime-Ngando et al., 2011). However, only one halophilic spindle-shaped virus (His1 virus infecting *Haloarcula hispanica*) has been isolated to date (Bath & Dyall-Smith, 1998). While studying an unpublished *Halorubrum* sp., we discovered both spindle-shaped particles and other virus-like particles (VLPs) in the supernatant of the cultures. Sequencing of the purified particles revealed two distinct viral genomes, a novel spindle-shaped virus with no homology to known viruses, and a novel Betapleolipovirus. Pleomorphic viruses of the family *Pleolipoviridae* also have morphologies unique to archaea and are nearly impossible to differentiate from membrane vesicles with microscopy alone. While trying to establish a stable virus-host system for the spindle-shaped virus, we observed interesting behavior that will be presented.

Prophage | Membrane vesicles | Gram-positive bacteria | Lacticaseibacillus casei

Contribution of prophages in extracellular vesicle production by the Gram-positive bacterium *Lacticaseibacillus casei*

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Bacterial cells release membrane vesicles (MV) which are nanostructures delimited by a lipid bilayer. The size and composition of those vesicles depend on the species, the environment and mechanism by which they are produced. In most cases, MV range from 20 to 400 nm and can carry a variety of macromolecules such as proteins, nucleic acids, virulence factors, quorum sensing molecules as well as other cellular components. The study of MV has gained considerable interest over the last 10 years due to the diversity of functions ensured by these structures. This diversity places MV at the crossroad of major research topics in today's microbiology, such as antibiotic resistance, horizontal gene transfer, inter- and intra-species communication, biofilm development, bacteriophage resistance, vaccination or pathogenesis.

MV research has mainly focused on Gram-negative bacteria and despite the increasing interest in Gram-positive MV, the mechanisms by which they are released remain to be unveiled. In this work, we established a link between the presence of a prophage and the release of MV by the Gram-positive bacterium *Lacticaseibacillus casei* BL23. By comparing a mutant harboring a defective prophage to the parental strain, we were able to quantify the contribution of *L. casei* BL23 prophages in MV production. We also demonstrated that prophages influence the membrane integrity of the bacteria, thus contributing to the formation of MV. Interestingly, prophages are only responsible for the production of a subset of the MV released by the bacteria, suggesting the existence of additional mechanisms involved in MV production in this strain. Overall, our results suggest a key role of prophages and provide new insight into MV production by Gram-positive bacteria. Finally, the study of MV biogenesis is of great interest as prophage-induced MV could potentially influence bacterial behavior and stress resistance.

bacteriophage L-forms Phage-bacteria interaction

Gram-positive bacteria evade phage predation through endolysin-mediated L-form conversion

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Bacteriophages kill bacteria by osmotic lysis towards the end of the lytic cycle. Under standard hypotonic culture conditions, peptidoglycan-degrading endolysins released at the end of infection cycles cause explosive cell death not only of the infected host, but can also attack non-infected bystander cells. Here, we show that in osmotically stabilized environments Gram-positive bacteria can evade phage predation by transient conversion to a wall-deficient L-form state. This L-form escape is triggered by endolysins disintegrating the cell wall from without, leading to turgor-driven extrusion of wall-deficient, yet viable L-form cells. Remarkably, in absence of phage predation, L-forms can quickly revert to the walled state, which is favorable under normal environmental conditions. These findings suggest that L-form escape represents a population-level persistence mechanism to evade phage killing. Furthermore, we demonstrate phage-mediated L-form switching of *Enterococcus faecalis* in human urine, which underscores that this escape route may be widespread and could have important implications for phage- and endolysin-based therapeutic interventions.

Staphylococcus aureus | bacteria-phage interaction | transcriptome | Kayvirus

Transcriptomic landscape of *Staphylococcus aureus* during phage K infection

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The treatment of infections caused by human and veterinary pathogen *Staphylococcus aureus* is becoming worldwide healthcare concern due to the increasing resistance to antibiotics. A promising alternative to currently used drugs is represented by lytic phages from genus Kayvirus, but their use is impeded by the lack of knowledge of phage-bacterium molecular interactions. We performed RNA sequencing of two *S. aureus* strains infected with Kayvirus bacteriophage K to decipher the transcriptomics of the phage lytic life-cycle and the host response. We found that the temporal transcriptional profile of phage K was comparable in both strains except for a few loci. The RNA-Seq data also revealed presence of phage non-coding RNAs, which may play a role in the regulation of phage and host gene expression. The response of *S. aureus* to phage K infection resembles a general stress response and involves upregulation of nucleotide, amino acid and energy synthesis and transporter genes and the downregulation of host transcription factors. The interaction of phage K with variable genetic elements of the host showed slight upregulation of gene expression of prophage integrases and antirepressors. The virulence genes involved in adhesion and immune evasion were only marginally affected. The study gives a comprehensive view on the phage-bacterium interactions that improves the knowledge of molecular mechanisms underlying the Kayvirus lytic action. We clarify the global transcriptional interactions between phage and host, which will ensure safer usage of phage therapeutics and may also serve as a basis for development of new antibacterial strategies.

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Prophage SOS-response Acinetobacter baumannii

The *Acinetobacter baumannii* SOS gene *ddrR* is crucial for prophage maintenance and induction

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Acinetobacter baumannii, considered a number one priority pathogen by WHO, is threatening hospitals due to its high ability to acquire antibiotic resistance. We have recently shown that mobile elements, such as prophages, are highly prevalent and encode several fitness/virulence-related genes, suggesting that they may serve as vectors for the spread of virulence. Here, we want to understand how stress factors influence the prophage behavior and disclose the role of the unique SOS response system, *umuDAb* and *ddrR*, in prophage induction. First, the *A. baumannii* ATCC 17978 strain was subjected to different sub-mic concentrations of mitomycin C (MMC), H_2O_2, and ciprofloxacin (cip), followed by incubation, RNA extraction and qRT-PCR analysis. After verifying the prophage behavior under these stress conditions, a *ddrR* knockout mutant was engineered using CRISPR-Cas9 to assess its influence on prophage expression. As expected, all conditions triggered an SOS response in the type strain as well as prophage induction, as both the *ddrR* and *umuDAb* genes, and the *cro* and capsid genes were overexpressed. Different levels of induction were observed between the conditions tested. For example, prophage induction was lower when challenged with cip than with MMC. In terms of growth curves, we observed that the *ddrR* mutant grew at faster than the type strain. Curiously, when challenged with MMC, the type strain showed significantly decreased CFUs in contrast to the *ddrR* mutant strain. Our findings show that each stress condition leads to different levels of prophage responses and that some can increase the fitness/virulence expression without detrimental effects on the host. The SOS gene *ddrR* is important for prophage induction and, consequently, its absence contributes to the bacterial robustness during growth. In conclusion, it is important to understand how prophages are affected by host genes under different stresses to improve antimicrobial efficacy.

Pesudomonas aeruginosa | wound infection | bacteriophage

Phage treatment efficacy in a *P. aeruginosa* mouse, wound-infection model, and associated innate immune system modulation

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Pseudomonas aeruginosa is an opportunistic, nosocomial pathogen that is correlated with persistent infections within wound environments, leading to delayed healing. These infections are often recalcitrant to antimicrobial treatments and host immune defenses. Due to the resulting limited treatment options, development of novel therapies is a critical research priority. Herein, we demonstrate the therapeutic potential of the well-characterized phage, PEV2, as a promising strategy for controlling *P. aeruginosa* infections in an *in vivo* wound infection model. This phage was found to lack toxicity as no morbidity or mortality was observed with phage administration. Phage monotherapy significantly improved *P. aeruginosa*-infection outcome, decreasing bacterial burden within the wound bed by 2.9 log, 72 h post infection. Moreover, bacterial infections substantially elevated neutrophil numbers, both in the blood and wounds, while phage treatment significantly lowered neutrophil circulatory levels and accumulation in wounds despite prior *P. aeruginosa* infection. This suggests that phage-related decreases in bacterial burdens caused lower neutrophil recruitment. Improved healing and overall reduced levels of inflammatory markers also were observed in phage-treated wounds relative to untreated controls, which is presumably correlated with reductions in bacterial loads and direct downregulation of the activity of cells engaged in proinflammatory processes. Besides wound beds, spleens and livers served as major sites of accumulation of phages and bacteria, presumably as a function of the roles of these organs in foreign-body removal from circulation. Our study demonstrates that topical administration of phage PEV2 has a promising therapeutic potential in *P. aeruginosa* wound infection treatment, including positively impacting wound healing processes and reducing immune and inflammatory responses.

Pseudomonas aeruginosa | Phage Therapy | Bacteriophage | antibiotics | phage/antibiotic combinations

Friends or Foes? High vs. low antagonism of *P. aeruginosa* phage infection activities by different classes of antibiotics

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Phage therapy employs viruses (phages) to treat bacterial infections. In the clinic, phage therapy often is used in combination with antibiotics, including against *Pseudomonas aeruginosa* infections. Antibiotics, however, interfere with critical bacterial functions, such as DNA replication and protein synthesis, that are required for phage infection. Nevertheless, the impact of antibiotics on phage replication is not commonly determined prior to phage therapies. Here we use an optical density-based 'lysis profile' assay to assess the impact of four classes of antibiotics – colistin, ciprofloxacin, meropenem, and tobramycin – on the bactericidal, bacteriolytic, and virion-production activities of *P. aeruginosa* phages. This is a rapid, high-throughput assay that provides results of phage-antibiotic interactions within a few hours, e.g., as could be employed prior to the start of phage-antibiotic combination treatments. In our study, phage-antibiotic combinations were more potent in reducing *P. aeruginosa* than phages or antibiotics alone, though colistin and tobramycin substantially interfered with phage bacteriolytic and virion-production activities even at minimum inhibitory concentrations (MICs). Ciprofloxacin and meropenem, by contrast, had no or minimal impact at clinically relevant concentrations (up to 9× MIC). We corroborated these lysis profile results by more traditional measurements: colony forming units, plaque forming units, and one-step growth experiments. To our knowledge, this is one of the first studies addressing the impact of antibiotics on *P. aeruginosa* phage activities over the duration of individual phage infections. Collectively, this indicates that ciprofloxacin as well as meropenem may be minimally antagonistic in phage therapy co-treatments of *P. aeruginosa* infections. More generally, our results point to a prospect of rapid, routine testing of antibiotic antagonism on phage infection activities prior to the initiation of phage treatments.

Receptor binding protein | Staphylococcus aureus | Phage receptor | wall teichoic acid | Bioinformatics | phylogeny | Computational host prediction

Predicting the clone-specific host range of *S. aureus* phages based on clustering of receptor-binding proteins

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Staphylococcus aureus poses a high risk of life-threatening, often antibiotic-resistant infections, and its phages might become helpful for diagnostics and disease treatment. The most important factor determining the host range of S. aureus phages is the species-specific structure of wall teichoic acids (WTA), which is the only know phage receptor in *S. aureus*. While most other *Staphylococcus* species carry a WTA composed of a glycerol-phosphate backbone, nearly all *S. aureus* strains possess a ribitol-phosphate WTA with GlcNAc-glycosylation in different conformations. These cell wall polymers cannot mutate as easily as proteinaceous phage-receptors of other bacteria such as Gram-negatives. Due to this slow-evolving nature of the phage-receptors, we aimed to investigate the degree of conservation, structure and function of the corresponding receptor-binding proteins (RBPs).

Through bioinformatic analysis of *S. aureus* phage genomes, we discovered various RBPs necessary for adsorption to the host. With the help of multi genome analysis of over 300 *S. aureus* phage genomes, we found that *S. aureus* ribitol-phosphate binding phages cluster in 10 different groups based on their predicted RBPs. To confirm the binding capabilities of these receptors, protein fusion constructs were created by addition of a fluorescent N-terminus to the phage RBPs. These constructs were then used to investigate the specific binding of these proteins to the host cells, which matched the in-vivo behavior of the corresponding phages. The created phage clusters will allow us to predict the success of phage adsorption to different WTA glycosylation types during the initiation of phage infection and may be useful for gaining more insights into the host range of both known and novel phages.

Bacteriophages | CRISPR interference | Bacteriophage biology

Using CRISPR interference technology to study biological characteristics in a CrAss-like phage

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CRISPR interference (CRISPRi) technology is a method that allows sequence specific control of gene expression. Based off the CRISPR Cas9 system, CRISPRi uses a single guide RNA (sgRNA) and a catalytically inactive version of the Cas9 protein (dCas9). Working at the transcriptional level, the sgRNA-Cas9 complex binds to the target DNA sequence and causes a steric block to RNA polymerase. In this way, it can provide reversible, targeted inhibition of genes of interest and can be a useful tool for understanding gene functions. So far, it has been demonstrated to work in bacterial systems, but has yet to be used as a tool in bacteriophages.

CrAss-like phages are extremely prolific and widespread bacteriophages in the human gut, accounting for over 86% of gut viral genomes. First discovered *in silico* in 2014, only five crAss-like phages have been isolated with their hosts thus far (ΦCrAss001, ΦCrAss002, DAC15, DAC17 and Φ14:2). While a number of intriguing genomic features have been observed *in silico*, many of their biological characteristics remain unknown. The aim of this work is to use CRISPRi to target putative head, tail, terminase, DNA and RNA polymerase genes in DAC15, in order to garner further insight into the structural and biological roles these genes play. This is carried out using a system already established in *Bacteroides thetaiotaomicron*, the host of DAC15.

Prophage | stx-phages | bacteria-phage interaction | motility

Characterization of the contributions a detoxified Stx-phage prophage makes towards the fitness of its bacterial host.

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Escherichia coli producing Shiga toxin (Stx) are called STEC. Stx-phages can horizontally transfer Shiga toxin-encoding genes between bacteria. The production of Stx by lysogens in the digestive tract can cause bloody diarrhea with intestinal cramping, diarrhoea, hemorrhagic colitis, HUS (Haemolytic uraemic syndrome), each disease being progressively worse and to some extent governed by the amount of Stx produced during the resulting STEC infection. In addition, Stx-phages are lambdoid phages, of which λ phage is the archetype. The size of Stx-phage genomes can be 50% larger than the size of the λ phage genome. However, there is very little research focusing on the function of this extra DNA.

One of the Stx-phges is $\Phi 24_B$. Previous work showed that the expression of two genes of unknown function from $\Phi 24_B$, *vb_24B_13c* and res, are only expressed whilst the prophage is integrated stably in the host cell. In addition, unpublished works done by Dr. Veses-Garcia showed that *vb_24B_13c*, *res* and *stk* are under the control of same promoter, p_{RM}, which drives C_I expression, and these three genes are likely to be transcribed as one transcript and have been shown to contribute to the motility of *E. coli* lysogen at 37°C.

Therefore, this project was aimed to investigate evidence, to prove these three prophages, *vb_24B_13c*, *res* and *stk*, can alter the lysogen's motility at the transcriptional level. Multiple approaches were included in this project, such as motility test, NanoString datastes, RNA-seq (from previous data), bioinformatics analyses. The production of genetic constructs, and the development of assays (both biochemical and molecular biological) were used to ascertain the function of *vb_24B_13c*, *res* and *stk*.

Bacterial virulence | Bacteriophage | Phage-bacteria interaction

Attenuation of phage induction during Listeria infection of mammalian cells involves domestication of phage repressor

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Temperate phages that associate with bacterial pathogens must be well adapted to the pathogenic lifestyle of their hosts. A lytic induction in the mammalian environment, if occurs, has to be tightly regulated, as it can lead to the elimination of both the bacteria and the phage. The *comK*-associated phage of *Listeria monocytogenes* (*Lm*) strain 10403S (ϕ10403S) is well adapted to the intracellular lifestyle of its host. During *Lm* infection of macrophage cells the phage, which is integrated within the *comK* gene, excises its genome, yielding a functional *comK* gene that promotes the escape of *Lm* from the macrophage's phagosomes to the cytosol, without triggering virion production and bacterial lysis. It was recently demonstrated that this phage has lost its main anti-repressor, and become fully dependent on the anti-repressor of another phage element that inhabit the chromosome, which responds to SOS conditions by producing phage tail-like bacteriocins. Here we show, that this evolutionary changeover of the phage anti-repressor was accompanied by the acquisition of a unique domain at its CI-like repressor, that attenuates phage induction at 37°C, hence preventing phage-mediated bacterial lysis in the mammalian niche. Using a set of experiments comparing ϕ10403S to a closely related listerial *comK*-phage that did not lose its anti-repressor, we demonstrate that the acquisition of the CI extra domain and the loss of the phage anti-repressor play an important role in the adaptation of this phage to the pathogenic lifestyle of its host.

Agro-food, veterinary and environmental biotechnology applications

phage therapy | Chicken model | Salmonella enterica

The effect of phage therapy on the level of immunological parameters in the chicken model

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Bacteriophages are viruses specific to bacteria. Due to the lack of appropriate receptors on the surface of eukaryotic cells, they were long considered neutral to animals. However, the increasing number of recent studies show that bacteriophages are capable of interacting with immune cells. Therefore, such interactions must be comprehensively investigated before phage therapy can be considered completely safe.

The main purpose of this study was to analyse the effect of phage therapy on immune cells and proteins in broiler chicken model. Therefore, the levels of inflammatory factors in serum and organs were evaluated.

Experiments were conducted on 8 groups of broiler chickens: 3 control groups (receiving saline, phage cocktail and *Salmonella* respectively), 3 *Salmonella*-infected groups treated with phage cocktail, 2 *Salmonella*-infected groups treated with antibiotics. The levels of cytokines such as: IFN-γ, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12 in serum and IL-6 and IL-10 in organs were detected by ELISA. Moreover, the levels of lymphocyte subpopulations such as: lymphocytes B, T helper cells, cytotoxic T lymphocytes in blood serum were determined.

No significant differences were observed in *Salmonella*-infected animals receiving phage cocktail 24 hours after infection, compared to the control receiving 0,9% NaCl. However, we observed that levels of cytokines and lymphocytes in *Salmonella*-infected animals receiving phage cocktail 4 days after detection of bacteria in the feces were increased compared to uninfected control groups.

Our results suggest that phage therapy does not increase the proinflammatory parameters in the chicken blood and organs, but, importantly, could reduce some of them, that may indicate their potential ability to modulate immune system. Furthermore, we showed that the appropriate timing of phage cocktail administration affects protein and cellular levels of the immune system.

biocontrol bioinformatics crop production

Genome characterization of novel lytic *Erwinia amylovora* bacteriophage KEY

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It has been reported about promising results on applying lytic phages along with a non-pathogenic epiphyte *Pantoea agglomerans* in biological control of fire blight (Lehman, 2007). Recently, the novel lytic bacteriophage KEY was isolated from quince with fire blight symptoms. Transmission electron microscopy revealed its resemblance to members of the family *Siphoviridae* (Faidiuk, 2015). Host range analysis indicated that this phage is able to lyse *P. agglomerans, E. amylovora* and *E. horticola* cells (Tovkach, 2013). Here, we report the genome sequencing and characterization of *E. amylovora* phage KEY.

Phage DNA was sequenced using the Illumina HiSeq 2500 platform at The Centre for Applied Genomics (Hospital for Sick Children, Toronto, Canada). The contigs were assembled using DNASTAR's SeqMan NGen12 software. Genes were predicted using DFAST with subsequent manual curation. tRNA encoding genes were identified using tRNAscan-SE and ARAGORN.

The obtained reads were assembled into four contigs. Contig_3 was the longest one (108.069 kb) and all of the contigs could not be assembled in an integral genome sequence. Their total length was approximately 119.089 kb. KEY shows high sequence identities to *P. agglomerans* phage AAS21 (MK770119), which has 97.56% identity and 96% query coverage to the KEY contig_3. Genomic analysis and annotation reveal 184 open reading frames (ORFs), of which 18 ORFs have no homologs in the databases, 91 ORFs encode hypothetical proteins and 75 ORFs have predicted functions involved with virion morphogenesis, DNA metabolism, adsorption and host lysis. Phage KEY also encodes 26 tRNA genes and putative EPS-depolymerase. No obvious toxins, phage lysogeny, antibiotic resistance or virulence factors are encoded by these phage genomes. According to the genome structure, it was classified as a T5-like phage.

The obtained data expand the knowledge of *Erwinia* phages and may be helpful for the biocontrol of *E. amylovora*.

bacteriophage biocontrol | lytic bacteriophages | Comparative genomics

Novel Lytic Bacteriophage of *Salmonella* Typhimurium; Suggestive of a New Genus in Subfamily *Vequintavirinae*

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MDR serovars of *Salmonella* Typhimurium are a major concern for food safety. Phage therapy can be an alternative for the treatment of such MDR *Salmonella* serovars. We have isolated and analyzed a novel S*almonella* phage (*Salmonella*-Phage-SSBI34). SSBI34 exhibited good lysis potential and restricted bacterial growth till 16h as compared to the control bacteria. It was stable at high temperatures and low pH. Genome analysis (141.095 Kb) indicated that its nucleotide sequence was novel as it exhibited only 1–7% DNA coverage. The phage genome features 44% GC content, and 234 putative open reading frames were predicted. The genome was predicted to encode for 28 structural proteins and 40 enzymes related to nucleotide metabolism, DNA modification, and protein synthesis. Further, the genome features 11 tRNA genes for 10 different amino acids indicating alternate codon usage and hosts a unique hydrolase for bacterial lysis. This study provides new insights into the subfamily *Vequintavirinae* and SSBI34 may represent a new genus.

Bacteriophage isolation | Clostridium | host range

Isolation and biological characterization of bacteriophages against Clostridium spp.

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The majority of *Clostridia* species are known as animal and human pathogens. Food borne illness caused by *Clostridium perfringens* is the most common illness resulting from the consumption of contaminated food (Scallan *et al*., 2011). *Clostridium difficile* is recognized to be antibiotic associated pathogen, it is one of the major triggers of gastrointestinal (GI) comorbidities. Bacteriophages (phages) can be considered as candidates to replace antibiotics medical practice.

The aim of this study was to isolate phages against *Clostridia* species and study their biological characteristics.

A total of 85 *C. perfringens* strains were used in this study (48 o isolated from the poultry, 37 - from patients). Bacteriology identification was performed using selective media (Kitt-Tarozzi, Wilson-Blair, etc.). Phages were isolated from the water samples using enrichment method. Phage susceptibility and host range was determined using spot-test analysis. Electron microscopy was performed on JEOL-100 SX. Newly isolated phage clones were purified and concentrated according to standard methods (Adams, 1961). *C. difficile* strains were obtained from Gent University, Belgium.

The strains collection was screened for phage susceptibility against 17, three (F3, Fk and F49) out of which were isolated from the river Mtkvari. *Cl. perfringens* # 49 and # 217 were used as host strains. The host coverage for different phages varied from 1.17% (14F) to 60 % (phage F1). Five clones (F1, F2, F3, Fk and F49) with overlapping host ranges were selected for further studies. These phages showed cross activity against *C. difficile* strains, ranging from 43% (for phage F2) to 90,5% (for Phage Fk).

F1 showed the broadest coverage of *C. perfringens* strains, we used it for further characterization. It was demonstrated that the adsorption of phage F1 on the host cell surface starts after 5 minutes and achieves its maximum after 20 min, by this time 99% of phages are attached to the appropriate cell receptors. The latent period is rather long and takes about 1.5h, cell burst size is quite low about 40-80 phage particles/cell.

Genetic studies of *Clostridia* phages are ongoing.

engineering host-range | synthetic biology | T4 phage

Engineering T4-like Phage for Alternate Host-range

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Antibiotic resistance in both medical and agricultural context is an increasingly emergent threat that might be addressed with phage-based antimicrobials. However, relatively narrow host-range and development of phage resistance are hurdles in the advancement of effective phage therapies. Even among phage with nearly a century of study in the laboratory, virtually all contain numerous gene products of unknown function. These phages are experimentally shown to have their ability to identify and adhere to their host primarily defined by the receptor binding proteins located at the distal tip of their short and long tail fibers. The diverse group of adhesins called gp38 recognize numerous different outer membrane proteins and functional sites on the surface of E. coli. The short tail fiber gp12 is responsible for irreversible binding to the host to begin the infection cycle. Within T4-like myoviruses there is a large diversity of receptor binding domains, as well as highly specific epigenomic features. For example, T4 which may bind to ompC or lipo-polysaccharides (LPS) produces glucosyl-hydroxymethyl cytosine (glc-5hmC). RB69 is a T4like phage that identifies ompF and which is shown to be unable to recombine with most other T4-like phage, likely due to arabinosylation of its DNA. Little is known what other functions this sugar modification may have. Here we use Lb-Cas12a mediated homologous recombination to exchange adhesins and short tail fibers of several T4-like phage with those of RB69. We assess the resulting change in host-range by plating the engineered phage on the 72 unique strains in the E. coli collection of reference (ECOR). From this we seek to unravel more of the impact of epigenetic modifications in phage and develop an approach for efficient host-range modification that is useful to create a broad host range phage with optimal therapeutic features.

phage therapy | leafy green | E. coli

Phage Therapy Against *E. coli* O157:H7 Contamination During Garden Cress Vegetation

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The number of outbreaks through contaminated fresh produces by foodborne pathogens has been increased worldwide. Foodborne pathogens are most frequently transmitted to fresh produce through contaminated irrigation water. After transmission, it is hard to eliminate foodborne pathogens on the fresh produce after harvest. Depending on the microbial contamination agent and load on the fresh produce, the foodborne illness and severity might be varied, respectively. For bacterial pathogen control, the most used sanitizer is chlorine as a disinfected agent. However, chlorine use has been linked to an increase in chemical risk. Therefore, alternative prevention methods that can be applied in the field should be investigated. One of the major foodborne bacterial causative agents is *Escherichia coli*. According to CDC, approximately 265,000 *E. coli* infection occur in the U.S. each year and *E. coli* O157:H7 causes 36% of them. In this study, the aim is to develop an alternative application to prevent the presence of *E. coli* O157:H7 on freshly consumed garden cresses. First, cresses contaminated with *E. coli* O157:H7 with a bacterial load 10^5 CFU/mL, according to the irrigation schedule which contains 6 different groups via spraying. Then, phage cocktail solution for *E. coli* O157:H7 mixed with 0.85% NaCl with the multiplicity of infection (MOI) = 100. Cresses that are contaminated by *E. coli* O157:H7 watering with this solution consider the irrigation schedule by spraying. After the harvest, enumeration for total *E. coli* and biofilm-forming *E. coli* count was conducted. Results were compared with their control groups. Moreover, adherence of *E. coli* on leaves was investigated with the Scanning Electron Microscope (SEM). Results demonstrated that there is a significant difference between control and therapy groups with an average 1,94 log reduction in total *E. coli* count and an average 2,35 log reduction in biofilm-forming *E. coli* count.

bacteriophage biocontrol | Pseudomonas syringae | Agriculture

Use of bacteriophges to control *Pseudomonas syringae* pv. *actinidiae* in kiwifruit orchards.

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Chile is one of the largest kiwifruit producers worldwide. This industry has been affected by the phytopathogen *Pseudomonas syringae* pv. *actinidiae* (Psa), for which control methods includes copper-based products and antibiotics; however, their use is heavily restricted due to the risk of resistance development and phytotoxicity.

Phages has been proposed as an alternative to control bacterial pathogens in agriculture due to their natural origin and specificity. Here we evaluate the use of a cocktail of four, previously characterized, phages to control Psa infections in real kiwifruit production conditions. The phages were evaluated in two fields (Peumo, Region of O'higgins and Linares, Region of Maule, Chile) where Psa was present, and during two seasons in independent trials (2019 and 2020).

As expected, no differences in symptomatology were observed between the plants at the beginning of the trials. Similarly, no differences were observed between the treatments during the first season in Peumo, but in Linares, plants that received a copper treatment showed an increased damage index (19.25%) in comparison to other treatments, including phage treatment (7.5%).

During the trials of the second season, plants that received phage treatments showed a damage index lower (12.5% in Peumo and 9.5% in Linares) than the damage index of plants that received copper treatment (29% in Peumo and 25.5% in Linares), and near 20% lower than the dame index observed in control plants with no treatment (32% in Peumo and 30% in Linares). These results were consistent in the two fields assayed. Phages were only detected in plants that received phage treatment through the experiment. The bacterial and Psa load were interrupted due to the pandemic restrictions.

These results showed that phages have the potential to combat Psa infections in real kiwifruit production conditions. We expect that this study will contribute to the development of new tools to control this phytopathogen.

Phage receptor | soft rot | plant pathogenic bacteria | adaptation | coevolution | fitness costs

Resistance of Soft Rot *Pectobacteriaceae* bacteria to lytic bacteriophages results in fitness tradeoffs during infection.

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Resistance to phage infections allows bacteria to survive and multiply in the presence of their viral predators. However, such resistance frequently results in direct costs for resistant variants linked with their ecological fitness, competitiveness for resources or/and virulence. Unfortunately, little is known about ecological fitness penalties paid by plant pathogenic bacteria in their natural habitats, including agricultural fields. Soft Rot *Pectobacteriaceae* (SRP), including *D. solani* and *P. parmentieri,* are agricultural pathogens causing increasing losses in the production of crops and nonfood plants globally. This study aimed to analyze fitness costs paid by the phage-resistant *Dickeya solani* and *Pectobacterium parmentieri* variants *in vitro* as well as during infection of their primary host plant - *Solanum tuberosum* L. (potato). Using Tn5-based mutagenesis, we identified and characterized bacterial loci that encode structures required for phage attachment and infection susceptibility to better understand the molecular determinants responsible for fitness alterations in phage-resistant *D. solani* and *P. parmentieri* strains. With this approach, we investigated a tradeoff hypothesis that phage-resistance causes fitness disadvantages in the environment. Our main finding is that the cost of phage resistance for SRP bacteria is context-dependent. Although phage resistance did not affect most of the phenotypes of SRP bacteria *in vitro*, all phage-resistant variants of both bacterial species were heavily impacted both in their ability to survive on the plant surface and cause disease symptoms *in planta*. This suggests that fitness costs due to phage resistance in plant pathogenic bacteria may be more often happening in nature than it has been reported from *in vitro* studies.

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bacteriophages | Avian pathogenic Escherichia coli | Poultry

Isolation, Characterization, and Genomic Analysis of three novel *E. coli* bacteriophages that effectively infect *E. coli* O18

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Escherichia coli (E. coli) is one of the most common pathogenic bacteria worldwide. Avian pathogenic *E. coli* (APEC) causes severe systemic disease in poultry (Colibacillosis) and accordingly has an extreme risk on the poultry industry and public health worldwide. Due to the increased rate of multi-drug resistance among these bacteria, finding an alternative therapy to antibiotics to treat such infections. Bacteriophages are considered one of those best solutions. This study aimed to isolate, characterize, and evaluate the potential use of isolated bacteriophages to control *E. coli* infections in poultry. Three novel phages against *E. coli* O18 were isolated from sewage water and characterized *in vitro*. The genome size of the three phages was estimated to be 44,776 bp, and the electron microscopic analysis showed that they belong to the *Siphoviridae* family, in the order Caudovirales. Phages showed good tolerance to a broad range of pH and temperature. The complete genomes of three phages have been sequenced and deposited into the GenBank database. The closely related published genomes of Escherichia phages were identified using BLASTn alignment and phylogenetic trees. The prediction of the open reading frames (ORFs) identified protein-coding genes that are responsible for functions that have been assigned, such as cell lysis proteins, DNA packaging proteins, structural proteins, and DNA replication/transcription/repair proteins.

Staphylococcus aureus | biofilms | phage susceptibility

Biofilm formation and susceptibility of different *Staphylococcus aureus* strains to phages and endolysin LysRODI

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Bacteriophages and their derived proteins are an attractive alternative for combating surface contamination with the Gram-positive pathogen *Staphylococcus aureus*. However, the success of this strategy will depend on the susceptibility of different strains to these antimicrobials. Within this context, understanding the different mechanisms that lead to resistance or decreased susceptibility to phages and phage-derived lytic proteins will help predict the outcome of their use as antibiofilm agents.

This work aimed to perform a comparative phenotypic and genotypic analysis of 21 *S. aureus* strains from different origins (clinical origin, milk from mastitic cows and food industry surfaces). On the one hand, we assessed several phenotypic traits of these isolates, including biofilm formation, staphyloxantin production, susceptibility to four staphylococcal phages (two myoviruses: phiIPLA-RODI, phiIPLA-C1C, and two siphoviruses: phiIPLA35 and phiIPLA88) and one endolysin (LysRODI) derived from phage phiIPLA-RODI. On the other hand, the genomes of all strains were compared in order to search for potential differences that explain the observed phenotypes, including putative susceptibility markers. To do that, some genomes were taken from public databases and others were sequenced de novo. The results of this analysis shed light on some possible mutations involved in the characteristics of these isolates, but also confirmed the complexity of the studied phenotypes, especially susceptibility to phages and endolysins. Indeed, further studies will be necessary to confirm if the genotypic changes detected are actually behind the differential ability of these antimicrobials to eliminate the analyzed strains.

noroviruses food safety bacteriophage

Development of an indirect method to detect infectious noroviruses in vegetables and oysters, using F-RNA phages.

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Human noroviruses represent ~ 20% of all gastroenteric infections, with an estimation of 685 million cases per year worldwide. Combined with oysters, it is the world second leading cause of pathogen/food outbreaks. Oyster production is heavily impacted by the withdrawal of noroviruses contaminated oysters from the market. To date, no regulatory criteria exists in Europe for the management of noroviruses in foodstuff. The presence of norovirus genomes in vulnerable foodstuff is only monitored by RT-qPCR in control plan or prevalence studies using the ISO 15216-1-2 standard methods. It is now commonly accepted that the presence of norovirus genome is not representative of the presence of infectious particles in the food tested. It is well admitted that viral genomes have a better persistence than infectious particles in the environment, only an unknown part of the detected genomes corresponds to infectious viruses. Noroviruses are extremely difficult to replicate and no reliable method exist to quantify the number of infectious particles in foodstuff. It is essential to find new ways to quantify infectious noroviruses in food samples instead of norovirus genome only as it is not economically sustainable for the stakeholders. F-specific RNA bacteriophages (FRNAPH-II) which are similar to noroviruses (*i.e.* morphology, structure, size), can be used to estimate the amounts of infectious noroviruses in food. The main objective of our work is to study the behavior of infectious FRNAPH-II to different environmental stimulus like UVs, filtration, salinity or temperature to confirm its better survival compared to infectious noroviruses. By using FRNAPH as a reliable indicator of infectious noroviruses, the number of oysters and vegetables batches removed from the European market could be drastically reduced. FRNAPH are a great tool to monitor viral pollution in prospective investigations and to prevent norovirus outbreak in shellfish and vegetables.

bacteriophage biocontrol | aquaculture | Pseudomonas aeruginosa | Escherichia coli | Salmonella enterica

Isolation and characterization of lytic phages from wastewater and their use for biocontrol of human pathogens *in vitro*

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Bacterial pathogens (specially ESKAPE group) are an important risk for human health on a national and international scale, causing mortality in susceptible people (immunocompromised and under other conditions) and even over healthy ones. These problems generate higher costs of hospitalization and maintenance of hospitals (cleaning and disinfection costs) for governments and institutions, requiring more strategies to controls pathogen populations not only in hospitals, but also in sites like wastewater treatment plants where these microorganisms are present. Also, wastewater treatment plants accumulate high numbers of microbial populations and could be a reservoir from resistant and multi-resistant bacteria, addressing special attention for control. For these points, phage treatment is an alternative that gained focus to control bacterial presence from different areas, because eliminates specific pathogens (without disturbing natural populations) even to strain level, and eliminates resistant and multi-resistant antibiotics bacteria. Considering all these aspects, the aim of this work was the isolation and characterization of lytic phages against *Escherichia coli*, *Pseudomonas aeruginosa*, Salmonella enterica, and Staphylococcus aureus strains; and their potential as biocontrol therapy during in vitro trials. Actually, we isolated lytic phages from different zones inside wastewater treatment plant at La Paz, BCS Mexico; with 20 phages for E. coli, 11 phages for P. aeruginosa, and 6 phages for S. enterica. All these phages will be evaluated by individual and cocktail treatments during in vitro trials to know their effectiveness to control bacteria populations.

bacteriophage biocontrol | phage cocktail | salmonella infantis

Selection of lytic phages for a cocktail against multidrug-resistant *Salmonella* Infantis.

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Salmonella spp. is a worldwide distributed foodborne pathogen. In the last decade, *Salmonella* Infantis (*SI*) has been reported as an emergent serovar with high levels of multidrug resistance (MDR) in isolates from chicken meat, pork, humans, and the environment. Thus, biocontrol of MDR *SI* is crucial for food safety. Bacteriophages are viruses that infect only bacteria. Due to capability for cause lysis, phages are an alternative to target emergent MDR SI. Here, we evaluated genomic and phenotypic characteristics of six phages that infect *SI*. Methods: We selected six phages (DR91, DR94, FD55, FD35, 4FA and RB01) from our library that infected *SI* (strain PUCV57). Phages were sequenced by Illumina, annotated with Rasttk, their images were obtained with TEM. Additionally, we determined the host range, using 14 serovars of *Salmonella spp.*, and evaluated their effect on 10 strains of *SI* with different resistant profiles. We selected RB01 to analyze the killing curve at MOI 10. Results: DR91, DR94, FD55, FD35 and 4FA were classified as *Myoviridae*, except DR91 that belongs to the *Siphoviridae* family. Neither of the phages had genes associated with antibiotic resistance, virulence or lysogeny. Host range results showed that DR091, DR094, FD55, FD35, 4FA and RB01 lyses, respectively 13, 13, 6, 11, 8 and 12 different serovars of *Salmonella spp.*, being DR91, DR94 and RB01 the phages with a broader host range. Additionally, DR091, DR094 and RB01 were able to lyses all evaluated MDR *SI*. FD55 and RB01 lyses 9, but phage FD55 cause partial lysis in 7 strains. Finally, we observed that RB01 inhibit the growth of MDR SI from the first to the seventh hour; and RB01 was capable to reduce in 1,23 log CFU/mL to strain tested. Significance: Genomic and phenotypic analysis of six phages identified phages DR091, DR094 and RB0 as possible candidates against MDR *SI*. Since the use of phages are an option against MDR bacteria, it is necessary further studies to develop a cocktail against MDR *SI*.

bacteriophages Salmonella enterica food safety

FageCapsuleS_*Salmonella*_phage formulation with targeted intestinal release, showed successful in chicken applications

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Introduction: *Salmonella* represents a significant problem for the chicken production . Therefore, Bacteriophage (phage) appears as an alternative for the biocontrol of *Salmonella* in chickens, phage cocktails need to be delivery to the intestine in an adequate concentration and viability. **Aim:** To micro-encapsulate a cocktail of lytic and specific phages against *S. enterica* with targeted intestinal release **Methods**: We sequenced and further characterized the 3 phages on their host range, their transduction ability, and their Scale-Up. Different micro-encapsulate formulations whit intestinal release, were tested. Subsequently, was selected as the most successful formulation (liposomes (lecithin-cholesterol)+alginate= L/A, to be applied in 3 groups of broiler chickens: A_B_C, kept in a production unit, with biosecurity standards, for 46 days. All Groups were inoculated orally (with 1 mL 10^7 CFU/mL *S. enterica)* at 40 of production-day. Subsequently, between 40-45 days, were applied the formulations (1mL:10^{10} CFU/cocktail) in Group (A): micro-encapsulated phage; Group (B): non-encapsulated phages; In addition, the control Group(C), was inoculated with 1 mL SM buffer. All experimental groups, were treated 5 times with the cocktail. **Results**: The FageCapsuleS formulation, was composed of 3 phages belonging to morphotypes 2 Siphoviral (Nº=2) and Myoviral (Nº=1), non-transducer of resistance genes, different host ranges, satisfactory Scale-Up and no antagonistic effect. The selected formulation L/A showed 95.36% encapsulation, an average of 66% SIF release at 60 min and 68-95% *in vitro* effectiveness in reducing *S.* enterica. This formulation in Group A showed a 1.8 Log10 reduction of *S. enterica*, compared to Group B (≤0.05). And a reduction of *S. enterica* (≤0.05), was also observed between experimental and Group C. **Acknowledgement:** FONDEF_IDEA_ID18I10235 FageCapsuleS.

Staphylococcus aureus endolysins biopreservatives

Activity of endolysin LysRODI and its derived lysin LysRODIΔAmi against *Staphylococcus aureus* in milk and fresh cheese

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The sustainability of food production is one of the future priority lines in all developed countries, where modern consumers demand safer and less-processed products. In this context, biopreservatives are of special interest, since they are antimicrobials that naturally extend the shelf-life of food and preserve its organoleptic properties. Among the recent studied biopreservatives, phage-derived lytic proteins (endolysins) have a relevant role. The potential of these proteins to eliminate both pathogenic and spoilage bacteria in different environments has already been demonstrated, including in food matrices.

Many foodborne disease outbreaks are caused by *Staphylococcus aureus*, a pathogenic bacterium that can contaminate milk at different stages along the dairy chain, starting from infected dairy cows, carrier handlers or contaminated equipment. For this reason, it is necessary to find new strategies that allow minimizing such risk. One possibility is the use of endolysins as biopreservatives, as findings to date indicate that they are innocuous for humans and do not readily select for resistant variants.

This study evaluates the potential of two proteins, LysRODI and its derivative LysRODIΔAmi (lacking the amidase domain), to successfully decrease the bacterial load in milk contaminated with *S. aureus*. Our results show that the engineered protein is much more efficacious than the parent endolysin under several conditions. Indeed, LysRODIΔAmi is a suitable candidate for biocontrol in milk due to its fast action and high killing effect, reducing contamination to below detection levels in most samples. Moreover, this protein was also effective during lab-scale fresh cheese coagulation with rennet, corroborating the high potential of these enzymes as antimicrobial food additives.

Avian pathogenic Escherichia coli | bacteriophage | phage cocktail | phage therapy | chick

Evaluation of phage cocktails to prevent avian colibacillosis

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Colibacillosis is the main bacterial disease in poultry and is treated mainly by antibiotics, which eventually led to the emergence of antibiotic resistant strains. Our objective was to evaluate the efficacy of two phage cocktails to prevent colibacillosis induced in chicks by the pathogenic strain BEN4358. The first cocktail was constituted of eight phages of different genus, four phages were able to replicate on BEN4358, leading to the death of the bacterial cells; three phages were not able to replicate on the strain, but were able to lyse bacterial cells by the action of virion-associated enzymes and one phage was not active on BEN4358. The second cocktail was composed of the four phages able to replicate on BEN4358. In a chicken embryo lethality assay, the cocktail of 8 phages allowed 90% of chicken embryos to survive an infection by BEN4358, in contrast to the control (BEN4358 only), which gave a survival rate of 0%. Then, embryonated chicken eggs were inoculated by the phage cocktails in the allantoic fluid at 16 days. At day 19, embryonated eggs were transferred from the egg incubator to isolators, where the chicks hatched. Strain BEN4358 was inoculated subcutaneously at one-day-old chicks. Even if phages were detected in chicks, the mortality between the non-treated and the phage-treated groups was 75% and 80%, respectively. There was no reduction in BEN4358 intestinal load. Same *in vivo* experimental scheme was conducted with the second cocktail and the chicks were challenged with a 10-fold reduced bacterial inoculum. There was no difference in the mortality rate between both groups; however, the mortality was delayed in the treated-group. Moreover, the intestinal BEN4358 load was statistically significantly lower in the phage-treated group ($2x10^7$ CFU/mL) compared to the non-treated group ($1x10^8$ CFU/mL).Thus, *in ovo* inoculated phage cocktails did not prevent colibacillosis but phages were transmitted to chicks and one cocktail reduced intestinal carriage.

receptor-binding protein | tailspike protein | depolymerase | carbohydrate-binding module | lectin-like domain | synthetic phages | chimeric proteins | Klebsiella pneumoniae

Unravelling the function of the carbohydrate binding domains in a highly specific tailspike of *Klebsiella* phage KP32

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Klebsiella pneumoniae is recognized as a critical pathogen by the WHO, featured by extensive and even pan-drug resistant strains. *Klebsiella* phages are often capsule-dependent with their receptor-binding proteins (RBPs) highly specific for capsule serotypes. The recently acquired crystal structure of one of the two RBPs of *Klebsiella* phage KP32 (KP32gp38) revealed a multimodular structure with a central enzymatic domain degrading the capsule and two additional C-terminal domains, i.e., a carbohydrate-binding module (CBM) and a lectin-like domain (LD). We analyzed the function of these domains by preparing C-terminally truncated proteins, chimeric protein fusions as well as phage particles lacking the domains of interest. Deletion of LD, located at the ultimate C-terminus, does not disturb the enzymatic activity of K32gp38 with a conserved specificity for serotype K21/KL163. However, synthetic phage particles lacking LD show a dramatic drop in the efficiency of plating, with no infection of the K21 serotype and only small plaques on the KL163 strain. The same infection pattern was noted when both LD and CBM domains have been removed from the phage particle, but also the enzymatic activity was lost on the protein level. There was no serotype specificity switch/extension due to C-terminus deletion nor when the CBM and LD domains were used in chimeric fusions with other RBPs. In addition, the CBM and LD are not able to bind to the surface of capsulated bacteria, whereas cell binding takes place with the full-length protein. In sum, the LD domain is essential for infection and the CBM domain for proper folding and/or enzymatic activity. The exact function of LD and CBM remains to be elucidated, requiring further investigation.

Poultry campylobacter Bacteriophages

Development and application of a phage cocktail to reduce intestinal Campylobacter load in broiler chickens

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Campylobacteriosis is one of the leading food-borne gastrointestinal diseases worldwide. Reducing intestinal *Campylobacter* concentrations in broiler chickens is considered an effective strategy to reduce *Campylobacter* entering the food chain. The aim of this study was (i) to develop a phage cocktail in a systematic in vitro approach and (ii) to investigate the in vivo efficacy in broiler chickens. The in vitro lytic activity of eight newly isolated group II phages and 18 group II phages, and the well characterized group III phage NCTC 12673, each, alone and in different combinations were examined using a Tecan Spark Multiplate reader for a planktonic killing assay (PKA). All combinations of group II and group III phages showed significantly higher in vitro growth inhibition against the used C. jejuni test strain than single ones or combinations of phages of the same group. For application in vivo, a combination of phage NCTC 12673 (group III) and vB_CcM-LmqsCPL1/1 (group II) was selected. One hundred and eighty Ross 308 broiler chickens were divided into a control and a treatment group. Ten days post hatch, seeder birds were orally inoculated with the test strain. Phages were administered via drinking water at a final concentration of 107 PFU/mL four, three, and two days before necropsy. Due to a lower Cmaplyobacter colonization ot the tretment group, the in vivo efficacy of the phage cocktail could only be estimated by the increasing difference of bacterial concentrations in cloacal swabd of the two groups, indicating a reducing effect. At necropsy, Campylobacter counts in colonic content of the treatment group were significantyl reduced by 1.3 log10MPN/g compared to the control group, while there was no difference observed in cecal concentrations. In accordance with these results, Campylobacter isolated from cecal content showed higher resistance rates of 23.6% (NCTC 12673) and 2.8% (vB_CcM-LmqsCPL1/1) compared to isolates from colonic conetent and cloacal swabs.

bacteriophage biocontrol | Bacteriophage isolation | plant pathogens

Impact of phages on plant-pathogen interaction in a gnotobiotic growth system

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With a rising world population and almost all arable agricultural land in use, we encounter a difficult situation. One major factor we could counteract is yield losses caused by plant pathogenic bacteria which are estimated to account for 10% of our total yield losses. But classically used pest control agents like antibiotics lose their effect since more and more bacteria become resistant due to over-usage. Therefore, phages provide a sustainable and targeted solution for biocontrol in agriculture.

For this reason, we isolated novel phages for economically relevant plant pathogenic bacteria. Many plant pathogenic microbes are soil-born and target the plant root as an early entry point. However, the role of viruses shaping the plant microbiome along the root is not well understood and a better understanding of phage influence within the rhizosphere could lead to new application strategies. In our project, we aim to enlighten that 'dark matter' in the plant-bacteria-phage interaction along the root on a molecular and physiological level.

Here, we present the isolation and phenotypic characterization of phage *Alfirin* infecting *Agrobacterium tumefaciens*, phage *Pfeifenkraut* infecting *Xanthomonas translucens*, and phage *Athelas* infecting *Pseudomonas syringae*. All phages show a lytic lifestyle, which is supported by genome sequencing and phage infection curves, making them suitable candidates to test their potential in planta.

To study the tripartite system, we are using a sterile fabricated Ecosystem the EcoFAB. Its gnotobiotic environment is constructed by fusing a fluidic camber on a microscope slide, both enclosed in a sterile container. This enables the investigation of the plant-pathogen interaction, as well as plant-phenotypic changes over time. Further, we used plaque assays, TEM, sequencing, and annotation of phage genes as well as infection experiments to validate our potential phage candidates in terms of suitability for biocontrol applications.

aquaculture bioinformatics Phage Therapy

High throughput annotation of a year worth of published Vibriophages genomes: a meta-analysis

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Vibrio spp. are responsible for major fish diseases, leading to heavy loss in aquaculture farms. They are a promising target for phage therapy. In-depth study of phages genomes is a key step to assess their safety before therapeutic use. However, phage genomes have different structures than their bacterial hosts, such as a higher coding density (CD) and smaller, overlapping genes. In this study, we assessed the quality of genome annotations of vibriophages published last year.

179 published Vibriophage genomes were analyzed using a high throughput pipeline designed to screen for therapy compatible phages in large collections. To ensure repeatable results, multiple heuristics and databases were used for structural and functional annotations.

The median genome size was 59 kbp. The average compute time per phage was 30 min on a single core. The published annotations yielded 24% of functionally annotated genes on average, and an 89% average coding density. After the new analysis, +6% new genes were detected on average, and 49% of the genes had functional annotation. The newly detected genes were small (215 bp on average; average overall gene size was 590 bp), and +9% overlapping genes were detected. Published mean intergenic region size (IRS) was 93 bp on average and decreased to 75 bp after the new study. Eventually, the average CD was +2.14% higher (92%) after our analysis.

Those results prove that the average quality of phage genome annotations can be strongly improved using our high throughput pipeline. 1) The high number of overlapping genes detected, 2) the smaller size of the newly detected genes, and 3) the reduced IRS suggests that our analysis takes better consideration of the characteristics of phage genomes. However, 49% of functionally annotated genes is not enough to prove the absence of harmful genes (such as antibiotic resistance or virulence) in a genome. This pipeline is best suited to screen a large collection, before a deeper annotation.

veterinary | Bacteriophage isolation | ETEC

Phage therapy as a solution to porcine post-weaning diarrhea

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Antimicrobial use in livestock largely contributes to multidrug-resistant bacterial infections in humans. The treatment of porcine post-weaning diarrhoea (PWD) caused by enterotoxigenic *Escherichia coli* (ETEC) is one of the cause of antimicrobials use, and the problem will increase with phasing out zinc oxide in Europe. Phages have been proposed as a promising alternative, but no products are available on the market yet.

By using the PHAGEBio ETEC collection of 84 diverse strains from European pigs with PWD, we tested more than 200 *Enterobacteriaceae* phages for infection, but none of them could infect any of the ETEC strains. We then screened for phages in more than 100 samples originating from wastewater, feces from pigs healthy or affected by PWD, and gut specimens from pigs died from PWD, using different enrichment conditions. We prepared 200 phage stocks and determined their Pulse Field Gel Electrophoresis (PFGE) and host range profiles. After excluding many phages due to similar PFGE or host range profiles, we sequenced and focused our efforts on four phages infecting 33% of the collection. Since phage ETEP21B infected most of these strains, we scaled up production and optimized its formulation. To test ETEP21B efficacy against ETEC in a controlled manner, we set up an animal model for PWD by inoculating 3-weeks old pigs with ET54, a strain from the PHAGEBio ETEC collection. The phages, formulated as a powder and mixed with feed, will be administered prior and post infection. As a control, a group of pigs will be inoculated only with saline, and another one only with ET54. We will monitor clinical signs, diarrhea development, ETEC and phage shedding. In addition, samples for microbiome analysis will be collected to monitor the impact of exogenous phages on the gut microbiome of the piglets. With this animal study, we will test phage efficacy in reducing PWD, and provide data essential to bring a phage product to the market.

bacteriophage biocontrol | Food Safety | root uptake

Potential bacteriophage application against *Listeria monocytogenes* in contaminated basil plants after root uptake

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Background

Listeria monocytogenes (*Lm*) is the causative agent of Listeriosis. It is well known that foodborne pathogens present in soil/irrigation water can internalize via root into edible plants, making the post-harvest intervention strategies insufficient to guarantee the consumer's health. The present study evaluated the potential ability of *Lm* to contaminate basil plant by root uptake and the efficacy of bacteriophages to reduce the contamination levels.

Method

Ten plants were cultivated in hydroponics for 2 weeks. One plant was used as negative control and 9 plants were subjected to internalization by immerging their roots in BHI broth containing *Lm* ATCC 7644 (9 $\log_{10}$ cfu/ml). After 24 h, roots of 6 plants were immersed in a decontamination solution containing ϕIZSAM-1 (10^6 pfu/ml) and roots of 3 plants were immersed in BHI broth (*Lm* internalization control), for 24 h period. At the end of the experiment, leaves and stems of all the plants were tested for *Lm* detection (UNI EN ISO 11290-1:2017) and enumeration (UNI EN ISO 11290-2:2017).

Results

Among the plants used as *Lm* internalization control, 2 out of 3 tested positive for *Lm* detection both in leaves and stems, with an average of 3 $\log_{10}$ cfu/g. The plants used to verify phage decontamination activity were negative to both *Lm* detection and enumeration in 5 out of 6 individuals; instead, 1 plant showed positive results only to *Lm* detection in leaves. The negative control plant did not show any *Lm* contamination.

Conclusions

The results presented in this study demonstrated the ability of *Lm* to internalize via roots in the plants. Moreover, these findings provide information about the potential of bacteriophages to control *Lm* contaminations in basil and could contribute to reduce the risk of human infections due to the consumption of raw/undercooked basil leaves. More research is needed to improve the efficiency of phage administration in horticulture as pre-harvest strategy to guarantee food safety.

Bacteriophages | Genetics | veterinary

Presence of production strain's DNA in BAFASAL® preparation - one aspect of phage commercial product analyses.

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Phage therapy has become a promising way of combating bacterial diseases. However, there are a number of issues that need to be explored in order to develop a good phage product. One of the most important is the selection of a bacterial host for the production of bacteriophages, which requires knowledge of its genome. It is also important in view of the characteristics of the final product, which must be free from bacterial residues, including DNA fragments involved in HGT (horizontal gene transfer). Such DNA fragments may carry genes encoding prophages or antibiotic resistance genes (AMR – Antimicrobial Resistance genes).

In order to register BAFASAL® preparation as feed additive, a lot of tests had to be carried out according to the EFSA (European Food Safety Authority) guidelines. "Guidance on the characterization of microorganisms used as feed additives or as production organisms" (2018) constitutes a document that may assist the applicant for preparation of an application permitting the usage of additives in animal nutrition. According to that document, the study on the presence of DNA from the production strain in BAFASAL® preparation was performed. In this study the presence of GalUniq3 – a fragment characteristic for *Salmonella enterica* serovar Gallinarum, SGphi11 – a fragment of prophage SG12A, AMG-res – a fragment of aminoglycoside resistance gene (aac-6'Iaa gene), and gyrA-res – a fragment of topoisomerase gene with point mutation responsible for quinolone resistance was checked by the PCR method.

In conclusion, none of the analyzed DNA fragments specific for the production strain was detected in any of three batches of BAFASAL® in detection threshold of 0.01 ng/µl. BAFASAL® preparation is deprived of DNA fragments specific for production strain: genes characteristic for *Salmonella* Gallinarum serovar, prophage

and antibiotic resistance genes.

bacteriophage Endolysin Lysis process

Identification and characterization of a novel endolysin from bacteriophage SSU5 infecting *Salmonella* Typhimurium

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Endolysins are peptidoglycan hydrolase produced by phages to lyse bacterial cell walls and release offspring phages. Most of the double-stranded DNA phages have a holin-dependent system to secrete endolysin into the periplasm while certain endolysins containing a secretion sequence or signal-arrest-release (SAR) sequence are exported via a holin-independent system such as Sec pathway. In this study, we characterized a lysis system of core oligosaccharide-specific phage SSU5. *In silico* analysis revealed that ORF26 and ORF27 of SSU5 are putative holin and endolysin (LysSSU5), respectively and that LysSSU5 contains two domains, one homologous to a virion protein D at the N-terminal and the other one homologous to a transmembrane domain (TMD) at the C-terminal. Overexpression of LysSSU5 alone in *Salmonella* Typhimurium LT2 resulted in host cell lysis, but co-expression of LysSSU5 and a holin showed better cell lysis. When Sec inhibitor (NaN_3) was treated in both experiments, the lysis activity was decreased concentration-dependently. The recombinant LysSSU5 could not be purified due to rapid cell lysis but LysSSU5 with truncating mutation of TMD (LysSSU5_ΔTMD) was successfully purified. In the presence of EDTA as an outer membrane permeabilizer, LysSSU5_ΔTMD (1 μM) showed significant reduction in optical density (600 nm) of *S*. Typhimurium LT2, indicating that the domain homologous to a virion D has a peptidoglycan hydrolase function. All these findings demonstrate that the SSU5 phage possesses a lysis system, in which endolysin can be secreted through either the Sec pathway or holin for maximum release of LysSSU5 to peptidoglycan layer. The virion protein D domain could be a valuable resource for the development of Gram-negative bacteria-targeting drug.

bacteriophage | Salmonella enterica | Poultry

The story of a phage cocktail: effectiveness of phage cocktail against *S.* Typhimurium on *in vitro* and *in vivo* models

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Bacteriophages are seen as alternative to antibiotics in fight against foodborne diseases both in animals and humans. They are also being used as antimicrobial agent in food industry.

Salmonellosis is second most common foodborne disease, with over 1 million of cases every year. *Salmonella* Typhimurium is the most common serovar causing salmonellosis in humans and is most commonly associated with contaminated poultry meat, eggs and other poultry-derived produce.

In our work, we have isolated two bacteriophages against *S.* Typhimurium, combined them into an experimental phage cocktail and assessed their effectiveness against this serotype using different models. We have analysed phage ability to infect *S.* Typhimurium under standard laboratory conditions in planktonic culture and in biofilm. We have also analysed the effectiveness of the cocktail using multi-species model of bacterial culture (planktonic and biofilm) and performed experiments in microaerophilic conditions to mimic the growth conditions inside of gastrointestinal tract. Finally, we have assessed the effectiveness of phage cocktail using *in vivo* poultry model, with different times of phage administration after *Salmonella* infection. We have also analysed the prevalence of phages in chicken feces and the spread of phages through the chicken body.

We have observed, that experimental phage cocktail was effective against *S.* Typhiumurium in all *in vitro* trials. Furthermore, it proved effective *in vivo*, regardless of the time of administration in chickens. We have also observed, that phages spread through chicken body as they were present in various organs such as brain or liver. However, the prevalence of phage in feces and organs seemed to depend on the phage and on the individual characteristics of the chicken.

Bacteriophages | Avian pathogenic Escherichia coli | Poultry

From a single plaque to an effective phage preparation preventing colibacillosis in poultry – the story of BAFACOL

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Nowadays, with the crisis of antibiotic resistance, a lot of research is focused on the use of bacteriophages to fight pathogenic bacteria. One of the important issues is to eradicate APEC (Avian Pathogenic *E. coli*) in poultry farming. APEC is the significant etiological factor of bird bacterial diseases causing colibacillosis. Therefore, it is significantly important to develop the phage preparations intended also for that animals. Taking up this challenge, we have developed an innovative bacteriophage preparation BAFACOL, which includes 5 virulent phages with a broad spectrum of specificity for APEC strains. The stability of the preparation and its effectiveness *in vitro* were confirmed. The aim of the study was to assess *in vivo* effectiveness of BAFACOL on productive performance of broilers challenged with APEC bacteria. The research material consisted of 600 broilers, randomly divided into 5 equal groups. The first two groups were negative and positive controls, not infected and infected by APEC strain, respectively. The next two groups were APEC strain infected, but broilers in one of them were given BAFACOL in drinking water every other day, while broilers in the other group were given enrofloxacin on days 9 to 13. The broilers in the fifth group were not infected by APEC strain but exposed only to BAFACOL, which was administered in drinking water every other day. During the experiment period, mortality and zootechnical parameters were monitored. The study showed that BAFACOL is effective in the prevention of colibacillosis and effectively reduces the mortality of birds. BAFACOL significantly improves performance of birds and it has a positive effect on birds welfare. It was demonstrated that prophylaxis with BAFACOL is better than antibiotic treatment, as a result BAFACOL could contribute to the reduction of the amount of antibiotics used in poultry.

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bacteriophage gel preparation | mastitis | tolerance study

Safe use of the new bacteriophage gel preparation BAFACAM, preventing *E. coli* and *S. aureus* udder infections in cows

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Mastitis in cattle is an infection of the mammary glands usually caused by bacteria, especially *Staphylococcus aureus* and *Escherichia coli*. Mastitis is one of the largest problems in the dairy industry in the world. Due to limitation of the use of antibiotics in dairy industry, it is necessary to invent new, alternative methods of controlling mastitis. The aim of the study was to prove the safety of developed BAFACAM gel bacteriophage preparation intended for the prevention of *S. aureus* and *E. coli* udder infections. BAFACAM contains 8 unique phages specific to *E. coli* and *S. aureus* causing mastitis. The bacteriophage cocktail showed specificity for 98% and 100% of different mastitis causing strains of *E. coli* and *S. aureus*, respectively. The phage composition was designed to maintain the lytic activity against resistant bacterial variants to individual phages contained in the preparation. Efficacy of phage cocktail in *in vitro* tests (biofilm destruction, activity in milk) were proved. A natural polymer used in pharmacy and food industry was used to prepare BAFACAM. The tolerance study of the bacteriophage preparation BAFACAM was carried out on the group of 20 cows (10 cows in test group, 10 in control group), in which BAFACAM was administered once to healthy cows during the lactation period. In the study there were assessed parameters such as animal health, serum immunological parameters, somatic cell count (SCC), microbiological analysis of milk, bacteriophage presence in milk, general and local side effects.

It has been shown that the preparation is safe for intramammary application in cows. The research results obtained so far prove the safe use of BAFACAM in cows and indicate its high potential in the control of mastitis in dairy cattle.

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phage ecology smear-ripened cheese

Bacteriophages infecting surface bacteria from a smear-ripened cheese are persistent and contaminate the dairy plant

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Smear-ripened cheeses are well known for hosting high densities of microorganisms, comprising mainly yeasts and bacteria. Bacteriophages, *i.e.* viruses infecting bacteria, are also present in dairy products but most studies currently available focus on phages infecting lactic acid bacteria (LAB) starter cultures since they can be responsible for fermentation failure. However, their effect on bacteria colonizing cheese rind over the ripening period is not clear thus far. Recently, the composition of the viral community present on the rind of smear-ripened cheeses was explored by using viral metagenomic and culture-dependent approaches. The results collectively revealed a great diversity of phages which, for the vast majority, have no sequence homology with known phages and, consequently, no predicted hosts. The hypothesis of the present study is that phages have an important ecological role in cheese ecosystem.

In order to explore this question, isolation of “indigenous bacteria” (e.g. non LAB starters) and their associated-phages from the surface of a French smear-ripened cheese was conducted. We obtained five virulent phages infecting *Brevibacterium aurantiacum*, *Glutamicibacter arilaitensis*, *Leuconostoc falkenbergense* and *Psychrobacter aquimaris* respectively. Their host range was very narrow since they were only able to infect a few isolates of the same species obtained from the same studied cheese. The analysis of their genome indicated that four of them are new, constituting at least new genera. Complementary analysis, performed using both microbiological (spot assays) and molecular (viral metagenomic) approaches, confirmed the persistence of these phages on cheeses sampled over a 6-years period and helped identifying three potential reservoirs within the dairy plant.

DNA assembly Pseudomonas SEVA vectors

SEVAtile: a standardized DNA assembly method optimized for *Pseudomonas*

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Over the past decade, the generation of genetic circuits has become a reproducible and efficient process due to standardized DNA assembly techniques. Several of these techniques are based on Type IIs restriction enzymes (RE), including BioBrick and VersaTile, which enable the rapid construction of transcription units and/or fusion proteins from standard DNA building blocks. While standard DNA assembly techniques originated in the field of synthetic biology (SynBio) for model organisms such as *Escherichia coli* and *Saccharomyces cerevisiae*, it would be beneficial to extend these approaches to biotechnological research with other, non-model species including *Pseudomonas*.

As *P. putida* is gaining attention as a valuable SynBio host, some SynBio standards and expression systems have already been optimized for this species. The Standard European Vector Architecture (SEVA) database offers a wide array of standard, modular vectors for Gram-negative species, especially *P. putida*. Due to their elegant design and the public accessibility, these SEVA vectors are considered the golden standard for the *P. putida* biotechnology research community. To introduce both SEVA vectors and DNA assembly standards in the *Pseudomonas* community in a low-threshold manner, we introduce the SEVAtile technique. SEVAtile is a Type IIs-based assembly approach, which enables the rapid and standardized assembly of genetic parts – or tiles - to create genetic circuits in the established SEVA-vector backbone. Contrary to existing DNA assembly methods, SEVAtile is an easy and straightforward method, which is compatible with any vector, both SEVA- and non-SEVA. To prove the efficiency of the SEVAtile method, fourteen genetic constructs with up to six building blocks were created with high efficiency (on average 5.39E+04 CFU per µg transformed DNA). Furthermore, a three-vector system was successfully generated to independently co-express three different proteins in *P. putida* and *P. aeruginosa*.

nanobody phage display Mycobacterium bovis

Nanobodies as a new diagnostic tool for *Mycobacterium bovis*

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Objective

In this study, we aim to improve the *M. bovis* diagnostics by using the single variable domains of heavy chain only antibodies (nanobodies), derived from bovine tuberculosis (bTB) naturally infected alpacas. The search of high affinity nanobodies targeting *M. bovis* will be performed by the phage display technology. This powerful technique allows high-throughput screening of protein-protein interactions by displaying proteins on the surface of phages.

Methods

As a first step, an alpaca nanobody displaying phage library was constructed with nanobody coding sequences derived from B-cells isolated from alpacas at the Salictum alpacafarm. To this end, blood samples were collected from alpacas and the peripheral blood mononuclear cells (PBMCs) were isolated. Subsequently, the total RNA was extracted and converted into cDNA. Based on literature, primers were optimized and used to amplify the regions of the cDNA encoding for the nanobodies. The nanobody fragments were cloned into a newly constructed phage display vector, transformed to XL1 blue cells, creating an alpaca nanobody library. In a last step, this library was infected by phages to create the alpaca nanobody displaying phage library.

Results

In total, 10^8 PBMCs were isolated from 50 ml blood and a total of 62 µg RNA was extracted. Higher yields of RNA were obtained by performing the RNA extraction immediately after PBMC isolation. All the RNA was converted into cDNA and resulted in 30 µg nanobody DNA fragments. An alpaca nanobody library with 10^6 individual transformants was obtained and sequencing analysis confirmed successful cloning of the domains in the phage display vector.

Conclusions

The optimization of the preparation of the alpaca nanobody displaying phage library will help to efficiently create such a library with nanobodies derived from bTB naturally infected alpacas. The phage display technology will be performed with this library to find a nanobody targeting *M. bovis*.

Vibrio Agriculture Aquaculture

Development of bacteriophage as feed additive in aquaculture feed for controlling pathogenic *Vibrio* spp.

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Vibriosis is the most common disease leading to large-scale losses and mortality of aquaculture which is considered as a significant problem with severe economic losses worldwide. *Vibrio* spp. are usually associated with aquaculture diseases in shrimps and fishes. Bacteriophage is a potential alternative treatment against *Vibrio* infection. This study aimed to develop a phage-based feed additive for applying in aquaculture feed. In our study, 57 isolates of *Vibrio* spp. were obtained form seafood including shrimps (*Litopenaeus vannamei*) and blood clams (*Tegillarca granosa*). The pathogenic and strain characteristics of each isolate were confirmed via *16s rRNA* gene. Only *Vibrio parahaemolyticus* isolates were used to confirm the presence of *pirVP* gene causing acute hepatopancreatic necrosis disease (AHPND). Of 21 *Vibrio* phages isolated from sea, blackish and fresh water samples (n = 51), only phage PVIB002 phage showed the highest phage lytic ability up to 36.8% on the given hosts and was characterized as polyvalent phage infecting a variety of species in the genus of *Vibrio*. Phage PVIB002 at the multiplicity of infection 10 and 100 showed the reductions of over 3 log CFU/mL (60.0±0.2%) and 5 log CFU/mL (100.0%) at 6 h post-treatment, respectively. For phage survivability test, phage PVIB002 survived at temperature up to 55°C (90.9% recovery), at pH between 5 to 11 (100% recovery), and in the presence of available chlorine up to 5% (v/v) (24.5% recovery). Phage PVIB002 was used to formulate the aquaculture feed. The phage number showed a slight reduction during storage for 30 days (6 to 5.6 log PFU/g). Finding here suggests that a phage-based feed additive is effective biocontrol alternative for controlling pathogenic *Vibrio* spp. in aquaculture production.

Bacteriophages Pseudomonas Milk

Isolation, characterization and application of phages for biocontrol of *Pseudomonas fluorescens* in milk

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Psychrotrophic bacteria are among the leading problematic microorganisms in dairy industry. *Pseudomonas fluorescens (P. fluorescens)* is frequently isolated from raw and pasteurized milk and a prominent species with its extracellular enzymes. Since these enzymes can remain active even after heat treatment, they cause problems such as gelation, taste and aroma deterioration in both pasteurized/UHT milk and dairy products. For this reason, it is vital for dairy industry to prevent *Pseudomonas spp.* from reaching the numbers that can produce these enzymes. Phages are an important alternative for combating pathogenic or spoilage bacteria in food industry and used successfully in many applications. The number of isolated and sequenced *P. fluorescens* phages in the literature is quite limited. The aim of the present work was the isolation, identification and comprehensive characterization of phages for biocontrol of *P. fluorescens* and to determine the elimination efficiency of these phages. For this purpose, *P. fluorescens* were isolated from raw milk. Then, phages were isolated from similar sources using the isolated bacteria as host. The host range of phages were determined, then their morphological characterization was carried out using TEM (transmission electron microscopy) and they were classified by RFLP (restriction fragment polymorphism analysis. In order to determine the replication parameters of phages, one-step growth curves were obtained.The elimination effectiveness of phages were carried out in a model milk environment. Thus, the efficacy of phages against *P. fluorescens* was elucidated and a method was established for the natural and economic solution of problems that cannot be overcomed by the existing methods in the milk and dairy industry.

phage therapy | enterotoxigenic E. coli | swine

Controlling ETEC colonization on cultures of an intestinal pig cell line with a T4-like phage

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Enterotoxigenic *Escherichia coli* (ETEC) colonizes the intestine of young pigs causing severe diarrhoea and consequently bringing high producing costs. The rise of antibiotic selective pressure together with on-going limitation on their use demands news strategies to tackle this pathology. The pertinence of using phages to tackle this problematic is being explored, and in this work, the efficacy of a T4-like phage vB_EcoM_FJ1 (FJ1) in reducing the load of ETEC O9:H9 (Sta, F5/F41) was assessed. FJ1 has a 170,053 bp genome, and of the 270 coding sequences none corresponds to identified undesirable proteins, such as integrases or transposases. Envisaging the oral application to piglets, FJ1 was previously encapsulated on $CaCO_3$/alginate. Assays were performed on 15-day cultures of the intestinal pig cell lineIPEC-1 seeded in transwell inserts. Phage treatment occurred 2 hours after ETEC infection, when, in average, $5x10^5 CFU.cm^{-2}$ were adhered to cultured cells. Encapsulated phage provided reductions of, approximately, 2.3 Log $CFU.cm^{-2}$ and 2.8 Log $CFU.cm^{-2}$ on adhered bacteria, respectively 3 and 6 hours after administration. The repeated exposure of the host to FJ1 led to the emergence of phage-insensitive mutants, phenotype that brought fitness costs to the host strain: they were 90% more vulnerable to the pig complement system and less efficient in adhering to cultured cells (in about 90%). Overall, FJ1 is presented here as promising to fight against ETEC infections through oral administration to piglets.

Salmonella Poultry bacteriophage biocontrol

Evaluation of UPWr_S134 phage cocktail for use in the control of *Salmonella* in poultry

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Salmonella remains the major cause of food-borne diseases worldwide and poultry products are recognized as the leading source of human infections caused by *Salmonella* contamination. Moreover, *Salmonella* form challenging biofilms that contribute to their virulence and antimicrobial resistance. In our study, we focused on bacteriophages infecting Salmonella isolated from the poultry environment. Five novel lytic UPWr_S phages were biologically and genetically characterized.

All phages showed strong antimicrobial activities against various Salmonella serovars, including the most prevalent serovars in poultry production.

Based on the great potential of UPWr_S phages we further evaluate in vivo effectiveness of phage cocktail UPWr_S134 composed of phages UPWr_S1, UPWr_S3 and UPWr_S4 to control Salmonella Enteritidis in experimentally infected animals. In a mouse model of acute typhoid fever, animals in eight groups treated with phage cocktail showed significant ($P < 0.05$) delay in death from sepsis caused by highly virulent S. Enteritidis 327 lux strain irrespective of dose and treatment schedule. The above findings correspond to in vivo phages' ability to reduce Salmonella in the chicken model. We demonstrated that UPWr_S134 cocktail significantly reduces ($P < 0.05$) the bacterial load by 2 - 3 log10 CFU/g in internal organs of the immune system such as bursa of Fabricius, cecal tonsils and spleen.

Additionally, the phage cocktail showed a prospective ability ($P < 0.05$) to reduce in vitro biofilm formed on poultry drinkers and eradicated Salmonella from the abundant bacterial load on poultry drinkers in an experimental chicken model.

Our results indicate that UPWr_S phages are effective in combating the highly invasive Salmonella serovars and support the future application of UPWr_S cocktail to target Salmonella infection in poultry.

Avian pathogenic Escherichia coli | Poultry | bacteriophage biocontrol

UPWr_E134 phage cocktail effectively control avian pathogenic *E. coli* in animal models

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Avian pathogenic E. coli (APEC) are responsible for severe economic losses in the poultry industry. APEC may cause a variety of diseases in the avian host, which are collectively termed avian colibacillosis. Moreover, avian pathogenic E. coli are frequently resistant to multiple drugs including antibiotics, entailing the treatment and control of these infections extremely difficult.

Four UPWr_E phages have been genetically and functionally characterized and have the potential for the prevention and treatment of infections caused by APEC and are also effective against ESBL Klebsiella pneumoniae strains. UPWr_E phages alone and mixed in cocktail exhibited great activity in biofilm removal. Moreover, gastric gavage with UPWr_E124 phage cocktail resulted in a highly reduced number of luciferase-expressing APEC strain in mice internal organs such as liver, spleen and kidney. Both intraperitoneal and intragastric administration of a single dose of UPWr_E124 phage cocktail effectively eradicated pathogens from the lungs. Interestingly, intraperitoneal injection of phage cocktail in mice infected with APEC strain did not affect the bacterial number. Phages were detected only in organs taken from mice from the group administrated with phages via gastric gavage. These data suggest that in the murine model the overall rate of phage absorption in the gastrointestinal tract seems to be more efficient.

Additionally, the UPWr_E124 phage cocktail's ability to eradicate APEC was confirmed in an experimentally infected chicken model. APEC load in the internal organs was substantially reduced by over 3 log10 CFU/g and the number of APEC isolated from the blood was lower than 5 log10 CFU/g indicating great application potential of UPWr_E124 phage cocktail in poultry production.

temperature | Phage-host interaction | plant pathogenic bacteria | Podoviridae

Too hot for infection: temperature affects interaction between plant pathogen *Dickeya fangzhongdai* and its bacteriophage

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Bacterial plant pathogens represent a major challenge for agriculture. Soft rots including those caused by *Dickeya* spp. greatly reduce the yield of agricultural crops used to sustain the nutritional needs of the increasing world population. There is no efficient chemical management strategies implemented and bacteriophage biocontrol strategies were proposed as a promising alternative. Nevertheless, bacteriophage biocontrol strategies are still faced with many challenges, including resistance development in bacteria and complex dynamics among bacteriophages, bacteria and their environment, which is still poorly understood. Among environmental factors temperature is critical in disease development caused by *Dickeya* spp. and can directly affect physiology of the host cells.

In our study, we investigated the influence of the environmental temperature on bacteria-bacteriophage interactions on a comprehensively characterised bacteria - bacteriophage system based on bacteria *Dickeya fangzhongdai* and a bacteriophage from family *Podoviridae* BF25/12. The bacteria – bacteriophage interactions were followed in the system using spot and plaque assay, adsorption assay and following bacterial growth kinetics. All tested susceptible *D. fangzhongdai* strains showed reduced bacteriophage susceptibility at higher temperatures 28 °C. However, bacterial growth kinetics did not differ significantly between tested temperatures. At 37 °C adsorption of bacteriophages could still be detected at low levels to strain *D. fangzhongdai* B16. However, adsorption did not lead to bacterial lysis despite bacterial growth.

The results of our study indicate the importance of the environmental conditions and the necessity of understanding relevant environments for bacteria-bacteriophage systems. This is especially of a great importance for bacteriophage biocontrol applications in the field of agriculture and plant health.

phytopathogenic bacteria | Xylella fastidiosa | Bacteriophages | biocontrol

Bacteriophages against the plant pathogen *Xylella fastidiosa*

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***Xylella fastidiosa* (Xf) is a worldwide important plant pathogen of the family *Xanthomonadaceae*, which can cause serious diseases in strategic crops with devastating effects. Its host range exceeds 500 plant s**pecies. Xf lives in the xylem of the plant, where it can form biofilms and obstruct sap flow, and also in the foregut of xylem-feeding insects that act as vectors. This bacterium presents a great genetic diversity that results in different subspecies and sequence types (ST). In Europe there are several outbreaks, most of them located in the Mediterranean basin, affecting different host plants, but mainly olive and almond trees, depending on the subspecies and ST of the pathogen present in each area. Since there are no effective therapeutic measures and antibiotics are forbidden in Europe, the control of *Xf* is mainly based on the eradication of infected plants and the use of chemical compounds against insect vectors, but this is not enough. Efficient alternative strategies that are environmentally friendly are needed. Biological control using bacteriophages can be a viable and sustainable tool in an integrated management of Xf diseases. Due to the inherent difficulty in culturing Xf under laboratory conditions, *Xanthomonas* spp. strains were used as surrogate hosts for phage hunting in plant, water and soil samples from areas with active Xf outbreaks, and also in wastewater samples. A total of 22 bacteriophages were isolated and amplified, and their lytic activity was tested against more than 50 strains of *Xanthomonas* spp. and also on strains of other phytopathogenic bacterial species. All bacteriophages were phenotypically and genomically characterized and four of them with lytic activity against different strains of Xf were selected for *in vivo* assays. Infected plants treated with phages showed less symptoms than control plants. These results are very promising and suggest that some of the selected phages could be used for control of Xf diseases.

Salmonella spp. Decontamination Bacteriophage

Efficacy of Bacteriophage-containing Microaerosol in Volumetric Decontamination

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Introduction

Volumetric decontamination of confined environment raised substantial interest because it allows to simultaneously decontaminating pathogens, including in hardly accessible sites that is absolutely impossible with conventional manual protocols widely accepted and practicing today. Use of bacteriophages for volumetric disinfection looks very promising for multiple scenarios.

Aim

The aim of this study was to investigate the possibility of using bacteriophage-containing microaerosol in decontamination of salmonella-infected surfaces with a special medical nebulizer that produces microaerosols.

Materials and Methods

The stock suspension of Salmonella phage cocktail of 10^8 PFU/ml and culture of *S. typhimurium* $1{,}1x10^4$ CFU/ml concentration were used in the experiments. 100 µl of fresh *S. typhimurium* cells were inoculated on a surface of solid nutrition medium and on different types of coupons. Coupons and plates were placed to enclosed plastic chamber (volume 0,08 m^3) and microaerosol composed of *Salmonella* bacteriophage was fumigated to the chamber using medical nebulizer attached to it to generate microaerosol (~2µm in diameter) and create phage droplets in the form of a cool fog. Multiple controls were applied, including cells survival on the coupons without treatment, fumigation of microaerosol not-containing phage, etc.

Results

Experiments revealed that the minimum time leading to complete elimination of the pathogen was 1h. both on different types of coupons as well as on solid nutrient medium. At 1h of exposure, 5 ml of phage concentrations completely eliminated the pathogen, and after application of 3 ml of phage, viable cells remained on coupons and the solid nutrient medium.

Obtained results demonstrates that the minimum exposure time and minimum phage concentration was 5 ml phage at 1 h exposure.

Conclusion

Our experiment data suggest that bacteriophages can be successfully used as a useful biocontrol of pathogenic microorganisms.

Food Safety | bacteriophage biocontrol | Listeria | Salmonella

Application of bacteriophages to enhance food safety from Pharm to Phork: Conquer the challenges!

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In the face of recurrent foodborne outbreaks and product recalls, the need for safer foods remains in the public interest. The extended overuse and misuse of antibiotics and other antibacterial agents in the food industry have further exacerbated the spread of foodborne pathogens with the rise in antimicrobial resistance. This increased demand for safer food products with clean label drives the continuous search for natural alternatives to control the growth of pathogenic bacteria. Bacteriophages have emerged as a legitimate antibacterial alternative with a wide scope of applications which continue to be discovered and refined. From farm to fork, bacteriophages have been shown as a viable option to treat diseases in animals and plants, reduce biofilm formation and contamination in food processing environment, and increase the safety and shelf-life of food products. In this presentation, our research group effort in application of lytic phages to tackle selected bacterial pathogens will be presented. Suggested approaches to overcome the challenges of phage applications as biocontrol tools throughout the food supply chain will also be discussed. This presentation will start with developing a spry dried phage-carrier biopesticide to control Erwinia amylovora, the causal agent of fire blight disease in apple and pear. Then, the potential of Listeria lytic phage to disperse Listeria monocytogenes biofilm will be evaluated in a simulated food processing conditions. Finally, a smart and high throughput approach for developing a phage cocktail to mitigate the risk of Salmonella in poultry products will be presented.

Erwinia amylovora | bacteriophage biocontrol | Agriculture

Evaluating candidate phages as biocontrol tools against fireblight disease in fruit trees

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Fireblight is one of the most important fruit diseases and has catastrophic consequences for apple and pear trees. The development of an innovative strategy for an effective fight against it is a priority challenge for fruit production in France and worldwide. This disease is caused by the bacterium Erwinia amylovora and a potential successful biocontrol strategy could be the use of phages, the viruses of bacteria. To achieve this goal, a collection of 16 new Erwinia phages isolated in the south of France were analysed to determine their morphology, their genome and their host range on a selection of 44 E. amylovora strains. This phage collection represents 5 genera, including 1 new, and 7 different species, including 4 new ones. The phages observed are tailed, with a majority of myovirus and a few podovirus morphological types. Phage lifestyle analysis determined that all phages are virulent, as reported before for Erwinia phages. Ten phages showed a large host range, targeting more than 20 E. amylovora strains, and 2 phages were capable of infecting 100% of the strains. A selection of Erwinia phage candidates based on genomic diversity and host range extent criteria was made for subsequent tests. Currently, the efficacy of the selected phages in controlling the bacterium in vitro and on apple tree seedlings is under evaluation. Overall, this project seeks to prove the potential of phages as an efficient biocontrol tool against fireblight disease.

RFLP Phage Therapy Escherichia coli O157:H7 Salmonella spp.

Classification and Characterization of Bacteriophages Effective Against Foodborne Pathogenic Bacteria

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Phages that are effective against *Salmonella* and *E. coli* can be used for different purposes when considering human health and food safety. One of these is to replace phages with antibiotics in the treatment of diseases caused by these pathogens. The others are the use of them in surface disinfection instead of chemical disinfectants, and/or the treatment of the food surface with phages in order to ensure food safety. For phage therapy to be more effective, more comprehensive, and productive, it is of utmost importance that the characteristics of the phages are determined. Within the scope of this study, *S.* Enteritidis, S. Typhimurium, and *E. coli* O157: H7 phages were classified according to their host specificities, morphological properties, and RFLP analyses. After that, they were characterized by determining their replication parameters, and the possible genotoxic regions of phages were tested. To perform all of these tests, phage titers were increased to a suitable level for the isolation of DNA and determination of their host spectra. The DNAs of these phages were then be isolated using isolation kits and RFLP analyses, which are the basis for the classification studies, were performed. The differences of the phages were determined by using the fragments that occur as a result of the enzyme digestion. Later, whether the phages contain certain toxic gene regions were checked. Additionally, the growth curves were subtracted, latent period and burst sizes were calculated, and the adsorption rates were determined. Finally, mixtures of phages were prepared according to the host spectra and RFLP results, and the elimination efficiencies of those mixtures were determined. Thanks to a particular phage collection that was established in this study, acquired phage products could be used both in human/animal experiments and that might be usable at any stage of the food safety process.

Aeromonas salmonicida | Bacteriophages | Aquaculture

Isolation and characterization of a new bacteriophage against *Aeromonas salmonicida,* the causative agent of furunculosis

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Aeromonas (A.) salmonicida, a Gram-negative bacteria belonging to the *Aeromonadaceae* family, is a primary fish pathogen that causes furunculosis in salmonids, carp and perch, as well as septicemia in a variety of fish. This species is considered as one of the main bacterial pathogens responsible of important economic losses in aquaculture industry. Large amounts of antibiotics such as oxytetracycline, quinolones and sulfonamides are used to treat this infection, which highly contributes to the emergence of antibiotic-resistant strains. The application of bacteriophages (phages) in aquaculture seems to be a promising solution to control pathogenic bacteria in this field because phages are well adapted to aquatic environments. The aim of this work was to isolate and characterize new lytic phages against *A. salmonicida.* The phage isolation was performed by enrichment method, against the ATCC 7965 strain of *A. salmonicida*. This method consists in mixing a centrifuged and filtered water sample with a bacterial culture in exponential phase. When clarification of the medium is observed, the supernatant of this mixture is spread on the surface of LB agar and covered with a bacterial overlay in exponential phase. Phage present in distinct clear lysis plaques is then purified three times by subculturing. For this purpose, a sampling campaign of water from fish farming ponds in the south of Belgium was carried out in January 2022. Out of 36 water samples, a new lytic phage was isolated. This phage remained active between 4 and 10 units of pH but shows a drop of activity at 37°C. A preliminary host spectrum test showed that this phage was not active against 3 other *A. salmonicida* strains. Further studies are now needed to analyse the genome and to assess the *in vivo* safety and efficacy of this phage.

Bacteriophage | Erwinia amylovora | bacteriophage biocontrol

Characterization and identification of lytic bacteriophages against *Erwinia amylovora*

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Fire blight is a bacterial disease that affect apple, pear and quince trees. Disease symptoms can be seen all over the plant including blossoms, leaves, fruits, shoots, branches and rootstock. *Erwinia amylovora* is a bacterium that causes fire blight disease. It is a Gram negative, motile, non-sporulating bacterium. *E. amylovora* is able to produce exopolysaccharides that gives it pathogenicity. The bacteria can penetrate plant tissue, which enables the disease to spread all parts of the plant. Antibiotics and chemicals are the most widely used control strategies for fire blight, but the recent problem of resistance to antibiotics and chemicals has triggered the search for new methods. Today, phage therapy is one of the methods seen as an alternative to antibiotics and other chemicals in the treatment of bacterial infections. Bacteriophages, called phages for short, are viruses that only act on bacteria. Today, lytic phages can be used against pathogenic bacteria that develop antibiotic resistance to protect human and animal health, as well as in the field of food and agriculture, as a reliable alternative to prevent bacterial contamination and thus infections. Therefore, it is possible and appropriate to use phages, a biological material, in a reliable and effective way, instead of expensive and environmentally harmful chemicals to protect plant health. It is of vital importance to characterize the phage to be used for phage therapy, both in terms of genomic and developmental parameters. In this study, the isolation characterization of phages effective against *E. amylovora* bacteria was performed. Phages were isolated using leaves and soil samples from areas where the disease was seen. One-step growth curve, adsorption kinetics, TEM image, RFLP and genomic characterization steps were performed. Thus, phage cocktails can be created for use in field applications.

Phage Therapy | Bacteriophage | Galleria mellonella | Escherichia coli K1

In Vitro Characterization and Assessment in *Galleria mellonella* of Newly Isolated Phages against *Escherichia coli* K1

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Extra-intestinal *Escherichia coli* express several virulence factors that increase their ability to colonize and survive in different localizations. The K1 capsular type is involved in several infections, including meningitis, urinary tract, and bloodstream infections. The aims of this work were to isolate, characterize, and assess the in vivo efficacy of phages targeting avian pathogenic *E. coli* (APEC) O18:K1, which shares many similarities with the human strains responsible for neonatal meningitis. Eleven phages were isolated against APEC O18:K1, and four of them presenting a narrow spectrum targeting *E. coli* K1 strains were further studied. The newly isolated phages vB_EcoS_K1-ULINTec2 were similar to the *Siphoviridae* family, and vB_EcoP_K1-ULINTec4, vB_EcoP_K1-ULINTec6, and vB_EcoP_K1-ULINTec7 to the *Autographiviridae* family. They are capsular type (K1) dependent and present several advantages characteristic of lytic phages, such as a short adsorption time and latent period. vB_EcoP_K1-ULINTec7 is able to target both K1 and K5 strains. This study shows that these phages replicate efficiently, both in vitro and in vivo in the *Galleria mellonella* model. Phage treatment increases the larvae survival rates, even though none of the phages were able to eliminate the bacterial load.

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Bacteriophages | Lytic bacteriophage | adsorption process

Effect of the rotating magnetic field exposition on the lytic bacteriophages life cycle

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Electric and magnetic fields influence the basic life processes of microorganisms. Electromagnetic fields influence enzyme activity, cell viability, metabolic activity, and proliferation rate. Additionally, the DNA synthesis, transcription, and translation processes can be overregulated. The use of the rotating magnetic field (RMF) to enhance and support the biotechnological processes is a promising idea. At this moment there are no field-based methods for the support and intensification of bacteriophages production. The aim of this study was to evaluate the influence of RMF on the selected bacteriophages properties.

Obtained data showed that the RMF modified the bacteriophage lifecycle and lytic activity. It was observed that the latent period of the lytic cycle of T4-phage was shortened from 20 min to 15 min (excluding the adsorption time). Additionally, the burst size significantly increased from 103 PFU per infected cell to approx. 330 PFU per infected cell. Additionally, the study showed increased lysis activity of this phage on liquid cultures of E. coli when temporary (1h) exposition was used. These preliminary experimental results showed the potential usage of RMF in bacteriophage research and process bioengineering.

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Bacteriophages smear-ripened cheese Brevibacterium aurantiacum

The impact of Brevibacterium aurantiacum virulent phages on the production of smear surface-ripened cheeses

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Although it is well documented that virulent phages negatively impact lactic acid bacteria, their impact on ripening strains, such as *Brevibacterium aurantiacum*, has been overlooked. Here, we aimed to study the effect of *B. aurantiacum* virulent phages on the production of smear-ripened cheeses. We used model cheeses at an industrial factory and monitored the development of the color of the cheese rind as well as of its microbial composition under two conditions: a control group with no added phages and a phage group, in which the virulent phage AGM9 was added. Our results show that the presence of *B. aurantiacum* phages significantly delayed the development of the orange rind color in the model cheeses. Surprisingly in the final days of ripening, phages were also detected in the control curds. Sequencing the genome of phage isolates from the control cheeses indicated that they were different than phage AG9, suggesting contamination from the manufacturing and storing environments. Our results highlight the risks of using a phage-sensitive strain in smear-ripened cheese production. To the best of our knowledge, this work is the first to report on the impact of *B. aurantiacum* phages in smear-ripened cheeses.

Mycoviruses | Fusarium culmorum | Wheat

High-throughput sequencing reveals novel RNA mycoviruses in *F. culmorum* isolates

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Fusarium culmorum is a causative agent of several diseases of cereals including fusarium head blight and foot and root rot. *F. culmorum* and its disease route has been studied extensively, however, mycoviruses infecting it remain an elusive factor potentially influencing its phenotype. While majority of the known mycoviruses do not appear to cause detrimental effects to their hosts, there have been published reports showing the possibility of hypovirulence and their use as biocontrol agents. With the objective to investigate hypovirulent mycoviruses, we isolated F. culmorum from several wheat (Triticum aestivum) cultivars and screened for mycoviruses. We extracted double stranded RNA (dsRNA) and performed RNA-sequencing. Based on the RNA-dependent RNA polymerase domain analysis, we found a number of dsRNA but also a few ssRNA mycoviruses. Using blast, we found a member of Hypoviridae family, two dsRNA viruses - within Unirnaviridae and Partitiviridae families. In addition, two negative sense ssRNA mycoviruses belonging to Mymonaviridae and one distantly related to Aspiviridae were detected. Further efforts to sequence full genomes, analyze phylogeny and characterize these viruses are still ongoing.

Bacteriophages antiphage Phagecontamination

Antiphage agents: Molecules, polymers and nanoparticles

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I plan on discovering or producing several antiviral agents that have vast areas of application. The aim is to aid industries that rely on bacterial products for commercial use in a broader aspect. Bacteriophages are ubiquitously available biological entities that are currently most predominant, capable of being friends or foes. Phage contamination was first reported by Whitehead and COX in 1935 in a dairy culture, paving the way to a brand-new field of research. My poster will broadly cover three categories of antiphage agents:

1. Molecules: In this project, I showed a new method for the inactivation of bacteriophages. We used a food additive to decrease active virions in phage suspensions. The main aim of the research is to explore antiphage agents that can be used directly in bioreactors. T

2. Polymers: Associations of bacteriophages and polymers find applications in different research fields, including targeted drug delivery, decontamination of microbial infections, as antibiotics alternatives, bacteriophage entrapping and encapsulation, and phage inactivation. The disruption of the bacterial cell wall by varying proportions of [+] and [-] ligands tethered to the nanoparticles has been studied in the past. Inspired by mixed charge nanoparticles against bacteria, my aim is to artificially produce polymers of mixed charges and amphiphilicity to inactivate bacteriophages

3. Nanoparticles: Nanoparticles are emerging in the field of microbiology with the revelation of their antimicrobial nature. I am exploring the efficiency of silver nanoparticles in the presence and absence of various ligands, along with silver, bismuth and antimony alloys against bacteriophages. Antimony and Bismuth have shown promising results. Moreover, I explore the effect of nZVI on bacteriophages, and establish how different phages react differently under the influence of the same nanoparticles.

Phage Therapy | Salmonella | Poultry

Evaluation of the prophylactic and therapeutic effect of a phage cocktail to control *Salmonella* Enteritidis

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Salmonella is one of the main causes of foodborne disease related to poultry product consumption and a public health concern. The use of lytic bacteriophages could be a novel, safe and effective approach to reduce the prevalence of *Salmonella* in poultry and subsequently reduce the incidence of foodborne salmonellosis in humans.

This study evaluates the preventive and therapeutic effect of phages, administered via drinking water, on *Salmonella* levels in chickens. A 6-phage commercial cocktail, SalmoFresh™ (developed and produced by Intralytix, Inc., Columbia, MD, USA), with demonstrated *in vitro* efficacy against a variety of *Salmonella* serotypes was used.

First, the phages were demonstrated to persist in chickens' gut for at least 3 days without *Salmonella* challenge. The prophylactic and therapeutic potential of the cocktail was then evaluated *in vivo.* 50 chicks were challenged by oral gavage with *Salmonella* Enteritidis LA5 at $5x10^4$ CFU/chick at 7 days of age. Phages were administrated before the challenge via drinking water during the first 6 days of the chicks' life and 2 days prior to the end of the trial. *Salmonella* enumeration and phage identification and counting were investigated during the 4-week trial.

Results showed that up to 4 days post infection, phages had a preventive effect and they significantly ($P<0.05$) reduced *Salmonella* colonization in the ceca and the feces by 2 to 4 logs. *Salmonella* levels increased 7 days post infection, after phage treatment was paused. During this period, only 2 out of 6 phages were detected in the different gut segments. Resumption of phage administration 2 days before the end of the trial reduced the *Salmonella* loads again by 1 log in the ceca.

This treatment showed encouraging results regarding the effect of phages on *Salmonella* levels in chickens during critical steps of poultry production; data suggest that administration and dosing regimen may need to be further optimized for optimal effect for the poultry industry.

bacteriophage biocontrol | Pseudomonas syringae | Carrier bacteria | Lytic Phages

Bioassays and carrier bacteria experiments toward an effective use of phages against *Pseudomonas syringae*

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Pseudomonas syringae species complex is rich with plant pathogens. *Pseudomonas syringae* pv. *actinidiae* (Psa, kiwifruit canker agent) and *Pseudomonas syringae* pv. *phaseolicola* (Pph, bean halo blight agent) are two economically important plant diseases. For now in Europe only copper treatments are used to prevent these pathologies. However, biocontrol using the natural bacteria enemies, bacteriophages, is currently used against other phytopathogens. In our previous work we identified16 Pph phages isolates and 5 Psa phages isolates collected, 3 of which representative of species novel to literature: 'Ceppovirus pphageB1', 'Mantavirus psageA1' and 'Nickievirus psageB1' (https://doi.org/10.3390/v13102083). Psa phages showed a wide host range, with the ability of infecting Pph strains too. 14 phages were without temperate life associated genes nor toxin or antibiotic resistance genes. In this work, we applied two different strategies: non-pathogenic *P. syringae* carrier strains, which seems to be able to prolong the stability of pphageB1 on bean phyllosphere, and biossays using pphageB1 and a cocktail of pphageB1 and psageB1, those consistently showed symptoms reduction in bean plants, if treated 1 h before inoculation with a Pph strain from Piedmont. Furthermore, preliminary studies were conducted on development of products based on 7 different endolysins derived from these phage genomes, as biocontrol methods for the Pph and Psa diseases.

Bacteriophage | Pseudomonas syringae pv. actinidiae | bacteriophage biocontrol

A lipopolysaccharide-dependent phage infects a *pseudomonad* phytopathogen and can evolve to evade phage resistance

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Bacterial pathogens are major causes of crop diseases, leading to significant production losses. For instance, kiwifruit canker, caused by the phytopathogen *Pseudomonas syringae* pv. *actinidiae* (*Psa*), has posed a global threat to kiwifruit production. Treatment with copper and antibiotics, whilst initially effective, is leading to the rise of bacterial resistance, requiring new biocontrol approaches. Previously, we isolated a group of closely related *Psa* phages with biocontrol potential, which represent environmentally sustainable antimicrobials. However, their deployment as antimicrobials requires further insight into their properties and infection strategy. Here, we provide an in-depth examination of the genome of ΦPsa374-like phages and show that they use lipopolysaccharides (LPS) as their main receptor. Through proteomics and cryo-electron microscopy of ΦPsa374, we revealed the structural proteome and that this phage possess a T=9 capsid triangulation, unusual for myoviruses. Furthermore, we show that ΦPsa374 phage resistance arises *in planta* through mutations in a glycosyltransferase involved in LPS synthesis. Lastly, through *in vitro* evolution experiments we showed that phage-resistance is overcome by mutations in a tail fiber and structural protein of unknown function in ΦPsa374. This study provides new insight into the properties of ΦPsa374-like phages that informs their use as antimicrobials against *Psa*.

Aeromonas hydrophila MDR Bacteriophages Phagetherapy

New set of *Aeromonas hydrophila* – specific bacteriophages for control of *Aeromonas* infections in fish

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A. hydrophila frequently causes disease outbreaks in wild and cultured fish worldwide. The use of antibiotics is still the most widely used approach to control fish infections. The alternative treatment strategies are strongly advised because of the emergence of MDR strains. Phage therapy can be an effective alternative to antimicrobial chemotherapy for control of *A. hydrophila* infections in aquaculture.

In our recent work (Janelidze et al, 2022) we demonstrated high therapeutic potential of *A. hydrophila* phage AhMtk13a in the experimental trials on Zebrafish. The aim of the presented work was to create a reserve collection of characterized virulent phages active to *A. hydrophila* that could guarantee a rapid response to emerging infections in fish farms.

Up to 90 *A. hydrophila* strains were isolated in 2017-2020 from different sources in Georgia (diseased fish, fish farm water and etc). Subtyping using ERIC PCR revealed a fairly high diversity among these strains. Majority of *A. hydrophila* isolates showed multiple resistance to antibiotics commonly used in aquaculture practices.

After cloning and propagation 12 *A. hydrophila* phages out of 30 primary phage isolates obtained from different water environments were selected for further characterization. According to the TEM studies, the virion morphology of 10 phages were consistent with the *Myoviridae* and 2 phages with the *Podoviridae* morphotype. Selected phages showed different lytic activity and all together covered majority of tested strains (77%) due to overlapping lytic spectrum. Phages demonstrated high lysis stability in liquid culture along with the low frequency of occurrence of phage-resistant bacterial mutants, also maintained the viability under changing environmental conditions (temperature, pH and etc). Genomic studies of the selected phages are ongoing. For further evaluation of the therapeutic potential of selected phages small scale field experiments on juvenile trout are planned.

bacteriophage spray survivability

The effect of spray parameters on the survival of bacteriophages

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There have been numerous studies highlighting the efficacy of various bacteriophages (phages) and phage cocktails in the reduction of pathogens in food. Despite approval from legislative bodies permitting phage use in food processing environments, applied via spray or dip, there is still no information on which spray parameters should be used for successful implementation. The study here investigates phage survival diluted to 1% in distilled water (dH_2O) and prepared bottled water (PBW), followed by a subsequent spray application through a fixed nozzle (530 µm) and strainer size (74 × 74 µm), with pressures of 3, 5, and 6 Bar. The survival of the phage was determined through sampling the outputs of the spray system and performing double agar overlay plaque assays. PBW decreased the phage concentration ($p = 0.18$) more than the dH_2O ($p = 0.73$) prior to spray application. It was found that the PBW phage solution was less affected by the various spray parameters ($p = 0.045$) than the dH_2O ($p = 0.011$). The study showed that unchlorinated water (dH_2O), as well as a pressure of 3 Bar, had the highest output phage concentration through the nozzle and strainer, providing valuable information for industrial implementation.

bacterial canker | kiwifruit | Pseudomonas syringae pv. actinidiae | phage biocontrol

Phages for the Biocontrol of Bacterial Canker of Kiwifruit

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Bacterial canker of kiwifruit (*Actinidia* spp.) caused by *Pseudomonas syringae* pv. *actinidiae* (Psa), causes significant yield and financial losses. The use of copper-based products and antibiotics are the current techniques for Psa control. These compounds are phytotoxic and also promote copper and antibiotic resistance. The isolation and characterization of (bacterio)phages for the control of Psa was the focus of this research, motivated by the demand for safe and effective biocontrol techniques against this disease. A Portuguese collection of Psa strains was characterized by molecular and phenotypic tests. Phages were isolated from branches, buds, leaves and flowers of kiwifruit plants in the North of Portugal. Phages were isolated by the enrichment procedure with Psa strains CFBP 7286 and P84 as possible hosts, and the lytic spectra of 6 selected one's were tested against the Psa collection. The two phages displaying broader host ranges (between 71% and 84% of efficacy among Psa strains) were stable between -20ºC and 50ºC, pH range of 3 to 11 and UV light at 366 nm. Transmission Electron Microscopy was used to characterize phages morphology. In vitro efficacy studies revealed that, with MOI=1, phage 177T decreased the number of CFUs after 4 hours of inoculation while maintaining a low bacterial load for up to 24 hours. Over 24 hours, phage VC3 maintained the bacterial growth stable. Preliminary *ex vivo* and *in vivo* assays on kiwifruit leaf discs and directly applied to the plant, showed differences between the phage application and the control after 12 days of inoculation. One phage has been sequenced and confirmed to be lysogenic, data that corresponded to the *ex vivo* results. Even so, this lysogenic phage showed potential to be used as a biological control agent against Psa.

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Salmonella spp. Bacteriophage stability

Silica Vesicles Increase Stability of Salmonella-Specific Phages in Environments Mimicking the Gastrointestinal Tract

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Non-typhoidal *Salmonella* (NTS) *enterica* serovar Enteritidis is one of the major causes of foodborne infections worldwide. This NTS serovar is mainly transmitted to humans through poultry products. Bacteriophages (phages) are a promising alternative to antibiotics to reduce NTS incidences in poultry farms. The ability to survive the harsh environment encountered in the chicken gastrointestinal tract (GIT), such as low pH, high temperature and enzymatic digestion, can be valuable in selecting phages with high therapeutic potential. In this study, we characterized 13 newly isolated Kenyan S. Enteritidis-specific phages for their ability to survive in pH-adjusted media, different temperatures, and simulated gastric and intestinal fluids (SGF and SIF, respectively). Furthermore, we evaluated the possibility of using silica vesicles (SV) to increase the stability of these phages in these environments. All phages were relatively stable from pH 4 to 12 and from 25°C to 42°C following 12 hours of incubation. At pH 3, phages lost up to 3 logs in viral titres after three hours of incubation. They remained more stable at pH 9, with phage titres 2 logs higher than at pH 3. In SGF, they were stable for 20 minutes; afterwards, they started losing their viability up to 5 logs, while they were relatively stable in SIF for up to two hours. Moreover, significant differences were observed among the different phages in surviving these environments. Encapsulating phages with SV demonstrated a slow but long rate of phage release upon adsorption for 96 hours. Preliminary data indicate that SV 140 C_{18} can protect phages longer than other silica vesicles tested. In contrast, free phages in SGF had an average reduction of 7 logs PFU/ml after 60 minutes of incubation. These data suggest that a number of these phages can potentially survive through the chicken GIT and that SV can be an ideal technology to prolong the stability of phages in acidic environments.

ETEC Piglets Endolysin Simulated intestinal fluid

Are endolysins promising agents in controlling *E. coli* associated post-weaning diarrhea in piglets?

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Enterotoxigenic *Escherichia coli* (ETEC) associated infections are the major cause of piglets' mortality in weaning and post-weaning period, resulting in significant economic losses to the swine industry. Furthermore, the increase of multidrug resistant ETECs have been recognized a public health danger due to the potential transfer of resistance into the food chain. Bacterio(phages) endolysins are enzymes produced in the end of phage lytic cycle that are responsible for cell lysis. So far, no resistance has been reported, which make endolysins an attractive alternative to antibiotics. In the present work, endolysins were exploited to tackle ETECs in piglets. Two enzymes previously cloned were tested against ETEC SP23 strain exponential cells. First, Lys68 (used herein as proof of concept) was tested alone or in combination with 5 different organics acids (already implemented in the piglets' diet) – citric, malic, formic, lactic and sorbic acids – in 20 mM HEPES, 149 mM PBS and 140.33 mM simulated intestinal fluid (SIF). Later, PlyF307 endolysin was also tested with malic and citric acids in SIF. Results demonstrated that both enzymes reduced ETEC concentration in more than 4 orders of magnitude in HEPES. Lys68 together with malic acid displayed the best antibacterial activity in PBS, being able to reduce approximately 1 order of magnitude. However, enzymes efficacy was drastically reduced when tested in buffers that mimicked physiological conditions. In SIF, Lys68 and PlyF307 did not display antibacterial activity. In summary, phage endolysins revealed to be ineffective to treat ETEC bacterial load in more complex environments.

soft rot | Bacteriophage isolation | Potato

Utilisation of bacteriophage to combat soft-rot disease in potatoes

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Soft-rot is a bacterial disease that primarily affects vegetable but also ornamental plants. Within the potato industry, worth £1.1 billion in the UK, soft-rot causes annual losses of £750 million world-wide. Spread through contaminated farming equipment, there is currently no commercially available treatment other than rigorous sanitisation procedures. Strict regulations severely limit artificial additions to crop and therefore it is critical that alternative treatments are investigated. Bacteriophages, viruses that infect bacteria, that are capable of infecting the offending bacteria seem a natural option. However the library of published bacteriophage that could be used to treat soft-rot-causing bacteria is limited and requires much expansion. Through collaboration with APS biocontrol, I have successfully identified over 30 novel bacteriophage against soft-rot-causing *Pectobacterium* and *Dickeya* species. Comprehensive host range analysis of these bacteriophage show wide host ranges, with many infecting greater than 50% of all strains screened. These novel bacteriophage will enable the optimisation of an already commercially available bacteriophage cocktail additive. Additional analysis of the bacteriophage cocktail dynamics will ensure no antagonistic effects. Cocktail effectiveness will be conducted *in vitro* and through a potato slice assay to ensure an optimal reduction in soft-rot disease progression and that any resistance development does not alter pathogenicity. The outcomes of this work will ultimately assist in the evolution of the first commercially available treatment for soft-rot disease. Furthermore, it'll lessen the economic burden of soft-rot disease, raise awareness of the importance of understanding bacteriophage cocktail dynamics and how bacteriophage resistance may alter disease progression.

bacteriophage biocontrol | Food Safety | Phage evolution

Evolution of *Listeria* phage LP-125 to improve Efficacy Under Specific Food Conditions

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Listeria monocytogenes is a foodborne pathogen that causes illness with a relatively high mortality rate (~20%). It is particularly challenging to control in food environments as it can grow at refrigeration temperatures. Currently, there are several phage products available for use in many countries as control agents for *L. monocytogenes*. Previous work has shown that *Listeria* phages can evolve in vitro to overcome challenging resistance types. The goal of this study is to (i) determine if evolution of *Listeria* phages can be exploited to improve their efficacy under specific food conditions and (ii) identify the phage mutations that are selected under food-specific conditions. One-step growth curves showed that LP-125 infection kinetics were significantly different in oat and whole milk (n = 3). The average burst size was 47 ± 2 phages per cell in oat milk, compared to 34 ± 2 phages per cell in whole milk ($P<0.05$). In addition, the latent period was reached at an average of 70 min in oat milk, compared to 87 min in whole milk ($p<0.05$). LP-125 was passaged through 10 rounds of infection in each milk condition; phage growth kinetics were observed for each round of infection, and we found that the resulting lysate concentrations trended up over time. Three phages were plaque purified from each of the final milk adapted lysates. DNA was extracted from each of the 6 milk-adapted phage isolates, and DNA was also extracted from each of the two milk-adapted phage lysates (representing the total mutant phage population). These DNA samples were sent for Illumina sequencing, and phenotypic assays are underway to compare milk-adapted phages to ancestral phages. We hypothesize that we will see significant differences between the ancestral phages and the milk-adapted phages, and that we will identify mutations responsible for conferring those differences. We anticipate that this knowledge may be used to improve the efficacy of phage applications in food safety.

Belgian Society for Viruses of Microbes | Inaugural BSVOM symposium

Belgian research and development network on viruses of microbes

Launch of the Belgian Society for Viruses of Microbes

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Belgium holds a long-standing tradition in microbial virus research, dating back to early works of André Gratia on *Staphylococcus aureus* phages and the first experimental therapeutic use of phages by Richard Bruynoghe and his student Joséph Maisin, both in 1921. Pioneering work continued with the discovery of positive regulation of gene expression in phage lambda by René Thomas and coworkers in 1966, the first viral genome (MS2) sequenced by the group of Walter Fiers in 1976,

and more recently, a highly advanced regulatory framework for phage therapy actively applied in Belgian hospitals, known as the Magistral Phage.

On May 11, 2022, the Belgian Society for Virus of Microbes (BSVOM) was founded. BSVOM aims to sustain and improve the dense Belgian research and development network on viruses of microbes by bringing together all stakeholders from academia, government and industry. In spite of the small geographical size of Belgium, at least sixteen entities are currently active in the field of viruses of microbes. Our society adopts the triple helix model, providing an interdisciplinary perspective ranging from basic research to industrial developments and biotechnological & clinical applications. BSVOM pursues the intensification of this fertile Belgian ecosystem on viruses of microbes, with a particular focus on supporting young and future generations in the field.

The inaugural BSVOM symposium scheduled on September 23, 2022, will be the first highlight of the society with an impressive line-up of (international) speakers, blended with talks from established research groups and a pitch session with young talent (www.bsvom.be). There will be ample and excellent opportunities to discuss most recent advances, including a social activity to foster the interactions.

Salmonella enterica | Poultry | Antibiotic resistance

New Bacteriophages from Kenyan Poultry Farms Reveal Ubiquity, Diversity and Capacity to Kill Field Strains of Salmonella

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Current methods of controlling *Salmonella enterica* infections in poultry farms include antibiotics. However, the emergence of multidrug-resistant (MDR) bacteria in poultry farms due to unrestricted use of antibiotics indicates the need for more sustainable nonantibiotic interventions. Bacteriophages infecting *S. enterica* are widespread in natural environments and offer a potential means for controlling this bacterium. Yet relatively little is known about these phages in Kenyan poultry farms. In our goal of developing phage therapy to tackle MDR in poultry farms, we examined the prevalence of *S. enterica* and its phages from chicken feces and water samples from poultry farms in Nairobi and Kiambu counties in Kenya in 2019. CRISPR-typing of the identified Salmonella strains revealed three common serovars, *S.* Enteritidis, *S.* Kentucky and *S.* Heidelberg. We investigated the presence of antimicrobial resistance genes in these isolates and found that at least 12.5% are multidrug-resistant. *Salmonella*-specific phages were isolated from 57 poultry farms and five slaughterhouses. Analysis of 631 crude lysates encompassing 103 water and 528 chicken samples revealed that 67% presented Salmonella phages. The most prevalent being against serovars *S.* Heidelberg (58%), *S.* Cholerasius (45%), *S.* Braenderup (29%), *S.* Brandenburg (20%), *S.* Enteritidis (12%), *S.* Pullorum (8%), *S.* Kentucky (5%) and *S.* Typhimurium (2%). Of these crude lysates, 63 phages were selected based on host range and restriction profiling. Preliminary whole-genome sequencing data reveal that these phages cluster within the *Myoviridae, Siphoviridae* and *Podoviridae* families. The selection of therapeutic phage candidates for poultry and their detailed characterization is ongoing and will be tested in a Salmonella infection model in chicken. Key results indicate that Kenyan poultry farms present a diversity of Salmonella phages that can be used to develop a phage-based strategy to control Salmonella infection in chicken.

Mycoviruses | Cryphonectria hypovirus | Chestnut blight

Natural Spread of Mycoviruses a Puzzling and Dazzling Issue for Biocontrol of Chestnut Blight by Cryphonectria Hypovirus

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Mycoviruses are widespread viruses in filamentous fungi considered ubiquitous in all groups. *Cryphonectria hypovirus* 1 (CHV1) of the Hypoviridae family is included in the genus *Hypovirus*. Hypovirus are well-studied biological control agent of chestnut blight a lethal disease of *Castanea sativa* Mill. Unlike many mycoviruses, which are cryptic or latent in their host, CHV1 reduce virulence, reduce pigmentation and sporulation and induce female sterility in its host the *Cryphonectria parasitica* fungus. Chestnut blight was introduced in Portugal and rapidly became a severe lethal disease in all chestnut regions in the 90's. Initial epidemics had a very rapid spread and no or few cases of hypovirulence was reported. Besides scientific issues are obtained therapeutic hypovirulence was introduced as a biological control to mitigate disease impact. Later, in 2013 chestnut blight appears in scattered stands of the coast north Minho region where natural spread is the dominant way of hypovirulence spread. In this study we investigate and specifically addressed the question of the massive natural spread of hypovirulence. We studied vegetative compatibility system (*vic genes*) of the host fungus, presence and characterization of CHV1 subtypes and hypovirus transmission capacity, issues that play a key role in hypovirus spread. Although some different results are obtained major key determinants and driven forces to improve natural hypovirus spread are still unclear and new approaches as (HTS) high throughput sequencing will be valuable to understand the driven forces of natural dissemination that will greatly increase field sustainability of therapeutic applications of hypovirulence and chestnut recovered

Endolysin Cytotoxity Purification Method

A method for the purification of non-pyrogenic bacteriophage-derived endolysins with an *in vitro* or *in vivo* application

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Introduction: Endolysins are a class of antimicrobials that are on the verge of break-through in (veterinary) medicine. Although the activity and engineering of these proteins is well-studied, insights regarding their stability, pharmaco-kinetics & -dynamics are limited in an eukaryotic cell environment or upon administration to either animals or humans. **Objective:** Our groups developed a method that allows the purification of non-pyrogenic endolysins purified from *E. coli* applied to our previous research in the context of bovine mastitis. **Materials, Methods and Results:** ClearColi® BL21 (DE3), an *E. coli* line knocked-out for immunogenic lipopolysaccharide (LPS), was used for the expression of a polyhistidine (his)-tagged endolysin. After sonication on ice, the centrifuged lysate was applied to a HisTrap column on an Äkta Pure system, which was pretreated with 1M NaOH to disintegrate residual LPS. The column was washed with lysis buffer containing 0.1% EMPIGEN® detergent to remove any residual LPS left in the sample. Next, this detergent was removed from the column by washing with lysis buffer, after which his-rich host cell proteins were likewise removed with 50 mM imidazole. An isocratic elution with 400 mM imidazole yielded the his-tagged protein. Finally, buffer exchange was executed by centrifugation in a Pierce™ protein concentrator, also correcting the volume to the desired molarity. As a proof-of-concept for non-pyrogenicity, bovine mammary epithelial cells (boMECs) and a limited number of mouse mammary glands (n = 6) were incubated or intramammarily injected with 8 or 2.5 µM of purified endolysin, respectively. Neutral red staining evaluated cytotoxicity on the boMECs and histology on the mouse mammary glands also confirmed safety. **Conclusion:** Our method successfully purified one selected endolysin without precipitation or disintegration loss. This purified endolysin was regarded safe both *in vitro* and *in vivo* upon its administration to boMECs and mice.

Disinfection | Food Safety | VBNC cells | Staphylococcus aureus

Bacteriophage treatment after chemical disinfection to tackle *S. aureus* VBNC cells adhered to food contact surfaces

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Food is often contaminated during processing and packaging through contact with equipment surfaces. To avoid it, an efficient sanitation is necessary. However, several studies have demonstrated that although sanitizers reduced the levels of foodborne pathogens they might induce cells of foodborne bacteria into a viable but non-culturable (VBNC) state. We already confirmed that sodium hypochlorite and peracetic acid induce VBNC state in *E. coli* and that those VBNC cells can be detected by flow cytometry. These VBNC cells can "resuscitate" and recover their virulence resulting in a serious risk to human health. Thus, it is urgent to establishing an efficient disinfection protocol to food contact surfaces.

The main aim of this work was to develop a targeted solution for controlling risks associated with *Staphylococcus aureus* VBNC cells in food processing surfaces. For that, clinical isolates of *S. aureus* were subjected to disinfection with sodium hypochlorite (SH) and the bacteriophage LM12 was assessed as an antimicrobial agent to target the remaining VBNC cells.

The results showed that to completely eliminate $1x10^8$ CFU/ml of *S. aureus* adhered to stainless steel surfaces it is necessary a concentration of 275 mg/l of SH with a contact time of 5 minutes. A 1 log reduction is observed to 200 mg/l of SH. An increase of contact time, as expected, leads to lower concentrations needed for reduction. When phage is used as the antimicrobial agent, at contact times of 30 and 60 minutes, it was observed that *S. aureus* presented susceptibility with more than 1 log reduction.

Despite some vulnerability of bacteriophage LM12 to high concentrations of SH, we are currently exploring the application of LM12 to tackle *S. aureus* VBNC cells after disinfection of stainless steel surfaces with sodium hypochlorite.

Paenibacillus larvae prophages | Bacterial fitness | Bacterial virulence

Analysis of intact prophages in genomes of *Paenibacillus larvae*, an important pathogen for bees

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Paenibacillus larvae is a highly contagious spore-forming bacteria, responsible for the American Foulbrood (AFB) disease, lethal to honeybee brood. Integrated in bacterial genomes, prophages are often able to provide new genes or to alter phenotypic characteristics of bacteria. The potential role of prophages in the performance of *P. larvae* has been studied.

A total of 55 intact prophage genomes from 11 *P. larvae* strains were annotated and analysed. The main focus was to infer the influence of their genes with some type of virulence trait (e.g.: toxins), or functions such as antibiotic resistance, metabolic function, germination/sporulation or transporter of nutrients, which could improve bacterial fitness. We also aimed at understanding if specific traits were provided to a given genotype (ERIC I-V).

A total of 67 putative genes with different functions were identified. Some were present in all genotypes, as for example, genes encoding phosphomannomutase, HicB and MazE antitoxins, while others were exclusive from a specific genotype. In ERIC I, were found genes encoding a DNA internalization protein or an enhancin-like toxin, in ERIC II, genes responsible for a SocA antitoxin or a DNA mismatch repair protein, in ERIC III, a gene for a lipid phosphatase, in ERIC IV, genes encoding proteins associated to iron–sulfur uptake and nitrogen fixation and in ERIC V, genes for an aromatic acid exporter family protein, for an epsilon-toxin type B or for an epithelial and chitin-binding protein.

Although several prophage-derived genes are closely linked to metabolic processes, only ERIC V strains appear to have a competitive advantage since prophages contained multiple genes that could contribute to a more aggressive infection.

Despite the low representativeness on *P. larvae* strains diversity, we definitely contribute to leveraging studies in a subject with recent and short knowledge.

biocontrol activated-sludge filamentous bacteria overgrowth

Bacteriophages have potential to control foaming caused by Rhodococcus erythropolis in WWTP

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Activated-sludge is the most widely used biological process to remove pollutants from wastewater worldwide, mainly due to its economic advantages. Bacteria sum up around 95 % of the total microbial community of the activated-sludge, being responsible for most of the water depuration. Filamentous bacteria are normal components of these artificial ecosystems but their excessive growth leads to potential problems, mainly on the sludge settling (filamentous bulking) or on the formation of scums (filamentous foaming), dramatically reducing the efficiency of the wastewater treatment plants (WWTP). *Rhodococcus erythropolis* is a gram-positive filamentous bacterium previously identified as one of the sources of foams in activated-sludge.

The present work aimed at isolating and characterizing phages infecting R. erythropolis, using sewage water and mixed liquor from an urban WWTP as phage source.

Two phages were isolated (one from mixed liquor and the other from sewage) and further characterized genetically and biologically. The TEM analysis revealed that both phages belong to the siphovirus morphotype but with different sizes. The one step growth curve, carried at 28 °C in LB medium, revealed that the *Rhodococcus* phage isolated from mixed liquor and the one isolated from sewage have significant differences with latent periods of 110 min and 35 min, respectively, and burst sizes of 5 PFU/infected and 106 PFU/infected cell, respectively. Both phages were stable between 4 and 28 °C and between pH values from 7 to 10, which suggests good stability in the WWTP environment. Moreover, phages were able to maintain a *R. erytropolis* suspension at low levels for up to 30 h post-infection, using MOIs of 0.1, 0.5, and 1. Infection with the mixed liquor phage, maintained the bacterial reduction for 48 h in all MOIs tested. These results demonstrate the potential of using phages to control the problem of bacterial foaming in WWTP.

Antibiotic resistance | bacteriophage biocontrol | in vivo

The use of bacteriophage to control antibiotic-resistant Pseudomonas aeruginosa infection in a rat model

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Antibiotic-resistant *P. aeruginosa* is one of the most common pathogens contributing to wound infections following surgery or injury, resulting in a high mortality and morbidity rate in severe cases and listed as a critical pathogen by the World Health Organization. However, multiple in vitro and in vivo studies have been shown that bacteriophage (phage) therapy could be a promising alternative to antibiotics against *P. aeruginosa*. Consequently, this study aims to isolate and characterize a novel phage (ZCPA1) from sewage water targeting *P. aeruginosa* followed by examining its efficacy both in vitro and in vivo. The infectivity profile showed that ZCPA1 could infect 58% of tested antibiotic resistance P. aeruginosa clinical isolates. Moreover, the ZCPA1 phage revealed high stability against high temperatures (<80 °C), a wide range of pH values (4–10), and UV for 60 min. Also, it displayed a significant lytic activity and elimination of biofilm against P. aeruginosa evidenced by inhibition of bacterial growth in vitro in a dose-dependent pattern with a complete reduction of the bacterial growth at a multiplicity of infection of 100. Regarding in vivo assessment, phage-treated *P. aeruginosa* inoculated wounds displayed 100% wound closure with a high quality of regenerated skin, evidenced through histological examination compared to the untreated and gentamycin-treated groups due to complete elimination of bacterial infection.

Bacteriophage LAMP Salmonella detection

Novel Same-Day method for viable *Salmonella* Enteritidis detection in chicken meat combining phage amplification and LAMP

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Salmonella enterica is a major foodborne pathogen worldwide. Poultry products, especially eggs and meat, are the main responsible for human salmonellosis cases. Culture-based methods require at least 3 days to detect *Salmonella* positive samples. To facilitate food chain processes and provide a rapid response to food outbreaks, a simple and rapid detection method is necessary. For this purpose, nucleic acid amplification-based techniques are a potential solution. Loop-Mediated isothermal AMPlification (LAMP) has emerged as an alternative to qPCR due to the simple equipment necessary to perform the analysis while allowing the detection of living cells when combined with bacteriophages. The aim of this work was to develop a same-day protocol based in the combination of LAMP and a *Salmonella* phage (vB-SenS_PVP-SE2) to detect viable *Salmonella* Enteritidis cells in chicken meat. Specific LAMP primers were designed to target the capsid and endolysin genes of *Salmonella* phage vB-SenS_PVP-SE2. Two different detection strategies were developed: real-time fluorescence; and colorimetric (naked-eye detection). The LAMP method developed could detect down to 0.2 fg/µL of pure phage DNA and concentrations of viral particles in buffered peptone water (BPW) of 10^2 pfu/mL. After optimization in spiked chicken samples, a 3 h sample pre-enrichment diluted 1/10 in BPW before phage addition to the samples followed by a co-incubation (with phage) of 4 h was established. The proposed method could determine the presence of *S.* Enteritidis in less than 8 h including sample processing, DNA isolation and LAMP analysis with a LOD_{50} of 1.5 cfu/25g and a LOD_{95} of 6.6 cfu/25g, both by fluorescence and naked-eye observation. The results were in close concordance with the reference method for *Salmonella spp.*, the ISO 6579-1:2017. The described method represents a promising alternative for the rapid detection of *Salmonella* in the food chain.

Biotechnology applications in health care

Enzybiotics | Phage Lytic Proteins | PhaLP database | protein engineering

Exploring PhaLP, a database of Phage Lytic Proteins, to engineer modular enzybiotics against *Enterococcus faecalis*.

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Enzybiotics are considered as one of the most promising alternatives to conventional antibiotics. They are derived from enzymes used by bacteriophages to degrade the bacterial cell wall, also called phage lytic proteins. We have created PhaLP (https://www.phalp.org/), an elaborated and comprehensive database of phage lytic proteins with 17,356 entries (and growing). PhaLP aims to serve as an open portal for the phage community, particularly those researchers interested in phage lytic proteins. The database is easily searchable through two user-friendly web interfaces and integrates nine data types originating from multiple source databases.[1]

PhaLP can be used as a starting point to explore the broad diversity of phage lytic proteins, for example to study their host-specific evolution. In addition, PhaLP can also serve as a resource to perform protein engineering with phage lytic proteins. Indeed, due to their modular architecture, they can be engineered by recombining protein domains of native phage lytic proteins to create new modular variants with improved properties. Here, we describe a dataset that describes a complete design space of 780 modular variants. Every possible variant was constructed in a rational way using the VersaTile technique, starting from a small number of diverse building blocks (six enzymatically active domains and three cell wall binding domains) selected from PhaLP. The muralytic activity of each variant against vancomycin-resistant *Enterococcus faecalis* was determined in synthetic urine as a surrogate condition for a urinary tract infection, yielding hits that by far exceed the activity of the bulk of the variants.

phage therapy | phage cocktail | Klebsiella pneumoniae

Designing a broad host range phage cocktail against *Klebsiella pneumoniae*

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The emergence of multidrug-resistant bacteria has positioned bacteriophage (phage) therapy as one of the most promising alternatives to antibiotics. However, phages are highly specific and this hampers their therapeutic use, especially against highly diverse bacteria. An interesting example is *Klebsiella pneumoniae,* a major threat pathogen, with more than 100 capsular types described. Here we design a broad-spectrum phage cocktail against *K. pneumoniae,* capable of combating multiple *Klebsiella* strains. For this purpose, we used the 77 reference capsular types (collection purchased at the Staten Serum Institute, Copenhagen, Denmark) to isolate novel phages from wastewater. Remarkably, the 82 isolated showed enormous phenotypic and genomic diversity. Each phage-bacteria combination was then tested to obtain a cross-infection matrix. The results showed that about 40% of the phages were capsule-dependent, and only seven were considered broad-spectrum phages (infecting at least 10 capsule types). Using the infection matrix the Cytoscape app PhageCocktail provided a candidate cocktail combining 12 phages that was modified to take into account some preference criteria, such as the incidence of capsular types in clinics. The resulting cocktail is expected to infect more than 70% of the capsular types. Experimental validation of the cocktail against reference and clinical strains of *K. pneumoniae* is currently under study, as well the evaluation of phage-phage interactions and the emergence of phage-resistant bacteria.

Acne vulgaris | bacteriophage | Antimicrobial Resistance | in vivo | in vitro | Acne | neutrophils

acne vulgaris phage therapy : in vitro coverage and in vivo application in a mouse model

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Cutibacterium acnes (C. acnes) plays a significant role in the pathogenesis of acne vulgaris. Recently, the prevalence of antibiotic-resistant C. acnes strains is on the rise. One promising alternative treatment against antibiotic-resistant bacteria is bacteriophage (phage) therapy.

Herein, we tested the in vitro susceptibility of 36 clinical isolates of C. acnes to five commonly used antibiotics. then, we assessed their susceptibility to eight new isolates of C. acnes phages. we have observed full coverage of the tested C. acnes strains by a combination of phages (88%) and antibiotics (69.4%). *in vivo* acne mice model was induced through the intradermal injection of C. acnes combined with a topical application of artificial sebum to ICR mice. Mice were assigned to two groups; each group was treated daily for five days topically with a vehicle gel with or without C. acnes phages. The presence of bacteria and inflammatory lesions at the site of C. acnes injection was evaluated. The treated mice had significantly superior clinical scores over the placebo group and showed a significant reduction in neutrophil percentage compared to the control group. These results demonstrate the prevelacne of antibiotic resistance strains in our region is on the rise, and that with phages full coverage might be achieved. more over, topically applied lytic phages on C. acnes-induced lesions lead to clinical improvement and a reduction of neutrophil migration, which is an essential part of acne vulgaris pathogenesis, thus having therapeutic potential in acne vulgaris treatment.

bacteriophage Bacteroides gut

Phage Therapy As Preventive Treatment Against Colon Cancers Caused By Enterotoxigenic Bacteroides Fragilis

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Phage therapy has been considered as an alternative solution to treat infections caused by antibiotic-resistant bacterial strains. Some bacteria are associated not only with severe infections but are also considered to be responsible for the development of various malignancies. *Bacteroides fragilis* is a commensal bacteria inhabiting the intestinal mucosa and is involved in various physiological processes such as digestion and maturation of the gut immune system. The carriage of enterotoxigenic strains of *B. fragilis* (ETBF) producing e-cadherin degrading metalloprotease toxin, increases the risk of developing colorectal carcinoma (CRC). We propose that phage therapy can be considered to diminish or eradicate the ETBF load in the colon and decrease the likelihood of CRC development. Bacteriophage Vb_bfrs_va7 active against ETBF strains was previously isolated from the wastewater in Tbilisi, Georgia, and morphologically characterized with TEM as phage with long non-contractile tail_ siphovirus. The sequence analysis of the phage genome revealed the absence of any undesirable genes associated with virulence, resistance, or horizontal gene transfer. HCT 116 human colon cell culture was used to evaluate the antibacterial as well as anti-cancerogenic activity of VA7. The IL-8 levels as one of the major cytokines involved in the mechanism of ETBF-induced CRC and the bacterial count of *B. fragilis* have been measured. The cell culture assay showed two log-phase lower bacterial counts after 3h of the phage application and lower IL-8 levels compared to the control. The low IL-8 levels could be decreased due to the antibacterial activity of the VA7. Based on the results of our preliminary study, we suggest that the phage VA7 carries a high potential to be used for the prevention or treatment of colon cancer. In the future more comprehensive in vitro as well as in vivo research is foreseen to define the mechanisms of anti-tumorigenic potency of the ETBF specific phage VA7.

Sonodynamic therapy bacteriophage ultrasound

Ultrasonic phages: engineering M13 phage as sonodynamic therapy vector for the selective killing of cancer cells

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Sonodynamic therapy (SDT) represents a promising non-invasive anticancer treatment for solid tumors, combining the use of sonosensitizer molecules and deeply penetrating ultrasound. Ultrasonic sonosensitizer excitation leads to the generation of reactive oxygen species (ROS), with consequent cytotoxicity to the target tumor cells. The specific targeting of sonosensitizers to cancer cell is fundamental for the development of safe approaches, representing one of the major translational challenges for SDT. Here, we conjugated an engineered M13 bacteriophage with sonosensitizer molecules to provide a potent anti-cancer SDT vector platform. M13 was genetically modified to display an Epidermal Growth Factor Receptor (EGFR) targeting nanobody fused at the N-terminal of the pIII protein. The phages were then chemically conjugated on the capsid surface with hundreds of Rose Bengal molecules. The efficient retargeting of phage to tumor cells was qualitatively and quantitatively proved through flow cytometry and confocal microscopy experiments on the epidermoid carcinoma EFGR overexpressing cell line A431. The efficacy of the phage vector in SDT was demonstrated *in vitro* by using a classical ultrasonic water bath as well as a medical approved ultrasound generator device. The results of this proof-of concept study reveal important features regarding the potential of filamentous phages to serve as modular vector platform for anticancer SDT.

biotechnology | Effectiveness of commercial phage cocktails | bacteriophage

New Form of Phage Preparation- Phage Pastilles

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Phage therapy covers a wide range of diseases. Different commercial phage preparations are available on market in Georgia, most of them are in a liquid form. High efficiency and effectivity of a Liquid form of phage preparations are confirmed by laboratory investigations and long-term experience.

However, for the effective treatment of certain bacterial infections, a new, solid form of phage preparations would be more effective. It has been established that the lozenges form of the drugs are always more effective against upper respiratory and oral cavity infections than the liquid form. According to this, we developed the production technology of a new solid form of phage preparation – Phage Pastilles.

Phage Pastille is a new preparation that as API contains (1) biologically and genetically fully characterized, wide host range *Staphylococcus* spp, *Streptococcus* spp, *E. coli* and *Enterococcus* spp specific phages. Those phages are part of commercial phage preparations of “Pyo Bacteriophage” and “Intesti Bacteriophage” produced by LDT “Eliava Biopreparations”; and (2) Eucalyptus extract- which is characterized with anti-inflammatory and antiviral activity.

Small-scale batches of Phage pastilles were produced and determined of good quality indicators. Study of Activity and Stability of phage preparation was performed as well and all the results indicated that pastilles meet the all requirements of commercial phage preparations.

Phage Pastilles represent bacteriophage depots releasing phages in a prolonged time period. Accordingly, their local action is also prolonged until complete disintegration of the form that itself increases treatment effect of the pastille. Due to its safety the Phage Pastille will be recommended to be used for both prophylactic and therapeutic purposes and can be used by any person regardless of age or physical condition.

Acknowledgements

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biofilm bacteriophage Staphylococcus aureus

Synergistic effect of phage phiIPLA-RODI and depolymerase Dpo7 for removing *Staphylococcus aureus* biofilms

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Multidrug resistance (MDR) in pathogenic bacteria is one of the major public health problems worldwide. *Staphylococcus aureus* is a Gram-positive bacterium that has the ability to form biofilms on both biotic and abiotic surfaces, thereby protecting itself from antibiotic treatments and antimicrobial disinfection agents. This microbe is currently considered a priority pathogen, according to the WHO, being necessary to develop new strategies to overcome and prevent staphylococcal infections. In this context, phage therapy is being proposed as an alternative safe strategy to conventional antimicrobials that could help to control the spread of antibiotic resistance. However, more research is still needed to maximize the efficacy of phage therapy in different settings. For instance, it would be useful to determine if the combination of phages with other antimicrobials or bacteriophage-encoded polysaccharide depolymerases, proteins that degrade the extracellular matrix, can enhance their ability to kill the target pathogen.

In this work, the potential synergistic effect between a bacteriophage-encoded exopolysaccharide depolymerase, Dpo7, and the virulent staphylophage phiIPLA-RODI was investigated. To do that, 24-old *S. aureus* biofilms were formed and then treated with the phage, the protein or a combination of both compounds. Samples were taken after 24 h of incubation and the results were compared to an untreated control. The number of viable cells in the biofilm was significantly reduced when using the combination of both antimicrobials; however, there was no reduction in biomass when the protein Dpo7 was applied alone.

Overall, the results show the existence of a synergistic effect between phiIPLA-RODI and the bacteriophage-encoded exopolysaccharide depolymerase Dpo7 in reducing bacterial density in *S. aureus* biofilms.

Antimicrobial Acinetobacter bacteriophage

A potent orthogonal phage vector platform for targeted photodynamic therapy of Gram-negative bacterial pathogens.

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The increase in bacterial antibiotic resistance has encouraged the revival of phage-inspired antimicrobial approaches. Also photodynamic therapy (PDT) is considered a very promising research area for the protection against infectious diseases. Yet, very few efforts have been made to combine the advantages of both approaches in a modular, retargetable platform. Here, we propose the M13 bacteriophage as a multifunctional scaffold enabling selective photodynamic targeting of bacterial cells. We took advantage of the well-defined molecular biology of the phage to functionalize its capsid with hundreds of photo-activable chemical sensitizers (Rose Bengal) and contemporarily target this suicide vector to different bacterial species via pIII-phage-display of bacteria-binding peptides or nanobodies. By this method, we managed to concentrate the number of sensitizers per binding event, thereby increasing PDT efficacy.

Chimeric phages were engineered to target specifically Gram (-) bacteria including the human pathogens *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Specificity was investigated with flow cytometry and furhter validated in PDT assays, in which photoactive phages displaying a LPS binding peptide killed *P. aereuginosa* and *A. baumannii* cells while causing minimal damage to *Staphylococcus aureus*, used as Gram (+) control.

The modularity and versatility of the M13 phage as delivery agent for antimicrobial PDT was further demonstrated by specifically retargeting M13 against *A. baumannii* through the display of a nanobody anti – BAP (Biofilm Associated Protein), a surface protein of *A. baumannii* bacteria.

Together, these results contribute to the development of new antimicrobial solutions, tailored to target selectively different pathogens with the same scaffolding platform.

Phage Therapy | Regulatory | Magistral Preparation

European regulatory aspects of phage therapy: magistral phage preparations

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Bacteriophages (phages) have been used for more than a century to combat bacterial infections, particularly in Poland and in the former Soviet Union. The capacity of phages to specifically target pathogenic strains (sparing commensal bacteria), to adapt to these strains, and to rapidly overcome bacterial resistance, makes them suitable for flexible therapeutic approaches. To exploit these advantages phages offer over conventional 'static' drugs such as traditional small molecule-type antibiotics, it is important that these sustainable phage products are not submitted to the traditional (long and expensive) medicinal product development and licensing pathways. This poster discusses the Belgian 'magistral preparation' phage therapy framework and the extrapolation of this framework to the European level, enabling an expeditious re-introduction of personalized phage therapy into Europe. The magistral preparation pathway is a short and feasible pathway allowing patients' access to personalized and sustainable phage therapy products. Physicians, pharmacists, phage Active Pharmaceutical Ingredient (pAPI) producers and EDQM reference laboratories each play their specific roles. Physicians prescribe personalized (tailored) phage preparations for use in specific patients. Pharmacists prepare these phage products according to the individual prescriptions, using pAPIs. Industry and non-profit players produce these pAPIs according to a phage monograph, and reference laboratories perform the QC release testing of these pAPIs. Industry can market these pAPIs. Pharmacists can also outsource the production of magistral phage preparations to industry. A general phage chapter, once included in the European Pharmacopoeia (Ph. Eur.), can (non-restrictively) guide the quality of the produced and released pAPIs. Further efforts are needed to incorporate general- and specific phage monographs into the Ph. Eur. This process should be driven by industry, if and when they feel the need.

photodynamic cancer engineering host-range PDT

Multiple advantages in the use of an engineered M13 phage as phototheranostic agent against cancer cells

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Photodynamic therapy (PDT), which combines the use of photosensitizer molecules and light, is gaining increasing interest as non-invasive anticancer treatment for solid tumors, with the main challenge being the specific targeting of photosensitizers. Accordingly, different proteins were used to create stable bioconjugates for the delivery of sensitizers to cancer cells. Among these, human serum albumin (HSA) was recently used as delivery agent of oligotiophenes for the photodynamic treatement of cancer cells. Here, we compare the theranostic properties of an engineered M13 phage with HSA. The M13 phage was genetically engineered to express an EGFR (Epidermial Growth Factor Receptor) binding peptide in fusion with the N-terminal of the minor capsid protein pIII. HSA and $M13_{EGFR}$ were then chemically conjugated with the oligotiophene molecule ECB04 and tested on different cell lines. $M13_{EGFR}$ demonstrated significantly better theranostic properties compared to HSA in terms of both targeting and killing of cultured cancer cells, as well as a major selectivity for the EGFR overexpressing cell line A431. These performances were confirmed also on 3D spheroids, in which conjugated phages demonstrated superior penetrance and killing. Furthermore, phage-mediated PDT triggered programmed cell death mechanisms on cancer cells, while HSA-mediated PDT mainly induced necrotic events. In conclusion, this study highlights multiple advantages in the use of engineered M13 bacteriophages as phototheranostic agents.

Receptor binding protein | biotechnology | tail fiber | bacteriophage

Rapid screening of *Burkholderia pseudomallei* colonies by a bacteriophage tail fiber-based latex agglutination assay

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Melioidosis is a life-threatening disease in humans caused by the Gram- negative bacterium *Burkholderia pseudomallei*. As severe septicemic melioidosis can lead to death within 24 to 48 h, a rapid diagnosis of melioidosis is critical for ensuring that an optimal antibiotic course is prescribed to patients. Here, we report the development and evaluation of a bacteriophage tail fiber-based latex agglutination assay for rapid detection of *B. pseudomallei* infection. *Burkholderia* phage E094 was isolated from rice paddy fields in northeast Thailand, and the whole genome was sequenced to identify its tail fiber (94TF). The 94TF complex was structurally characterized, which involved identification of a tail assembly protein that forms an essential component of the mature fiber. Recombinant 94TF was conjugated to latex beads and developed into an agglutination-based assay (94TF-LAA). 94TF-LAA was initially tested against a large library of *Burkholderia* and other bacterial strains before a field evaluation was performed during routine clinical testing. The sensitivity and specificity of the 94TF-LAA were assessed alongside standard biochemical analyses on 300 patient specimens collected from an area of melioidosis endemicity over 11 months. The 94TF-LAA took less than 5 min to produce positive agglutination, demonstrating 98% (95% confidence interval [CI] of 94.2% to 99.59%) sensitivity and 83% (95% CI of 75.64% to 88.35%) specificity compared to biochemical-based detection. Overall, we show how a *Burkholderia*-specific phage tail fiber can be exploited for rapid detection of *B. pseudomallei.* The 94TF-LAA has the potential for further development as a supplementary diagnostic to assist in clinical identification of this life-threatening pathogen.

prophages | P. aeruginosa | diagnostics | persistent infections | fitness

Exploring the role of active prophages in the fitness of *P. aeruginosa* persistently colonizing cystic fibrosis airways

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It remains unclear how much the accessory genome contributes in the processes of establishment and virulence of bacterial infections. Meanwhile, more and more people suffer from persistent infections. *P. aeruginosa*, a common opportunistic human pathogen, is a major cause of persistent infections associated with severe morbidity and mortality in cystic fibrosis (CF) patients. Genome-integrated viruses, aka prophages, are frequent elements of this bacterium's large accessory genome and can contribute to the virulence of *P. aeruginosa* via the expression of non-essential genes. However, the effect of long-lasting prophage-host relationships to the evolution and fitness of the ubiquitous *P. aeruginosa* in different environments has not been clarified systematically. This ongoing study sets out to investigate the role of active prophages in the in-patient fitness of clinical *P. aeruginosa* isolates. We have assessed a collection of more than 500 clinical airway isolates of *P. aeruginosa* to single out a cohort of 12 young CF patients with a high-resolution history of persistent infection. Nanopore MinION technology was used to sequence one early isolate per CF patient to generate whole-genome sequences of high contiguity. To trace potentially intact prophages and determine their in-patient frequency, we scanned assembled genomes and genomes of longitudinal, follow-up isolates with a machine-learning based tool. We then evaluated prophage activity by performing manually-curated genome annotations followed by antibiotic-assisted prophage inductions. Two different CRISPR-Cas systems are currently being employed to cure active and longitudinally-frequent prophages from their host's genome. Finally, we plan to perform competition experiments to quantify the relative fitness of cured versus wild type versions of host *P. aeruginosa* isolates. Besides elucidating the role of active prophages, we expect findings to assist in developing novel diagnostics of *P. aeruginosa* persistence.

Phage Therapy | Complement system | Pseudomonas aeruginosa

Human complement system inhibits binding of phages of the *Myoviridae* family to *Pseudomonas aeruginosa*

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Bacteriophage (phage) therapy is gaining momentum as an alternative to antibiotics. However, the interactions of phages with our immune system might limit their success as therapeutic agents. Here, we have studied the activity of phages targeting *Pseudomonas aeruginosa* in presence of human serum.

We developed a method based on a fluorescent dye that stains the DNA of both phages and damaged bacteria, emitting a signal as bacteria lyse and new phage progeny is produced. Using *P. aeruginosa* strain PAO1 as a host, we screened a panel of phages in presence of serum. Our results reveal that human serum reduces the ability of phages from the *Myoviridae* family to infect bacteria. The effect was observed, among others, with phage 14-1, known to be used in therapeutic cocktails. Phage activity was not compromised when using heat-inactivated serum, which suggests an involvement of the complement system. Compstatin, a C3 cleavage inhibitor, can also counteract the negative effect of serum on phage activity.

Next, we fluorescently labelled *Pseudomonas* myovirus PB1 to monitor its binding to PAO1 by means of flow cytometry and confocal microscopy. In this way, we were able to detect that human serum prevents phage PB1 from binding to its host.

We conclude from our data that human serum has an inhibitory effect on phages of the *Myoviridae* family. This effect is likely mediated by the early stages of the complement system, and takes place at the stage of host recognition and binding. Phages currently used in the clinic to treat *P. aeruginosa* infections could be rendered ineffective as a result of this phenomenon. Our findings highlight the importance of considering the human immune system when applying phage therapy, and may impact the design and content of future phage cocktails.

Klebsiella pneumoniae | bacteriophage | Galleria mellonella | urinary tract infection

Characterization and *in vivo* assessment of two newly isolated bacteriophages against a *Klebsiella pneumoniae* strain

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Antibiotic resistance represents a major public health concern nowadays. New alternatives are needed, and phage therapy seems to be a promising one. *Klebsiella pneumoniae* belongs to the "ESKAPE" bacteria and can cause urinary tract infections (UTIs). In this work, two bacteriophages were firstly isolated against a ST13 *K. pneumoniae* isolated from a UTI and identified as a K3 capsular type by the *wzi* gene PCR. Both bacteriophages were characterized *in vitro* and, then, their *in vivo* efficacy was assessed in the *Galleria (G.) mellonella* larvae model. Genomic analysis showed that these bacteriophages, named vB_KpnP_K3-ULINTkp1 and vB_KpnP_K3-ULINTkp2, belong to the *Drulisvirus* genus. They showed resistance to different temperatures and pH. The bacteriophage vB_KpnP_K3-ULINTkp1 had the narrowest host spectrum (target only K3) compared to the second which also infected other *Klebsiella* types. Short adsorption times and latent periods were observed for both bacteriophages. *In vivo* experiments showed their ability to replicate into *G. mellonella* larvae and to decrease the bacterial titers. Moreover, both bacteriophages improved the survival of the infected larvae. In conclusion, these two bacteriophages had different *in vitro* properties and showed *in vivo* efficacy in the *G. mellonella* model with a better efficiency for vB_KpnP_K3-ULINTkp2.

diagnostics Engineering Bacteriophages

Rapid detection of bacterial urinary tract infections using reporter phage-mediated bioluminescence

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Bacterial urinary tract infections (UTIs) are among the most common microbial diseases and major contributors to the injudicious use of antibiotics. Rapid and accurate pathogen identification directly from urine improves clinical UTI management, alleviates the socioeconomic impact and facilitates antibiotic stewardship. Highly specific and rapid pathogen detection can be achieved through bacteriophage-mediated delivery and host-dependent expression of luciferase reporter genes, a diagnostic approach known as “reporter phage”. Here, we engineer six distinct reporter phages and develop a urinalysis assay that detects and differentiates the three predominant UTI pathogens *E. coli*, *Klebsiella* spp. and *E. faecalis*. Our phages positively identified 89% of clinical isolates from pure cultures. Diagnostic assay performance was assessed alongside routine clinical testing of ~200 fresh patient urine samples collected from two local hospitals in Switzerland over five months. The reporter phage assay led to reliable detection of *E. coli*, *E. faecalis*, and *Klebsiella* spp. with high specificity (97, 98, 98%) and sensitivity (66, 81, 81%) at a resolution of $\geq 10^3$ CFU/mL. This phage-based diagnostic platform offers opportunities for prompt bacterial UTI diagnosis in point-of-care settings and is currently being investigated as a companion diagnostic to determine patient eligibility for phage therapy.

virulence mycobacteriophage infection dynamics

Comparative study on the virulence of mycobacteriophages

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The global tuberculosis (TB) epidemic affected 10 million people and caused 1.4 million deaths in 2020 alone. Multidrug-resistant TB is successfully treated in less than 60% of cases by long, expensive and aggressive treatments. One vaccine protecting children has been developed but despite recent developments, none is currently effective for adults. Additionally, diagnostic technologies are limited by low sensitivity, long processing, specialized infrastructure and cost. Mycobacteriophages have the potential to redefine TB immunization, treatments and diagnostics. Additionally, the engineering of mycobacteriophages is a powerful approach to further improve their properties for these applications. In order to reliably assess the performance and quality of phage-based products, one needs to effectively evaluate many parameters related to phage physiology and infection, including virulence. In previous work, our group developed the virulence index, a metric for quantitative analysis of phage virulence. In this method, reduction curves of bacteria inoculated at different multiplicities of infection (MOI) are used to establish a quantitative assessment of phage infection dynamics under various conditions. In this work, we adapted the virulence index method to static cultures and biofilms. We then evaluated the virulence index of different mycobacteriophages, used alone and in combinations, against *Mycobacterium smegmatis* (a model bacterium for TB) under different growth conditions. The results inform us about mycobacteriophage infections and their dynamics in cocktail formulations, serve as a basis for the proposal of mycobacteriophage engineering strategies to enhance virulence, and are the starting point for the development of a standardized method for evaluation of the virulence of phages when targeting biofilms.

Endolysin Enzybiotics Bioluminescence Burn Wound Infections

Development of a bioluminescent *ex vivo* wound model to characterize novel phage-inspired enzybiotics

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Phage-derived lysins are currently being tested in phase III clinical trials as a promising alternative to the conventional antibiotics. Advanced engineering of these enzybiotics improving pharmacological properties such as half-life and immunogenicity may unlock their clinical potential even further. Such engineering approaches result in so-called third-generation lysins. Protein engineering requires appropriate screening approaches that strike a balance between the best possible mimic of the eventual application and a feasible throughput. As *in vitro* screening conditions differ significantly from the final *in vivo* settings, the activity of the component might be lost under those *in vivo* conditions. Therefore, we developed a medium throughput bioluminescent *ex vivo* wound infection model based on pig skin. In this way, the potential of a wide variety of enzybiotics can be assessed in more realistic settings, which will improve clinical translatability of promising, *in vitro* developed lysins.

Bacteriophages | Phage Therapy | Database

Creating a database for phage therapy requests in Hadassah Medical Center in Israel

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Bacterial antimicrobial resistance is a global problem and efforts to achieve an alternative therapeutic approaches are in need. Bacteriophages are re-emerging as a potential treatment option, especially when considered as a personalized therapy with minimal collateral damage of antimicrobial resistance. Aiming to find a phage for each patient's bacterial infection, we developed a database to process requests for phage therapy. From January 2019 until March 2022, a total of 109 requests from Israel and abroad, including Switzerland, Australia and the US were reviewed in the Israeli Phage Therapy Center (IPTC). Of them, 8 Israeli patients received intravenous (IV) phage therapy (7.3%). *Pseudomonas aeruginosa, Staphylococcus aureus* and *Acinetobacter baumannii* were amongst the top organisms of interest in requests, compromising 80% of cases, with *Pseudomonas aeruginosa* as the predominant pathogen. Respiratory and skin and soft tissue infections were amongst the most common infections, accounting for 52.2% of requests. In 35 cases, phage hunting process resulted in a potent phage, yet only 8 received therapy due to lack of phage availability for IV use. In 21 cases no suitable human pharmaceutical-grade preparation was available and thus were not treated even when a potent phage was found. For this reason, we focused on requests in which bacterial strains had an available human-grade phage preparation. 6 out of the 8 patients had MDR/XDR *Pseudomonas aeruginosa*, and received Pa14NøPASA16 as the available potent phage. Phage treatment was administered as an adjuvant to standard of care antibiotic therapy. Treatment period ranged between 14 - 60 days with a twice daily regimen of IV injections. Half of the patients had an outcome of partial to full recovery. Developing a database for phage therapy requests can serve a useful tool for identifying patients who may benefit the most from such treatment and will enable better processing framework and analysis of each request.

Phage Therapy | Formulation of smart phage cocktails | Enterobacter cloacae complex

Intelligent phage cocktail for the trεatment of infections with multidrug-resistant *Enterobacter cloacae* complex

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Enterobacter cloacae complex (ECC) have been associated with several hospital outbreaks reported worldwide that are often linked to multi-drug resistant clones, and more importantly, carbapenem-resistant ECC clones, with patients often requiring critical care and having a high mortality rate. Given the limited antibiotic treatment options for such infections, bacteriophages (phages) can serve as an alternative avenue for treatment. However, phages are very specific to their host and in vitro screens for lytic activity are typically required prior to therapeutic use. This presents a challenge for the phage treatment, causing delays in the delivery of treatment and substantial personalisation when preparing viable phage therapeutics. Therefore, we aim to develop a phage cocktail using broad-spectrum phages, each with distinct receptors for strains of the targeted pathogen, that could be used as empirical therapy. We isolated 25 novel phages across 36 genetically diverse ECC, which were clinically isolated from hospital-acquired infections. We tested the lytic activity of phages against all ECC using plaque assay, which revealed broad-spectrum phages that lysed up to 50% of tested strains. Based on these results, we combined three phages, which had 65% coverage across the hosts. All three phages are lytic as observed from genetic and morphological features and have high killing efficiency. Our molecular analysis revealed each phage in the cocktails targeted distinct receptors, including glycosyl-transferase (LPS), uridyl-transferase (cell wall) and siderophore. Combining these three characterised phages into a single treatment cocktail has produced a phage cocktail, with broad-spectrum activity, reduced frequency of phage resistance that has been tailored to combat nosocomial, multi-drug resistant ECC infections. The cocktail implements concepts of intelligent design and will be used as empirical therapy in the treatment of multidrug-resistant ECC infections.

crAssphage qRT-PCR Human Fecal Contamination

Development and Evaluation of a Real-time PCR Method for Rapid Detection of CrAssPhage in Human Fecal Contamination

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Water-borne diseases such as diarrhea, cholera and typhoid fever were caused by contaminated fecal pathogens and reported to be transmitted generally through fecal-oral route. Currently, coliforms and enterococci are used as indicator bacteria, but it is difficult to differentiate the original source between human and animal feces. Recently, CrAssphage was discovered only in human fecal samples and it is generally abundant only in human gut environment, suggesting it could be used as an alternative indicator of human fecal contamination. In this study, 139 human fecal samples and 89 animal fecal samples including chicken (n=3), cow (n=7), dog (n=15), pig (n=20), pigeon (n=7) and mouse (n=37) were collected. For rapid identification of fecal sources, TaqMan Real-Time PCR methods have been developed to detect CrAssphage in the fecal samples. Five different primer-probe sets (RQ, CPQ056, and CrAssBP, previously published; CrAssPFL1 and CrAssPFL2, this study) were selected. The CrAssPFL1 and PFL2 primer sets were designed targeting to ORF00018 encoding DNA polymerase and ORF00044 encoding hypothetical protein in the CrAssphage genome (GenBank accession no. JQ995537.1), respectively. Of the 139 human samples, detection rates of CrAssphage are as follows: 23% by RQ, 30.2% by CPQ056, 28.8% by CrAssBP, 20.1% by CrAssPFL1, and 30.9% by CrAssPFL2, respectively, suggesting it revealed the highest detection rate and the highest detection sensitivity. (up to $>10^{-4}$ ng/ul). However, no CrAssphage was detected in all animal fecal samples, suggesting this method works for detection of fecal sources from humans. In addition, Real-Time PCR was performed with sewage samples collected from five sewage treatment plants. Interestingly, CrAssphage was detected in all sewage samples, suggesting that they were contaminated with human feces. Therefore, newly developed Real-Time PCR targeting CrAssphage would be a useful method for differentiation of human fecal contamination in foods.

Antibiotic resistance | Phage Lytic Proteins | Yeast Surface Display

Hacking Yeast Cells With Phage Lysins to win the Fight Against Antibiotic Resistant Bacteria

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With uncontrolled antimicrobial resistance on the rise, developing new and improved antibacterial strategies is imperative. Today, bacteriophage-encoded lysins are a highly promising new class of antibacterials that respond to this call. These bacteriolytic enzymes are featured by their rapid mode-of-action based on active peptidoglycan degradation. Additionally, due to their modular nature and wide abundance, they are ideal candidates for protein engineers. By recombining different lysin building blocks with varying functions, the lysin activity, stability and specificity can be modified. Hence, we can envision creating application-specific lysins for virtually any bacterial species, both Gram-positive and Gram-negative. An increasing number of such modular lysin variants is analyzed. Yet, time-consuming protein expression, purification and high-throughput evaluation of these lysin variants still impede us from reaching this goal. A recent proof-of-concept paper has shown that lysins can be displayed in active form on the yeast cell surface. Baker's yeast *S. cerevisiae* is engineered to associate or *display* the lysins on its cell wall and as such, each yeast cell becomes 'armed' with about 10^4 to 10^5 copies of bacteriolytic enzymes that can collectively dismantle their target pathogen. Here, we evaluate this new and innovative strategy for lysin selection based on yeast surface display of specific lysins. Creating a population of yeast cells, all displaying unique lysin variants, will avoid laborious and time-consuming protein expression and purification, representing a big step forward on the road towards tailor-made lysins.

Endolysin | Staphylococcus aureus | Antibiotic resistance

Lytic enzymes for treatment of staphylococcal infections involving abscesses, bloodborne and intracellular bacteria

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Staphylococcus aureus is an important human pathogen causing a broad spectrum of diseases. Many of these infections are difficult to treat with conventional antibiotics for several reasons, including antibiotic resistance and the ability of *S. aureus* to form abscesses, biofilms, and intracellular persisters. Peptidoglycan hydrolases (PGHs), including phage endolysins, are promising novel antimicrobials featuring rapid bactericidal activity, high pathogen specificity, and a low probability of resistance development. Despite these advantages, systemic administration of PGHs has been hampered by several limitations, including a lack of cell-penetrating properties, insufficient accumulation at infection sites, and short serum circulation half-life. Our lab has compiled a large collection of engineered PGHs, which can be rapidly screened for enzymes active under relevant conditions. To target intracellular staphylococci, we identified PGHs exhibiting high activity under intracellular conditions and fused them to cell-penetrating peptides (CPPs), thereby enhancing their cellular uptake. These PGH-CPPs killed intracellular *S. aureus* in multiple eukaryotic cell lines and proved effective in a murine subcutaneous abscess model. When coupling PGHs to osteoblast-specific cell-penetrating homing peptides (CPHPs) identified by phage display, the enzymes were targeted to the bone upon systemic administration and reduced *S. aureus* burden in a murine model of osteomyelitis. PGHs highly active in blood were fused to an albumin-binding domain (ABD) to extend their circulation half-lives in mice. Upon intravenous injection, the ABD mediates high-affinity binding to serum albumin, thereby reducing renal filtration and lysosomal degradation of the complex. In a murine bacteremia model, we showed that half-life extension translates into increased treatment efficacy. Overall, our results corroborate the high potential of PGHs as therapeutics for treatment of *S. aureus* infections.

filamentous phage | biotechnology | analysis

Filamentous-phage-derived nanorods as detector particles

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Ff (f1, fd and M13) filamentous phage of E. coli have been the workhorse of phage display technology over the past few decades. Their use has expanded in recent years into nanotechnology, where they serve as filament-like-templates (≥890 x 6 nm) for assembly of nanostructures, nanomaterials, and as carriers of agents used for diagnostic and therapeutic purposes. Ff-derived nanorods, on the other hand, are protein-DNA complexes that cannot replicate independently and contain no coding sequences in contrast to standard Ff-derived vectors that replicate in *E. coli* and contain antibiotic-resistance genes. Helper-phage-assisted nanorod production is laborious and time-consuming, making it difficult to upscale for obtaining a high yield of nanorods free of contaminating full- length phage. We developed a novel method high-efficiency production and purification of short Ff-derived nanorods and showed their use as detector particles in the immunodetection assays.

Pseudomonas aeruginosa | bacteriophage | cytokine effects | phage immunization | phage efficacy

Immunogenicity and Therapeutic Efficacy of Diverse *Pseudomonas aeruginosa* Phages

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Differences in the structure of phage particles may correlate with different immunogenicity. The purpose of this work was to assess the immunogenicity of phylogenetically diverse phages and effect of phage immunization on subsequent therapeutic efficacy in mice. *Pseudomonas aeruginosa* myophages, siphophages and podophages were tested *in vitro* for cytokine effects using human whole blood samples and for human Toll-Like Receptor (TLR) activation in HEK-Blue TLR Reporter Cell Lines. To evaluate phage effects on immune system *in vivo*, mice were immunized with single phages or a 6-phage cocktail (PAM3). Dorsal wounds were created both in immunized and naïve mice, infected with *P. aeruginosa* PAO1::*lux* and treated with phage or saline. Testing human blood samples showed that all phages mildly induce production of pro-inflammatory cytokines IL-1β, IL-6, IL-10, and TNF-α. Both LPS control samples and all phage preparations activated TLR4, TLR2/6, and TLR3 but not TLR8. We noted down-regulation of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in murine plasma 24 h after immunization with PAM3. In parallel, upregulation of anti-inflammatory cytokines IL-4 and especially IL-10 was detected. There were also some phage-specific effects. Sera of 7/20 (35%) immunized mice caused significant reduction of phage plaques, only at low serum dilutions. Infected wounds in all mice treated with the PAM3 cocktail showed no luminescence and closed on day 15 compared to prolonged luminescence and delayed wound healing in mice treated with single phages or saline. There was no significant difference in phage therapeutic efficacy between immunized and naïve mice. These results suggest that phages can be administered repeatedly, without a risk of their notable inactivation by the immune system and reduction of the therapeutic effect, which is important for the development of phage therapeutics.

bacteriophage | diagnostics | Phage Therapy | Staphylococcus aureus | Engineering

Detection and Discrimination of Bacteria and Pathological Analytes using Photoacoustic Flow Cytometry

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Infection with resistant bacteria has become an ever-increasing problem in modern medical practice. Bacteremia is a serious and potentially lethal condition that can lead to sepsis without early intervention. *Staphylococcus aureus* is a leading cause of bacteremia and methicillin resistant *S. aureus* (MRSA) accounts for more than a third of the cases. Rapid diagnostics for each of the "superbugs" has been a priority for health organization the world over. Bacteria can be tagged with dyed phage and processed through a photoacoustic flow cytometer where they are detected by the acoustic response. We have previously demonstrated that *Salmonella* can be detected and discriminated from *E. coli* using this method. This demonstrates the capabilities to discriminate and enumerate any bacteria using a bacteriophage tag. *Staph. aureus* is a leading cause of bacteremia and methicillin resistant *Staph. aureus* (MRSA) accounts for more than a third of the cases. Compared to methicillin sensitive *Staph. aureus*, MRSA is more than twice as likely to be fatal. We describe the use of our system to identify antibiotic resistant infections and characterize those bacterial populations based on the penetrance of antibiotic resistance. This research presents an innovative way of identifying and differentiating bacterial strains, sub populations, and antibiotic sensitivity. This method can be further developed for use with other bacterial pathogens in blood cultures representing a major step forward in clinical practice. Often the limiting factors for treatment of patients is the time spent waiting for results. Bacterial plate cultures and Gram staining are 19th century technologies that have been the gold standard for decades, but current trends in resistant bacteria have necessitated a move towards more rapid and quantifiable diagnostic tools.

Bacteriophages | Phage Therapy | Bacteriophage isolation | Phagogram

Phage therapy initiative in Helsinki

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Helsinki Phage Therapy Initiative was launched in 2013. The aim of the initiative is to set up a phage therapy unit that could provide personalized phage cocktails to patients for whom antibiotics do not help. The work packages in the Phage Therapy Initiative include: 1) Setting up a large phage collection, 2) The development of methods for fast phage susceptibility screening, 3) The development of methods for production of clinical-grade phage products, 4) Setting up a quality system and guidelines to promote regulatory framework of phage therapy, and 5) The preparation of phage cocktails for compassionate treatments. We currently have a phage collection of ~500 phages, most of which infect ESKAPEE species bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp*., and *Escherichia coli.* We have set up a phage susceptibility assay that can be performed in 4-5 h total time, and are currently optimizing analysis methods for quality control for therapeutic phage products. Until now, we have produced phage cocktails for eight patients, two of whom had infected burn wounds, three had chronic sinusitis, two had chronic/recurrent infections in lower respiratory tract, and one had chronic urinary tract infection. The etiological agents were *P. aeruginosa, K. pneumoniae, S. aureus*, and *E. coli.* The efficacies of the treatments were variable, but only minor side effects were reported.

antibiotics lysins discovery platform functional metagenomics

Towards a high-throughput lysin discovery platform based on functional metagenomics

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The rapid emergence and spread of multi- and extensively drug-resistant bacteria are a major public health threat. Antibiotic-resistant bacteria are projected to kill 10 million people by 2050, thereby outnumbering cancer-related deaths. Today, no new antibiotic classes have been introduced since over 50 years. The once successful Waksman platform for the discovery of natural antibiotics is largely depleted. So, the spreading antibiotic resistance and the unprecedented void in the discovery of novel antibiotics increasingly leads to a highly reduced number of therapeutic options. Therefore, new discovery platforms are needed to identify new antibacterials to replenish the portfolio of antibiotics. Lysins or bacteriophage-encoded peptidoglycan hydrolases are a novel, alternative potential class of antibiotics. They are generally highly specific at the species level and thus have a narrow spectrum. Yet, we and others claim that more novel lysin candidates must be discovered to feed the (pre)clinical pipeline. Given the natural abundance of bacteriophages, lysins can be found against any bacterium. The largely unexplored metagenome from uncultivable bacteriophages is still a practically infinite reservoir of potentially powerful lysins. In this regard, we elucidate a concept about the development of a highly performant discovery platform for novel lysin-based antibiotics. The platform is driven by functional metagenomics, starting from the untapped metagenomic reservoirs.

enterococci | Phage Lytic Proteins | Microbiome | antibiotics

Phages in feces: developing a platform for discovery of *Enterococcus* lysins

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While broad-spectrum antibiotics are effective in treating acute bacterial infections, the detrimental effects of broad-spectrum antibiotics on the microbiome are becoming increasingly clear. Therefore, a current paradigm shift focuses on the elimination of the disease-causing bacteria while conserving and even restoring a healthy balance in the microbiome during the treatment of infections.

Lysins are enzymes encoded by bacteriophages that degrade the peptidoglycan layer of the bacterial cell wall, resulting in osmotic lysis of the bacterial cell. Because lysins are specific to certain bacteria, they could play a role in meeting the need for new, narrow-spectrum antibiotics. The first lysins are currently tested in clinical trials, emphasizing their potential as a new class of antibiotics. However, certain hurdles need to be taken to make lysins applicable as an antibiotic treatment against a variety of bacterial infections. One of these hurdles is the establishment of a platform enabling a fast discovery of lysins targeting diverse bacteria.

The aim of this research project is to adapt a platform for high-throughput discovery of lysins targeting *Enterococcus* in a representative gut environment. Because enterococci are natural inhabitants of the gut, feces will be used as start material. However, the platform will be adjustable to use diverse start materials and to target diverse bacteria as well. Therefore, such a generic platform could contribute to the establishment of lysins as a new, narrow-spectrum antibiotic class with a multitude of possible applications.

bacteriophage | diagnostics | Receptor binding protein | Y. enterocolitica

Tail proteins of bacteriophages as dual-function molecules

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The results of recent years showed that tail proteins of phages (TPs) are not just structural components of that part of the phage. They are dual-function molecules that may exhibit additional biological features such as anti-biofilm and hydrolytic activity, adhesion, and recognition of the bacterial host cell. Among the bacteriophage tail proteins, there are the tail tubular and the tail fiber proteins, abbreviated as TTP and TFP, respectively. These proteins are the result of the co-evolution of phages and their hosts. *In silico* studies of the phage genes encoding polysaccharides tail hydrolyses showed that 120 of the 160 different phage hydrolyses are coded in the same open reading frame as structural proteins (tail and fibers proteins). Analysis of the genomes of *Klebsiella pneumoniae* (KP) and *Yersinia enterocolitica* (Ye) phages confirmed the presence of three tail proteins, which are most likely involved in the process of bacterial infection. Proteins appeared in the UniProt database only as hypothetical proteins, and the only way to learn about their properties was through experimental studies. Summarizing our efforts, to characterize the phage tail proteins we described ten proteins belonging to KP and Ye phages. All TTPs from KP phages were exopolysaccharide depolymerases and had bacteriostatic activity. TTP from Ye phages showed inhibition of bacterial biofilm formation. We described the 3D structure for KP TTP and determined the binding site of the saccharide moieties. The binding and the catalytic domain of TTPA were predicted using *in silico* analysis and confirmed by the point mutations. We also found that one of the *Y.enterocolitica* TFP is very selective adhesin toward the pathogenic O:3 serotype and can serve as a diagnostic tool.

Phage Therapy | Resistance mechanisms | Pharmacodynamics | Modelisation | in vivo | Escherichia coli | Pneumonia

Combination of *in vivo* phage therapy data with *in silico* model highlights key parameters for treatment efficacy

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The clinical development of phage therapy faces the challenge of deciphering the dynamics of phage-bacteria interactions in the *in vivo* context. In particular, the self-amplification of phages and the rapid development of phage-resistant clones are rarely address *in vivo*.

By coupling an *in vivo* imaging tool to longitudinally follow animals over time with a mathematical model, we characterized the interplay between phages and bacteria during the treatment, by a single dose of the virulent phage 536_P1, of a murine pneumonia induced by the Escherichia coli strain 536. The model highlights several key parameters for phage therapeutic efficacy, such as the dose and route of administration, the burst size, the synergy with the immune system and importantly, emphasizes the critical role of a refractory population of bacteria that include bacteria inaccessible to phages and/or bacteria resistant to phages (1).

In infected and treated mice, we found that the phage resistance rate reached as much as 44% at 48 hours post-infection. Resistant clones carry a single modification of cell surface components, with 75% of mutations located in genes involved in LPS biosynthesis and 20% in genes coding the K15 capsule. The fitness of these mutants was moderately affected in vitro, while a virulence assay in mice showed that LPS-related but not capsule-related mutants lost their virulence. Ongoing work aims to refine the mathematical model by integrating the data from phage-resistant bacteria. Ultimately, the model will help taking decisions on the design of phage therapy clinical trials as well as for compassionate use.

gram-negative bacteria peptoid Endolysin potentiating effect

Potentiating effect of peptoid on endolysin activity against the Gram-negative bacteria

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As the emergence of multidrug-resistant bacteria has caused a public health problem, the demand for an alternative to antibiotics has been increasing. Endolysin, a bacteriophage-derived cell wall-degrading enzyme, is drawing attention as a promising solution for controlling them because it lyses cell rapidly without the problem of resistance development. However, endolysin cannot control the Gram-negative bacteria effectively because the outer membrane of the Gram-negative bacteria prevents external endolysin from accessing the peptidoglycan. Peptoids, *N*-substituted glycines, are a class of peptidomimetics that can have antibacterial activity without cytotoxicity. Some peptoids are known to have membrane permeabilization activity that we studied the possibility of peptoids as a novel potentiator that can extend the use of endolysin to the Gram-negative bacteria. Peptoids 2 and 3, in which *NLys-Nspe-Nspe* residues are repeated 2 or 3 times, respectively, were selected as the candidates because they had low antibacterial effects but could penetrate the bacterial outer membrane with little hemolytic activity. Maximal antibacterial activities were 4 log reduction in the presence of 500 μM of peptoid 2 and 5 log reduction in the presence of 62.3 μM of peptoid 3 against *Escherichia coli* MG1655. Endolysin LysB4 that is derived from *Bacillus cereus* bacteriophage B4 cannot lyse *E. coli* due to its outer membrane. However, the combination of 545 nM of LysB4 and 25 μM of peptoid 2 treatment showed 2.5 log reduction even though 25 μM of peptoid 2 alone showed 0.3 log reduction of *E. coli* growth. The similar effect was seen with peptoid 3; the combination of 545 nM of LysB4 and 12.3 μM of peptoid 3 exhibited 3.43 log reduction even though 12.3 μM of peptoid 3 alone reduced 1.7 log of *E. coli* growth. These results suggest that peptoids can potentiate the antibacterial effect of endolysin against the Gram-negative bacteria.

bacteriophage | Engineering | Staphylococcus aureus | Phage Therapy

CRISPR-Cas assisted engineering of virulent *S. aureus* bacteriophage for the detection of clinical *S. aureus* isolates.

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Bacteriophages are ideal alternatives to target pathogens where conventional antimicrobial treatment has failed. While naturally occurring phage variants are a viable option for phage therapy, steady advances in synthetic biology and genomic engineering allow for targeted alterations in functionality to improve phage therapeutics, e.g., through the introduction of structural changes and/or antimicrobial genetic payloads. *S. aureus* is an important Gram-positive opportunistic pathogen, causing a variety of diseases that are difficult to treat due to circulating antimicrobial resistant variants, including methicillin- and vancomycin-resistant isolates. Members of the *Herelleviridae* family of strictly lytic, broad host-range *Staphylococcus* phages are currently the most promising therapeutic candidates. However, due to their large genome sizes, these phages are notoriously difficult to engineer. Using bacteriophage K as a model, we developed a simple and efficient engineering platform based on homologous recombination and CRISPR-Cas9 assisted counterselection. To this end, we engineered a nanoluciferase-expressing reporter phage, K::*nluc*. While the plaquing host range of phage K::*nluc* covered ~80% of 73 clinical strains tested, the bioluminescence detection range covered >98 % of isolates. Using a rapid (<5 hours) K::*nluc*-based protocol, we detected 173 CFU/mL *S.aureus in vitro* and ~10^3 CFU/mL in a complex matrix such as whole human blood. Our engineering technology can provide novel diagnostic applications and opens avenues for design and engineering of *Herelleviridae* phages to combat circulating and newly emerging pathogenic *S. aureus* strains.

gastrointestinal microbiome | phage therapy | Experimental models of disease

Screening of donor faecal filtrates for lytic bacteriophages against bacteria associated with necrotizing enterocolitis

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Necrotizing enterocolitis (NEC) is a severe gastrointestinal disease affecting premature infants, characterized by gut inflammation and dysbiosis. It was demonstrated in a preterm piglet model that donor faecal filtrate transplantation from a healthy donor fully prevented NEC and decreased relative abundance of mucosal Proteobacteria. Based on this, we hypothesize that specific phages in the filtrates may provide protection against NEC by reducing the abundance of Proteobacteria.

Bacterial isolates were obtained from the ileum of premature piglets following NEC induction by formula feeding. Pathological tissue specimens were aseptically collected for mucosa isolation, and serial dilutions of homogenized mucosa were plated on MacConkey agar. Individual bacterial colonies were identified at species level by MALDI-TOF mass spectrometry and further differentiated by Pulsed-Field Gel Electrophoresis (PFGE). Faecal filtrates were prepared from colon contents of 10-day old healthy pigs from various Danish farms by centrifugation and filtration. Suspensions of single bacterial isolates and individual filtrates were incubated, with continuous monitoring of the bacterial growth up to 18 hours. Single plaques were obtained from the medium showing growth inhibition and further purified to prepare phage stocks.

In total, 33 bacterial isolates were obtained from nine animals, and further separated into 21 different PFGE profiles, most representing *Enterobacter cloacae* and *Escherichia coli*. The screening against 18 donor faecal filtrates showed a quick decrease in the bacterial density from four filtrates, suggesting bacteriolysis by one or more phages. Our ongoing work has resulted in successful isolation of 23 phages for two *E. coli* isolates and six phages for two *E. cloacae* isolates.

Further isolation and characterization will provide a consortium of phages from donor faecal filtrates with the potential for therapeutic effect against NEC, and to test in vitro and in vivo.

Phage Therapy | Microscopy | phage-host ineractions

Visualizing phage - bacteria Interactions on a population and single cell level

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In an era of unprecedented emergence of antibiotic resistant bacteria, alternative therapeutics are being urgently sought. There is a renewed interest in phage - viruses that replicate within and lyse bacteria cells - therapy to combat pathogenic infections. Phage therapy has been demonstrated to be effective against bacterial infection. However, a better understanding of phage-host interactions and how they are affected by the environment is still needed in order to improve the prospects of future treatments.

We investigate how spatial barriers effect the rate of phage attachment to host cells using the *Pseudomonas aeruginosa* pathogen. We have developed a system to track infection events both on population and single cells levels using fluorescent and bioluminescent markers. Bacteria used for these experiments express the fluorescent protein GFP. We can also detect infection events through a nano luciferase reporter gene system, in which the nanoLUC gene has been introduced into the genome of a phage. When the genome of the phage enters the host cell, the luciferase gene is expressed, and the enzyme is produced, it breaks down its substrate to create a bioluminescent signal that can be detected. This allowed us to record two indications of infection : the disappearance of the fluorescent signal when cells die upon phage infection, and the appearance of the bioluminescent signal indicating that the cells are being infected by the phage. We have shown that we can detect luminescence from different bacteria infected by the luciferase producing phage in liquid culture using a microplate reader. We also detected infection events on a single cell level using a high throughput confocal microscope.

Pseudomonas aeruginosa | Phage receptor | Phage resistant mutants

A dual role of P. aeruginosa LPS O chain in its interactions with phages, and its application for human phage therapy

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Phage resistance emergence during phage therapy is a phenomenon that clinicians, regulatory agencies, and researchers want to avoid. Unfortunately, the phage-bacteria co-evolution is continuous and answers to the natural selection pressure that each partner exerts on the other. We studied the frequency of appearance of phage resistant mutants of *Pseudomonas aeruginosa* against four therapeutic phages, two myoviruses and two podoviruses. It led to the discovery that *psl* genes, encoding an exopolysaccharide, were essential for the synthesis of the surface receptor for the two podovirus phages, which both belonged to the Luz24 species, a well-known phage which receptor was previously unknown. However, although adsorption of the Luz24 phages depended only on the presence of this receptor, their virulence also depended on the LPS O chain. The LPS O chain impeded Luz24 phages to form plaques in rich medium. In parallel, and as expected, the same LPS O chain was found to be the receptor of the two myovirus phages, which belonged to the Ab27 species.

These results are very interesting in a phage therapy perspective, as strains that were first susceptible to the two myoviruses and resistant to the podoviruses, upon becoming resistant to the myoviruses became at the same time, and due to the dual role of LPS O chain, susceptible to the podoviruses. A combination of phages from the two families decreased the resistant mutant frequencies.

Endolysin | Drug delivery | Otitis media | Transtympanic

Exploring endolysin-loaded liposomes for a transtympanic treatment of *S. pneumoniae* otitis media

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Otitis media, the main reason antibiotics are prescribed in childhood, is often caused by Streptococcus pneumoniae. The exogenous use of recombinantly produced endolysins, peptidoglycan hydrolases encoded by bacteriophages at the end of their lytic cycle, has shown effectiveness against this pathogen. The endolysins' bioavailability could be increased if they could be directly applied to the ear. This would also reduce the probability of recurrent or chronic middle ear infection. However, the endolysins need to be encapsulated into delivery systems with permeation enhancing characteristics that can surpass the barrier provided by the tympanic membrane. Therefore, this work aimed to develop a novel endolysins delivery system for a transtympanic treatment of pneumococcal otitis media.

The MSlys endolysin was encapsulated into deformable liposomes composed of L-alpha-lecithin and sodium cholate (L:SC:MSlys) or PEG2000 PE (L:PEG:MSlys) with an efficiency of approximately 35% on average, being released in a controlled manner. Liposomes loaded with MSlys showed no cytotoxicity against keratinocyte and fibroblast cell lines. Moreover, MSlys-loaded liposomes interacted with S. pneumoniae cells, significantly reducing planktonic and biofilm cells. Transtympanic permeation studies demonstrated that PEGylated liposomes significantly enhanced the transport of MSlys through human tympanic membranes in an ex vivo model, showing antipneumococcal effect after 2 hours. Nevertheless, degradation of MSlys occurred during extended incubation at 37 ºC, which affected its effectiveness.

In conclusion, endolysin-loaded liposomes are promising for transtympanic treatment of otitis media caused by S. pneumoniae. Nevertheless, further optimization is required in order to increase effectiveness.

Phage Therapy | Phage Cocktails | Phage-Phage Synergy

Synergistic Interactions Reveal a Novel Therapeutic Role for Lysogenization-Capable Phages

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The increasing prevalence and worldwide spread of multidrug resistant bacteria is an imminent danger to public health and threatens virtually all aspects of modern medicine. Particularly concerning are opportunistic, hospital-transmitted pathogens such as the members of the *Burkholderia cepacia* complex (Bcc), which cause life-threatening infections in immunocompromised patients and have a high degree of both innate and acquired antimicrobial resistance.

One potential solution to this crisis is the use of phages, bacterial viruses which target and destroy specific strains of bacteria, in what is known as phage therapy. Phages are dichotomously categorized as either lytic or lysogenic, with the latter category having canonically been considered therapeutically suboptimal since these phages, if they form stable lysogens, can potentially transfer antimicrobial resistance or virulence factors to their lysogens and do not always kill the cells they target. Recognizing that the tendency to form lysogens is not predicated solely on the ability to do so, we propose the term lysogenization-capable (LC) to describe phages which have the genetic capacity to form lysogens and occupy points on a gradient in terms of their tendency to form stable lysogens, and we introduce lysogenization frequency ($f_{(lys)}$) as a novel metric with which to quantify this variable.

We found that among Bcc phages, $f_{(lys)}$ varies substantially with environmental conditions such as host, temperature, and infection medium, and is inversely correlated with the Growth Reduction Coefficient (GRC), a novel metric used to quantify the antibacterial effects of phages. Moreover, many high-$f_{(lys)}$ LC Bcc phages engage in mathematically defined synergistic interactions with low-$f_{(lys)}$ counterpart phages to produce powerful antimicrobial effects, in both *in-vitro* and *in-vivo* settings. Taken together, these findings re-examine our understanding of lysogenization, and reveal a novel therapeutic role for LC phages.

Endolysin AMP Gram-negative pathogens bacteriophage

Improved Antimicrobial Efficacy of *E. coli* Endolysins through Fusion with Antimicrobial Peptide

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An endolysin is a bacteriophage-encoded enzyme that degrades bacterial cell wall when a bacteriophage bursts out in a lytic replication cycle. Recombinant endolysins provided extracellularly could serve as alternative to antibiotics. While wild-type endolysins are promising, protein engineering offers many opportunities to increase its efficacy. An endolysin's activity might be hampered due to the presence of outer membrane in Gram-negative bacteria when provided from outside. Antimicrobial peptide cecropin A crosses the outer membrane by self-promoted uptake, causing distortions of the membrane. We fused Cecropin A to endolysins from *E. coli* phages 10-24(13), PBEC30, and PBEC56. Cecropin A-fused endolysins showed improved efficacy against various Gram-negative bacteria including ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp.*) with minimum inhibitory concentrations as low as 4 μg/ml. The distortion of the outer membrane may facilitate uptake of cecropin A-fused endolysins as well as cecropin itself, leading to a much higher antibacterial efficacy of the fusion protein. As a conclusion, engineered endolysin could be used strong candidates that replace traditional antibiotics.

bacteriophage Endolysin Gram positive bacteria gram-negative bacteria

Synergy Effect of Bacteriophage Endolysins in Combination with Antibiotics against Gram-positive and negative Bacteria

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For decades, numerous antibiotics have been developed to kill Gram-positive and Gram-negative pathogens, but bacteria continued developing resistance to the drugs. Bacteriophage endolysins are enzymes that are emerging as alternatives to antibiotics. Endolysins are enzymes used by bacteriophages at the end of their replication cycle to break down the peptidoglycan of the bacterial host from the inside, resulting in cell lysis and release of progeny virions. In this experiment, whether bacteriophage endolysins synergistically acted with antibiotics to combat bacterial pathogens was tested. Endolysins derived from *Staphylococcus aureus* phages and those derived from *Pseudomonas aeruginosa* phages were tested. In order to determine whether synergistic effects are shown between various antibiotics and endolysins, minimal inhibitory concentrations (MICs) were measured against selected pathogens. Although not all antibiotics showed synergistic effects, combination of some antibiotics and endolysins showed synergistic effects in antibacterial efficacy. In fractional inhibitory concentrations (FICs) tests, *Staphylococcus* endolysin SA11 showed synergistic effects with ampicillin or streptomycin. *Pseudomonas* endolysin LNT103 showed synergistic effects with chloramphenicol, sulfamethoxazole, ciprofloxacin, or trimethoprim. Sometimes the synergy was shown depending on which target bacteria was tested.

RFP molecular imaging phage circulation

RFP - Labelled Phage Circulation in a Murine Model

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Interactions between bacteriophages and mammals strongly affect possible applications of bacteriophages. This has created a need for tools that facilitate studies of phage circulation and deposition in tissues. We propose red fluorescent protein (RFP)-labelled *E. coli* lytic phages (HAP1) as a new tool for the investigation of phage interactions with tissues and organs. RFP-labeled phages were applied in a murine model of phage circulation in vivo. Phages administered by three different routes (intravenously, orally, rectally) were detected through the course of time. The intravenous route of administration was the most efficient for phage delivery to multiple body compartments: 20 min after administration, virions were detected in lymph nodes, lungs, and liver; 30 min after administration, they were detectable in muscles; and 1 h after administration, phages were detected in spleen and lymph nodes. Oral and rectal administration of RFP-labelled phages allowed for their detection in the gastrointestinal (GI) tract only.

functional metagenomics | Klebsiella pneumoniae | Antibiotic resistance

Delivering functional metagenomic libraries into pathogenic bacteria by genetically reprogrammed bacteriophage particles

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Functional metagenomics is a key tool to identify antibiotic resistance genes (ARGs) in the environment and the routes where these ARGs transfer. However, functional metagenomic studies usually use a single expression host, most commonly a laboratory strain of *Escherichia coli*. This limitation results in a lower coverage of resistomes as certain genes cannot be expressed in this strain. To find a solution this problem, our aim was to extend the host range of functional metagenomics by developing a new method called DEEPMINE. This method utilizes genetically reprogrammed bacteriophage particles, with altered host specificity that is achieved by exchanging the phage tails between different bacteriophages. In our study these particles were used to introduce metagenomic libraries into three pathogenic hosts besides *E. coli*. Interestingly, one of the host bacterium, a *Klebsiella pneumoniae* strain identified more than half (62%) of the ARGs, indicating that this species might have a higher potential to express environmental genes than the conventional hosts, in agreement with a previous study. Therefore, our next aim is to create *K. pneumoniae* specific bacteriophage particles to involve more *K. pneumoniae* strains in the study. By generating and screening functional metagenomic libraries from diverse environments we intend to investigate the potential role of *K.pneumoniae* in trafficking ARGs from the environment to the clinic.

Phage Therapy | Staphylococcus epidermidis | phage efficacy

Developing Representative Mouse Model for Bacteriophage Therapy of Periprosthetic Joint Infection

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The growing number of joint replacement surgeries is a consequence of increasing life expectancy and obesity, as well as improvements in surgical techniques. Prosthetic joint infection (PJI) is a devastating post-operative complication with a consistent rate of 1.4–3.3% over the years. The effectiveness of antibiotic treatment is limited by increased antibiotic resistance, and the formation of bacterial biofilms, which virtually eliminate the possibility of treatment success without removal of the implant. Bacteriophages are being considered as a supplementary treatment for PJI and are currently used as expanded access therapy. Their use would be more reliable, if guidelines were established for the optimal treatment dose, treatment duration, and means of administration. We aim to develop a mouse model to assess the efficacy of bacteriophages against PJI. Here we present initial steps in model optimization starting with characterisation of clinical strains of Staphylococcus epidermidis and evaluating various parameters after bacteriophage treatment in vitro and in vivo. As frequent pathogen of PJI, the model strain Staphylococcus epidermidis was selected from a panel of clinically relevant isolates that were characterized for antibiotic resistance, biofilm-forming ability and MLST. Bacteriophages against these strains were isolated from environmental samples and their safety and lytic potential was evaluated. The behaviour of the selected bacteriophage-host pair was tested using established mouse models. Pharmacokinetics of the phage was evaluated using local and systemic administration of bacteriophage COP-80B at different doses. The selected strain was further tested in several modes to establish chronic infection of a prosthetic mouse joint. Different materials and shapes of implants as well as different routes of inoculation were tested. Presented results will lead to the development of clinical protocols for treatment of PJI with bacteriophages in humans.

Phage Therapy | Diabetic Foot | Staphylococcus aureus | Pseudomonas aeruginosa | Clinical Study

DUOFAG® and its journey to conquest diabetic foot infections

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In Czechia, the history of phage therapy research dates back to interwar Czechoslovakia, but currently, phage therapy is almost unavailable there. Over the last decade, MB PHARMA has been working on phage research and has developed the phage cocktail DUOFAG®. This cocktail comprises phages active against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. DUOFAG® is intended for diabetic foot infections treatment but has the potential for curing other types of infections too.

DUOFAG® comprises two phages active against *S. aureus* and one phage active against *P. aeruginosa*. The phages characterized on morphological and genomic levels have their host ranges examined on hundreds of clinical isolates of bacteria. DUOFAG® is manufactured in a Czech GMP-certified facility. Elaborate release testing discussed with the State Institute for Drug Control ensures the quality of DUOFAG®. The stability of phages is evaluated regularly. MB PHARMA has established a seed lot system for phages and propagation bacteria.

DUOFAG® was examined in preclinical animal studies. Acute dermal toxicity study on rats and local dermal tolerance study on rabbits confirmed the safety of DUOFAG®. Follow-up preclinical studies focusing on subcutaneous administration of DUOFAG® are currently underway. Single and repeated dose toxicity and toxicokinetics studies of DUOFAG® are performed on rats.

According to data from Czech hospitals, *S. aureus* and *P. aeruginosa* are the main causative agents of diabetic foot infections. The upcoming clinical evaluation of DUOFAG® focuses on proving the safety, tolerability, and efficiency of multiple doses of DUOFAG® in subjects with infected diabetic foot ulcers. The first cohort of patients will be observed for safety evaluation of DUOFAG®, and possible adverse effects will be monitored. In Cohort 2, the study will continue as a double-blind, randomized add-on study of DUOFAG® vs. placebo. The wound healing rate and the microbiological load will be evaluated.

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bacteriophage phageome Human Gut Microbiota

Exploring and characterizing the genetic characteristics of bacteriophages associated with the human system

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The rapid spread of antibiotic resistant pathogenic species is an ever-growing healthcare threat claiming millions of victims throughout the globe. Studies predict it will become the leading cause of deaths by 2050 if effective measures are not taken. One of the most promising approaches to ward off the spread of antibiotic resistance is the therapeutic application of bacterial viruses (bacteriophages or phages), the so-called phage therapy. However, there are numerous aspects of phage therapy about which we have insufficient understanding. Critical among these our incomplete knowledge how phages interact with the human body. This can ultimately undermine phage-based therapeutic approaches, since not all phages equally tolerate or are tolerated by the human organism. While it is well-known that the human body, especially the gut, is richly inhabited by phages, the molecular determinants of this relationship remain underappreciated and understudied. Importantly, (i) how the phages and the immune system interact, (ii) how phages are able to enter and remain in the body and (iii) which are the niches of the body that can be inhabited by phages, among others. These therapeutically crucial features are hypothesized to be governed by certain structural formations prevalent on phage structural proteins. Despite their potentially key therapeutic value, the exact characteristics of these domains are gravely uncharted. Our aim is to screen bacteriophages isolated from human samples for such domains, in a high throughput manner, utilizing three types of human cell lines as model-system followed by bioinformatical analysis. The research focuses on discovering and characterizing domains responsible for the adherence to gut mucosal or mucus-free cell surface and/or invasion into human cells. We expect to reveal therapeutically important domains that could be subsequently integrated into phages with therapeutic potential and thus aid efforts aiming to improve phage therapy.

Pseudomonas aeruginosa | mucus | mucosal phages

Interactions between phages and *Pseudomonas aeruginosa* in mucosal conditions

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Bacteriophage (phage) therapy is considered a promising solution to the antimicrobial resistance crisis. Conventional phage therapy is focused on the curative properties of phages, which are used to treat already established infections. However history shows that phages also have a potential for prophylaxis by preventing bacterial diseases. Given the positive impact a preventive phage therapy approach could have for mortality and morbidity related to bacterial diseases, it is puzzling why this is not pursued with more effort nowadays. The main reason might be the lack of a mechanism to justify and improve phage-based prophylaxis in modern standards. The bacteriophage adherence to mucus (BAM) model proposes an ancient symbiosis between phages and metazoans, in which phages attached to mucosal layers protect animals from invading bacteria. Inspired by this model we were able to show that phage binding to mucus mediates preventive protection against Flavobacterium columnare infections. To expand these finding to other targets we evaluated mucosal interactions between Pseudomonas aeruginosa and a phage collection isolated at the Eliava Institute. Even though the tested phages do not differ regarding binding to mucins in vitro, we verified that some respond differently to infections in the presence of mucins or not. Following the trend seen for F. columnare phages, two P. aeruginosa phages had improved growth if the host was exposed to mucins before infection. This indicates that mucosal associated phages can be found for different bacterial species, and that the biological mechanisms behind phage-bacteria mucosal interactions have potential of being exploited to achieve prophylactic phage therapy.

orthopedic implants | Bacteriophages | Pseudomonas aeruginosa | phage-antibiotic synergy | biofilm

Phage-Antibiotic Synergy for the Treatment of Biofilm-related Infections on Orthopedic implants

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BACKGROUND: On top of the emerging antibiotic resistance crisis, bacterial infections are often complicated by the formation of biofilms, especially in the setting of orthopedic implants. These (poly)microbial micro-communities, are known to be much more resistant when compared to their planktonic counterparts, thereby causing difficult to treat infections.

AIM: Devising a novel treatment modality for biofilm-related infections on orthopedic implants by investigating the synergy between bacteriophages and routinely used antibiotics.

METHODS: 79 phage clones active against *P.aeruginosa* were de novo isolated from a wide variety of environmental sources. 20 phages with broad host range and lytic activity against *P.aeruginosa* PAO1 were identified and tested on PAO1 biofilms. Five phages showing antibiofilm activity, have been tested in combination with ciprofloxacin, meropenem and ceftazidime for their effects on CFU counts, biomass and metabolic activity. Scanning electron microscopy was performed for PAO1 biofilms grown on titanium coupons to gain more insight on biofilms structure after different treatment protocols. Genetic sequencing identified the phages as a Yuavirus, a Pbunavirus and a Bruynoghevirus.

RESULTS: When using antibiotics at their MIC in combination with bacteriophages, significant reductions are seen for both CFU counts as for biomass evaluations. When increasing antibiotic concentrations to 10xMIC, an even higher decrease on biomass & viable population was observed (biofilm reduction of more than 50%). These findings have been confirmed with metabolic assays (Omnilog Biotyper) and scanning electron microscopy for PAO1 biofilms grown on titanium coupons.

CONCLUSIONS: A combination of phages and antibiotics is capable of reducing CFU counts, thereby reducing biofilm respiratory rate as seen with Omnilog assays, as well as the biomass of PAO1 biofilms grown in-vitro and on titanium coupons more efficiently than each type of agent alone.

Phage Therapy | multidrug-resistant bacteria | Mycobacterium abscessus

Isolation and characterization of novel phages against *Mycobacterium abscessus*

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The misuse of antibiotics has fostered the emergence of multidrug-resistant bacteria prompting the search for alternative therapies such as the use of phages, bacteria-specific viruses. *Mycobacterium abscessus*, an opportunistic mycobacterium that causes serious complications, especially in patients with cystic fibrosis. Here, we searched for phages against *M. abscessus* using *Mycobacterium smegmatis* mc^2 155 as an intermediate host. For this purpose, more than 300 samples from different environments including wastewater were used and a total of 33 *M. smegmatis* phages were isolated, while none of the samples gave rise to direct lysis of *M. abscessus*. Of these, 4 phages showed lytic activity against various clinical strains of *M. abscessus* from patients with cystic fibrosis enrolled at the Hospital Universitari i Politècnic La Fe (Valencia, Spain) although with a lower efficiency of plating (EOP), supporting the use of an intermediate strain. Plaque morphologies, transmission electron micrographs, and genomic analyses, showed a high variability of the isolated phages. Moreover, a phage cocktail will be tested and subsequently optimised by directed evolution, to improve lytic activity in *M. abscessus* clinical strains.

diagnostics | Receptor binding protein | pathogen detection

Recombinant receptor binding proteins of bacteriophages as versatile tools for detection of highly pathogenic bacteria

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For highly pathogenic bacteria, such as *Yersina pestis* or *Bacillus anthracis*, rapid and unambiguous detection is crucial for timely antibiotic therapy of infected patients. Polymerase chain reaction (PCR) is the gold standard for diagnostics of most infectious diseases. While detection of specific genes by PCR can be achieved within a few hours, the presence of intact bacteria can only be verified after one to several days usually by culture based methods. For decades, plaque assays using highly specific bacteriophages are being widely used as low-tech alternatives to PCR. However, phage susceptibility assays are time consuming and typically require pure cultures of suspect pathogen isolates. Using phage receptor binding proteins (RBPs) in lieu of whole phages can facilitate and accelerate phage-based pathogen detection. RBPs, which may be, e.g., phage tail-fibers or -spikes, are employed by the virus to recognize specific surface structures on bacterial host cells. Therefore, RBPs typically determine phage specificity. Here, we identified various RBPs of several commonly used diagnostic phages specific for *Y. pestis, B. anthracis* and other highly pathogenic bacteria. Recombinant RBPs were produced from genetic fusions with different reporter proteins, such as fluorescent proteins or enzymes. From these RBP-reporters, we developed a set of novel tools for the facile, rapid and highly specific detection of these notorious pathogens not only in pure cultures but also in clinically relevant matrices. In addition, RBPs were coupled to magnetic beads to serve as highly specific capture molecules for bacterial pathogen enrichment or isolation approaches. Recently, an outbreak of an anthrax among cattle in southern Germany provided a first time, real-life opportunity to apply and successfully validate these new RBP-based tools for rapid anthrax diagnostics and outbreak investigation.

Staphylococcus Microbiome lytic bacteriophages phage-resistance bacterial community Bacterial immunity

Bacteriophages of *Staphylococcus* spp. isolated from human skin

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Human skin is colonized by a wide variety of microbial communities that individually or collectively impact the skin health. Species of *Staphylococcus* are highly abundant in different region of the skin. The coagulase-negative *S. epidermidis* is among the most frequently found bacteria on skin. While previous studies have shown that the relative abundance of *Staphylococcus spp.* differs across skin sites, the temporal factors that influence their abundance are unknown. Studies have shown that the skin virome influences the dynamics of bacteria in the skin microbiome.

Our study aims to investigate the contribution of skin bacteriophages in regulating the *Staphylococcus spp*population dynamics on the skin in health and disease. For that, we collected swabs from over 80 healthy volunteers across different skin sites to isolate cutaneous phages that infect different *Staphylococcus* spp. So far, we have isolated, purified and investigated 42 phages that infect 8 different *Staphylococcus* species present on the skin. We sequenced the genomes of the skin-isolated bacteriophages using Oxford Nanopore Technology. We assessed qualitatively the degree of phage resistance using a wide host range of 122 *Staphylococcus* strains of the 8 species. Our results identify that, among the coagulase-negative staphylococci, *S. hominis* exhibits high resistance to phage infection. Based on our observation, we hypothesize that *S. hominis* encodes a phage resistance mechanism to limit phage infection, and we will examine this further in our studies. In addition, we determined that *S. aureus* is highly resistant against the phages isolated from the skin that infect coagulase-negative staphylococci.

We will examine interactions of the phages with staphylococci to better understand their infection lifecycles and seek to interrogate metagenomic data to inform the potential contributions of the identified bacteriophages to population dynamics and dysbiosis.

Phage Therapy | clinical application | Safety

Current and future clinical phage product quality control in Belgium.

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As antibiotic resistance become a world health issue, new interest in phage therapy has risen all around the world. In Belgium, phage therapy has a special status as phages are considered “Active Pharmaceutical Ingredients” (APIs) and as such can be prepared and combined according to a monograph by an hospital pharmacist [1]. This special status has made Belgium the most active country in the EU regarding phage therapy [2]. This monograph states that clinical phage product (API) have to be prepared in a GMP facility (Queen Astride Military Hospital) and their quality controlled by a national approved laboratory (Sciensano). This process starts with the construction of a genetic passport containing information regarding the phage’s lifestyle, genome size and content, and capacity for horizontal gene transfer. The bacterial production host’s genome is also checked for the presence of active prophages and phage-inducible chromosomal islands. Secondly, each production lot is checked for microbial contamination, endotoxin levels, pH and presence of the specific active component. The obtained results are condensed in a Certificate of Analysis which is returned to the manufacturer, and then transferred to the hospital pharmacy to enable preparation of the formulation upon a physician’s prescription. To this day, this process has insured the quality and safety of more than 40 different phage product lots, and prevented the administration of contaminated products to patients.

Overall, we will present this phage product quality control process in details, acknowledge its limitations and discuss its evolution in the future.

1. Pirnay, J.-P. *et al.* The Magistral Phage. *Viruses* **10**, 64 (2018).

2. Djebara, S. *et al.* Processing Phage Therapy Requests in a Brussels Military Hospital: Lessons Identified. *Viruses* **11**, 265 (2019).

Bacteriophage collection | bacterial hypervirulent clones | capsule

The GENeva PHage (GENPH) collection against multidrug resistant *Klebsiella pneumoniae*

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Multi-drug resistant *Klebsiella pneumoniae* is a major health threat because of its increasing incidence, high infection mortality rates, and lack of alternative treatment. Phage therapy is gaining interest as therapeutic option against this pathogen. The GENPH Collection aims to provide a characterized phage bank, including microscopy, DNA sequencing, and phage life cycle, and as a resource for researchers and clinicians. Using a large and sequenced *K. pneumoniae* collection, that includes mostly carbapenemase-producing isolates with a broad diversity of sequence types (ST) and capsules type (KL), we recover phages targeting these clinically relevant strains. We have isolated 100 phages against 15 international clones. The majority display a myoviral morphology, assed by transmission electron microscopy. The host range was determined by challenging the phages against selected *K. pneumoniae* clones, identifying five phages with a broad host range. These were able to infect up to seven different ST/KL. Three phages isolated against the highly virulent lineage ST23-KL1, display podoviral morphology, show specific activity against the isolation strain and produce haloes around the plaque, likely due to depolymerase activity. The phages are being sequenced. Seven siphoviral phages against the hypervirulent lineage ST14-KL2 were also isolated mainly showing specific activity against these strains. For the 15 phages showing activity against isolates ST258-KL106, ST258-KL107 and ST512-KL107 we selected 80 strains belonging to the clonal complex (CC)258 (i.e., ST11, 258, 512, 437) to test their host range within the CC. Phages displayed varying degrees of specificity, ranging from low activity evidenced by turbid plaques to high activity in up to 71% (n=54) of the strains used. In parallel to the phage sequencing, we are selecting and sequencing spontaneous resistant *K. pneumoniae* variants to determine naturally occurring mechanisms of phage resistance.

serological profiling | antibodies | bacteriophage epitopes | phage display

Serological profiling of humans and identification of epitopes in bacteriophages recognized by specific antibodies.

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Phages are immunogenic and induce specific immune responses, including phage-specific antibodies. This applies to both phages present in the human body (phageome) and phages delivered by various routes in phage therapy. To successfully and safely conduct phage therapy, it is essential to understand the natural phage interactions in the human microbiome. Antibodies induced by one phage may cross-react with another phage. Phages are composed of multiple proteins displaying multiple epitopes; thus phage-specific antibodies are in fact the sum of the responses to various phage proteins. Is it possible to identify common phage epitopes, especially those that are highly immunogenic and predispose the phage to be highly visible to the immune system? So far, there is no data or methodology to trace the history of phage exposure in our lives, and in particular, the typical phage epitopes that are most often recognized by the human immune system are unknown. However, such epitopes are well recognized in the case of eukaryotic viruses, and the epitope databases have recently been supplemented thanks to the use of high-throughput technology, the so-called VirScan. We have modified VirScan to PhageScan that enables identification of antigenic epitopes for which specific antibodies can be found in human patients. This analysis method combines immunoprecipitation and mass sequencing (NGS) of phage display libraries containing peptides representing tested antigens. The main goal of the research was to create the first database of identified bacteriophage epitopes in the context of entire phage populations occurring in human organisms. The created database allowed for: (1)providing key information for personalized phage therapy; (2)tracing the history of phage exposure. This work was co-financed by the European Union under the European Social Fund (Interdisciplinary Environmental Doctoral Studies KNOW in the field of Biotechnology and Nanotechnology, grant POWR.03.02.00-I037/16-00).

nanoparticles capsid cryo-EM immunization

Using bacteriophage T5's capsid as a therapeutic nanoparticle

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Bacteriophage T5 capsid is an icosahedral structure 90nm in diameter. This capsid auto-assembles from a major protein which makes the faces and vertices of the icosahedron, a portal protein that forms the gate at one vertex for the genome transport, and a maturation protease. Following capsid assembly and DNA encapsidation, the decoration protein pb10 binds onto 120 sites on the capsid outer surface[1,2]. These nanoparticles can be functionalized thanks to pb10, which can be fused to heterologous proteins of interest, thus allowing their exposure onto the capsid surface[3]. Building on this property, we fused the model antigen ovalbumin (Ova) to pb10 and we assessed the potential of T5 capsids displaying the chimera pb10-Ova as a vaccination platform. We showed that mice immunised with these nanoparticles elicited strong and long-lasting immune responses without the need for external adjuvant. These results suggest a natural adjuvant effect of the capsids and demonstrates the strong immunogenicity of Ova multimerised onto the capsid. By their ability to expose a “modular” decoration protein, T5 empty capsids constitutes a polyvalent platform for the development of therapeutic applications, notably for vaccination. In order to facilitate the production of T5 empty capsids, we set up a capsid assembly system independent of phage infection by expressing the genes encoding for the capsid proteins in E. coli cells. This biosynthetic system yields Capsid Like Particles (CLP), which show similar characteristics as the capsids assembled during phage infection. New vaccination trials are underway to assess the capacity of this recombinant T5 CLP to trigger immune responses against pathogens of public health concern.

Human Gut Microbiota | phage cocktail | Phage Therapy

Can a phage cocktail be beneficial for the gut microbiota?

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The gut microbiome is a dynamic and complex community of microorganisms that work together allowing humans to maintain health. Antibiotic treatment of gastro-intestinal infections can have long-lasting effects on the microbiome composition, which can result in a state of dysbiosis. Bacteriophage (phage) therapy is a potential alternative to combat the infection and several preparations can be purchased “over-the-counter” in certain countries, but little is known about how these preparations affect the native members of the gut microflora. To investigate the effects of phage therapy and simulate the dynamics, we modelled the gut using batch culture fermenters seeded with faecal samples from three healthy adults. The faecal slurries were sampled over time, total microbial DNA was extracted and sequenced. The resulting sequences were analysed with various bioinformatic profiling tools including MetaPhlAn3 and Kraken2, to observe changes in the microflora. Upon seeding, each donor sample contained a unique combination of detectable bacterial species. At the bacterial genus and species level, limited differences between phage-treated and control samples were observed. Some of the phages increased and decreased in abundance over the time course in a donor-dependent manner, suggesting productive infection. Overall, our experiment shows that addition of the phage cocktail had minimal effects on the healthy gut microbiota at the bacterial species level. The donor-dependent phage bloom further indicates phage-host strain-level effects that warrant further investigation.

Bacteriophage collection | phage therapy | fixed phage cocktails | Pseudomonas aeruginosa | Klebsiella pneumoniae

Global Collection of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* Bacteriophages for Durable Therapeutic Cocktails

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Pseudomonas aeruginosa and *Klebsiella pneumoniae* are Gram-negative bacteria responsible for many infections globally, and increasingly are multidrug-resistant (MDR) with few treatment options. These infections also often involve biofilms that make the bacteria recalcitrant to antibiotic activity. New antimicrobials, including bacteriophages (phages), are needed as additional treatment options combined with standard-of-care antibiotics to improve patient outcomes. To further the development of robust fixed cocktails of lytic phages against MDR-bacteria, Walter Reed Army Institute of Research (WRAIR) and its overseas laboratories implemented phage collections from various sources in Thailand, Kenya, and Georgia to add to an established inventory of therapeutic phage candidates isolated from the Washington DC region. Of the 446 lytic phages isolated by the WRAIR Overseas Labs, 279 were specific for *P. aeruginosa* and 223 for *K. pneumoniae*. Initial whole genome sequencing of 40 phages (24 from AFRIMS/Thailand and 16 from USAMRD-A/Kenya) increased the Army *P. aeruginosa* phage panel to 128 therapeutic candidates that span five virus families and 13 genera, adding the genus *Phikzvirus* (a "jumbo" phage). Eight of the phages isolated in Thailand and Kenya had broad host ranges (40 - 63% coverage) in plaque assays against a diverse global *P. aeruginosa* panel of 100 MDR clinical isolates. Potential cocktail formulations of these phages had predicted lytic spectra of up to 89% of this MDR panel. Whole genome sequencing of 30 initial *K. pneumoniae* phages from the WRAIR Overseas Labs (21 from Thailand, 9 from Kenya) revealed two new genera and increased the Army *K. pneumoniae* phage panel to 133 therapeutic candidates spanning five virus families and 11 genera. Twelve promising phages from Thailand and Kenya were selected and tested against a 100-strain MDR *K. pneumoniae* diversity panel. A theoretical cocktail of eight of these was predicted to cover 56% of this strain panel.

phage engineering | biosensors | biotechnology

Systematic Evaluation of a Portfolio of Promoters in Engineered T4 Phage

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The inherent properties of phages – such as high host specificity, rapid replication rates, high number of progenies, and low-cost production – make them an excellent biotechnological tool for a wide range of applications. Phage engineering provides opportunities to further expand phage properties and accelerates the development of phage-based technologies toward phage therapy, pathogen detection, biocontrol, and more. Better approaches to phage engineering require a better understanding of phage genetics and of the design of genetic parts and expression cassettes. In most cases, we want to ensure that modified phages retain or improve their ability to recognize and kill their host while imparting the desired new trait or expression levels of a gene of interest for a given application (e.g., biosensors). The present study presents a systematic evaluation of the activity of 8 different promoters in expression cassettes in engineered T4 phages carrying reporter genes (*gfp, nanoLuc*, modified *cobA*). Phage variants were created using the acriflavine gene selection method. We demonstrate these promoters can be used to modulate gene expression. Moreover, we evaluated the impact of expression on phage infection through the assessment of the virulence index for the different phage variants developed. This works provides valuable information on the impact of phage genetic modification on virulence and establishes a portfolio of promoters for the modulation of heterologous gene expression.

phage therapy | individualized medicine | critical infections

Phage therapy of critical bacterial infections: single-center experience

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The growing need for the treatment of infections caused by multidrug-resistant pathogens is forcing both clinicians and potential patients to look for alternative therapeutic agents. Currently, phage therapy is considered as an effective alternative or addition to standard antibiotic therapy in cases of critical infections for patients. However, given the growing popularity of this approach and only emerging international legislation, timely and adequate recommendations for the rational use of bacteriophages for therapeutic purposes are required. On the basis of the Hannover Medical School, we organized a Center for Phage Therapy. Here we present our experience including processing requests for phage therapy, assessing the validity and possibility of using bacteriophages for medicinal purposes, isolating and characterizing new bacteriophages, preparing and using individualized preparations for patients.

endolysins DNA dyes bacterial cell lysis

Differences in susceptibility of bacterial strains to phage lysin detected with DNA dye and mathematically quantified.

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Reason - WHO calls for development of new antibacterial treatments alternative to traditional antibiotic. Endolysins (phage lysins) are potentially useful proteins that cause lysis of bacteria through destruction of cell-wall main component – PEG (peptidoglycan). This leads to dissolution of the cell-wall and usually imminent death. We address the need to understand how endolysins work and how they can be used for medical purposes.

We provide a way to measure bacteriolytic activity with use of DNA dyes:

- rapidly (few minutes of measurements),

- in a wide range of bacteria concentrations (from over 10^7 CFU/sample to under 10^3 CFU/sample),

- in real time,

- by measuring metabolically active bacteria,

- in a wide range of lysin concentrations (from 200 mg/l to 0.2 mg/l)

We've published this use of DNA dyes (Harhala et al., 2021, DNA Dye Sytox Green in Detection of Bacteriolytic Activity: High Speed, Precision and Sensitivity Demonstrated With Endolysins)

We expand our work and show how various strains of Streptococcus sp. respond to the same concentration of bacteriolytic agent (phage lysins: Pal and Cpl-1). Our results show significant differences in susceptibility of bacteria strains to bacteriolytic agents that go far beyond simple susceptible/not susceptible labels. We also propose a mathematical way to quantify detected differences to simplify comparisons between strains and lysins.

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Global Health | Developing Countries | Antimicrobial Resistance

Phages for Global Health - Facilitating Phage Applications in Africa & Asia

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Phages for Global Health is a nonprofit organization that facilitates phage applications in Africa and Asia, where 90% of the deaths from antimicrobial resistance are expected to occur. We accomplish this mission by working in four areas:

(1) Capacity Building Programs

Since 2017 we have delivered 4 in-person laboratory training workshops in East and West Africa (including participants from 11 countries), plus our first virtual workshop in 2022 for scientists in Southeast Asia (with participants from 5 countries). Participants learn key essentials of phage biology, techniques for isolating and characterizing phages, and potential applications in people, agriculture and aquaculture. They also develop relationships with other phage researchers in their region and with instructors from around the world. The participants have already taught phage biology to >1200 others through their home institutions, initiated >50 phage projects, and won grants totaling >$945,000.

(2) Product Development Projects

We collaborate with international, multidisciplinary teams to co-develop phage products for public health problems in developing countries. Our goal is to create products that are both technically effective and socially accepted within the local cultural context.

(3) Promoting Phage Banks

We have been advocating for the establishment of centralized phage banks in developing countries, beginning in East Africa. Local governments could potentially use such banks to rapidly and cost-effectively respond to antibiotic-resistant outbreaks in their countries.

(4) Communicating About Regulatory Systems

Regulatory agencies in many countries have begun clarifying how phages might be evaluated through existing or adapted drug regulatory systems, but that process has not yet begun in most developing countries. Thus, we are gathering input from regulatory leaders in North America, Europe and Oceania, aiming to share that information with regulatory authorities in Africa and Asia.

nebulisation nanofibrics human therapy Pseudomonas aeruginosa Staphylococcus aureus

Nebulisation and controlled release - development of novel methods of administration of bacteriophages

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Bacteriophages are getting closer to a regular application in human clinical care. This requires the development of various ways of administration of drug products based on bacteriophages to the human body. MB Pharma focused on two perspective methods of administration for two different purposes.

The first way was nebulisation of phage lysate and distribution into the human respiratory system. The aim is to target pathogens causing lower respiratory tract diseases, like pneumonia, cystic fibrosis etc. Commercially available nebulisers of various types were used for the experiments. The presentation describes the survival of the anti-*Pseudomonas aeruginosa* phages after the nebulisation, and the viability of the phages in several time intervals after the nebulisation to determine, whether the phages would be viable after administration. Moreover, the biodistribution of phages in 3D printed models of the human respiratory system was tested.

The second way of application was the incorporation of the anti-*Staphylococcus aureus* bacteriophages into nanofibrous materials. The main goal of such application was to reach a controlled release of the bacteriophages to infected areas like topical wounds, skin burns etc. Polymers selected for phage incorporation were used and behaviour of bacteriophages in the designed conditions was tested.

Burkholderia cepacia complex | Phage collection | Israeli Phage Bank

Phage collection for *Burkholderia cepacia* complex (BCC)

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The term Burkholderia cepacia complex (BCC) refers to a group of 24 Burkholderia species that are notorious for their drug resistance. BCC infections are a serious problem, especially among persons with cystic fibrosis (CF) in whom they can cause life-threatening respiratory tract infections.

Phage therapy is currently a promising solution for treatment of BCC infections. Tailored phage therapy with accurate Clinical Phage Microbiology for BCC infections may allow effective treatment with minimal effects on the microbiome, effective respiratory biofilm decomposition (a major problem with BCC), and when needed, can be adapted and “trained” specifically to the patient's bacterial isolate.

In order to create an infrastructure for phage therapy for BCC infections we first created a unique phage library that aims to target clinical isolates of BCC. To this end, we screened 35 BCC isolates, most from people with CF, from the collections of the of the Hebrew University and the CFF BCC lab in Michigan , with hundreds of environmental samples for phage isolation.

So far, our screen yielded 20 phages from different sources. Phage characterization included whole-genome sequencing, electron microscopy, host range determination, lytic activity profile, plaque morphology, and more. We found that some of these phages have unique characterizations. Five of them are megaphages with a genome size of up to 200 Kb, and one has been identified as belonging to the unique family of PurZ that uses the 5th nucleotide in their genome and was recently described.

In our screen, we found at least one matched phage to 80% of the clinical isolates (40/50), where some of them matched only after adaptation by “phage training”. Currently, several of the phages are in various steps towards clinical application.

This project provides new hope for numerous CF patients with BCC infections around the world.

Bacteriophage | Undergraduate Research | Training model

Undergraduate Training Model for Active Bacteriophage Research Engagement

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Without a doubt, undergraduate involvement in active research is a catalyst for stronger interest in science. For a budding research field like bacteriophage biology, the bounds of knowledge cannot be explored if research engagement is not encouraged at all levels of learning. The seeming disinterest of undergraduate students in pursuing a scientific career has been blamed on its poor financial prospects. An aspect of this discussion gradually coming to the fore is the design of structured training models that encourages active participation and a sense of scientific ownership for undergraduates, regardless of their prior knowledge and academic strengths. Bacteriophage research in particular will profit greatly from such training models owing to a higher number of undergraduates, easy to tailor research minds, and the inherent inquisitiveness displayed at the prospects of discovery. A successful undergraduate training in bacteriophage research should be designed for voluntary participation, incorporate hands-on laboratory experimentation, be reflective of a well-laid out research process to address day-to-day research questions, afford a degree of independence for self-learning, outline the prospects of continued participation, emphasize the impact of the research on global health and more importantly afford sustainable mentor-mentee relationship. A major obstacle to the success of this model would be the seeming idea of abstraction that comes with concerns about the immediate impact of research findings on humanity. Purposeful research intent can be achieved in bacteriophage research by emphasizing the available translational pipelines. The gains of research discovery should also not be replaced by grades or publications. This might shift the attention of the students from the sense of fulfilment to a silent 'means to end mindset'. If bacteriophage research is going to achieve its fullest potential, this integral aspect of training must not be jettisoned.

Bacteriophage | phage amplification | bacteriophage diversity

Optimization of the plate wash method used for amplification of bacteriophages

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Bacteriophages (phages) are considered one of the most important alternatives to conventional antibacterial drugs. In recent years, interest in application of phages in medicine and different fields of industry has been constantly growing. Therefore, researchers try to establish new strategies of exploiting phages as a therapeutic treatment through in-depth analysis of their nature.

Working with phages entails the need for seeking practical solutions and techniques, which makes this effort easier and more effective. One of the most important and necessary methods used in phage-related laboratories is amplification of phage particles. Because currently methods of phage stabilization are not widely available, and time-dependent decrease in phage titer is inevitably associated with phage storage, amplification must be routinely performed prior to experiments.

Considering the above, the objective of our studies was to develop the optimal method of phage amplification. In our previously published research, we pointed out the difficulties of selecting universal method of phage amplification due to many variables which may influence this process. In the present study, we have taken a step further and tried to optimize one of the previously investigated methods - the plate wash method. We optimized the technique for four phages including *E. coli* specific T4, LO5 (specific to *Enterobacter cloacae*) and TO1/6F and TO1/7F (both specific to *Enterococcus faecalis*). The efficacy of the technique was challenged at different volumes of the washing broth as well as at the different washing times. Optimal volume of the washing broth used in the experiments was 10 mL for three out of four tested phages and the optimal washing time for all phages was 20-30 min. While the initial phage titer in preparations used in the experiments was 1×10^4 pfu/mL, all the combinations applied for the proposed protocol resulted in obtaining phage titers not lower than $\wedge 10^8$ pfu/mL.

Phage Therapy antibiotic-resistance phagetherapy

Retrospective analysis of bacteriophage treatment at the Eliava Phage Therapy Center

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The Eliava Phage Therapy Center (EPTC) in Tbilisi, Georgia is one of only a few phage therapy facilities worldwide. EPTC was established in 2009 as a part of the Eliava Consortium, which, apart from the well renowned G. Eliava Institute of Bacteriophages, Microbiology and Virology includes spin-off companies focused on diagnostic services and phage production. EPTC clinicians collaborate closely with Eliava scientists.

Most patients visiting EPTC come from different countries but with similar stories: a failure to clear chronic infections after numerous conventional (antibiotic) treatments. Such patients suffer from infections of different organs and systems (e.g., genitourinary and respiratory systems, skin and soft tissues). Some are surgical patients with chronic wounds, prosthetic-associated infections, diabetic foot ulcers, etc. Others contact EPTC because of microbiome disorders (SIBO, IBS) and also, due to secondary infections (cystic fibrosis patients).

Bacterial pathogens, which are logically the most frequently targeted with phages at EPTC include E.coli, P.aeruginosa, A.baumannii, E.cloacae, different species of Klebsiella, Enterococci, Proteus, Streptococci and Staphylococci. Phages used for treatment are not limited to readily available polyvalent bacteriophage cocktails, but also include individual phage preparations targeting specific clinical strains.

Substantial knowledge and data have been accumulated in more than 20 years of EPTC's experience with phage-based therapy addressing various infectious complications. In this work we provide a visual representation of a summary of such data, which were collected in the years 2018-2021.

Patient data have been analyzed according to their country of origin, gender, disease and bacterial pathogens targeted. Phage susceptibility of bacterial strains also has been considered. In total, 4295 EPTC patients from the 4 years (2018-2021) have been considered for the preparation of presented visualizations.

bacterial cell lysis | Endolysin | protein engineering | human gut microbiome

Biochemical characterization of endolysins targeting *Ruminococcus gnavus*

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Ruminococcus gnavus is a strictly anaerobic Gram-positive bacteria, which is a member of the healthy human gut microbiome. However, an overabundance of this bacteria has been observed in patients with irritable bowel disease (IBD). IBD is an incurable chronic inflammatory illness of the gastrointestinal tract caused by an atypical response to gut microflora whose treatment is mainly focused on the management of symptoms. A high level of *R. gnavus* could be treated using antimicrobials with a specific or narrow spectrum of activity, removing the undesired bacteria, and conserving the desired gut population. A technology that has this capability is endolysins, enzymes with lytic activity used by bacteriophages at the end of their replication to degrade the peptidoglycan of its specific bacterial host. The goal of this study was to characterize four endolysins against *R. gnavus*: Lys 84, Lys 210 (*in silico* derived lysins), Lys 507/2-1 (phage derived lysin) and Lys 84/2-1 (a chimeric lysin created by combining the enzymatic active domain of Lys 84 and the cell wall binding domain of 507/2-1 to narrow spectrum of activity to *R. gnavus* alone). Biochemical characterization of these endolysins was achieved by turbidity reduction assays using *R. gnavus* dead cells to determine the endolysin concentrations, pH, temperatures and NaCl concentrations optimal for lysis. All four endolysins display a broad range of activity for the conditions tested. Activity was observed for pH's between 6-11, temperatures 25°C-50°C, NaCl concentrations up to 150mM and across endolysin concentrations between 1-0.25µM. From these experiments it is clear that Lys 84, 210, 507/2-1 and 84/2-1 are active across a broad range of conditions, and therefore should remain active in the gut environment (pH 5.9 - 7.8; 36.5 - 37.5 °C; and NaCl concentration ~150 mM) reducing *R.gnavus* numbers.

Bacteriophage isolation | Cutibacterium acnes | biocontrol

Isolation and primary characteristics of bacteriophages specific to *Cutibacterium acnes*

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Acne treatment often requires long-term use of antibiotics, which contributes to the formation of resistant strains not only *C. acnes* but also other bacteria inhabiting the skin. As a result, bacteriophage therapy is now considered to be a promising treatment substitute. This study aimed to isolate, characterize, and evaluate the potential use of bacteriophages to control *C. acnes.*

C. acnes bacteriophages were isolated from the skin swabs of healthy volunteers. Standard double-layered agar method coupled with anaerobic cultivation was used for phage isolation. The host range of the isolated phages was examined using the spot assay method against a variety of clinical isolates and type strain of *C. acnes*. The size and phage morphology were determined using TEM.

Three lytic bacteriophages infecting C. acne were successfully isolated in this study. These phages showed effective lytic activity against target bacteria. The electron micrograph revealed that the three phages under study had typical morphology of *Siphoviridae* family with an icosahedral head and long thin tail. Isolated bacteriophages displayed activity against a wide range of clinical isolates and type strain of *C. acnes*.

Considering strong lytic activity, broad spectrum, and stability, phages isolated in this study are considered as potential candidates for *in vivo* investigations. The biological properties of these phages allow us to propose them as potential biocontrol agents for use in the treatment of *C. acnes*-driven pathology. *C.acnes* bacterophages have been previously described in different laboratories, however this is the first attempt to isolate these viruses in Ukraine and newly isolated viruses could usefully replenish the phage collection**.**

Endolysin Engineering Gram negative efficacy

Characterization of an Engineered Phage Endolysin LNT103 Effective Against Gram-Negative Pathogens

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Objectives

Few antibiotics against Gram-negative pathogens have been newly introduced over the last two decades. The rapid development of antimicrobial resistance to existing antibiotics is threatening human health, and thus, new antibiotics are urgently needed. Phage endolysins are a potential candidate, but the presence of an outer membrane in Gram-negative bacteria hinders the attack of recombinant endolysin from outside. Here, we sought to mine an endolysin effective against Gram-negative pathogens from a bacteriophage bank and improve its efficacy by protein engineering.

Methods

Putative endolysin genes from 400 different phages were cloned, expressed, and the proteins purified and subjected to antibacterial activity tests. An endolysin encoded by phage PBPA90 was selected and its antibacterial activity was improved by substituting 15 amino acids and by fusing cecropin A to its N-terminus.

Results

The resulting engineered endolysin, LNT103, showed a strong antibacterial activity against various Gram-negative pathogens including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes*, with minimum inhibitory concentrations as low as 8 mg/ml. The engineered endolysin rendered bacterial membrane permeable and combination use with colistin showed a synergistic effect in antibacterial efficacy. Minimal cytotoxic effect and hemolytic activity were observed from LNT103. Unlike colistin, bacterial resistance to LNT103 was none to minimal in vitro. Its in vivo efficacy was verified in a mouse infection model.

Conclusion

LNT103 is an engineered endolysin highly effective against Gram-negative pathogens with a wholly different mode of action mechanism from existing antibiotics, making it a strong candidate for development as a novel antibiotic.

phage therapy Acinetobacter zebrafish

Unraveling the efficiency of phage therapy in combating *Acinetobacter baumannii* infection of zebrafish embryos

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Carbapenem-resistant *Acinetobacter baumannii* has been announced in 2017 by the World Health Organization as a critical priority pathogen, with an urgent demand for the development of new therapeutic approaches. Bacteriophages have regained attention as promising antimicrobial agents, aiming to achieve efficient eradication of life-threatening infections. The zebrafish (*Danio rerio*) model has emerged as a powerful preclinical animal model for studying various infectious diseases and for discovery of novel safe and effective antimicrobial drugs.

In this study, we investigated therapeutic potential of phage vB_AbaM_ISTD using the *A. baumannii*-zebrafish model of systemic infection. We aimed to compare the effect of phage treatment in relation to that of clinically used antibiotic meropenem, and to address the efficacy of their combined application in relation to individual treatments.

The systemic infection with *A. baumannii* was established by microinjection of fluorescently labelled cells of strain 6077/12 into the circulation valley of 48 h-old zebrafish embryos, and exposed to different treatments including ISTD phage (at doses of 10^4 and 10^5 PFU/ml), sub-MIC dose of meropenem (32 μg/ml), and various phage and meropenem combinations. Infected embryos were assessed for survival, morphological malformations and bacterial burden during a period of 3 days.

Data obtained in this assay revealed higher survival of *A. baumanii*-infected embryos upon treatment with meropenem and phages than in the untreated (control) group (survival rate of 59%), whereas solely applied phages appeared as more potent agents than antibiotic (survival rate of 93% at the dose of 10^5 PFU/ml vs. 67%, respectively). Assessment of viable cell count indicated that the administered phages effectively eradicated *A. baumanii* infection *in vivo,* since no bacterial colonies have been recorded after plating crushed embryos at 24 h post treatment, contrary to $6x10^3$ CFU/embryo in untreated group.

capsule | human gut microbiome | competitor | Microbiome engineering | e coli | Bacteriophage | Lytic Phages | gut

Phage therapy to exclude multidrug-resistant encapsulated pathogenic *Escherichia coli* from the gut

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The gut can serve as a reservoir for opportunistic pathogens, such as multidrug-resistant *Escherichia coli* that are responsible for severe extraintestinal infections, including recurring urinary tract infections and life-threatening sepsis in patients at risk, while being innocuous in healthy hosts. Many of these strains are protected by a polysaccharide capsule that promotes resistance to the host's humoral immune response by biological mimicry, and it is thus crucial to develop new strategies specifically targeting the capsule to exclude these pathogens from the gut microbiota.

While the capsule is a major virulence factor in these pathogens, it can also be a weak point as it makes them a target for bacteriophages that specifically use the capsule as a receptor to infect and kill the encapsulated bacteria. In this study we aim to use a cocktail of capsule-dependent phages to exclude encapsulated pathogenic bacteria from the gut. Our phage cocktail targets clinical isolates of multidrug-resistant *E.coli* from neonate sepsis patients and has been shown to exert selective pressure on the encapsulated bacteria in the murine gut, rapidly excluding the target and driving the emergence of capsule-less mutants. The remaining population that has lost its protective capsule is now vulnerable to the host immune system and we are developing vaccine-based and probiotic-based strategies to exclude the remaining pathogens, in combination with the phage therapy approach targeting the capsule. We also address the efficiencies of the combined therapies and potential applications and targets.

Klebsiella | Bacteriophage collection | phage susceptibility | host range

The biology, morphology and genomic characteristics of *Raoultella* phages from the Hirszfeld Institute collection

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Bacteria of the genus *Raoultella* are known to inhabit natural environments but can be also found in laboratory samples. Recently, the pathogenicity of *Raoultella* strains in human has become increasingly important. Reports on isolation of *Raoultella* bacteriophages are scarce mostly due to inconsistent taxonomy within *Enterobacteriaceae* family. The genus *Raoultella* was separated from *Klebsiella* in 2001, but difficulties in its identification caused an underestimation of its incidence and pathogenic role.

For more precise identification our *Raoultella* strains have been identified using MALDI-TOF mass spectrometry system. This approach led us to identify bacterial hosts for 7 *Raoultella* phages isolated from environmental samples (all of them were isolated from sewage samples, including one phage from historical collection isolated in 1963). Based on transmission electron microscopy results, phages represent two families *Myo*- and *Podoviridae*. A ShortBRED profiling was used to search the virulence factors and toxins in predicted ORFs against the Virulence Factor Database (VFDB). The presence of antibiotic resistance genes was verified with the Comprehensive Antibiotic Resistance Database (CARD) and PHACTS web tool was employed to determine phage lifestyle probability. Among 6 tested phages, 5 turned out to be lytic phages. Further, in 4 lytic phages no homologs of known virulence factors and determinants of antibiotic resistance were found.

The stability of phages with the broadest lytic spectra was tested in different storage conditions. Phages retained their lytic activity under broad range of environmental conditions. Interestingly, our *Raoultella* phages reveal lytic activity towards a plethora of *Klebsiella* strains isolated from hospitalized patients. Their stability and broad lytic spectra make them promising candidates for further research and potential therapeutic applications.

Cutibacterium acnes | Phage Therapy | Endolysin

Bacteriophages and Endolysins as Potential Treatments for *Cutibacterium acnes*.

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Cutibacterium acnes, formerly known as *Propionibacterium acnes*, is a Gram positive, aerotolerant, opportunistic pathogen commonly associated with the skin condition Acne Vulgaris. This condition is estimated to effect approximately 9.4% of the world's population, making it the eighth most prevalent disease worldwide. It is regularly treated with topical and oral broad-spectrum antibiotics that can damage the natural skin microbiome, and over time can result in the emergence of antibiotic resistant strains of *C. acnes.* Phage therapy has gained a lot of traction in recent years as an alternative to antibiotic treatment regimes. Phages and their endolysins allow us to specifically target bacteria and precisely manipulate microbiome composition. A previous screening study in the lab led to the isolation of five phages targeting *C. acnes*. This study is investigating the efficacy of these phages to tackle *C. ances* in a complex microbial setting. A number of endolysins have also been identified in the genomes of these phages, and we are in the process of recombinantly expressing these peptidoglycan-degrading proteins to assess their suitability as precision antimicrobials.

Endolysin Biofilm Ruminococcus gnavus

Lytic activity of endolysins targeting *Ruminococcus gnavus* biofilms implicated in inflammatory bowel disease

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Inflammatory bowel disease (IBD) which includes Crohn's disease and ulcerative colitis, is a chronic and uncurable disorder characterised by inflammation of the gastrointestinal tract. The overgrowth of biofilms of a key pathobiont, *Ruminococcus gnavus*, is associated with the development of this syndrome. However, is unclear if the overrepresentation of this bacteria is causal or associative. The use of antibiotics for the treatment of IBD is contraindicated due to their lack of specificity, which can result in collateral damage to the gut microbiome. In this light, the use of highly specific bacteriophages and/or their antimicrobial proteins, endolysins, represent a promising alternative. In this study, we evaluate the ability of *R. gnavus* JCM 6515^T to form biofilms in different culture media and investigate the capacity of endolysins Lys 84, Lys 210, Lys 507/2-1 and Lys 84/2-1 to eradicate them. The four endolysins used for these studies were selected for their high specificity and efficient lytic activity against planktonic cells of *R. gnavus* JCM 6515^T. The ability of *R. gnavus* JCM 6515^T to form biofilms in different culture media (ABB, BHI+, CIM, WC, YCFA, YCFA+ and LYHBHI) was evaluated in a 96-well plate by crystal violet assay. Similarly, the biofilm eradication ability of the endolysins was assessed in the same way. This strain demonstrated a strong ability to form a biofilm when grown in YCFA+ culture media, with strong lytic activity observed for endolysins 210, 507/2-1 and 84/2-1. Significant biofilm disruption (p<0.001) was observed even at a low concentration (1 μM) following a single hour of exposure at 37 °C. The potency and selectivity of these endolysins constitute a promising alternative treatment for IBD, avoiding the problems associated with antibiotic resistance and preserving beneficial commensal bacteria in the gut environment.

Vibrio | Bacteriophage | phage susceptibility

V. cholerae Eltor strains from early decades of the 7th pandemic are susceptible to later isolates of *V. choleare* phages

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The disease cholera continues to be a challenge for the global health. Recent outbreaks in different parts of the world demonstrated growing variability of *V. cholerae* and its high capability to cause massive epidemics. Bacteriophages play important role as natural controllers of *V. cholerae* in aquatic environments. At present phages gained again the interest of medical practitioners as a potential alternative or complementary treatment of cholera patients, and also as a biological mean for sanitation of drinking water reservoirs in endemic areas. We studied lytic activity of 2 sets of characterized bacteriophages: i) set 1 (11 phages), isolated from marine and freshwater sources in Georgia in 2007-2010 and ii) set 2 (7 phages), isolated in 2016 from Haiti water samples. Earlier both phage sets showed mild to high lytic activity against *V. cholerae* strains of different origin (Georgia, Asia, Central America, Haiti, Africa). The present study involved 47 strains of Eltor biotype from the collection of Georgian NCDC, Georgian isolates from 70's (beginning 7th pandemic), 80s and 90s, from patients and environment. The screening was done by phage spot test on bacterial culture lawns and lines using standardized phage suspensions (107 PFU/ml), followed by verification of negative plaque formation by agar overlay method. Eleven Georgian phages (set 1) lysed 93.6 % of NCDC's strains with the best result for the phage Vch24K (80,1%). Considerably low lytic activity (74.5%) was shown for the Haiti phages with the best result for the phage Vch20B (68.1%). These 2 sets of phages showed overlapping lytic spectrum. The fact that considerably recently isolated phages express high lytic activity towards the strains from early decades of 7th pandemic may sound promising in terms of creation of therapeutic phage mixture(s) for cholera control with stable lytic properties and broad coverage of potential subtypes of V. cholerae epidemic strains.

Pseudomonas aeruginosa | Tail fiber engineering | multidrug-resistant bacteria

Development of synthetic phages for delivery of custom cargo to MDR/XDR high-risk clones of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic human pathogen causing a wide range of acute and chronic infections, particularly in immunocompromised patients. This makes *P. aeruginosa* one of the most frequent and severe drivers of chronic respiratory infections in patients with cystic fibrosis or other chronic underlying diseases, and one of the top pathogens causing ventilator-associated pneumonia. Typically, antibiotic susceptible isolates of *P. aeruginosa* show a high clone diversity while, MDR/XDR strains are limited to a number of widespread clones, so-called high-risk clones (HiRiCs). The prevalence of nosocomial infections resulting from HiRiCs strains is at alarming rates globally, highlighting the importance of novel anti-*Pseudomonas* strategies.

To address the need for novel drugs targeting these HiRiCs strains, we developed a fully synthetic phage platform that can carry and deliver custom-made DNA cargo, by re-purposing an *Escherichia coli* phage. This SNIPR platform, allows the design of anti-*Pseudomonas* cargo in avirulent *E. coli* and delivery to different *P. aeruginosa* strains through engineered chimeric fibers. These cargos include genes of interest such as CRISPR-Cas circuits, lytic phage cassettes, biofilm degrading enzymes, or QS-quenchers.

Using comparative genomics on *P. aeruginosa* strains (n:59), we created an array of *E. coli* production strains for non-replicating synthetic phage constructs with different delivery profiles. We show successful delivery of GFP marker with profiles dictated by the chimeric fibers.

Through this process not only a fully synthetic platform was established but also new tools for the targeting and management of MDR/XDR strains of *P. aeruginosa* have been tested.

Bacteriophage S. aureus Stability

Stability of Refrigerated Commercial Bacteriophage Cocktails Over 1-Year Period

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BACKGROUND

Antimicrobial resistance is an immense challenge in public health. Thus, there is a strong interest in bacteriophages as a promising alternative to widely used antibiotics. It is worth reviewing the reliable storage of phages while researching their bacteriolytic activity. The objective of this study was to assess changes in the titer of commercial bacteriophage cocktails when stored at 4^{o}C over a 1-year period.

METHODS

The study sample was composed of *S. aureus* ATCC 25923 served as a reference strain and 2 phage stocks – Staphylococcal Bacteriophage and Pyo Bacteriophage (Eliava Biopreparations Ltd). Phage titer was determined by plaque assay. To attain a higher titer of each bacteriophage stock, propagation on *S. aureus* reference strain was performed. Phage lysates were stored in tryptic soy broth (TSB) at 4^{o}C for 1 year. The plaque assay was repeated for each propagated bacteriophage cocktail every 3 months from January to December 2020. All experiments were run in duplicate.

RESULTS

Staphylococcal Bacteriophage had an original titer of 6,2x10^{5} PFU/mL, whereas Pyo Bacteriophage - 1,0x10^{6} PFU/mL. After propagation, both phage stocks reached a three-fold rise in titer, namely Staphylococcal Bacteriophage (9,0x10^{8} PFU/mL), while Pyo Bacteriophage (4,0x10^{9} PFU/mL). From January to December 2020, titers of both propagated commercial phage stocks were not reduced by more than 1 log. In December, the titer of initially propagated Staphylococcal Bacteriophage was 1,2x10^{8} PFU/mL, whereas Pyo Bacteriophage - 2,0x10^{9} PFU/mL.

CONCLUSIONS

Preserved stability of refrigerated commercial phage stocks was observed over a 1-year period. Thus, TSB at 4^{o}C exhibits an optimal environment for maintaining the stability of phages. Although the findings of this study are assuring, further investigations compared with other liquid environments should be devoted to exploring the best long-term storage of phages to apply them in prophylactic and therapeutic interventions.

CRISPR-Cas | Antimicrobial | Klebsiella pneumoniae | Escherichia coli

Design rules for efficient chromosomal cleavage and killing of pathogenic enterobacteria using CRISPR-Cas

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The main outcome of efficient CRISPR-Cas9 cleavage in the chromosome of bacteria is cell death. This can be conveniently used to eliminate specific genotypes from a mixed population of bacteria, which can be achieved both in vitro, e.g. to select mutants, or in vivo as an antimicrobial strategy. The efficiency with which Cas9 kills bacteria has been observed to be quite variable depending on the specific target sequence, but little is known about the sequence determinants and mechanisms involved. Here we setup plasmids and assays to evaluate the efficiency of Cas9 or Cas12 cleavage in the chromosome of *E. coli* and *K. pneumoniae*. Genome-wide screens are used to determine the efficiency with which each guide RNA kills the cell. These datasets will be used to identify design rules of guide RNAs for efficient killing of target bacterial species and strains.

bacteria-phage interaction | multidrug-resistant bacteria | biofilm

Controlled release of bacteriophages for the treatment of multi-resistant *Pseudomonas aeruginosa* bone infection *in vivo* and *in vitro*

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Multidrug resistant bacterial infections and biofilm infections are challenging to treat with conventional antibiotic therapy. Combination of antibiotics and/or bacteriophages, may prevent the development of resistance and display increased activity. Biodegradable bio-materials like hydrogels, could then be employed for local delivery of the selected antimicrobial combinations. In this study we tested the activity of meropenem in combination with two Pseudomonas specific lytic bacteriophages (R9 and R3) embedded within alginate-chitosan microbeads and hydrogel. Antibiotic stability and phage activity were assessed *in vitro* over a period of 10 days. *In vivo*, the same material was tested in treatment of a 5-day old *P. aeruginosa* infection of a tibial plate osteotomy in mice. Treatment involved debridement and 5 days of systemic ciprofloxacn and meropenem (0.4 mg/kg) therapy in all groups, plus: i-saline, ii-phages and antibiotics loaded into hydrogel and microbeads (n=7 mice/groups). The infection load was monitored during revision surgery after 5 and 13 days (euthanasia) by CFU and PFU quantification. *In vitro* testing showed that bacteriophage R9 is active against *P. aeruginosa* 09, but phage R3 is not. Isolates that developed resistance to phage R9 *in vitro* were then found to become susceptible to phage R3. The activity and stability of both bacteriophages and meropenem in hydrogel was not affected over 10 days at 37°C, indicating the suitability of the carrier. A daily release of 10^9 phages particles from the hydrogel was observed over 9 days. The *in vivo* study showed that all mice receiving bacteriophages and antibiotics loaded into a hydrogel presented a reduction of the bacterial load in the soft tissue compared to untreated controls. Active bacteriophages could be recovered from the infected site at euthanasia (10^4 PFU/g). Bacterial cells that developed resistance towards the R9 bacteriophage but remained susceptible to phage R3 were also identified.

Acinetobacter baumannii | capsule degrading depolymerase | Antibiotic resistance | Acinetobacter baumannii phage

Building a collection of phage-derived capsular depolymerases to tackle relevant *A. baumannii* capsular types

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A. baumannii is the major cause of nosocomial and drug-resistant infections, its capsule representing a major virulence factor. This pathogen evolved to display a high variety of capsular types for evading host defenses and protecting themselves from predators. Some bacteriophages also evolved to produce capsular depolymerases, enzymes that specifically bind and degrade the bacterial capsules, allowing these phages to overcome this barrier and proceed with the infection.

In this study, 94 carbapenem-resistant *A. baumannii* clinical isolates (Northern region of Portugal, 2005-2012), were screened for their resistance genes by PCR. Genes *Oxa-23, Imp-like* and *Oxa-24* were present in 76%, 20% and 16% of the isolates, respectively. Based on their resistance gene profile, the genomes of 23 strains were sequenced. Using *in silico* typing with Kaptive, we found 4 prevalent capsular types, namely, KL2 (39%), KL7 (30%), KL9 (4%) and KL120 (26%).

Aiming at implementing an effective depolymerase-based anti-virulence strategy to control *A. baumannii* infections, we isolated novel capsular depolymerases from lytic and prophages, ending with an in-house collection of enzymes targeting 10 capsular types (KL1, KL2, KL9, KL19, KL30, KL32, KL38, KL44, KL45, KL67). Experiments using a human serum model proved that all capsular depolymerases can effectively sensitize *A. baumannii* to the host complement killing activity, that otherwise were resistant. Therefore, capsular depolymerases have demonstrated to be a very powerful anti-virulence weapon and an emerging solution to treat *A.baumannii*-related infections.

As a result, the collection of capsular depolymerases available was expanded to 17 K-specific depolymerases, advancing the prospects of application of these enzymes to control *A. baumannii* infections.

Virion-associated lysin | Endolysin | Enzybiotics | Antibiotic resistance | protein engineering

A VAL-based enzybiotic displaying strong bactericidal action against *Staphylococcus aureus* in growth supporting media

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Antibiotic resistance is a major threat to human health at a global scale [1]. Tackling this problem involves the exploration of alternative antimicrobial agents with mechanisms of action that minimize resistance development. Among promising alternatives are bacteriophage lytic enzymes, which cleave peptidoglycan (PG), the major component of the bacterial cell wall (CW) [2].

Phages employ two types of these PG-degrading enzymes. Virion-associated lysins (VALs) promote a local digestion of the PG to facilitate phage tail penetration for viral DNA injection. Endolysins promote massive PG degradation to cause host cell lysis for phage progeny release [2]. VALs have the advantage of being naturally designed to act from the outside of live bacteria, but they are often large proteins and usually lack a dedicated cell binding domain (CBD). Endolysins have this CBD that confers high affinity and specificity to the CW. However, in their natural context endolysins act from the cell inside and they rely on the membrane-depolarization action of another phage protein, the holin, for full lytic activity [2]. This has been proposed to explain some tolerance of actively growing bacteria to endolysins added extracellularly [3,4].

We have explored the best features of these phage lytic proteins *i.e.*, the superior lytic performance of VAL catalytic domains, and the high cell affinity of endolysin CBDs, to generate chimeric enzybiotics (VALue fusions) displaying strong lytic activity in conditions that support bacterial growth. As an example, we present the results with a VALue fusion that shows potent bactericidal activity against antibiotic-resistant *S. aureus* strains, and which clearly outperforms the reference endolysin LysK.

[1] Antimicrobial Resistance Collaborators, 2022. doi: 10.1016/S0140-6736(21)02724-0

[2] São-José, 2018. doi: 10.3390/antibiotics7020029

[3] Proença et al., 2015. doi: 10.1007/s00253-015-6483-7

[4] Gouveia et al., 2022. doi: 10.1038/s41598-022-05361-1

Pseudomonas aeruginosa | phage therapy | Bacteriophage isolation | hospital wastewater

Pseudomonas aeruginosa bacteriophages isolated from hospital wastewater and the effect on healthcare-associated strains

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The drug-resistant strains *Pseudomonas aeruginosa* are the cause of serious and even life-threatening infections. In this case, the application of bacteriophages is hopeful alteration of antibiotic therapy. New phages for therapy could be obtained from environment, when the one of the suitable sources for phage isolation are wastewaters. In this study, we have focused on isolation and characterisation of new bacteriophages with therapeutic potential from wastewater that has same origin (i. e. hospital) as phage host strains of *P. aeruginosa*.

Firstly, we isolated five new pseudomonal bacteriophages from samples of wastewater acquired from UHO (University Hospital Ostrava). We determined the lytic patterns of these phages against 26 UHO isolates of *P. aeruginosa* together with nineteen pseudomonal phages from FAGOFARMA company collection. Overall, the 80.8 % of strains were sensitive (i. e. at least one of the assessed phages formed plaques with these strains). After that, we used sequencing, PCR, and transmission electron microscopy for phage taxonomic classification. The phages from hospital wastewaters were characterised as members of *Pbunavirus* and *Nankokuvirus* genera. According to literature, similar phages are commonly use in commercial therapeutic cocktails.

In conclusion, we proved the possible isolation of bacteriophages with therapeutic potential from Czech hospital wastewaters. In the future, these phages can be components of cocktail for treatment of infections caused by drug-resistant strains of *P. aeruginosa*.

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Helicobacter pylori | Prophage | Phage Therapy

Screening and *in silico* characterization of prophages in *Helicobacter pylori* genomes

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Temperate bacterio(phages) play an important role on the evolution of pathogenic bacteria. Nevertheless, information on their role in *Helicobacter pylori* (an important gastric pathogen bacterium) is scarce.

The present study developed a workflow for the identification of prophages in Portuguese *H. pylori* clinical strains, proposing the use of a new PCR-based screening method. The genome of strains with different PCR profiles were then sequenced.

In the fourteen genomes analysed, nine intact prophages were identified by PHASTER. These prophages were annotated by analogy with other identified phages, where seven contained the integrase gene, corroborating the results obtained in the PCR screening, with only one exception. Still, in PCR screening, the holin gene was identified in 75 % of the strains containing intact phages, but BLASTp homologies only recognized this gene in one of the prophages. Fifty-six percent are podovirus, while in 44 % it was not possible to assign any family, according to the VirFam tool. Using the Resistance Gene Identifier of CARD it was identified the *Acinetobacter* mutant Lpx gene conferring resistance to colistin in two intact prophages. The BLASTp search identified a putative ABC binding cassette transporter in one of the intact prophages. On the bacterial genomes, 71 % have the CRISPR-Cas system classified as evidence level 1 by CRISPRCasFinder, which typically indicate potentially invalid CRISPR arrays.

The use of an initial PCR screening method increased the identification of intact prophage-containing strains from 20 % to 57 %. Furthermore, the few virulence factors identified in prophages, and the possible inactivity of CRISPR-Cas in the bacterial genomes, allow the choice of strains for the isolation of phages for future studies. Overall, our results represent a significant contribution to the knowledge of prophages in *H. pylori*, and provide valuable insights into their potential use in phage therapy.

Acinetobacter baumannii | Antibiotic resistance | Bacteriophage | phage resistance

Comparative genomics of *Acinetobacter baumannii* and therapeutic bacteriophages from a patient undergoing phage therapy

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In 2016, a 68-year-old patient with a disseminated multidrug resistant *Acinetobacter baumannii* infection was treated using lytic bacteriophages in one of the first modern human clinical uses of phage therapy in the United States. Due to the emergency nature of the treatment there was little time to thoroughly characterize the phages used in this intervention, or the bacterial pathogen itself. Here we report the complete genomes of the nine phages used for treatment and three strains of *A. baumannii* isolated prior to and during treatment. The eight myophages used for initial treatment were found to be T4-like members of the *Twarogvirinae* subfamily. A ninth phage introduced at the end of treatment, AbTP3Φ1, was a podophage of the genus *Friunavirus*. Analysis of 19 *A. baumannii* isolates collected before and during phage treatment showed that resistance to the T4-like phages appeared as early as two days following the start of treatment, and that these phages could be categorized into two groups based on patterns of host resistance, which correlated with sequence clustering of the predicted phage tail fibers. Three *A. baumannii* strains (TP1, TP2 and TP3) collected before and during treatment were sequenced to closure, and all contained a 3.9 Mb chromosome of sequence type 570 with a KL116 capsule locus and identical 8.7 kb plasmids. Most changes observed in the three strains could be attributed to genetic drift or the internal mobilization of IS elements, except for a 6.7 kb insertion sequence acquired by TP2 and TP3 which carried an aminoglycoside O-phosphotransferase and an NDM-1-like metallo-beta-lactamase. Phage-insensitive mutants of *A. baumannii* strain TP1 were generated *in vitro* using five phages from the phage cocktails. The presence of the same mutations in both the *in vitro* mutants and in phage-insensitive *in vivo* isolates (TP2 and TP3) indicate that *in vitro* investigations can produce results that are relevant and predictive for the *in vivo* environment.

Burkholderia cepacia complex | Phage Therapy | phage engineering

Engineering of temperate *Burkholderia* phage Milagro for use as a therapeutic

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As the increase in bacterial resistance to antibiotics has become a major public health problem, alternative strategies must be evaluated to combat multiple drug resistant bacterial infections. In Cystic Fibrosis (CF) patients, end-stage pulmonary disease often requires lung transplantation. However, pre-transplant colonization with antibiotic-resistant strains of *Burkholderia* is predictive of poor post-transplant outcomes. Unlike many pathogenic bacteria, *Burkholderia* has a limited virosphere dominated by temperate phages that are unsuitable for phage therapeutics. Moreover, the few virulent phages that have been isolated have limited host ranges. This situation points to the need for engineering approaches to render temperate phages virulent and to expand their host ranges. Here we report the isolation of *B. cenocepacia* phage Milagro, a temperate phage related to the previously described phage KL3 and distantly related to the P2-like phages of *E. coli.* Lysogens of phage Milagro were found to produce an auto-plaquing phenotype, in which phage plaques appear on lawns inoculated with the lysogen alone. Genomic analysis of these Milagro *vir* mutants showed that all mutations are located in or near the predicted phage promoter P_R, which we hypothesize drives expression of the phage lytic program. As previously noted, most *Burkholderia* phages have narrow host ranges. Using gene exchange, we have replaced the recognition components (tail fiber and tail fiber assembly) of the narrow host range phage Milagro *vir* with those from the related P2-like broad host-range tailocin BceTMilo. The chimeric phage exhibits the broad host range of the tailocin, indicating that this is a viable strategy for phage host range expansion in *Burkholderia* phages. This work represents initial steps for the use of phage Milagro as a platform for engineering therapeutic phages active against *Burkholderia* found in the CF lung.

bacteriophages endolysins enzybiotics antibacterial proteins

peptidoglycan hydrolases

MB Pharma's Microbial Collection as a Source of New Enzybiotics

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MB Pharma and FAGOFARMA are trying to build up a collection of microbes that could be a source of new antimicrobials. Not just phages from this collection, killing strains of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, *Aeromonas salmonicida*, *Cutibacterium acnes* etc., but also their specific enzymes such as peptidoglycan hydrolases could be used. Moreover, genes encoding bacteriocins, bacterial cell wall-degrading enzymes and prophage endolysins were found in the genomes of clinical bacterial isolates and propagation strains from the collection. All these enzymes could be used as enzybiotics – a tool to fight microbial infections.

More than 40 microbial genomes from the company's collection were automatically annotated using RAST and analysed. Hypothetical peptidoglycan hydrolases were found and compared with already identified endolysins. Tools such as HHpred, InterProScan and SMART were used to characterize their functional domains in more detail. Relatively low diversity of endolysins was observed in *S. aureus*, *C. acnes* or *P. aeruginosa* phages, whereas higher diversity was in *K. pneumoniae* phages. Some endolysins have been well described previously, such as LysK of *S. aureus* phage K, but others are novel. In addition, prophage endolysins were found in the bacterial genomes. In *Paenibacillus larvae*, where only one endolysin was already described, a different hypothetical endolysin was identified in this work.

Bacterial and phage genomes represent a great source of new antibacterial proteins whose activity needs to be proven experimentally. Several proteins, which could be used as enzybiotics, were identified in this research. Their modifications, such as addition of tags for destabilization and transmembrane transfer, could improve their activity, especially against Gram-negative bacteria.

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Biofilm Dormant cells S. lugdunensis

Characterisation of two novel bacteriophages specific against *Staphylococcus lugdunensis* biofilms

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Staphylococcus lugdunensis is a coagulase negative staphylococci (CoNS) and an emergent pathogenic biofilm-forming agent, responsible for causing a severe form of native valve endocarditis and infections of prosthetic heart valves, intravascular catheters, prosthetic joints, and ventriculoperitoneal shunts, among other hospital-acquired infections.

Biofilm formation is a virulence factor of *S. lugdunensis*. Inside biofilms, dormant cells can survive antibiotic therapy, due to their low metabolic activity. The survival of these bacterial cells to antibiotic treatments, results in biofilm relapse and consequent infectious illness recalcitrance.

Bacteriophages (phages) are an antimicrobial potential that can be used as alternative or in a complement of traditional methods. However, to data, there are no reports of *S. lugdunensis* phages.

The main goal of this study was the isolation and characterization of new *S. lugdunensis* phages and the evaluation of their activity against biofilms and dormant cells to select promising candidates for therapy.

The two isolated *S. lugdunensis* phages (Lud1 and Lud2) exhibited good antimicrobial properties against *S. lugdunensis* I439 and U867 biofilms. Phage Lud2 showed the rare ability to infect dormant bacteria. In addition, both phages characterization demonstrated that both exhibited a broad lytic spectrum, high stability at different pH and temperature conditions and potential safety, as genome analysis did not identify any virulence-associated nor antibiotic resistance genes.

As far as we know, these are the first well-characterized *S. lugdunensis* phages. Overall, both phages demonstrated that they can be promising agents to combat infections caused by this important novel pathogen.

bacteriophages | lysate purification | tangential flow filtration | ion exchange chromatography | monolithic column

Comparison of modern purification approaches and its effects on different bacteriophage lysates

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Bacteriophages (phages) are used in a broad range of biotechnological applications. With the arising problem of multidrug resistance leading to untreatable bacterial infections, they are becoming intriguing target for use as antimicrobial agents in human and veterinary medicine, food industry, agriculture, etc. Due to the demanding purity of bacteriophages for human use, need for high-quality purification protocols emerges.

We present comparison of three single step purification methods for one *Pseudomonas aeruginosa* and two *Staphylococcus aureus* phages. These bacteriophages are commonly used as antimicrobial organisms against *P. aeruginosa* and *S. aureus* in our laboratory. Altogether, three different procedures were tested – chromatographic method using a CIM® QA-1 ml tube monolithic column and tangential flow filtration with or without detergent addition.

Cleared and filtered lysates were loaded on previously mentioned purification systems. Purification efficiency was determined by bacterial proteins and endotoxins removal and total phage recovery. Infectious viral particles were determined by plaque assay. Host cell proteins were determined by Bradford NanoQuant (Roth). Endotoxins were quantified by Pierce™ Chromogenic Endotoxin Quant Kit.

Based on the results of the compared methods, it is not possible to accurately determine which of the tested methods is best for a specific phage. It depends mainly on the purpose for which the phage is being purified, so the method or combination of methods must be chosen accordingly. In most cases, a suitable compromise between phage recovery and phage purity must be made.

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Endolysin Bacteriophage Enzybiotics Staphylococcus aureus

Novel endolysin of *S. sciuri* phage S10 and its antimicrobial effect on *S. aureus*

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A lot of novel bacteriophages were isolated, characterised and added to the microbial collection of Fagofarma (and MB Pharma) recently. Some of these phages are unique and their genomes encode potentially novel peptidoglycan hydrolases. Such as the phage *S. sciuri* S10, which was isolated from wastewater and its genome showed low similarity to sequences found in public databases. In its genome, two genes encoding hypothetical endolysin (LysS10) or tail peptidoglycan hydrolase (TPH) were identified. In this study, we focused on examination of potential activity of these two enzymes.

Both genes were cloned, the proteins were expressed in *E. coli* and purified using chromatography. Zymogram was used as the first method for determination of activity on bacterial cell walls isolated from *S. sciuri* and *S. aureus*. This test proved activity of endolysin LysS10 on both types of cell walls. Activity of TPH was not proven, therefore, we focused mainly on LysS10 which activity was verified using spot assay and turbidity reduction assay. Moreover, LysS10 activity was compared with the LysK and the LysF1 on different strains and species of *Staphylococcus*, including MRSA. LysS10 had antimicrobial effect on every tested bacterium, but it was less active compared to LysK and LysF1.

Antimicrobial effect of novel endolysin LysS10 was proven by different methods on several *S. aureus* strains and other staphylococcal species. Although the enzyme is not as efficient as LysK, there is potential for improving its properties by changing domains, protein design of the catalytic domain or increasing the solubility of the protein.

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Acinetobacter baumannii | Klebsiella pneumoniae | phage cocktail

Healthcare-associated infections | phage therapy | Bacteriophage isolation

Isolation of new bacteriophages against *Klebsiella pneumoniae* and *Acinetobacter baumannii* clinical isolates

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Klebsiella pneumoniae and *Acinetobacter baumannii* are known as emerging opportunistic pathogens associated with hospital facilities. Clinical isolates are a threat due to multidrug resistance, virulence factors, and toxins. These bacteria can be fatal for immunocompromised patients, as well as they play key role in secondary infections of severely ill COVID-19 patients. Bacteriophages (also known as phages) are natural predators of bacteria. Nowadays, because of increasing cases of MDR infections, phage therapy has the potential to be used as an alternative or supplement to antibiotics.

In cooperation with the University hospital Ostrava bacteriophages against clinical strains of *Klebsiella pneumoniae* and *Acinetobacter baumannii* were isolated. Fagofarma company thus expanded its collection by more than 40 *Klebsiella* and 9 *Acinetobacter* phages. Phages were isolated from wastewater and sludge, including wastewater from University hospital Ostrava. Host ranges of isolated phages were determined using more than 60 clinical isolates of *K. pneumoniae* and 30 of *A. baumannii*. Combination of phages covers more than 90% of tested *Klebsiella* and *Acinetobacter* strains. Isolates with a broad host range or with specific features were characterized by whole genome sequencing and TEM. Some of them are unique, others have other have very similar genomes to previously described phages. Large scale production was optimized for each characterized phage, and long-term stability testing is under way. At present, downstream processes are optimized. The final liquid form of bacteriophage cocktail will have the potential of therapeutic usage in the future and could be used for various dosage forms.

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Bacteriophage collection | phage therapy | phage characterization | veterinary pathogens | human pathogens

Phage collection against human and veterinary pathogens

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When the world is facing an antibiotic crisis, it is necessary to investigate the development of alternative strategies. Phage therapy enables the specific treatment of bacterial infections. It is also possible to create highly efficient phage mutants to combat pathogenic bacterial strains that were initially resistant. The great diversity of bacterial strains, even at the species level, requires a variety of phages in collections that would be easily and quickly accessible in case of emergency. We aim to establish a collection of well-characterized phages applicable in human and veterinary medicine and to develop a quick phage isolation protocol, to be able to react to the actual needs of patients. Currently, our collection comprises phages against *S. aureus*, *S. sciuri*, *P. aeruginosa*, *E. coli*, *A. salmonicida*, *A. hydrophila*, *K. pneumoniae*, *A. baumanii*, and *C. acnes*. The phages were isolated using bacterial strains from the hospital environments, animal farms, and Masaryk University collection. The antimicrobial efficiency of phages was examined by spot test. The growth conditions of phages were optimized, and the stability of selected phages was tested. Selected phages were characterized in detail, and their genomes were sequenced. Chosen phages were purified, and their virions were depictured using transmission electron microscopy.

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Phagetherapy | qPCR phage titer | Electron microscopy | Culture based quantification

Transmission electron microscopy to study differences in quantification of phages by the culture based method and qPCR

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Background

Bacteriophages can be used to treat multidrug resistant bacterial infections, caused by for example *Staphylococcus aureus* (SA)*, Pseudomonas aeruginosa* (PA) or *Acinetobacter baumannii* (AB). It is important to have a good estimate of the number of infective phage particles for successful treatment. Traditionally, the double-agar overlay method (DAO) is used for quantification but this is labor intensive and takes multiple days to obtain results. Therefore, we developed a qPCR platform to quantify five phages against SA, PA and AB. We showed before that quantification of ISP and Acibel007 with qPCR resulted in a respectively 7.27 ± 0.21 and 67.41 ± 20.40 times higher concentration, compared to DAO.

Methods

To investigate this difference, transmission electron microscopy was used. ISP and Acibel007 phage stocks were blotted on maze Ni-grids with formvar/Ca-coating and glow discharge treatment and stained with 1% (*w/v*) uranyl acetate. Additionally, ISP was visualized with its host SA (ATCC6538) at varying incubation times between 0 to 45 min. Immunogold labeling was used to detect the presence of DNA outside the phage.

Results

The infection cycle of ISP could be visualized from the initial absorption until the release of new phage particles. The visualization of the individual ISP phage particles showed that ISP forms clusters and that these phages often have a contracted tail. This indicates that DNA is released in the phage stock where it can be detected with qPCR and not with DAO. Immunogold labeling showed indeed positive staining for DNA fibers outside the phages.

The visualization of Acibel007 showed that there are vesicles present in the phage stock, most likely formed by the bacterial host during phage propagation. These vesicles can act as a decoy to inactivate the phages.

Conclusion

The visualization of ISP and Acibel007 indicates that there can be multiple causes for the discrepancy between quantification of phages with qPCR and DAO.

Phage training | biofilms | Pseudomonas aeruginosa | cystic fibrosis

Adaptive evolution of phages towards *Pseudomonas aeruginosa* biofilm control

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Pseudomonas aeruginosa is the leading cause of chronic lung infection in patients with Cystic Fibrosis (CF). The main reason for the persistence of *P. aeruginosa* in CF lung environment is its biofilm mode of growth, associated with increased tolerance to antibiotics and host immune defenses. Phage therapy is a promising approach to treat biofilm-related infections such as CF. However, the complete eradication of biofilms is almost impossible. Given the natural ability of (bacterio)phages to evolve and counterattack the bacterial defense mechanisms, the aim here was to improve the anti-biofilm activity of phages through adaptive evolution.

The phage evolution was stimulated for 8 days in 24h-old biofilms formed by a *P. aeruginosa* clinical isolate recovered from a CF patient. The biofilms were treated with phage PE1, a Pseudomonas PB1-like phage. After 24h of infection, the phages were recovered from the wells and added to a fresh 24h-biofilm. This procedure was repeated daily in 24-well plates and the final biofilm-adapted phages were recovered for phage production and characterization.

The evolution process resulted in an increased anti-biofilm activity of the adapted phages compared to the wildtype phage, leading to a greater biofilm reduction. The two adapted-phages with the best anti-biofilm activity revealed an increased efficiency-of-plating against several *P. aeruginosa* clinical strains and *P. aeruginosa* colonies isolated from the biofilm. When comparing the phage genomes, it was possible to identify two SNPs in genes encoding a tail-fiber and a baseplate.

The emergence of mutations in genes involved in bacterial recognition and binding, together with the increased efficiency-of-plating, indicate that the biofilm evolution process improves phage host range and infectivity efficiency. Given the common heterogenicity of biofilms, the enhancement of bacterial recognition may be the key for the increased anti-biofilm activity of the evolved phages.

Amyloid-beta bacteriophage diagnostics

A phage-based platform to detect amyloid-beta oligomers

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Amyloid-beta (Aβ) is a prime suspect to cause Alzheimer's disease (AD).Aβ peptides can aggregate into soluble oligomers and fibrils, to finally deposit in insoluble plaques. Even though the plaques are main hallmarks of Alzheimer's, before plaque formation, the still-soluble Aβ oligomers and fibrils are the culprits that trigger a loss in synapses and memory dysfunction. However, whereas we are well-able to identify Aβ in plaques, we lack the tools to selectively target oligomeric/fibrillar Aβ in the brain.

Peptides with high affinity for Aβ may be the answer to this problem, since they are already known to selectively bind oligomeric and fibrillar Aβ, and even can inhibit Aβ aggregation. However, peptides are very unstable and do not cross the blood-brain barrier (BBB). To overcome these limitations, we have used a M13 filamentous phage as vector to display Aβ-specific peptides. Our results show that these engineered phages efficiently recognized Aβ aggregates in brain samples of AD-model mice and of AD patients.

This work demonstrates the unique ability of engineered M13 phages to selectively detect oligomeric/fibrillar Aβ in brain samples, thus having a great potential for application in the diagnostics of early Alzheimer's disease.

phage engineering | Pseudomonas aeruginosa | detection | bioluminescence

Phage engineering for the detection of *Pseudomonas aeruginosa*

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Maria João Costa and Luciana Meneses contributed equally to this work

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium. Due to its high antibiotic resistance and capacity to adapt and survive in hostile conditions, *P. aeruginosa* is responsible for a wide range of human infections, such as surgical site infections, bacteremia, urinary tract infections, and mostly, pneumonia. In COVID-19 patients, *P. aeruginosa* is a common co-infecting pathogen, associated with increased disease severity and worse clinical outcomes. Considering the slow turnover of conventional diagnostic methods and the problems associated with the molecular and immunogenic methods, this study aimed at assembling a bioluminescence-based reporter phage for the fast and sensitive detection of P. aeruginosa in clinical care.

Phage vB_PaeP_PE3 was genetically engineered using the yeast-based phage engineering platform. The genome of this phage was previously reduced by deleting genes with unknown function, and here, this phage genome was used as a scaffold for the insertion of the NanoLuc® luciferase. The gene encoding NanoLuc was swapped with gene gp55, encoding a hypothetical protein with unknown function. The sensitivity of this phage-based detection system was evaluated through the infection of serial dilutions of *P. aeruginosa* suspensions with the synthetic phage, and subsequent quantification of luminescence (in relative light units, RLU). Our data showed that the reporter phage was able to reliably detect 10^2 CFU in 1 mL of contaminated sample in less than 8 h.

Overall, the NanoLuc-based reporter phage allows for the rapid and sensitive detection and differentiation of viable *P. aeruginosa* cells using a simple protocol, 45 h faster than culture-dependent approaches. Therefore, this phage-based detection system is a promising alternative to the common methods for the accurate detection of *P. aeruginosa* in clinical settings.

cell-free phage expression | in vitro DNA replication

host-independent production platform

Cell-free production of personalized therapeutic phages targeting multidrug-resistant bacteria

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Bacteriophages are a promising therapeutic approach to combat rapidly increasing numbers of infections with multidrug-resistant bacteria. However, the broad implementation of bacteriophage therapy is currently impeded by a lack of safe production standards and insufficient phage characterization. We utilized a cell-free expression system to produce high titers of bacteriophage, fully omitting the use of living bacteria.

This synthetic system is able to perform all steps of phage replication, which we show by use of different technologies: phage DNA is replicated (shown by qPCR), phage proteins are expressed from the DNA template, which we show using mass spectrometry, and finally expressed phage proteins self-assemble into fully functional phages as verified by plaque assay.

We further developed this system into a host-independent platform for the production of phages against both gram-positive and gram-negative bacteria. At a microliter-scale, our *E. coli* derived cell-free expression system produces effective doses of phages against *E. coli*, including enteroaggregative *E. coli* (EAEC), *K. pneumonieae*, *Pseudomonas* sp. and *Y. pestis*. By co-expressing suitable host factors, we were furthermore able to extend the range of our platform to include phages of gram-positive bacteria like *B. subtilis*. The use of our cell-free system for phage production in a clinical setting offers the advantage of significant reduction of impurities such as endotoxin and prophage contaminations, compared to a bacteria-based approach.

Finally, we showcase a pipeline for personalized phage therapy of a multidrug-resistant ESKAPE pathogen from isolation to *in vitro* production of a phage against a clinical isolate of *K. pneumoniae*. We expect our cell-free methodology to enable and accelerate safe and tailor-made phage therapies against the growing number of pathogenic MDR bacteria.

Antimicrobial Resistance CRISPR ESBL phage engineering synthetic biology decolonization

Addressing antibiotic resistance via CRISPR-mediated decolonization or in *situ gene* editing

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We are witnessing a worrying increase of 3rd generation cephalosporin (ESBL+) and Carbapenem-resistant (CR) Enterobacteriaceae, particularly *E. coli* (Ec) and *K. pneumoniae* (Kp). An average of 20% of solid organ transplant recipients are carriers of ESBL+/CR+ Ec and/or Kp. As a consequence, these patients do not respond to classic antibiotic treatment and have an increased risk of developing difficult-to-treat bacteremia or surgical site infections that increase the length and cost of hospitalizations as well as mortality rates. We developed an antimicrobial strategy where CRISPR nucleases are used to either mediate sequence specific elimination of target strains or to inactivate their antibiotic resistance genes by gene editing. Engineered phage-derived particles, Eligobiotics®, are used to efficiently deliver a DNA payload programmed to target and kill ESBL Ec ST131 and Kp ST258 strains or to edit and inactivate a beta-lactamase (*bla)* AbR gene. We demonstrate the efficacy of this approach *in vitro* with epidemiologically relevant ESBL+/CR+ strains as well as in animal models: Eligobiotics® are able to significantly reduce colonization of ST131 and ST258 strains in a mouse gut colonization model, as well as to achieve >90% base editing and re-sensitization of AbR-carrying strains to antibiotics *in situ.* We also demonstrate that Eligobiotic® treatment of cecal contents from Ec ST131-colonized axenic mice completely abrogates sepsis and completely restores survival upon i.p. injection into BALB/c mice. Altogether, these results highlight the potential of CRISPR-Cas decolonization and base-editing strategies against ESBL+/CR+ and other pathogens.

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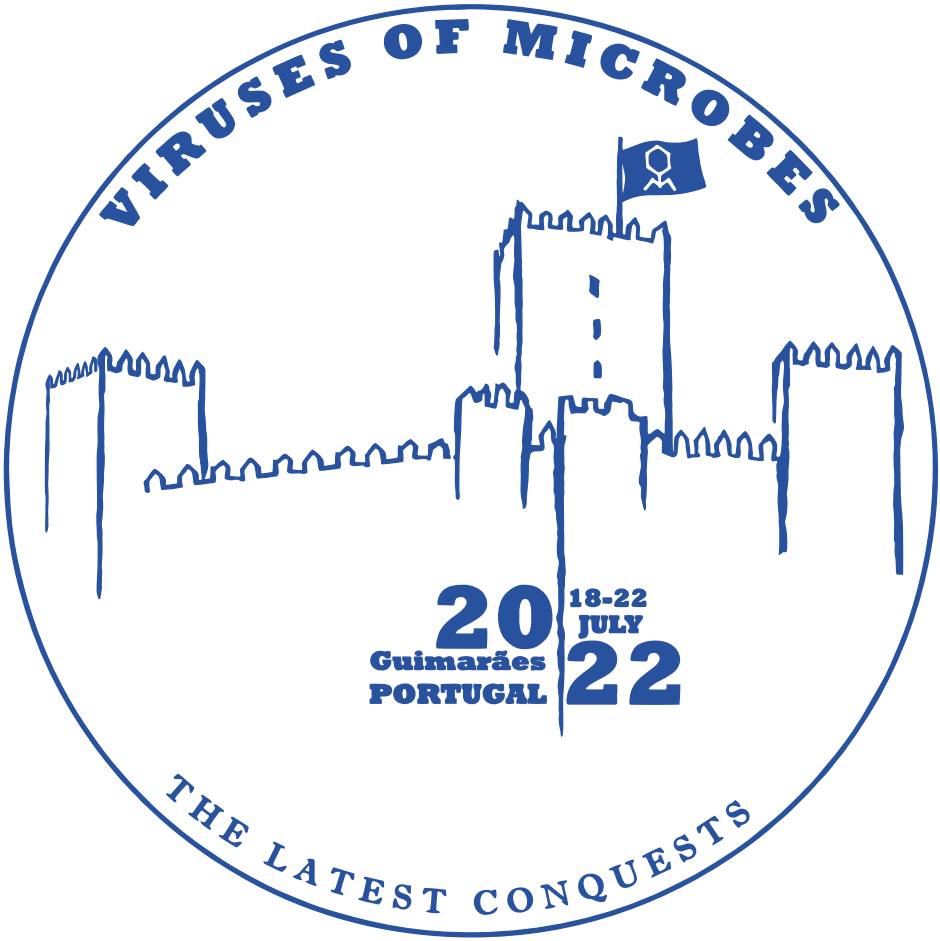




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