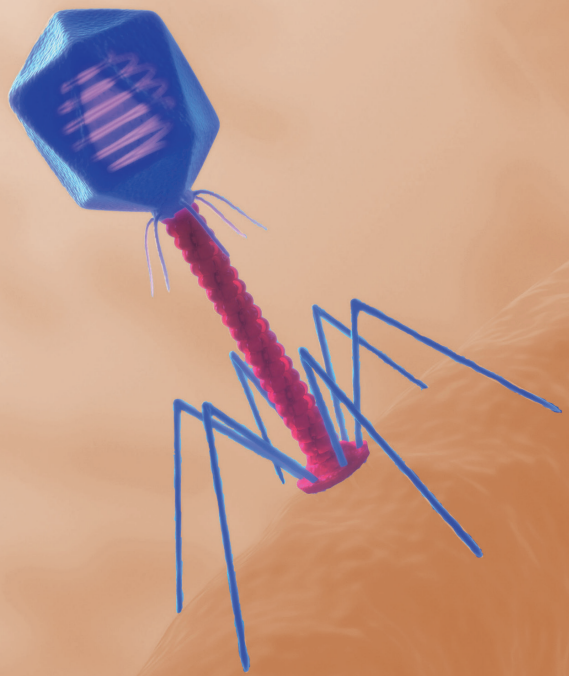
UNIVERSITY OF HOHENHEIM

2nd German Phage Symposium

Program and Abstract Book

23 – 24 May 2022

2ND GERMAN PHAGE SYMPOSIUM



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How to reach the venue

Public transportation

From Stuttgart Airport

From the arrival terminal's building, turn to the right to reach the bus station in approximately 3 minutes. Please follow the sign with the bus. Take bus 65 (Obertürkheim Bhf) to the final stop "Universität Hohenheim". From there, follow Heinrich-Pabst-Straße a few meters to the South and turn left after the "Mensa" into "Garbestraße". After 300 meters you reach Hohenheim Palace.

Travel time: approx. 25 minutes.

From Stuttgart Railway Station

From the main station at Stuttgart take the tram U7 (Ostfildern). At station "Ruhbank" change to bus 70 (Plieningen) until the final stop "Plieningen Garbe". From there, follow the "Garbestraße" eastbound to reach Hohenheim Palace. Travel time: approx. 30 minutes.

Alternative route:

Take trams U5 (Leinfelden BF), U6 (Fasanenhof) or U12 (Dürrlewang) to "Möhringen Bahnhof" and change to tram U3 to arrive at the final stop "Plieningen Universität Hohenheim".

Walk from there towards Hohenheim Palace

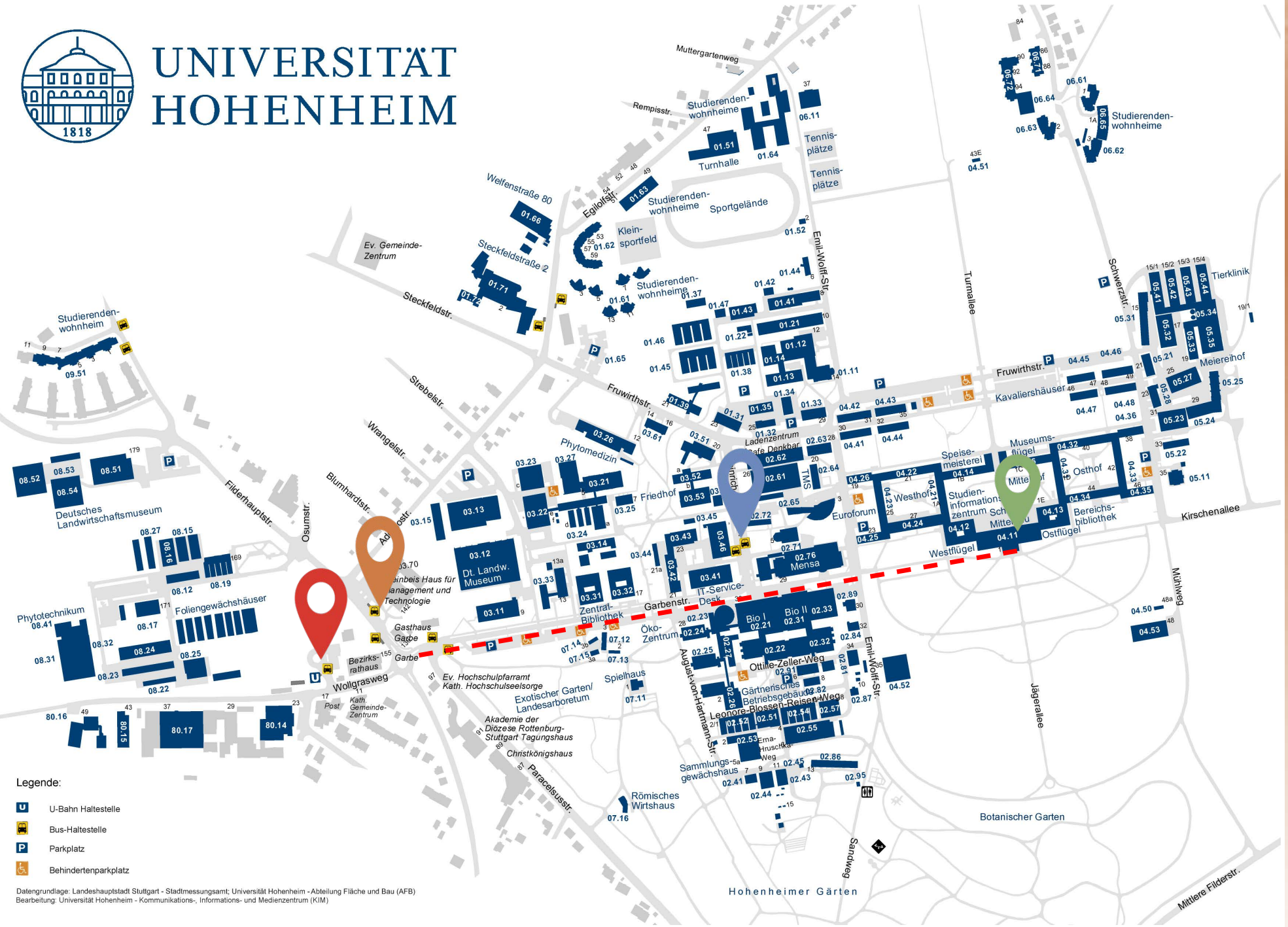
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UNIVERSITÄT HOHENHEIM



Legende:

- U-Bahn Haltestelle
- Bus-Haltestelle
- Parkplatz
- Behindertenparkplatz

Datengrundlage: Landeshauptstadt Stuttgart - Stadtmessungsamt; Universität Hohenheim - Abteilung Fläche und Bau (AFB)
Bearbeitung: Universität Hohenheim - Kommunikations-, Informations- und Medienzentrum (KIM)

2ND GERMAN PHAGE SYMPOSIUM

Welcome to the 2nd German Phage Symposium

23–24 May 2022, University of Hohenheim, Stuttgart

On behalf of the organizing and scientific committees I warmly welcome you to the 2nd German Phage Symposium!

Lysogenic infection of bacteria and archaea by phages is considered a 'temperate state' where phages engage in long-term associations with their hosts that can lead to mutually beneficial interactions and niche-specific fitness effects, of which the full extent is presently unknown. Temperate phages play a pivotal role through their ability to regulate their host's behavior. They drive the evolution of members of bacterial communities at both, individual and population levels. Being the most abundant gene carriers, transduction by phages is generally considered to be the most important mechanism of horizontal gene transfer between bacterial cells and an important evolutionary force underlying bacterial adaptation. Moreover, viral-mediated lysis of microbial cells shapes microbial communities like microbiomes, biogeochemical cycles, and global ecosystems.

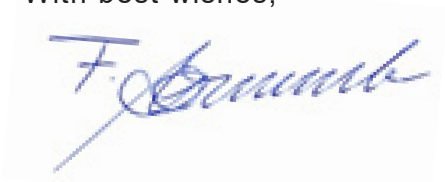
Phage-host interactions, their drivers and modulators need to be better understood in natural as well as in technical ecosystems such as bioreactors and for medical applications. To this end, knowledge about structural features and their spatiotemporal dynamics will be of crucial importance.

Relatively little is known about involvement of host factors in the infection and assembly process of phages at the molecular level. Phages can also modulate the cell wall structures of their hosts through mechanisms that are still poorly understood. However, understanding these processes at the molecular level is fundamental to analyze phage-host interactions in detail and consequently to describe more complex systems or adaptation dynamics of the host-virion interaction. Moreover, phage nanostructures like the DNA delivery or injection machines may be used in the future for medical and therapeutic applications such as the cell-specific injection of drugs.

While the 1st German Phage Symposium held in 2017 attempted to cover the whole kaleidoscope of phage research and application sectors, the 2nd German Phage Symposium will be mainly dedicated to molecular mechanisms underlying the control of life cycles of temperate and lytic phages, phage genomics, structural biology of phages and phage-host interactions.

The symposium provides scientists engaged in this fascinating research field the opportunity to meet colleagues, to find new partners for future collaborations and to discuss their scientific achievements and hypotheses in an open-minded atmosphere and a beautiful environment.

With best wishes,



Prof Dr Fabian M. Commichau
Scientific Director of the 2nd German Phage Symposium

University of Hohenheim

Being the oldest and most beautiful university campus in Stuttgart, today the University of Hohenheim is most famous because of its strong thematic specialization.

Founded in 1818 after devastating famines, the University of Hohenheim traditionally is committed to develop innovative solutions for some of society's most pressing problems, e.g., food security and sustainable agricultural production in times of climate and sociocultural change. For that, fundamental research and applied scientific disciplines are nurtured under one roof to cover the whole agricultural system in its socioeconomic and natural context.

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For several years now, University of Hohenheim is the leading German University in agricultural research and food sciences and with this specification also ranks in the top 10 of Europe. Complemented by natural, social, business, economic, and communication sciences, this unique combination makes it possible to find solutions for the global challenges of today and our future.





Scientific Board Members

SCIENTIFIC DIRECTOR



Prof Dr Fabian M. Commichau
University of Hohenheim
Institute of Biology, Department of Molecular Microbiology

Fabian Commichau studied biology at the RWTH Aachen and obtained his Ph.D. in microbiology at the University of Göttingen. After postdoctoral work at the Universities of Göttingen and Basel, he stayed for 2 years in the industry, R&D, at DSM Nutritional Products Ltd. In 2011, he returned to the University of Göttingen, where he became a group leader at the Department of General Microbiology and obtained his habilitation. In 2019 he accepted a professorship for Synthetic Microbiology at the BTU Cottbus-Senftenberg and since March 2022 he is heading the Department of Molecular Microbiology at the University of Hohenheim. Trained as a microbiolo-gist, his research group focuses on the physiology of bacteria. He is especially interested in metabolism, molecular mechanisms underlying gene regulation, second messenger-dependent signaling and bacterial evolution.

SCIENTIFIC BOARD



Prof Dr Julia Frunzke
Research Centre Juelich
Institute of Bio- and Geosciences, Department of Bacterial Networks and Interactions

Julia Frunzke leads the Department of Bacterial Networks and Interactions at the Research Center in Juelich and holds a professorship on the same topic at Heinrich-Heine University in Duesseldorf. Trained as microbiologist, her research interest focusses on the regulation of bacterial populations by bacteriophages and their exploitation for practical purposes. She was awarded an ERC Starting grant in 2017 to study the "Impact and interaction of prophage elements in bacterial host strains of biotechnological relevance". Since 2020, she is coordinating a DFG Priority Program (SPP) to investigate virus-host interactions in prokaryotes both, on single cell level and in the context of microbial communities.



Prof Dr Herbert Schmidt
University of Hohenheim
Institute of Food Science and Biotechnology, Department of Food Microbiology and Hygiene

Our research focuses on lambdoid Shiga toxin-encoding and non-Shiga toxin-encoding prophages from enterohemorrhagic *E. coli* (EHEC). We are in particular interested in the function of bacteriophage genes not directly being involved in the bacteriophage replication cycle. Current research supports the hypothesis that such genes may be involved in competition and maintenance of EHEC bacteria in the gut.



PD Dr med vet habil Wolfgang Beyer
University of Hohenheim
Institute of Animal Sciences, Department of Livestock Infectiology and Environmental Hygiene

The research of Wolfgang Beyer has been focused on the role of temperate phages in the live cycle and of lytic phages in the control of pathogenic members of *Bacillus cereus* sensu lato. Topics included were phage regulated changes in sporulation and biofilm formation as part of the environmental life cycle of *Bacillus anthracis* and its near relatives. Such features influence the persistence and pathogenicity of the pathogens.



Dr Dorothee Kiefer
University of Hohenheim
Institute of Biology, Department of Molecular Microbiology

Dorothee Kiefer is a senior scientist at the Department of Microbiology at the University of Hohenheim and works together with Andreas Kuhn on structure-function relationships of microbiological model systems. Her 'phage biology' group is particularly interested in the central spike protein complex of bacteriophage T4 and related adhesion and membrane transfer processes. Along these research lines, her group also targets the assembly and function of membrane protein complexes and comparative studies between eubacteria and archaea are performed.



Program

MONDAY, 23 MAY 2022 (DAY 1)

REGISTRATION AND WELCOME

08:00 **Registration**

09:00 **Introduction to the Symposium**
Prof Dr Fabian Commichau, Scientific Director, University of Hohenheim

SESSION 1:
Temperate phages and their role in biological systems

Host: PD Dr Wolfgang Beyer, National Phage Forum (NFP)

09:15 **Keynote: Bacteria-Phage cooperation during mammalian infection**
Prof Dr Anat Herskovits, Tel Aviv University, Israel

10:00 **Plasmidial tectiviruses infecting the *Bacillus cereus* group**
Prof Dr Jacques Mahillon, UC Louvain, Belgium

10:25 **The antiviral properties of aminoglycoside antibiotics**
Prof Dr Julia Frunzke, Research Centre Juelich, Germany

10:50 **Coffee break**

11:00 **Poster Session**

12:00 **Lunch break**

Host: Dr Dorothee Kiefer, University of Hohenheim

14:00 **Towards understanding Virus-Microbe-Host interactions and its potential as phage-based therapeutic tools**
Prof Dr Li Deng, Technical University Munich & Helmholtz Center Munich, Germany

14:25 **The molecular mechanism of headful packaging: a mechanical sensor to trigger a non-specific endonuclease cleavage**
Prof Dr Paulo Tavares, University Paris-Saclay, France

SESSION 2:
Structure-function relationships in phage-host interaction

Host: Prof Dr Fabian Commichau, University of Hohenheim

14:50 **In Memoriam of Prof. Lindsay Black, University of Maryland, Baltimore, USA**
Prof Dr Andreas Kuhn, University of Hohenheim

15:00 **Keynote: Towards a complete molecular description of infection initiation by phage T7**
Prof Dr Ian J. Molineux, University of Texas, USA

15:45 **Coffee break**

16:00 **Molecular prerequisites for phage infection initiation at the Gram-negative outer membrane**
Prof Dr Stefanie Barbirz, Medical School Berlin, Germany

16:25 **Post-translational protein modifications in *E.coli* during T4 phage infection**
Dr Katharina Hoefer, Max Planck Institute for Terrestrial Microbiology, SYNMIKRO

16:50 **Tail tip complex of phage T5: native structure and snapshot of the structural rearrangement occurring upon cell wall perforation**
Prof Dr Cécile Breyton, University Grenoble Alpes, France

17:15 **Farewell Address by Prof Dr Jan Frank, University of Hohenheim**
- Prof Dr Andreas Kuhn, University of Hohenheim
- PD Dr Wolfgang Beyer, University of Hohenheim

18:00 **Poster Session**

19:00 **Reception (Hohenheim Palace)**

TUESDAY, 24 MAY 2022 (DAY 2)

SESSION 2 *(continued)*:
Structure-function relationships in phage-host interaction

08:50 **Introduction to the 2nd Day**
Prof Dr Fabian Commichau, Scientific Director, University of Hohenheim

09:00 **Keynote: Dual Pathogenicity Island Transfer by a Novel Form of Lateral Transduction**
Prof Dr John Chen, National University of Singapore

09:45 **Insertion of the phage M13 procoat protein through a YidC-thinned membrane**
Prof Dr Ross E. Dalbey, Ohio State University, USA

10:10 **Coffee break**

10:30 **Announcement of Young Scientist's Poster Prize Winners**

10:40 **Presentation: Poster Prize Winner 1**

10:55 **Presentation: Poster Prize Winner 2**

11:10 **Presentation: Poster Prize Winner 3**

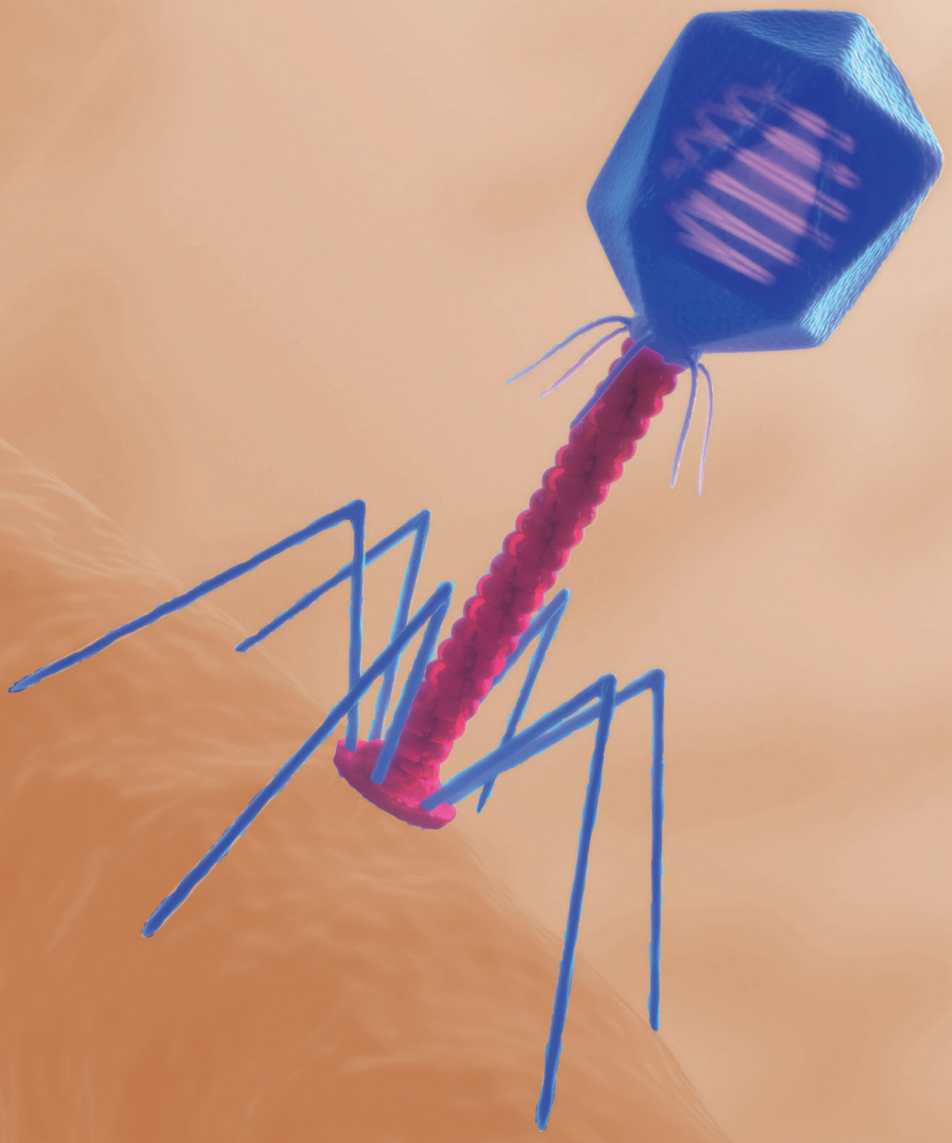
11:25 **Discussion**

12:00 **Wrap-up and Outlook to the Strategic DZIF Symposium for the Promotion of Translational Phage Research "Bacteriophages in Science and Clinical Use"**
Dr Christine Rohde, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH

12:15 **Young Scientist's Networking Lunch - Meet the Speakers and Potential Cooperation Partners**

14:45 **Guided Tour of the Botanical Garden**

2ND GERMAN PHAGE SYMPOSIUM



ABSTRACTS

Keynote Lectures



Session 1: Temperate phages and their role in biological systems

K1 Bacteria-phage cooperation during mammalian infection

ANAT A. HERSKOVITS
Shmunis School of Biomedicine and Cancer Research, George S. Wise Faculty of Life Sciences, Tel Aviv University, Israel
Corresponding author: anathe@tauex.tau.ac.il

Most bacterial pathogens are lysogens, namely carry prophages within their genome, in many cases more than one. While these prophages have the potential to turn into lytic production under stress conditions, and hence trigger bacterial lysis, it is unclear how pathogens manage to survive this internal threat under the stresses imposed upon their invasion into mammalian cells. Several years ago, we uncovered an intriguing pathogen-phage interaction, in which a prophage acquired an adaptive behaviour that supports the virulence of its host, the intracellular pathogen *Listeria monocytogenes (Lm)*.

The prophage, which is integrated within the *comK* gene, excises its genome, yielding an intact *comK* gene that promotes bacterial invasion into the host cell cytosol, without proceeding into the lytic cycle. More recently, we discovered that the prophage, though fully infective, is non-autonomous, completely dependent on another phage element that inhabit the genome, which encodes phage tail-like bacteriocins (monocins). Our studies reveal intimate cross-regulatory interactions between the two phage elements that promote bacteria-phage cooperative behaviours, supporting *Lm* survival in the mammalian niche.

Relevant publications:

1. Rabinovich L., Sigal N., Borovok I., Nir-Paz R., and Herskovits A.A.* Prophage Excision Activates Listeria Competence Genes that Promote Phagosomal Escape and Virulence.
Cell, 2012, August; 150(4):792-802,
2. Argov T, Sapir SR, Pasechnek A, Azulay G, Stadnyuk O, Rabinovich L, Sigal N, Borovok I, Herskovits A.A.*
Coordination of cohabiting phage elements supports bacteria phage cooperation.
Nature Communications, 2019 Nov 21;10(1):5288. doi: 10.1038/s41467-019-13296-x.
3. Pasechnek A, Rabinovich L, Stadnyuk O, Azulay G, Mioduser J, Argov T, Borovok I, Sigal N, Herskovits A.A.
Active lysogeny in Listeria monocytogenes is a bacteria-phage adaptive response in the mammalian cells.
Cell Reports, 2020 Jul 28;32(4):107956. doi: 10.1016/j.celrep.2020.107956
4. A dual function phage regulator controls the response of cohabiting phage elements via regulation of the SOS response.
Azulay G., Pasechnek A., Stadnyuk O., Ran Sapir S., Fleisacher A.M., Borovok I., Sigal N. and Herskovits A.A.*
Cell Reports, 2022, In press

Relevant reviews:

1. Feiner R., Argov T., Rabinovich L., Sigal N., Borovok I., and Herskovits A.A.
A new perspective on lysogeny: prophages as acive regulatory switches of bacteria.
Nature Reviews Microbiology, 2015, Oct;13(10):641-50.
2. Argov T., Azulay G., Pasechnek A., Stadnyuk O., Ran-Sapir S., Borovok I., Sigal N., and Herskovits A.A.
Temperate bacteriophages as regulators of host behavior.
Curr Opin Microbiol, Aug;38:81-87, 2017.

Session 2: Structure-function relationships in phage-host interaction

K2 Towards a complete molecular description of infection initiation by phage T7.

IAN MOLINEUX[1], CHUNYAN WANG[2], TAEHYUN PARK[1], JUN LIU[2]

[1]Department of Molecular Biosciences, LaMontagne Center for Infectious Disease, University of Texas at Austin, USA

[2]Department of Microbial Pathogenesis, Yale Univeristy, USA

Corresponding author: molineux@austin.utexas.edu

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 phage infection. Using high-resolution single-particle cryo-EM analyses of both mature phage and infection intermediates we have elucidated how tail fibers rotate from a retracted conformation to the extended state, revealed how the genome terminus is held by the portal in a conformation that allows the internal core proteins to be ejected into the infected cell, and show two of these proteins in a partially ejected state. We further delineate the sequence-specific binding of amino acids that recognize the packaged genome end in T7-like and many other cos-type phages.

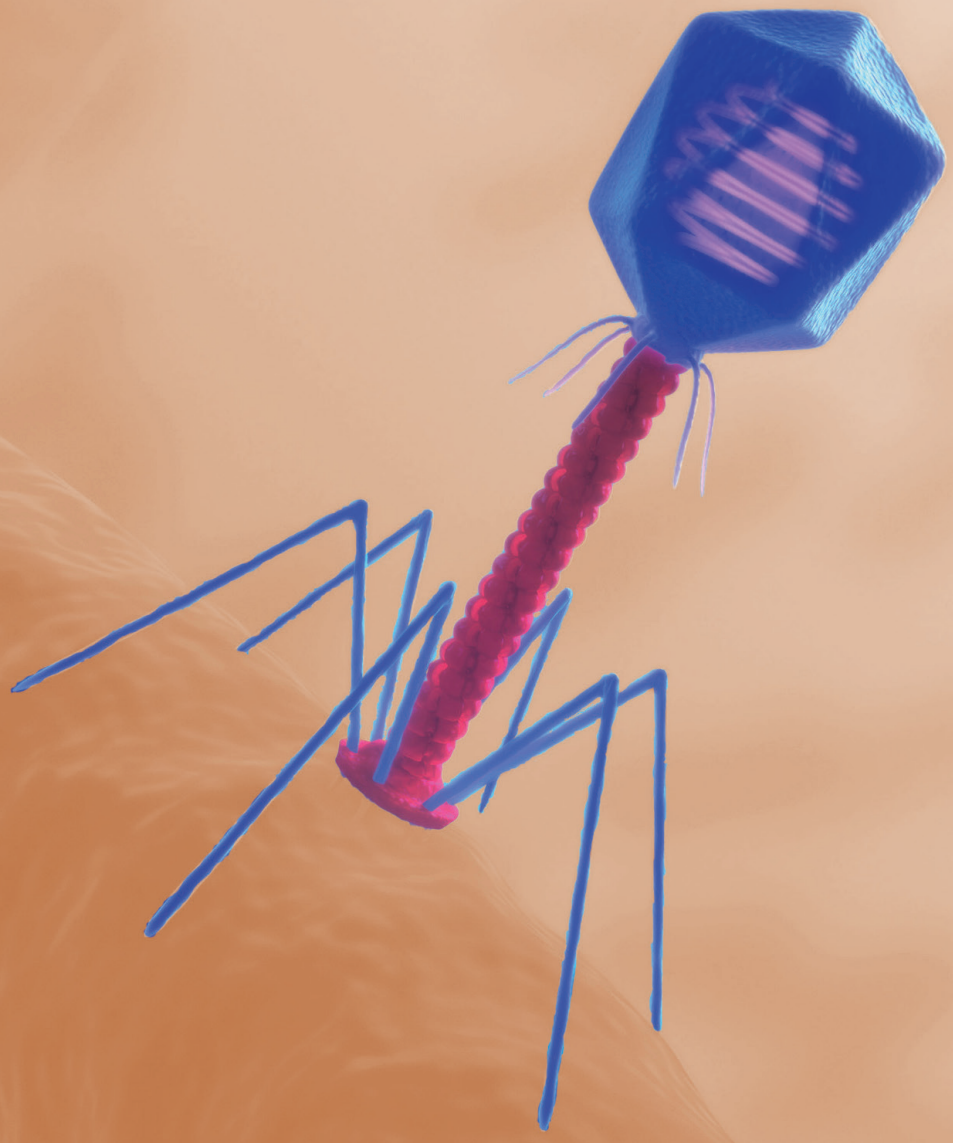


K3 Dual Pathogenicity Island Transfer by a Novel Form of Lateral Transduction

JOHN CHEN
Department of Microbiology and Immunology, Infectious Diseases Translational Research Programme, Yong Loo Lin school of Medicine, National University of Singapore, Singapore
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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 vSaα 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.

2ND GERMAN PHAGE SYMPOSIUM



ABSTRACTS

Oral Presentations



O1 Plasmidial tectiviruses infecting the *Bacillus cereus* group

ANNIKA GILLIS, JACQUES MAHILLON
Laboratory of Food and Environmental Microbiology, Earth and Life Institute, UCLouvain, Louvain-la-Neuve, Belgium
Corresponding author: jacques.mahillon@uclouvain.be

Bacteriophages (phages) are an important repository of genetic diversity and source of unexplored genes. In the particular case of tectiviruses infecting the *Bacillus cereus* group, these phages represent part of the bacterial *mobilome* and they are, so far, the only ones able to behave as linear plasmids during their lysogenic cycle. Recently, several novel tectiviruses have been found infecting diverse strains belonging to this bacterial lineage. Among them, Sole and Sato, isolated from non-emetic and emetic *B. cereus* strains, respectively, uncovered a highly variable region potentially involved in lysogeny maintenance. The genome of Sole spans 14,444 bp with 30 putative CDSs, whereas that of Sato has 14,852 bp and 32 putative CDSs. Phylogenetic analyses suggested that both isolates represent novel tectivirus species. Hence, tectiviruses in Gram-positive bacteria are more diverse than those infecting Gram-negative bacteria, as the latter ones display a very high level of sequence identity. In order to assess the host range of Sole and Sato against emetic *B. cereus* and *Bacillus cytotoxicus*, two species involved in food poisoning, 50 strains of each species devoid of tectiviral-like elements were tested. The results showed that Sole and Sato have a narrow host range, only infecting particular strains. The primary step that initiates bacteria-phage interactions is the recognition and adsorption of phages onto host bacteria. This stage relies on strong and specific interaction(s) between a receptor on the bacterial surface and the receptor binding protein(s) present on the phage. Diverse structures on the surface of bacteria can act as phage receptors and the vast majority of phage receptors described so far, are those found on the surface of Gram-negative bacteria. The primary interactions taking place between *Bacillus thuringiensis* sv. *israelensis* and tectiviruses were therefore investigated. This bacterium was chosen as model since it is used worldwide in environmentally friendly programmes to control dipteran insects, vectors of important human diseases. Using lytic variants of temperate tectiviruses, spontaneous phage^R mutants were obtained for which the phage absorption process was impaired. Using whole-genome-sequencing, the mutations in five of these strains were mapped to the same gene coding for a putative glycosyltransferase (GT; dubbed GT2). Further *in silico* analyses revealed that this GT is encoded as part of a gene cluster named *swcp2* that has recently been shown to code for enzymes required to produce the secondary cell wall polysaccharide (SCWP) in *Bacillus anthracis*. To link the phage^R phenotype to the identified mutations in the gene coding for GT2, a *marker-less in-frame gt2* deletion mutant was constructed. In addition, complementation strains were constructed by introducing a plasmid containing a wild type (WT) copy of the *gt2* gene into the *gt2* mutant strains (KO and spontaneous phage^R). The WT, *gt2* mutant and complementation strains were then evaluated, confirming the involvement of GT2 in the tectivirus-bacterium recognition process.

O2 The antiviral properties of aminoglycoside antibiotics

JULIA FRUNZKE
Institute of Bio- and Geosciences, Forschungszentrum Jülich, Germany
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Bacterial populations face the constant threat of viral predation exerted by bacteriophages (or phages). In response, bacteria have evolved a wide range of defense mechanisms against phage challenges. 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. Strikingly, we show that acetylation of the aminoglycoside antibiotic apramycin abolishes its antibacterial effect, but retains its antiviral properties. Altogether, these 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.



O3 Towards understanding Virus-Microbe-Host interactions and its potential as phage-based therapeutic tools

LI DENG
Chair of Microbial Disease Prevention, Technical University of Munich and Helmholtz Centre Munich, Germany
Corresponding author: li.deng@tum.de

Bacteriophages, viruses that infect bacteria, regulate the abundance, diversity, and metabolism of bacterial communities in a variety of ecosystems, including the human gut. Recent studies on phages in the human gut have suggested they play a central role in human health, and changes in their community structure are associated with several gastrointestinal and non-gastrointestinal diseases and conditions. Leveraging the unique combination of cultivation-free and single-phage technology, we aim to decipher the cross-talk between members of the human virome (community of viruses and phages), the microbiome (community of bacteria and fungi), and its impact on the human hosts. The understanding of functional role of human virome will allow us to ultimately catalyze its translations in disease preventions beyond infection.

O4 The molecular mechanism of bacteriophage headful packaging: a mechanical sensor to trigger a non-specific endonuclease cleavage

S. BRASILÈS[1], E JACQUET[2], MEHDI EL SADEK FADEL[1], DELPHINE NAQUIN[1], KÉVIN GORRICHON[1], M.C. VANEY[3], YVES D'AUBENTON CARAFA[1], P. TAVARES[1]

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[2]Institut de Chimie des Substances Naturelles, Campus CNRS, 91198 Gif-sur-Yvette, France
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In numerous tailed phages, DNA packaging is initiated at a *pac* sequence within a genome concatemer and terminated by an endonucleolytic cut when a threshold amount of DNA is reached inside the capsid. This implies that a sensor measures a physical parameter (the level of capsid headfilling) and triggers the chemical hydrolysis reaction.

Genetic and structural data identified the portal protein, through which DNA enters the viral capsid, as the headful sensor (1-5). Several single amino acid changes in the bacteriophage SPP1 portal protein (gp6) lead to undersizing of the packaged DNA (*siz* or sensor mutations) (1,4). They are localized in the gp6 core that we propose to be the primary headful sensor. A second set of amino acid substitutions, which alone do not affect the size of packaged DNA, amplify the undersizing effect of *siz* mutations. The position of these amplifier mutations in the gp6 structure reveals a path of headful signal transmission across the portal stem and clip regions to its interface that interacts with the SPP1 endonuclease TerL (gp2). The subsequent headful cleavage mediated by TerL is independent of the packaged DNA substrate origin (phage, plasmid or bacterial chromosome), has a ~2 kbp imprecision and shows no sequence preference. Collectively, our studies suggest a molecular model for the mechanism of headful-triggered DNA cleavage.

[1]J Mol Biol 224, 1055 (1992); [2]J Mol Biol 225, 81 (1992); [3]Nature Struct Biol 6, 842 (1999); [4]Mol Microbiol 51, 949 (2004); [5]Virology 505, 127 (2017).



O5 Molecular prerequisites for phage infection initiation at the Gram-negative outer membrane

NINA K. BRÖKER[1], MAREIKE STEPHAN[2], TIM KOLLENKIRCHEN[3,4], YVETTE ROSKE[5], TOBIAS IRMSCHER[4], VALENTIN DUNSING[4], SALVATORE CHIANTIA[4], STEFANIE BARBIRZ[3]

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[2]Department of Colloid Chemistry, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany.
[3]Department of Medicine, Science Faculty, MSB Medical School Berlin, Germany.
[4]Physical Biochemistry, University of Potsdam, Potsdam, Germany.
[5]Structural Biology of Membrane-associated processes, Max-Delbrück-Center for Molecular Medicine, Berlin, Germany
Corresponding author: stefanie.barbirz@medicalschool-berlin.de

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, often with dual roles as protective shields or as phage receptors. The diderm architecture of Gram-negative bacteria with an inner and an outer membrane (OM) represents a unique molecular environment for bacteriophage attack, with the OM as the main barrier. Interactions with OM components regulate bacteriophages: In intact cells, the OM serves as infection control point, OM vesicles mediate extracellular phage concentrations. In addition, membrane fragments alone can 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 with OM components in vitro to release their DNA. We show how bacteriophage enzymatic activity, OM properties and phage tail architecture lead to opening of the phage particle, illustrating the high regulatory power of the Gram-negative OM environment upon interactions with bacteriophages.

References:

Broeker, N.K., and Barbirz, S. (2017) Not a barrier but a key: How bacteriophages exploit host's O-antigen as an essential receptor to initiate infection. *Mol. Microbiol.* **105**: 353-357.

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O6 Post-translational protein modifications in *E. coli* during T4 phage infection

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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 NAD-capped RNA dramatically increases the protein size. Western blot experiments showed that the RNAylation also occurs i*n 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.

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O7 Tail tip complex of phage T5: native structure and snapshot of the structural rearrangement occurring upon cell wall perforation

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The vast majority (96%) 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 siphocoliphage 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 at 3.5 and 4.2 Å resolution, respectively. We could trace all the proteins that compose the tail tip complex [2, 3] (except the RBP): we will discuss structural homologies with other phages and phage-derived complexes, and the conformational changes induced by receptor binding.

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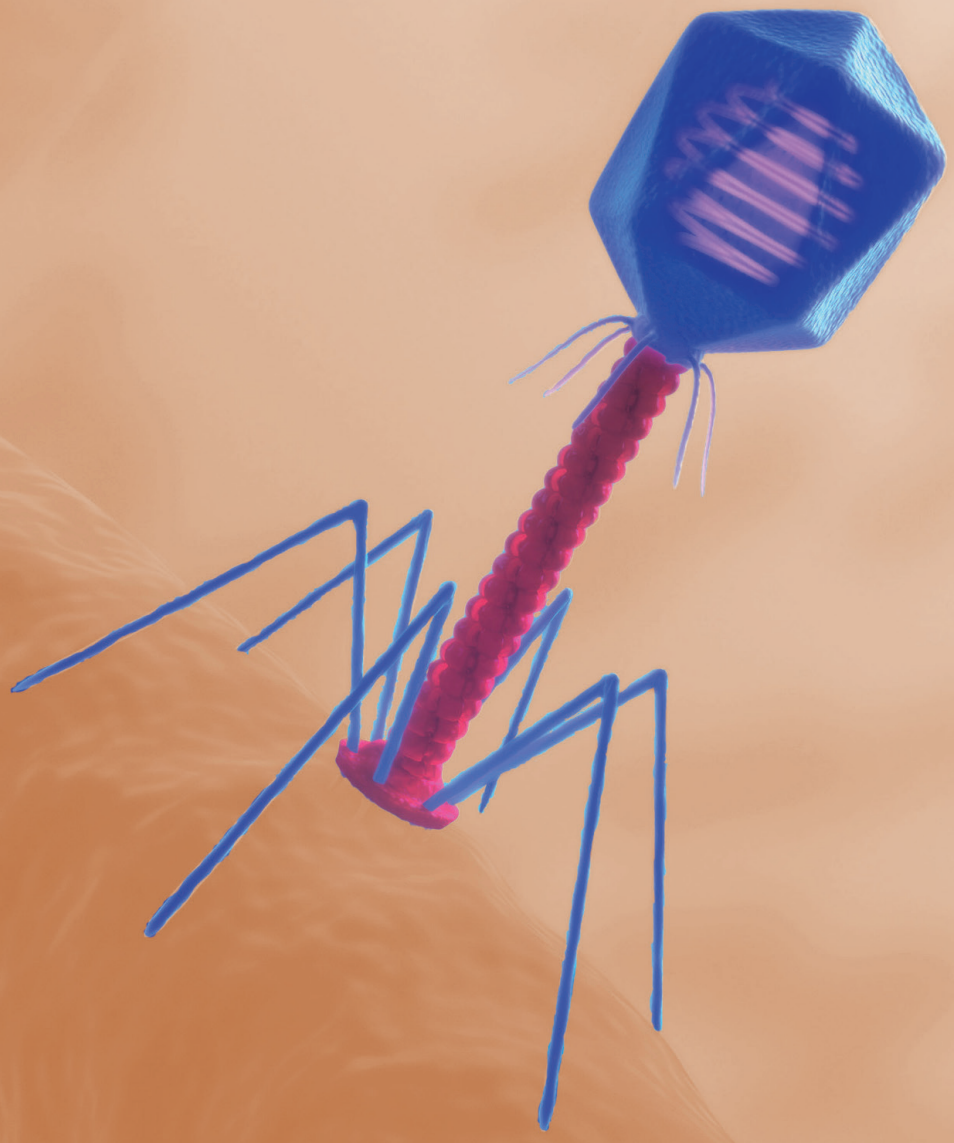
O8 Insertion of the phage M13 procoat through a YidC-thinned membrane.

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The SecYEG complex in bacteria inserts the majority of proteins into the inner membrane together with the YidC insertase. Interestingly, YidC mediates the insertion of some membrane proteins independent of the SecYEG machinery. For this latter function, YidC catalyzes membrane translocation of short hydrophilic regions via a membrane exposed hydrophilic groove that thins the adjacent membrane. In contrast, SecYEG catalyzes translocation of hydrophilic domains of membrane proteins through a membrane spanning hydrophilic pore. To decipher the features of membrane proteins that determine whether they insert by a YidC only or YidC/Sec pathway, we have used the phage M13 gene VIII coat protein as a model system.

The M13 coat protein is made in a precursor form, termed procoat, and requires YidC to translocate its twenty-residue long periplasmic region. We find that if the periplasmic region is below a certain polarity threshold it can insert across the membrane by a YidC-only mechanism. Increasing the polarity of the periplasmic region further results in insertion occurring by a YidC/Sec mechanism. If the polarity is too high it is not inserted even by a YidC/Sec mechanism. Taken together, the study shows that the YidC insertase has a limited translocase function and that YidC and SecYEG must be surveying the inserting polypeptide chain and acting cooperatively as the protein is inserting into the membrane.

2ND GERMAN PHAGE SYMPOSIUM



ABSTRACTS
POSTER



P1 Bacteriophage-therapy against canine otitis externa and pyoderma

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Background: Otitis externa and Pyoderma in dogs mainly caused by *Pseudomonas aeruginosa* and *Staphylococcus pseudintermedius*. Treatment is often performed by repeated and prolonged use of antimicrobial agents, which carries an increased risk of multi-resistance. Bacteriophages could be used as an alternative therapeutic option.

Aims: The aim of this project is to investigate the phage-skin-interaction ex vivo with infected dog skin and in vitro in a 3D canine skin model, before using it as an agent in diseased dogs. For the better permeability through the stratum corneum a nanoformulation with phages is tested.

Methods: Bacteriophages were isolated from environmental samples and characterized. To test bacteriophages-skin-interaction ex vivo, skin from euthanized dogs was examined in Franz-type diffusion cells. A concentration of bacteriophages was added to the artificially infected skin after a period of 16 hours. Samples were collected after 8 hours of additional incubation and the colony-forming units per milliliter were determined. The skin was analyzed using immunohistochemistry and electron microscopy. To establish the canine skin model fibroblasts, endothelial cells and keratinocytes were co-cultivated in an insert-system, to form a differentiated stratum corneum. The skin model can be used for infection experiments with bacteria and later with bacteriophages. The amount of mRNA and protein was determined and the skin was analyzed histologically.

Results: Bacteriophages were isolated and the host range was determined. First results of the Franz-type diffusion cells showed a reduction in the detected colony forming units. The 3D skin model had differentiated keratinocytes, although adjustments in the protocol are needed for an improved formation of the epidermis.

Conclusion: First results show that Franz-type diffusion cells can be useful for investigations of phage-skin-interactions. Further experiments are needed to investigate the interaction and the improvement of penetration with the nanoformulation.

P2 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, leading to the loss of 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.

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P3 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 different economically relevant plant pathogenic bacteria. Many plant pathogenic microbes are transmitted via seed or soil born and targeting 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 life-style, which is supported by genome sequencing and phage infection curves, making them suitable candidates to test their potential in planta. Furthermore, we present first results on phenotypic changes in Brachypodium root length during interaction with plant pathogenic bacteria.
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 functional annotation of phage genes as well as infection experiments to validate our potential phage candidates in terms of suitability for biocontrol applications.

P4 Establishment of a click chemistry based method for detection and characterization of phage-host systems in anaerobic digestion

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Motivation and aims: The performance of anaerobic digestion (AD) depends on the microbial community degrading the biomass to biogas. Bacteriophages are present in AD, as previously shown by metaproteomics and metagenomics approaches. However, neither the exact phage-host ratios nor the impact of phages on process performance has been characterized in more detail so far. To understand the impact of phages in AD, purification of phages together with their hosts is essential. Therefore, a model workflow using biorthogonal noncanonical amino acid tagging (BONCAT) and click-chemistry was established and tested to co-purify *Escherichia coli* and phage *lambda* in pure cultures.
Methods: The lysogenic *phage lambda* was induced in *E. coli* and labeled via BONCAT. Liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS) was used to verify the success of BONCAT labeling. Fluorescent dyes were attached via click-chemistry to the *lambda* phages. Labeled phages were incubated with *E. coli. Lambda* phages attached to *E. coli* cells were detected using fluorescence microscopy and FACS analysis. In addition, BONCAT labeled native *lambda* phages were coupled with biotin probes via click-chemistry and submitted to affinity separation using monomeric avidin beads.
Results: In LC-MS/MS measurements, about 346 from a total of 4952 phage peptides were identified as labeled. Fluorescent dyes were successfully attached to *lambda* phages. Phage-host complexes were successfully detected by fluorescence microscopy (up to 40 % positive fluorescence) and flow cytometry (up to 30 % positive fluorescence). Biotin-labeled native phages could be purified by subsequent affinity chromatography.
Conclusions: Using the *E. coli-lambda* system as a model, a workflow for detecting and co-purifying phage and host species from AD bioreactors was established. Next, enriched phage-host complexes from AD will be submitted to DNA sequencing and metaproteome analysis to elucidate the role of phages in AD.



P5 Development of a rapid detection method for *Burkholderia spp.* using recombinant receptor binding proteins of bacteriophages

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Burkholderia is a genus of gram-negative bacteria that includes, among others, the pathogens *B. mallei* and *B. pseudomallei*, the causative agents of glanders and melioidosis, respectively. The high genetic similarity of these two pathogens, as well as the similarity of the disease manifestations compared to, e.g., tuberculosis or pyemia, make accurate diagnostics challenging. The emergence of antibiotic resistances and the lack of vaccines further increase the need for precise and rapid diagnostics to ensure effective subsequent treatment of infected patients. The gold standard for *Burkholderia spp.* detection is polymerase chain reaction (PCR). Although highly specific, PCR is susceptible to inhibitors from clinical samples and only detects the presents of nucleic acids regardless of the presence of intact bacterial cells. In contrast, bacteriophage sensitivity assays using highly specific phages can be employed to detect viable cells of the pathogens. However, these tests require a pure bacterial culture and are therefore too time-consuming for rapid diagnostics. Alternatively, phage receptor binding proteins (RBPs) can be utilized for rapid pathogen diagnostics. RBPs can be, for example, tail-fibers or –spikes, and typically determine the host range of the phage by mediating the specific binding of the phage to the host cell.

Here, we identified RBP-genes of *Burkholderia* specific (pro-)phages φE125, φX216, φ1026, φE067 and φE058, expressed them in *E. coli* as fusions with the red fluorescent reporter mScarlet and purified the fusion proteins by affinity chromatography. Using these recombinant RBP-reporters as molecular probes we were then able to develop a fluorescence microscopy-based assay for identification of *B. mallei* and *B. pseudomallei*. This novel diagnostic tool enables the rapid detection of these notorious pathogens from bacterial culture or clinically relevant matrices in less than 10 minutes.

P6 Molecular multitasking – Aminoglycoside antibiotics protect bacteria from phage infection

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Bacterial populations face the constant threat of viral predation exerted by bacteriophages (or phages). In response, bacteria have evolved a wide range of defense mechanisms against phage challenges. 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. Here, we show that aminoglycosides, a well-known class of antibiotics produced by *Streptomyces*, are potent inhibitors of phage infection in diverse bacterial species. Using qPCR, RNA-sequencing and fluorescence microscopy, we could show that inhibition of phage infection most likely occurs early on, between phage DNA injection and replication. Inhibition of a *Streptomyces* phage was also achieved using the supernatants from natural aminoglycoside producers, hinting at a broad physiological significance of the antiviral properties of aminoglycosides. Strikingly, we show that acetylation of the aminoglycoside antibiotic apramycin abolishes its antibacterial effect, but retains its antiviral properties. Altogether, this study expands 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.



P7 SPβ c2 – Heat brings light into lysogeny management of SPβ-like phages

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The temperate *Bacillus* phage SPβ is known for half a century. Yet, only individual aspects of its biology are understood. SPβ-like phages exhibit a novel lysogeny management system regulated at various levels. The lysis-lysogeny decision and phage-host recombination are well-investigated. However, SPβ prophage establishment, maintenance and switch to lytic replication is not understood and requires further investigation (Kohm and Hertel, 2021). To gain more insides into the lysogeny management system of SPβ, we study the genotype of its c2 mutant. This mutant cannot maintain its lysogenic status upon heat induction, implying alteration of a core regulatory element.

We discovered the genetic origin of the SPβ c2 phenotype to locate on the prophage. Furthermore, a genome comparison of SPβ c2 and SPβ revealed four point mutations, of which two affected core genes of SPβ-like phages, namely *yopR(G136E)* and *yokI(G49S)*. Moreover, prophage transcriptome analysis exposed the conserved genes *yopR, yokI* and *yopQ* to be transcribed from the dormant prophage. Deletion experiments of these genes revealed involvement in the SPβ lysogeny management. Deletion of *yokI* led to an elevated spontaneous virion release but not to prophage activation. We hypothesize a connection of YokI to the arbitrium system (Kohm *et al.*, 2021). Complementation experiments demonstrated that YopR(G136E) is responsible for the heat-inducible phenotype of SPβ c2. Deleting *yopQ* was only possible with the wild type yopR present. In the presence of *yopR(G136E)*, the *ΔyopQ* mutants showed a clear-plaque phenotype indicating a lytic life cycle. By isolation and sequencing of heat insensitive suppressors of the c2 mutant, we identified YosL as an additional component of the induction cascade. Deletion mutants of *yosL* neither react to Mitomycin C nor heat treatment and prevent the excision of the prophage from the host genome.

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P8 Viral proteins involved in the adsorption process of phage Deep-Purple infecting members of the *Bacillus cereus* group

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The phage life cycle is initiated by the interaction between viral proteins and specific receptor at the bacterial surface. This adsorption step is a key determinant of the phage host spectrum and the main target of resistance mechanisms developed by bacteria to overcome phage infection. Thus, in the light of the increase interest for phages in medical and industrial applications, it is paramount to characterize the molecular mechanisms underlying phage-host primary interactions. Commonly, the central players in the adsorption step, called Receptor Binding Proteins (RBP), are responsible for the specific recognition of the bacterial host and subsequent triggering of genome delivery. However, recently, Carbohydrate-Binding Modules (CBM) have been identified in several tail proteins (*i.e.* Distal tail and Tail lysins) of siphoviruses (*i.e.* phages with long non-contractile tails) infecting Lactic Acid Bacteria and are believed to also be implicated in phage adsorption. In this work, we aimed to identify and characterize the structural proteins involved in the adsorption of Deep-Purple, a siphovirus infecting members of the *Bacillus cereus* group [1]. Bioinformatic analysis of Deep-Purple tail protein-encoding region allowed to identify Gp28 and Gp29 as the putative distal tail protein and RBP, respectively. Interestingly, both proteins possess a CBM, suggesting that they interact with host associated carbohydrates. When fused to a fluorescent tag, the CBM proteins were able to decorate the bacterial surface but displayed differences in binding spectra. The CBM of the RBP-Gp29 only recognized strains that are sensitive to the phage whereas that of the Dit-Gp28 bound to many strains including some that are insensitive to Deep-Purple, highlighting the fact that they have different implications in during phage adsorption. A detailed the characterization of both RBP-gp29 and Dit-gp28 will be presented, together with their involvement in Deep-Purple adsorption.

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P9 *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*.

P10 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 modes of action have 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 different proteins. Using HMM profiles of those orthologs, we searched for transposable phages in the database of uncultivated viral genomes IMG/VR. After dereplication at the species level, we established a set of 426 putative transposable phages, explored their genomic relationship using vContact2, and reconstructed their phylogeny based on their portal protein using RAxML.

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 phage genomes were assembled from viromes derived from various environments, such as water, soil, and living hosts. This work greatly expands the known diversity and host range of transposable phages, and sets the stage for further functional characterization of this enigmatic group of phages.



P11 Bacteriophages may influence the dynamics and functional resilience of an enriched hydrogenotrophic community in a starvation event

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Communities enriched for biomethanation of hydrogen are functionally resilient against starvation. Despite the importance of viruses in microbial communities, microbiologists have overlooked their role in this process. We hypothesized that viruses influence the resilience of hydrogenotrophic communities during intermittent starvation. To test this hypothesis, we evaluated the composition and diversity of prokaryotes and viruses in anaerobic bioreactors inoculated with a community performing biomethanation of CO_2 and H_2.

We operated three controls and three treated bioreactors for approximately 100 days and sampled at nine time points. Samples were taken in three experimental phases: stabilization (0 h and 1008 h after stable operation); starvation (1176 h and 1464 h), during this phase the treated bioreactors were starved; and recovery (1464 h, 1464.75 h, 1468 h, 1470 h and 1486 h), during this phase regular feeding was restored.

We recovered metagenome-assembled genomes (MAGs) using MetaWrap and uncultivated virus genomes (UViGs) using VirSorter, VirFinder, and VIBRANT in 53 metagenomic libraries (one failed preparation). We dereplicated the MAGs into prokaryotic operational taxonomic units (pOTUs) based on an average nucleotide identity of 0.99 (a proxy for strain level) and assigned pOTU taxonomy using GTDB-tk. The quality of the UViGs was assessed using CheckV and their taxonomy using vConTACT2. After dereplication, high-quality and complete UViGs were defined as viral operational taxonomic units (vOTUs). Potential pOTU hosts of vOTUs were predicted using WiSH.

We recovered 877 MAGs and dereplicated them into 30 pOTUs. Phylogenetic analysis indicated that 60% of our pOTUs represent novel species, including species of *Methanobacterium* and *Microbacterium*. We recovered about 45,000 viral contigs and identified 284 vOTUs. Among these, 59 (20.77%) may encompass novel viral taxa. WiSH analysis identified 7 (pOTU) hosts for 27 vOTUs. Some of the recovered MAGs may host several phages, among them *Aminobacterium colombienses, Clostridium ultunense,* and a *Bradyrhizobium* sp.

We are currently determining the life cycle of the vOTUs using DeePhage. After, we will assess the presence of genes for metabolic pathways relevant to our biomethanation system in the MAGs and vOTUs. The outcome of our study may open new doors for the use of phages in anaerobic bioprocesses.

P12 *Bacillus thuringiensis* receptors recognized by Vp4 tail proteins upon adsorption

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Adsorption is a key stage in phage recognition of sensitive host cell implying the specific interaction between Receptor Binding Proteins (RBPs) often located at the distal part of the phage tail (*i.e.* tail fibers, spikes or baseplate proteins) and receptors on the surface of their bacterial host. Since phages are obligate intracellular parasites, successful injection of its genome into its bacterial host is an essential condition for continuation of their life cycle. 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 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 [1]. These mutants displayed an impaired phage adsorption which suggested an alteration in the receptor. Analysis of their chromosomal sequences pinpointed a mutated collagen triple helix repeat domain protein in all four mutants. Knock-out of this gene in wild-type strain is under way and should clarify its potential implication in the adsorption process. Further confirmation will be obtained by complementation experiments in which the phage susceptibility should be regained.

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P13 Stability of Bacteriophage MS2 in Spaceflight Conditions

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Bacteriophages pose a range of dangers in spaceflight. From threatening cell cultures for biotechnology, to transferring pathogenicity genes between bacteria. On space missions, due to the limited resources available to the astronauts, bacteriophages can cause major problems related to health like infections of astronaut symbiotic microbiota, or allowing bacterial infections through gene transduction. Also, bacteriophage contamination of cyanobacterial cultures aimed for food and oxygen production would undoubtedly prove devastating on deep-space missions. Therefore, it is significant 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, a widely accepted model ssRNA bacteriophage, 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.

P14 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 foodborne gastrointestinal diseases worldwide. Reduction of 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 III phages, and the well characterized group III phage NCTC 12673, each, alone and in different combinations were examined using 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 10^7 PFU/ml four, three, and two days before necropsy. Due to a lower *Campylobacter* colonization of the treatment group, the *in vivo* efficacy could only be estimated by the increasing difference of bacterial concentrations in cloacal swabs of the two groups, indicating a reducing effect. At necropsy, *Campylobacter* counts in colonic content of the treatment group were significantly reduced by 1.3 $\log_{10}$MPN/g compared to the control group, while there was no difference 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-LmqsCP1/1) compared to isolates from colonic content and cloacal swabs.

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P15 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* and is one of the most complexly built phages, whose genome is composed of 169 kbp and encodes 273 proteins [1]. Bacteriophage T4 does not possess its own expression machinery and overtakes control over the expression machinery of *E. coli*. The phage infection occurs 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 requires only ~25 min to lyse the bacterial cell. Even though phage T4 had strongly 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. The recent developments in multi-omics approaches could be employed to unravel the molecular basis of the bacteriophage T4 infection and 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.

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P16 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.



P17 Recombinant receptor binding proteins of bacteriophages as versatile tools for detection of *Bacillus anthracis*

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Bacillus anthracis is the etiological agent of the zoonosis anthrax. Due to the fulminant course of anthrax infections, rapid and unambiguous detection is crucial for timely antibiotic therapy and thus, patient survival. *B. anthracis* is typically diagnosed by classical microbiological and molecular methods such as polymerase chain reaction (PCR). Alternatively, mass spectrometry techniques may aid in confirming the presence of the pathogen or its toxins. However, because of the close genetic relationship between *B. anthracis* and other members of the *B. cereus sensu lato* group (such as *B. cereus* or *B. thuringiensis*) mis- or questionable identification frequently occurs in diagnostic laboratories. Bacteriophages, such as phage gamma, have been in use for anthrax diagnostics due to their high specifity for *B. anthracis.* Since it usually takes several days to obtain results from plaque assays, faster methods are needed to ensure timely pathogen identification. For this, we used phage receptor binding proteins (RBPs) instead of whole phages. These proteins are typically utilized by the virus to recognize specific surface structures on bacterial host cells and thus, determine the specificity of the phage. Here, 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 *B. anthracis* 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 *B. anthracis* enrichment or isolation approaches. Recently, an outbreak of 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.

P18 Isolation and characterization of novel *Bordetella avium* bacteriophages

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Objectives: Bordetellosis, caused by *Bordetella avium*, is a common respiratory disease in poultry of all ages. Diseased animals show reduced body weight gain due to their poor general condition, causing high economic losses in commercial poultry farms. A future approach for the treatment of bordetellosis could be the use of bacteriophages instead of antibiotics. However, little is known about *B. avium* phages and none have been isolated from the environment of poultry farms to date. Therefore, the objective of this study was to isolate and characterize *B. avium* phages.

Methods: Drinking water and fecal samples from hobby and commercial poultry farms were used for the screening of phages capable of lysing *B. avium* in plaque assays. Phages were purified by repeated plating and were concentrated by plate lysate and polyethylene glycol sodium chlorid precipitation. Isolated phages were characterized by whole-genome sequence analysis and electron microscopy. To reveal the binding sites of bacteriophages on the surface of bacterial cells, treatment of bacteria with either sodium periodate or proteinase K followed by phage infection was performed.

Results: In this study, isolation and characterization of seven different phages is described. Sequence analysis revealed a size range of 39-43 kbp and showed considerable similarities in genes coding for structural proteins and differences in genes responsible for lifecycle regulation. Despite genetic evidence for lysogeny, the seven phages showed strong bacteriolytic activity. Electron microscopy analysis revealed that all analyzed phages belong to the *Myoviridae* family (order *Caudovirales*). Treatment of bacteria with sodium periodate inhibited phage absorption, indicating that all seven phages bind to lipopolysaccharide (LPS) structures of *B. avium.*

Conclusion: The isolation and characterization of these phages showed the in vitro effectiveness of phages against *B. avium* field isolates. Further development could provide an alternative to known antibiotic therapy in the future.



P19 Understanding phage dynamics and function in marine sponges

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Bacteriophages are known modulators of community composition and activity in environmental and host-associated microbial habitats. Sponges, as filter feeding animals, are continuously exposed to diverse microorganisms from the marine environment, and are able to maintain a unique and complex profile of bacterial and viral communities. Altogether, the eukaryotic host with its bacterial and viral counterparts forms a multicomponent functional unit (metaorganism) which maintains homeostasis through the regulation of tripartite interactions. While most studies until now focused on bacterial symbionts, little is known about the individual effect of phages within such complex multispecies communities.

We present the first results on the lifestyle of marine phages relevant to a local Baltic sponge, based on their morphological and genomic characterization. We also demonstrate the first attempts at manipulating natural microbial communities in controlled sterile conditions, i.e. phage therapy in sponges. With the application of specific marine/sponge phage isolates with a putatively narrow host range, we observed that the individual effect of specific marine phages on the bacterial community likely depends on the phage lifestyle and host abundance. Detectable differential effects in the total bacterial load, as well as total numbers of bacterial and viral particles, vary with methods of phage delivery and of used particles at the application start. However, each phage treatment resulted in the increase of total bacterial load in sponge tissues over time, which was positively correlated with an increased release of bacterial particles into surrounding seawater.

Based on the lifestyle of susceptible bacterial hosts, lytic phages could likely induce short-term changes in the total sponge microbial profile by breaking up polymicrobial biofilms and releasing nutrients, which lead to a fortified increase in bacterial growth. However, due to the overall complexity of such open systems and intricate trophic networks, further experiments are needed to better define the direct causes of individual phage effects.

P20 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 base-plate, 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.



P21 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 *Pseudomonas* 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 direct and indirect 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.

P22 Combination of a phage cocktail and an organic acid cocktail to reduce *Campylobacter* in broiler chickens – Examination of possible synergistic effects

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The incidence of human bacterial gastrointestinal diseases caused by *Campylobacter* has continued to rise in recent years. Transmission of this foodborne zoonosis occurs most often by the consumption of chicken. Therefore, health agencies emphasize the need for preventive measures. In order to minimize the introduction of *Campylobacter* into the food chain, different approaches have been examined to date. One possible mitigation strategy is to focus on primary production and to minimize the prevalence of *Campylobacter* in chicken before slaughter, thus reducing cross-contamination afterwards.
The use of bacteriophages (phages) administered to broiler chickens via drinking water has already shown positive results in previous studies. Using the same application form, organic acids were shown to reduce *Campylobacter* in primary production in some studies. However, based on the results, it can be concluded that the use of both individual measures alone would not reliably lead to a sufficient reduction of *Campylobacter* in chicken products. To investigate possible synergistic effects of phages and organic acids, the two measures were tested *in vitro*, each alone and in combination. The combination of phage NCTC 12673 and phage vB_CcM-LmqsCPL1/1 was used as a phage cocktail. The organic acid cocktail consisted of benzoic acid, sorbic acid, propionic acid and acetic acid. One *Campylobacter coli* field isolate (Cc7) and one *Campylobacter jejuni* field isolate (Cj18) were used as model bacteria. The phage cocktail, the organic acid cocktail and the respective *Campylobacter* isolate were transferred into the wells of a microtiter plate. The microtiter plate was then incubated with double orbital shaking in a Tecan Spark Plate Reader® for 24 hours. Bacterial growth was monitored hourly by optical density (OD) absorbance measurements at 600 nm (OD_{600}). The results of this study show that the combined use of phages and organic acids resulted in higher reduction rates compared to single testing.

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P23 Recombinant reporterphage rTUN1::*nLuc* enables rapid detection of *Klebsiella pneumoniae* K64 in clinical matrices

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The emergence of multi drug resistant (MDR) *Klebsiella pneumoniae (Kp)* strains poses a huge threat to global health as MDR associated treatment failure causes high mortality rates in nosocomial infections. Rapid pathogen detection and antibiotic resistance screening is therefore crucial for successful therapy and thus, patient survival. Established methods for *Kp* detection, such as PCR or MALDI-TOF, often rely on bacterial culture and isolation and are therefore too time consuming in critical cases. Reporterphage-based diagnostics represent a promising alternative as integration of reporters, such as luciferases, into highly specific phages enable real time detection of phage infections and thus, rapid pathogen detection. *Kp* specific phages use the host's capsule, a major virulence factor of *Kp*, as receptor for adsorption. To date, 80 different *Kp* capsule types (K-serotypes) have been described with predominant capsule types varying between different countries and continents. Therefore, reporterphages need to be customized according to the locally prevailing variant. Recently, we described the phage TUN1, which specifically infects *Kp* K64 strains, the most predominant strain at the military hospital in Tunis (MHT) that is also associated with high mortality rates, especially at the intensive care unit (ICU).

In this work, we constructed a luminescent reporterphage rTUN1::*nLuc* that enables the rapid detection of *Kp* K64 directly in clinical matrices, such as urine or blood, featuring a detection limit of only 10 CFU/ml. Since *Kp* infections coincide with high bacterial titers (10^5 - 10^7 CFU/ml), *Kp* detection using rTUN1::*nLuc* can be achieved within 2-4 hours. Furthermore, antibiotic resistances of the respective *Kp* strain can be determined simultaneously.

P24 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.



P25 Inventory of phages in a large collection of *Staphylococcus aureus* bacteremia isolates

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Background: *Staphylococcus aureus* is a pathogen causing a variety of diseases ranging from skin infections to life-threatening sepsis. The intense worldwide use of antimicrobial agents led to the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), limiting therapeutic options. Bacteriophages are natural killers of bacteria and offer a wide range of new possibilities for the treatment of bacterial infections.
Methods: To inventory bacteriophages that are possible candidates for the therapy of *Staphylococcus aureus* infections, we first sequenced 496 *Staphylococcus aureus* bacteremia isolates with the Illumina and Oxford Nanopore techniques and generated hybrid-assemblies with unicycler. Intact bacteriophage genomes were predicted using the "PHASTER" web application. *S. aureus* isolates predicted to harbor a prophage were experimentally tested for mitomycin C-inducible prophages. Additionally, we tested the susceptibility of these *S. aureus* isolates to different bacteriophage strains and analyzed for genes of the bacterial defense system CRISPR-Cas with the "CRISPRloci" tool.
Results: Overall 475 intact phages were predicted to be present in 361 out of 496 *Staphylococcus aureus* isolates. We identified 50 different bacteriophage strains with highly different frequencies of occurrence. Nine prophages could be induced with mitomycin C from the isolate collection and were used for susceptibility testing with a focus on *S. aureus* isolates that showed bacterial defense systems in the genomic analysis.
Conclusion: *S. aureus* isolates from bacteremic patients frequently harbour prophages, mainly from a small pool of different bacteriophage strains. CRISPR-Cas-systems were rarely found. This finding is consistent with previous data and suggests that although bacteriophages frequently infect *S. aureus*, CRISPR-Cas systems do not play a major role in host defence.

P26 Beyond predator-prey: phages in bacterial communities

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Co-cultures of a bacteriophage and its host are a powerful model system for predator-prey co-evolution. However, in nature the phage-bacterium relationship is far more complex. Most phages can integrate their DNA into the bacterial host, linking their evolutionary success. Moreover, specific bacterial genetic actively hijack the phage machinery for horizontal gene transfer. However, we lack systematic experimental studies, as such events do not occur in mono-cultures and it is notoriously difficult to obtain stable multi-species co-existence due to resource competition. We have developed an assay consisting of separately cultured bacteria, but with phages periodically surging through the entire bacterial metapopulation. We have used this assay to evolve hundreds of clinically relevant bacteria and roughly equally many phages. We use this assay in combination with high throughput sequencing and phenotypic assays to map phage-host networks and track gene flow between bacteria. Our work reveals how phages govern evolution in bacterial communities, such as the spread of antibiotic resistance genes.



P27 Phage-plasmids are widespread and produce antibiotic resistant lysogens.

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Bacterial evolution is mainly driven by phages and plasmids that are typically clearly distinguished in the literature. After infection, temperate phages tend to integrate the chromosome, whereas plasmids are DNA elements that stay episomal. Phage-plasmids infect bacterial cell as phages and are maintained in host cells as plasmids, blurrying the classical distinctions between these types of elements.

In our study, we established a workflow to find phage-plasmids in bacterial genomes and found them to be numerous and widespread (5% of >25k phages/plasmids). We used their gene repertoire relatedness to group and characterize them. Whereas some phage-plasmids are similar to each other, most groups are not related. The fact that these large, unrelated groups have phage and plasmid functions in common suggests phage-plasmids to be ancient elements. Some encode interesting traits such as antibiotic resistance genes (ARGs). ARGs are often found in plasmids, but are very rare in phages. Since phage-plasmids are phages as well, we thus wondered if phage-plasmids can spread ARGs as phages. We conducted experiments on phage-plasmids which we isolated from resistant strains retrieved 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 are capable to transfer accessory genes typical of phages and of plasmids. They may thus have a remarkable impact on bacterial evolution.

P28 Diversity of viral defence systems in the cow rumen microbiome

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Phages can regulate bacterial and archaeal diversity, which is of great ecological and biotechnological interest. They seem to play a crucial role in the gut microbiome's health and function, however, the competitive mechanism between the gut microbiome and the phages are still poorly described. For that reason, we explored the diversity and abundance of viral defence systems (VDS) in ~6000 publicly available genomes from the cow rumen. Briefly, 5578 metagenome assembled genomes (MAGs) from the MGnify catalogue and 412 isolates from the Hungate1000 collection were downloaded, taxonomically assigned and its proteins associated with VDS were annotated. Overall, the dataset was composed of mainly *Firmicutes, Bacteroidota* and *Proteobacteria* members. Out of all analysed genomes, 70% harbour proteins associated with VDS, while only the isolates reach 92%. On average, most of the genomes carried up to 5 VD proteins. In contrast, more than 30 VD proteins were found in some members of the *Lachnospiraceae* family. In total 11 different VDS were found across all genomes, Abi, Wadjet and CRISPR-Cas being the most abundant ones. VDS diversity between the MAGs and isolates was fairly similar. Instead, VDS from archaeal genomes were dominated by CRISPR-Cas systems (70%), while Abi (33%) and CRISPR-Cas (35%) were more evenly distributed among the bacterial genomes. Additionally, the Zorya and Wadjet system were only found in bacterial genomes, while mza only in archaeal genomes. Our data indicate that bacterial and archeal immunity in the cow rumen microbiome is mainly driven by degradation of phages (CRISPR-Cas) and abortive infection mechanism (Abi). Finally, VDS are highly diverse and distributed across the cow rumen microbiome and its description can shed light on phage's role on the animal's gut health.



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