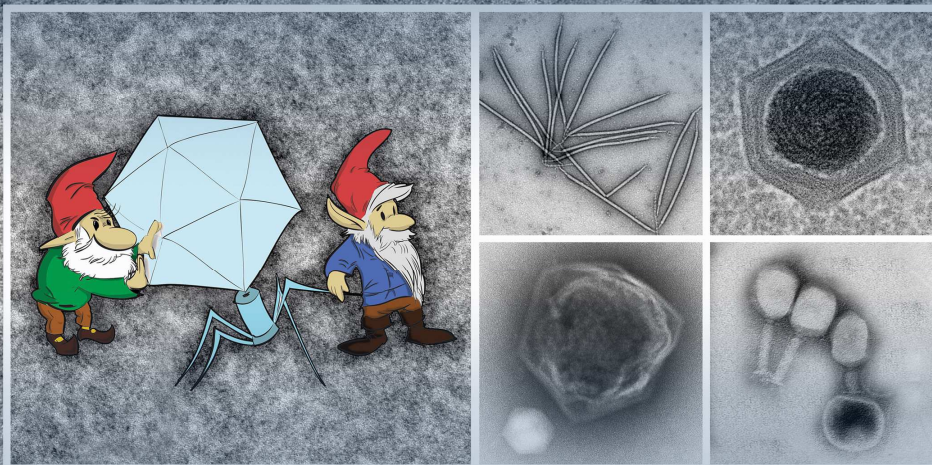


EMBO
Workshop



Viruses of Microbes 2018

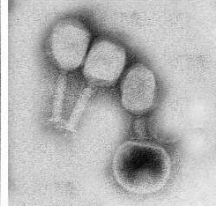
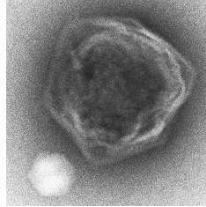
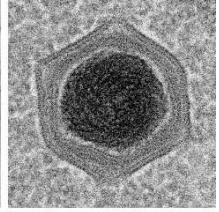
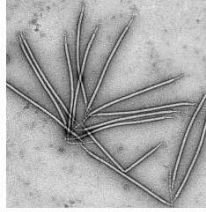
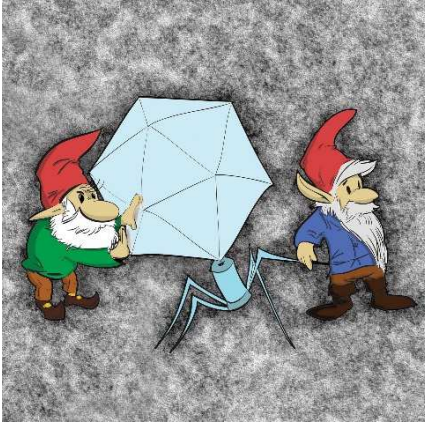
09 – 13 July 2018 | Wrocław, Poland

Abstract Book

meetings.embo.org/event/18-virus-microbe



Wrocław miasto spotkań



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1. WELCOME ADDRESSES

Welcome to the Viruses of Microbes 2018 EMBO Workshop!

We are happy to welcome you to Wrocław for the 5th meeting of the Viruses of Microbes series. This series was launched in the year 2010 in Paris, and was continued in Brussels (2012), Zurich (2014), and Liverpool (2016). This year our meeting is co-organized by two partner institutions: the University of Wrocław and the Hirsfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences. The conference venue (University of Wrocław, Uniwersytecka 7-10, Building D) is located in the heart of Wrocław, within the old, historic part of the city. This creates an opportunity to experience the over 1000-year history of the city, combined with its current positive energy.

The Viruses of Microbes community is constantly growing. More and more researchers are joining it, and they represent more and more countries worldwide. Our goal for this meeting was to create a true global platform for networking and exchanging ideas. We are most happy to welcome representatives of so many countries and continents. To accommodate the diversity and expertise of the scientists and practitioners gathered by VoM2018, the leading theme of this conference is “Biodiversity and Future Application”. With the help of your contribution, this theme was developed into a program covering a wide range of topics with the strongest practical aspect.

We are grateful to members of the VoM2018 scientific committee, local co-organizers, and organizers of previous VoM meetings for the help, support, and creativity that they offered to VoM2018. We are also grateful to the International Society for Viruses of Microorganisms (ISVM) and to the European Molecular Biology Organization (EMBO) for the opportunity to organize this meeting, and also for all their help and support.

We are sure you will enjoy the EMBO Viruses of Microbes 2018 conference and you will have a great time in beautiful Wrocław!

Krystyna Dąbrowska and Zuzanna Drulis-Kawa

(On behalf of the VoM2018 organizing and Scientific Committees)

2. INTRODUCTION

The central theme of this EMBO Workshop is Viruses of Microbes V: “Biodiversity and future applications” of viruses infecting microbes (algae, archaea, bacteria, fungi, protozoa and viruses). Viruses have always been a key element of microbial diversity and evolution, as well as a tool for molecular biologists to learn more about how the host cell functions. This information has also been put to productive use more recently to control infections and fouling in many areas of our modern life. The conference talks are grouped into sessions covering key areas of ecology, host-virus dynamics, biotechnological, medical aspects, and structural biology. A main objective of this EMBO Workshop is to stimulate new understandings of the role that viruses of microbes play in ecosystems and in the sustainable development of human technologies.



ORGANIZERS



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Experimental Therapy PAS,
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University of Wrocław,
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Warsaw, Poland



Marcin łoś
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Gdansk, Poland



Jakub Barylski
Adam Mickiewicz University,
Poznan, Poland

SCIENTIFIC COMMITTEE

1.	Zuzanna Drulis-Kawa	University of Wrocław, Wrocław Poland
2.	Krystyna Dąbrowska	Institute of Immunology and Experimental Therapy PAS, Wrocław, Poland
3.	Rob Lavigne	KU Leuven, Leuven, Belgium
4.	Tessa Quax	University of Freiburg, Freiburg, Germany
5.	Laurent Debarbieux	Institut Pasteur, Paris, France
6.	Andrey Letarov	Winogradsky Institute of Microbiology, RAS, Moscow, Russia
7.	Ye Xiang	Tsinghua University, Beijing, China
8.	Peter Fineran	University of Otago, Dunedin, New Zealand
9.	Alejandro Reyes	University of Los Andes, Bogota, Colombia
10.	Ramy Aziz	Cairo University, Cairo, Egypt
11.	David D. Dunigan	Nebraska Center for Virology, Lincoln, NE, USA
12.	Joana Azeredo	University of Minho, Braga, Portugal
13.	Mart Krupovic	Institut Pasteur, Paris, France

INVITED SPEAKERS



Matthew Sullivan
Ohio State University,
Columbus, OH, USA



Stan Brouns
Delft University of
Technology, Delft,
Netherlands



Petr Leiman
University of Texas
Medical Branch,
Galveston, TX, USA



Ye Xiang
Tsinghua University,
Beijing, China



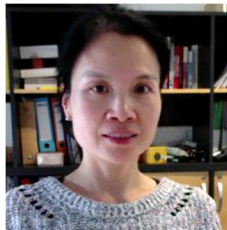
Valérie de Crécy-Lagard
University of Florida,
Gainesville, FL, USA



Minna Poranen
University of Helsinki,
Helsinki, Finland



Rob Lavigne
KU Leuven,
Leuven, Belgium



Xu Peng
University of Copenhagen,
Copenhagen, Denmark



Peter Fineran
University of Otago,
Dunedin, New Zealand

INVITED SPEAKERS



Barbara Maciejewska
University of Wrocław,
Wrocław, Poland



Joana Azeredo
University of Minho,
Braga, Portugal



Daniel Nelson
University of Maryland,
Rockville, MD, USA



Venigalla B. Rao
The Catholic University of
America,
Washington, DC, USA



Paulina Miernikiewicz
Institute of Immunology and
Experimental Therapy PAS,
Wrocław, Poland



Laurent Debarbieux
Institut Pasteur,
Paris, France



Jean-Paul Pirnay
Queen Astrid Military
Hospital, Brussels, Belgium



Andrzej Górski
Institute of Immunology and
Experimental Therapy PAS,
Wrocław, Poland



Elizabeth Kutter
Evergreen State College,
Olympia, WA, USA

INVITED SPEAKERS



Harald Brüssow
Nestlé Research Center,
Lausanne, Switzerland



Jerome Gabard
Pherecydes Pharma,
Romainville, France



Alejandro Reyes
University of Los Andes,
Bogota, Colombia



Andrew M. Kropinski
University of Guelph,
Guelph, ON, Canada



Daohong Jiang
Huazhong Agricultural
University,
Wuhan, China



Keizo Nagasaki
Kochi University,
Kochi, Japan



David Prangishvili
Institut Pasteur,
Paris, France



Andrey Letarov
Winogradsky Institute of
Microbiology RAS,
Moscow, Russia



Matthias Fischer
Max Planck Institute for
Medical Research,
Heidelberg, Germany

INVITED SPEAKERS



Ruth-Anne Sandaa
University of Bergen,
Bergen, Norway



Martin J. Loessner
ETH Zurich,
Zurich, Switzerland



Nina V. Tikunova
Institute of Chemical Biology
and Fundamental Medicine
RAS,
Novosibirsk, Russia



Ramy K. Aziz
Cairo University,
Cairo, Egypt



James Rodney Brister
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Bethesda, MD, USA



Mzia Kutateladze
G. Eliava Institute of
Bacteriophages,
Microbiology and Virology,
Tbilisi, Georgia

3. ACKNOWLEDGMENTS



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Medicine



Wrocław miasto spotkań

4. GENERAL INFORMATION

Theme of Conference

More than a century of research on viruses of microbes led to major discoveries in biology. Scientific interest in viruses of microbes is constantly growing, opening the way to new findings and applications. This conference series is intended to bring together researchers involved in all fields related to viruses of microbes and provide an opportunity to exchange and inspire ideas.

The central theme of the meeting is 'Biodiversity and future applications' of viruses infecting microbes: algae, archaea, bacteria, fungi, protozoa and viruses. Viruses have always been a key element of microbial diversity and evolution, as well as a tool for molecular biologists to learn more about how the host cell functions. This information has also been put to productive use in recent times to control infections and fouling in many areas of our modern life. The conference talks are grouped into sessions covering key areas of ecology, host-virus dynamics, biotechnology, medical aspects, and structural biology.

One of the main objectives of the meeting is to stimulate new understanding of the role that viruses of microbes play in ecosystems and in the sustainable development of human technologies. This conference will also promote the actions of the International Society for Viruses of Microorganisms (ISVM) and functions as the official bi-annual meeting of this society.

Wi-Fi

A Wi-Fi connection will be available in the auditorium and in the hall of building.

Network: [emboworkshop](#) | Password: [0913072018](#)

Certificates of Attendance

Certificates for participants will be send automatically by email.

Photography and Filming

The EMBO may carry out filming and photography throughout the conference. The videos and images will be used to promote the conferences and other activities of EMBO. They may be used online, in EMBO publications, or for other PR and marketing purposes. Only recording and photography of presentations are allowed with prior permission from the authors.

Social Media

Find us on Facebook: <https://www.facebook.com/events/558390551168563/>

Program changes

While every effort will be made to keep program changes to a minimum, any changes will be advised at the registration desk. Please note that the website is mobile optimized and any changes will appear as soon as possible.

<http://meetings.embo.org/event/18-virus-microbe>

Posters

The poster should be printed in portrait orientation at a size no larger than B1 (70 cm (W) x 100 cm (H)). Poster presentation will take place in strictly defined locations. The order of the presentation will be consistent with the **submission ID** (**please check it on your EMBO profile or find your abstract in this book**).

City Tour

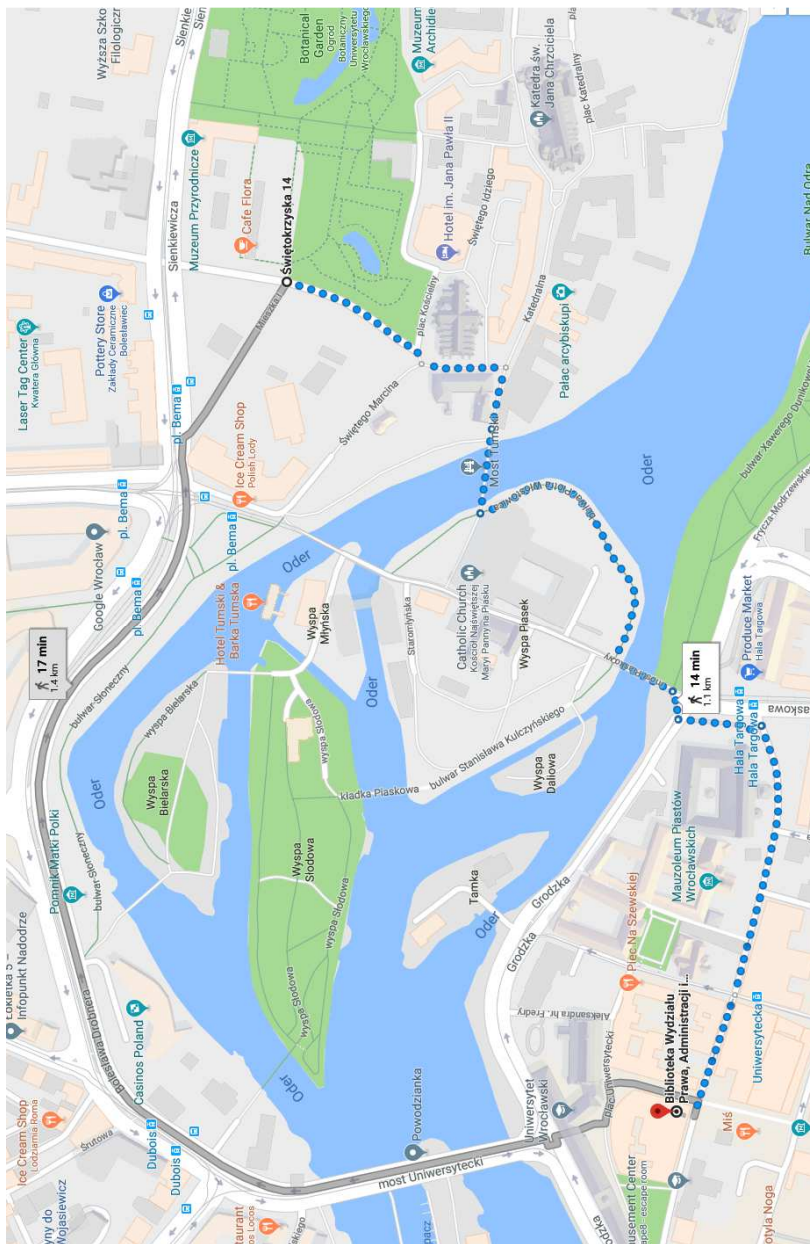
During the conference, a facultative one-hour city tour will be organized during the lunch break on 11th of July (from 14:00 to 15:00). Please feel free to participate and sign up at the reception during the registration

Registration Desk

For the convenience of VoM 2018 participants, the registration desk will be located on the ground floor. The reception will be open from 8:00 to 19:00 (Monday - Thursday) and from 8:00 to 17:00 (Friday).

Social Program

Each conference participant is entitled to participate in two social events free of charge (**badge required!**). The Welcome Reception will take place on 9th of July (19:00 - 21:00) at the **Oratorium Marianum** (Main Building of the University of Wrocław, Plac Uniwersytecki 1). On the fourth day of the conference (12th of July), an outdoor event (barbecue) will take place at the **Botanical Garden** of the University of Wrocław (side entrance, ul. Świętozrzska 14) (see the map on the next page).



Public Transport

From 9th to 13th of July the public transport will be free for every EMBO VoM2018 participant (**badge mandatory!**).

Trams & buses timetables: <https://www.wroclaw.pl/en>

Special Issue, Viruses, MDPI

Special Issue "Viruses of Microbes V: Biodiversity and Future Applications" will be dedicated to the scientific results presented during the conference.

http://www.mdpi.com/journal/viruses/special_issues/Viruses_of_Microbes_2018

Emergency Information

In case of emergency dial the general emergency number for mobile phones: **112**. If the direct contact with particular emergency unit is needed dial **71 999** for ambulance, **71 998** for fire brigade and **71 997** for police (71 is the local code of Wrocław, for mobile phones only).

Venue



University of Wrocław
Faculty of Law, Administration and Economics
Uniwersytecka 7-10, Building D
50-145 Wrocław, Poland

Getting Around and Dining Out

The conference venue is located in the Old Town, just a five-minute walk from the 13th century Main Market Square (Rynek). Wrocław is a relatively large city, but the historic city center and surrounding area – including Ostrów Tumski, the oldest part of the city – can be easily explored by foot. The Old Town, featuring beautiful tenement houses, the Old Town Hall and several churches, is also the center of entertainment as many night clubs and pubs are located near the Rynek and in the nearby Niepolda passage.

Public transportation has been covered by the conference organizers for all participants from July 9 to 13. Importantly, when traveling by city bus or by tram, participants must have their conference badges and should present the badges if there is a ticket inspection. Those who do not have a conference badge during travel must purchase tickets. Tickets can be purchased at vending machines at most stops (cash and credit/debit card payments are accepted), in selected kiosks and aboard every tram and bus (mobile app or contactless card payments only – upon payment, an electronic ticket is assigned to the card). Single-ride tickets as well as a variety of short-term tickets are available. To plan your trip within Wrocław you may use a planner <https://www.wroclaw.pl/en/public-transport-route-planner> or a map https://www.wroclaw.pl/files/komunikacja/dzienne_schemat_bus_tram_02_06_2018.pdf.

The venue is close to the beautiful Ostrów Tumski and newly renovated boulevards along the Oder river, which are nice places to walk. Also in proximity is Słodowa Island – belonging to the Oder archipelago located to the north of the Rynek, it is a perfect spot to relax and enjoy the sunny weather.

A wide selection of restaurants and takeaways can be found directly in the Rynek and surrounding streets. The price range for dining out and having drinks in Poland is quite reasonable. Typical price ranges are 30-60 PLN per person for dinner – or 50-100 PLN if ordered with a starter and a dessert. Taxes are included in the prices and tipping is not habitually required, but appreciated, especially in higher end establishments (usually 10-15%). Cocktail prices are in the range of 15-25 PLN per drink.

The conference organizers have booked one full meal per day for each participant from July 10 to 13; these meals will be served in exchange for tickets provided with conference materials. The meals will be available from 8 am until 6 pm in the restaurant Lepione on the ground floor of our conference venue.

Further information about the city attractions, restaurants, pubs, and interesting places in Wrocław can be found at <https://visitwroclaw.eu/en>, <https://www.wroclaw.pl/en>, or e.g. Trip Advisor.

5. SCIENTIFIC PROGRAM

DAY 1 (9.07.2018)

	Presentation title	Presenter	Affiliation
09:00-17:00	Arrival and Registration		
Preconference workshop (facultative)			
09:30-11:00	High-throughput sequencing methods and their impact on phage genome & transcriptome analysis	Rob Lavigne	KU Leuven, Leuven, Belgium
11:00-11:15	Break		
11:15-12:45	Subsystems-based phage resources	Ramy K. Aziz	Cairo University, Cairo, Egypt
12:45-13:00	Break		
13:00-14:00	NCBI Virus resources	James Rodney Brister	National Institutes of Health, Bethesda, MD, United States
14:00-16:45	Break		
Plenary session			
16:45-17:15	Welcome Addresses		
17:15-18:00	How studying ocean viruses may help save the Earth and cure disease	Matthew Sullivan	Ohio State University, Columbus, OH, United States
18:00-18:45	How microbes keep their CRISPR memories functional and up to date	Stan Brouns	Delft University of Technology, Delft, Netherlands
19:00-21:00	Welcome Reception Oratorium Marianum, Main Building of the University of Wrocław, Plac Uniwersytecki 1		

DAY 2 (10.07.2018)

	Presentation title	Presenter	Affiliation
Session 1: Viral entry in the variety of microbial hosts			
08:30-08:40	<i>Introductory overview by the session leader</i>	Mark van Raaij	Spanish National Center for Biotechnology (CNB), Madrid, Spain
08:40-09:05	Structure and function of the branched receptor-binding complex of bacteriophage CBA120	Petr Leiman	University of Texas Medical Branch, Galveston, TX, United States
09:05-09:30	Structural assembly of a tailed bacteriophage	Ye Xiang	Tsinghua University, Beijing, China
09:30-09:45	<i>Selected from abstracts:</i> An atomic model of phage-host recognition by the long tail fiber adhesion tips	Matthew Dunne	Swiss Federal Institute of Technology in Zurich (ETHZ), Zurich, Switzerland
09:45-10:00	<i>Selected from abstracts:</i> Not a barrier but a key: O-antigen as an essential receptor to initiate infection in <i>Salmonella</i> and <i>E. coli</i> phages	Stefanie Barbirz	University of Potsdam, Potsdam, Germany
10:00-10:15	<i>Selected from abstracts:</i> Structure and function of haloarchaeal pleomorphic virus spikes	Elina Roine	University of Helsinki, Helsinki, Finland
10:15-10:45	Coffee break		
Session 2: Transcription and replication in viruses of microbes			
10:45-10:55	<i>Introductory overview by the session leader</i>	Rob Lavigne	KU Leuven, Leuven, Belgium
10:55-11:20	7-deazapurines, novel modifications of bacterial and phage DNA stolen from the RNA world	Valérie de Crécy-Lagard	University of Florida, Gainesville, FL, United States
11:20-11:45	Genome replication and transcription by double-stranded RNA bacteriophages	Minna Poranen	University of Helsinki, Helsinki, Finland
11:45-12:00	<i>Selected from abstracts:</i> A single phage protein efficiently disrupts the host physiology by targeting sigma factors	Mathieu De Jode	Institut Pasteur, Paris, France; Sorbonne Universités, Paris, France

	Presentation title	Presenter	Affiliation
12:00-12:15	<i>Selected from abstracts:</i> Phage genes inactivate CRISPR-Cas via diverse mechanisms	Beatriz A. Osuna	University of California San Francisco, San Francisco, CA, United States
12:15-12:30	<i>Selected from abstracts:</i> The <i>E. coli</i> global regulator DksA attenuates transcription during T4 infection	Deborah Hinton	National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States
12:30-15:00	Poster session / Lunch break		
Session 3: Host-virus interactions: structure and function			
15:00-15:10	<i>Introductory overview by the session leader</i>	Grzegorz Węgrzyn	University of Gdansk, Gdansk, Poland
15:10-15:35	Tinkers, tailors, soldiers, spies? Elucidating phage ORFan gene functions in <i>Pseudomonas</i>	Rob Lavigne	KU Leuven, Leuven, Belgium
15:35-16:00	Host-bacteria-virus interactions: our experience with phage-derived enzymes degrading carbohydrate barriers	Barbara Maciejewska	University of Wrocław, Wrocław, Poland
16:00-16:15	<i>Selected from abstracts:</i> Temperate Stx-bacteriophage 24B subverts fatty acid synthesis pathways that promote a bacterial response to antimicrobials	Darren Smith	Northumbria University at Newcastle, Newcastle upon Tyne, United Kingdom
16:15-16:30	<i>Selected from abstracts:</i> Host metabolic reprogramming of <i>P. aeruginosa</i> by phage-based quorum sensing modulation	Hanne Hendrix	KU Leuven, Leuven, Belgium
16:30-17:00	Coffee break		
Session 4: Host-virus interactions: microbial resistance to viral infection			
17:00-17:10	<i>Introductory overview by the session leader</i>	Stan Brouns	Delft University of Technology, Delft, Netherlands
17:10-17:35	CRISPR-Cas-mediated phage resistance enhances horizontal gene transfer by transduction	Peter Fineran	University of Otago, Dunedin, New Zealand

	Presentation title	Presenter	Affiliation
17:35-18:00	Discovery and characterisation of anti-CRISPR proteins encoded by archaeal viruses	Xu Peng	University of Copenhagen, Copenhagen, Denmark
18:00-18:15	<i>Selected from abstracts:</i> Phage ΦKZ's nucleus-like structure confers pan-resistance to bacterial immune systems	Senén Mendoza	University of California San Francisco, San Francisco, CA, United States
18:15-18:30	<i>Selected from abstracts:</i> Functional and structural characterization of phage enzymes involved in combating the Restriction-Modification defense system	Annika Söderholm	Uppsala University, Uppsala, Sweden
Lecture from Session 10: Diversity and evolution, change for special request of the Speaker			
18:30-18:55	Exploring fungal DNA virus to control stem rot of oilseed rape caused by <i>Sclerotinia sclerotiorum</i>	Daohong Jiang	Huazhong Agricultural University, Wuhan, China

DAY 3 (11.07.2018)

	Presentation title	Presenter	Affiliation
Session 5: Microbial virus encoded enzymes: fundamentals and application			
08:30-08:40	<i>Introductory overview by the session leader</i>	Zuzanna Drulis-Kawa	University of Wrocław, Wrocław, Poland
08:40-09:05	Bacteriophage polysaccharide depolymerases, their role and biotechnological applications	Joana Azeredo	University of Minho, Braga, Portugal
09:05-09:30	Structure-guided mutagenesis of the bacteriophage endolysin PlyC cell binding domain	Daniel Nelson	University of Maryland, Rockville, MD, United States
09:30-09:45	<i>Selected from abstracts:</i> Bacteriophage receptor binding proteins can deliver endolysins across the Gram-negative outer membrane	Lone Brøndsted	University of Copenhagen, Frederiksberg, Denmark
09:45-10:00	<i>Selected from abstracts:</i> Engineering of phage endolysin-based proteins for improved serum circulation half-life and efficient killing of <i>Staphylococcus aureus</i>	Anna Sobieraj	Swiss Federal Institute of Technology in Zurich (ETHZ), Zurich, Switzerland
10:00-10:15	<i>Selected from abstracts:</i> The phage encoded depolymerase DpoL1 causes a strong synergistic inhibitory effect on <i>Erwinia amylovora</i> if applied with capsule independent phages	Lars Fieseler	Zurich University of Applied Sciences, Wädenswil, Switzerland
10:15-10:45	Coffee break		
Session 6: Biotechnology and molecular engineering of microbial viruses			
10:45-10:55	<i>Introductory overview by the session leader</i>	Joana Azeredo	University of Minho, Braga, Portugal
10:55-11:20	Engineering the bacteriophage T4 DNA packaging machine for delivery of genes and proteins into mammalian cells	Venigalla B. Rao	The Catholic University of America, Washington, DC, United States
11:20-11:35	<i>Selected from abstracts:</i> Synthetic biology and CRISPR-Cas: New tools to engineer phages that infect Gram-positive pathogenic bacteria	Samuel Kilcher	Swiss Federal Institute of Technology in Zurich (ETHZ), Zurich, Switzerland
11:35-11:50	<i>Selected from abstracts:</i> High-throughput design and analysis of modular endolysins to develop lead candidates using VersaTile shuffling	Yves Briers	Ghent University, Gent, Belgium

	Presentation title	Presenter	Affiliation
11:50-12:05	<i>Selected from abstracts:</i> A phage-inspired antibacterial peptide targeting bacterial motility	You-Hee Cho	CHA University, Seongnam, Republic of South Korea
12:05-14:00	Poster session		
12:05-15:00	Lunch break		
14:00-15:00	Facultative: City tour		
Session 7: Phage therapy I, fundamentals			
15:00-15:10	<i>Introductory overview by the session leader</i>	Krystyna Dąbrowska	Institute of Immunology and Experimental Therapy PAS, Wrocław, Poland
15:10-15:35	Pulmonary phage therapy and host immune response	Laurent Debarbieux	Institut Pasteur, Paris, France
15:35-15:50	Modulation of LPS effect on innate immunity reaction by T4 phage tail adhesin <i>in vivo</i>	Paulina Miernikiewicz	Institute of Immunology and Experimental Therapy PAS, Wrocław, Poland
15:50-16:05	<i>Selected from abstracts:</i> Frequent administration of bacteriophage does not adversely affect therapeutic efficacy in an <i>A. baumannii</i> mouse wound infection model	Michael Rouse	Naval Medical Research Center/HJF, Silver Spring, MD, United States
16:05-16:20	<i>Selected from abstracts:</i> Use of a regression model to study host-genomic determinants of phage susceptibility in MRSA	Henrike Zschach	Technical University of Denmark, Lyngby, Denmark
16:20-16:35	<i>Selected from abstracts:</i> Prophages of <i>Roseburia intestinalis</i> : the enemy from within	Jeffrey Cornuault	Micalis Institute, INRA, Agroparistech, Université Paris-Saclay, Jouy en Josas, France
16:35-17:00	Coffee break		
Session 8: Phage therapy II, application			
17:00-17:10	<i>Introductory overview by the session leader</i>	Andrzej Górski	Institute of Immunology and Experimental Therapy PAS, Wrocław, Poland

	Presentation title	Presenter	Affiliation
17:10-17:35	Phage therapy: beyond the antibacterial action	Andrzej Górski	Institute of Immunology and Experimental Therapy PAS, Wrocław, Poland
17:35-18:00	Phage therapy – research and application at the Eliava Institute	Mzia Kutateladze	G. Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia
18:00-18:25	Phage therapy research in the Brussels Military Hospital	Jean-Paul Pirnay	Queen Astrid Military Hospital, Brussels, Belgium
18:25-18:50	From a phage therapy trial to microbiome analysis in childhood diarrhea	Harald Brüßow	KU Leuven, Leuven, Belgium
Phage therapy discussion panel: Direction to the future			
18:50-19:05	Opening talk: PhagoBurn: efficacy and tolerability of a phage mix produced according to Good Manufacturing Practices to treat <i>Pseudomonas aeruginosa</i> infected burn wounds	Jerome Gabard	Pherecydes Pharma
19.05-19.30	Discussion	Andrzej Górski, Elizabeth Kutter, Laurent Debarbieux, Harald Brüßow, Jean-Paul Pirnay, Mzia Kutateladze, Jerome Gabard	

DAY 4 (12.07.2018)

	Presentation title	Presenter	Affiliation
Session 9: Diversity and evolution (part I)			
08:30-08:40	<i>Introductory overview by the session leader</i>	Matthew Sullivan	Ohio State University, Columbus, OH, United States
08:40-09:05	The quest for phage genomic signatures	Alejandro Reyes	University of Los Andes, Bogota, Colombia
09:05-09:30	What is ICTV doing to the classification of my phages?	Andrew M. Kropinski	University of Guelph, Guelph, Canada
09:30-09:45	<i>Selected from abstracts:</i> The human virome: Toward better diversity and functional annotation	Ali H.A Elbeherly	Helmholtz Zentrum München GmbH, Neuherberg, Germany
09:45-10:00	<i>Selected from abstracts:</i> K-mer approaches provide valuable insight into mobilome evolution in the domain <i>Archaea</i>	Ariane Bize	Irstea, UR HBAN, Antony, France
10:00-10:15	<i>Selected from abstracts:</i> Expanding knowledge of gene order conservation in viral genomes	Ksenia Arkhipova	Utrecht University, Utrecht, Netherlands
10:15-10:50	Coffee break		
Session 10: Diversity and evolution (part II)			
10:50-11:00	<i>Introductory overview by the session leader</i>	Małgorzata Łobocka	Institute of Biochemistry and Biophysics PAS, Warsaw, Poland
11:00-11:25	Enthusiastic algal-virus-hunting days and afterwards - What is the raison de'tre of viruses?	Keizo Nagasaki	Kochi University, Kochi, Japan
11:25-11:40	<i>Selected from abstracts:</i> Exploring cyanophage genomes by genetic engineering	Dror Shitrit	Technion - Israel Institute of Technology, Haifa, Israel
11:40-11:55	<i>Selected from abstracts:</i> Novel archaeal viruses from different anaerobic environments – isolation, characterization and environmental abundance	Martin Fischer	University of Kiel, Kiel, Germany
11:55-12:10	<i>Selected from abstracts:</i> Diversity of the most abundant human gut virus, crAssphage	Stephen Stockdale	Teagasc Moorepark, Fermoy, Ireland; APC Microbiome

	Presentation title	Presenter	Affiliation
			Ireland, Cork, Ireland
12:10-15:00	Poster session		
12:10-15:00	Lunch break		
Session 11: Ecology and systems biology (part I)			
15:00-15:10	<i>Introductory overview by the session leader</i>	Laurent Debarbieux	Institut Pasteur, Paris, France
15:10-15:35	How to package DNA: lessons from archaeal viruses	David Prangishvili	Institut Pasteur, Paris, France
15:35-16:00	How horses help us to ride phages: bacteriophage diversity, evolution and phage-host interactions in the equine intestinal community	Andrey Letarov	Winogradsky Institute of Microbiology RAS, Moscow, Russia
16:00-16:15	<i>Selected from abstracts:</i> Differential phage infection dynamics in the mammalian gut	Marta Lourenco	Institut Pasteur, Paris, France
16:15-16:30	<i>Selected from abstracts:</i> Co-regulation of infective and defective prophages promotes <i>Listeria monocytogenes</i> virulence	Anat Herskovits	Tel Aviv University, Tel Aviv, Israel
16:30-17:00	Coffee break		
Session 12: Ecology and systems biology (part II)			
17:00-17:10	<i>Introductory overview by the session leader</i>	Tessa Quax	University of Freiburg, Freiburg, Germany
17:10-17:35	Endogenous virophages in protists: indicators of a defense system against giant viruses?	Matthias Fischer	Max Planck Institute for Medical Research, Heidelberg, Germany
17:35-18:00	Diversity of marine prymnesioviruses and their strategies for stable co-existence with their phytoplankton hosts	Ruth-Anne Sandaa	University of Bergen, Bergen, Norway
18:00-18:15	<i>Selected from abstracts:</i> Functional characterisation of a cyanophage encoded psbA	Tamsin Redgwell	University of Warwick, Coventry, United Kingdom
18:15-18:30	<i>Selected from abstracts:</i> Viral dynamics within three aquifers of differing ages and origins in the Fennoscandian shield terrestrial deep biosphere	Karin Holmfeldt	Linnaeus University, Kalmar, Sweden
20:00-24:00	Botanical Garden social event (get together barbecue) Side entrance of the Botanical Garden, ul Świętokrzyska 14		

DAY 5 (13.07.2018)

	Presentation title	Presenter	Affiliation
Session 13: Application to market: food production, agriculture, environmental, diagnostics, health (part I)			
08:30-08:40	<i>Introductory overview by the session leader</i>	Heather Allison	University of Liverpool, Liverpool, United Kingdom
08:40-09:05	Cool tools made by evolution - how bacteriophage enables rapid diagnostics and efficient control of pathogenic bacteria	Martin J. Loessner	Swiss Federal Institute of Technology in Zurich (ETHZ), Zurich, Switzerland
09:05-09:20	<i>Selected from abstracts:</i> The impact of horizontal gene transfer on the evolution and function of cellulose-degrading microbial communities	Steven D. Quistad	ESPCI, Paris, France
09:20-09:35	<i>Selected from abstracts:</i> Bacteriophage biodistribution and infectivity – from honeybee to bee larvae	Ana Oliveira	University of Minho, Braga, Portugal
09:35-09:50	<i>Selected from abstracts:</i> Broad-spectrum antiviral agents for phage infections in industry	Łukasz Richter	Institute of Physical Chemistry PAS, Warsaw, Poland
09:50-10:25	Coffee break		
Session 14: Application to market: food production, agriculture, environmental, diagnostics, health (part II)			
10:25-10:35	<i>Introductory overview by the session leader</i>	Tobi Nagel	Phages for Global Health, Oakland, CA, United States
10:35-11:00	Phage therapy in localized infections	Nina V. Tikunova	Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia
11:00-11:15	<i>Selected from abstracts:</i> Combatting Lyme disease: phages for diagnosis and treatment	Jinyu Shan	University of Leicester, Leicester, United Kingdom
11:15-11:30	<i>Selected from abstracts:</i> Phages to treat and control cholera in the Democratic Republic of Congo	Daniel De Vos	Queen Astrid Military Hospital, Brussels, Belgium

	Presentation title	Presenter	Affiliation
11:30-11:45	<i>Selected from abstracts:</i> Getting from bench to bedside: Addressing challenges for clinical development of phage products	Susan Lehman	AmpliPhi Biosciences, San Diego, CA, United States; Westmead Hospital, Sydney, Australia
11:45-12:00	<i>Selected from abstracts:</i> Functional characterization of Lysin A&B from a New Delhi Mycobacteriophage-PDRPxv, virulent against <i>M. tuberculosis</i>	Urmi Bajpai	University of Delhi, Delhi, India
12:00-14:00	Lunch break		
14:00-16:00	Poster awards (& Ackermann's Award), speed presentations of prize winners, ISVM Assembly and Announcements		
16:00-16:30	Closing remarks		

6. ABSTRACTS (ORAL PRESENTATIONS)



[ID: 421] High-throughput sequencing methods and their impact on phage genome & transcriptome analysis

Rob Lavigne

Laboratory of Gene Technology, Division of Animal and Human Health Engineering, KU Leuven, Leuven, Belgium

This lecture focuses on the theoretical background on state-of-the-art sequencing technologies available to us today and in the near future. We introduce of the basic principles of Illumina, Nanopore and PacBio sequencing technologies and their sample preparation procedures. Furthermore, the lecture focuses on the implementation of these sequencing technologies towards phage genome sequencing and phage transcriptome analysis, assessing specific strengths and weaknesses.

The class will be presented as a theoretical lecture, interactive through questions. Course material slides will be made available to participants beforehand.

[ID: 311] Subsystems-based phage resources

Ramy K. Aziz

Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo Univesity, Cairo, Egypt

The accumulation of genomic and metagenomic data is creating an annotation bottleneck. As thousands of phage genomes are de novo sequenced or assembled from metagenomic data sets, consistent annotation of these genomes to generate biological knowledge is becoming even more challenging. Various genomic databases and tools have been developed over the years, but most of these tools are biased towards eukaryotes or bacteria. Here, subsystems-based databases (The SEED, PATRIC) are introduced, and their use for genome annotation through the RAST server is discussed. Optimizing the RAST annotation pipeline for phage annotation is demonstrated. A set of novel phage therapy-oriented tools and databases are being developed to respond to the emerging need for therapeutic phages that are well characterized and deemed safe for human use.

Links:

SEED: <http://pubseed.theseed.org/>

RAST: <http://rast.nmpdr.org/>

PATRIC: <https://www.patricbrc.org/>

[ID: 279] NCBI Virus resources

James Rodney Brister

National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, United States

The rapid increase in publicly available viral sequence data promises to fundamentally alter our understanding of viral ecology and evolution. Currently, there are more than 2.7 million viral sequences now available from GenBank and the other International Sequence Database Collaboration resources. While this number is likely to rise quickly in the coming years, inconsistent data quality, sample metadata, sequence annotation, and taxonomy descriptors can make this data difficult to use. To combat this problem, the NCBI Virus group validates publicly available data and creates value added datasets designed to meet researcher's needs. This talk will focus on our activities to improve the quality and accessibility of viral sequence data and will describe the datasets available from NCBI and how these can be used by the scientific community.

[ID: 142] How studying ocean viruses may help save the Earth and cure disease

Matthew Sullivan

Ohio State University, Columbus, United States

Microbes are recently recognized as driving the energy and nutrient transformations that fuel Earth's ecosystems in soils, oceans and humans. Where studied, viruses appear to modulate these microbial impacts in ways ranging from mortality and nutrient recycling to extensive metabolic reprogramming during infection. As environmental virology strives to get a handle on the global virosphere (the diversity of viruses in nature), we face challenges to organize this 'sequence space' (create a sequence-based viral taxonomy), link these viruses to their natural hosts (who infects whom), and establish how virus populations are structured (ecological drivers) and impact natural ecosystems (their impacts). Here I will share current thinking on how to study viruses in complex communities and how these efforts are revealing new biology in the clinic that will help enable a new generation of medical treatments.

[ID: 253] How microbes keep their CRISPR memories functional and up to date

Stan Brouns

Delft University of Technology, Department of Bionanoscience, Kavli Institute of Nanoscience, Delft, Netherlands

The CRISPR immune system protects bacteria and archaea from invading viruses and plasmids. Immunity depends on protein complexes that use small RNA molecules to find matching viral or plasmid DNA. I will show how viruses escape immunity by mutating target sites or glycosylating their DNA. I will highlight a mechanism called priming that takes care of these mutated viruses and will quickly update the memory of the immune system leading to rapid co-evolution between host and phage. I will present a new mechanism catalyzed by Cas proteins that increases the chances of selecting functional CRISPR memories that increase the efficiency CRISPR targeting. All of these insights into the biology of CRISPR gathered over the last 10 years have led to some of the most revolutionary molecular genetics tools to date, with Cas9 being the most well known example. I will review some new CRISPR tools for genome engineering approaches to edit genomes and to knockdown gene expression.

[ID: 289] Structure and function of the branched receptor-binding complex of bacteriophage CBA120

Michel Plattner ¹, Mikhail Shneider ², Nikolay Arbatsky ³, Alexander Shashkov ³, Sergey Nazarov ⁴, Nicholas Taylor ⁵, Yuriy Knirel ³, Petr Leiman ¹

¹ *University of Texas Medical Branch, Galveston, United States*

² *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia*

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⁴ *University of Basel, Basel, Switzerland*

⁵ *University of Copenhagen, Copenhagen, Denmark*

The host range of a bacteriophage is determined by the spectrum of the ligands to which its tail fiber and tailspike proteins can bind. In addition to binding, tailspikes degrade or modify the surface polysaccharides of the bacterial cell thus creating a passageway for the phage to reach the cell surface. Bacteriophages that carry multiple tailspike and tail fibers are thought to have a wider host range than their less endowed relatives. In that aspect, viunalike bacteriophages (distant relatives of phage T4) are champions with up to six different tailspikes and/or tail fibers per particle, several of which form a branched or hand-like structure. However, little is known about the actual host range for any of these bacteriophages. CBA120 is a rare representative of viunalike bacteriophages in that it specifically targets many pathogenic *Escherichia coli* O157:H7 strains and does not infect common *E. coli* B and K12 strains. It carries four tailspike proteins (TSP1 through TSP4, orf210-orf213), an additional fiber (orf215) and a VrlC-like tailspike or tail fiber (orf209) on the particle. Here, we report the crystal structures of TSP2, TSP3 and TSP4 proteins, and show that TSP2 is responsible for digesting the O157 exopolysaccharide and, consequently, for *Escherichia coli* O157:H7 infection, and that TSP3 and TSP4 digest the O77 and O78 exopolysaccharide, respectively. NMR analysis of the original polysaccharides and their digested products showed that all the three enzymes are hydrolases. We also report here the structure of TSP2 in complex with the repeating unit of O157 polysaccharide. Finally, we analyzed the assembly pathway of the TSP1-TSP2-TSP3-TSP4 complex and imaged it with electron microscopy. The experimental and bioinformatic data suggest that TSP4 is responsible for attachment of the entire complex to the baseplate.

[ID: 293] Structural assembly of a tailed bacteriophage

Ye Xiang, Jingwei Xu, Miao Gui

Beijing Advanced Innovation Center for Structural Biology, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Center for Infectious Disease Research, School of Medicine, Tsinghua University, Beijing, China

The mature virion of the tailed bacteriophage $\phi 29$ is a ~ 33 MDa complex that contains more than 450 subunits of seven structural proteins assembling into a prolate head and a short non-contractile tail. Here we report the near atomic structures of the $\phi 29$ pre-genome packaging head (prohead), the mature virion and the genome emptied virion. The prohead structure shows a ring of five prohead RNAs (pRNAs). At the final stage of genome packaging, the expansion of the capsid shell and the connector narrow end disrupts the packaging motor and generates sites for attaching the funnel shaped tail lower collar. The tail spikes form an interlocked assembly attaching the tail around the collar. The narrow tube stem of the lower collar is a 180 Å long 24 strand β barrel that attaches the tail knob through a mini barrel enlarge and undergoes contraction to trigger genome release. The membrane active long loops of the tail knob exit at the late stage of infection and form a cone-shape tip of largely a hydrophobic helix barrel ready for membrane penetration.

[ID: 138] An atomic model of phage-host recognition by the long tail fiber adhesin tips

Matthew Dunne¹, Jenna M Denyes¹, Martin J Loessner¹, Petr G Leiman², Jochen Klumpp¹

¹ *Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland*

² *Department of Biochemistry and Molecular Biology, Sealy Center for Structural Biology and Molecular Biophysics, Galveston, United States*

The first stage of any bacteriophage infection is adsorption to a bacterial host. For T-even bacteriophages (e.g., T2 and T4), the adhesin tips of long tail fibers (LTFs) initiate host adsorption by recognising specific cell surface receptors. The adhesins are thus primary determinants of phage host range. Despite their biological significance, very few high-resolution structures of tail fibers are available. Using X-ray crystallography, we present the multipart structure of the T2-like adhesin tip of the LTF from the broad host range Salmonella phage S16. The adhesin consists of a single copy of gp38 attached via hydrophobic interactions to a trimeric gp37 β -helical end of the LTF. Gp38 consists of three distinct domains, with the least conserved forming the distal receptor-binding tip. Here, rare polyglycine type II helices pack tightly into a highly ordered “polyglycine sandwich”. Sequence plasticity along the surface of exposed helical residues and their distal connecting loops form the putative binding sites that dictate host specificity. We describe how this unusual domain coordinates reversible polyvalent binding to the outer membrane protein C (OmpC), via an exposed Asn-Ala-Arg motif on extracellular loop 5, and the lipopolysaccharide outer core of Salmonella with variable affinity. Bioinformatics analyses reveal the prevalence of the S16 LTF architecture across the T-even family, excluding the well-characterized phage T4. Overall, the atomic structure of S16 LTF makes it finally possible to describe the unusual modular structure of the T-even adhesins and establishes a framework for sequence-structure-function studies, as well as for accelerating the engineering of adhesin specificity for many biotechnologically important phages.

[ID: 221] Not a barrier but a key: O-antigen as an essential receptor to initiate infection in Salmonella and E. coli phages

Nina Broecker, Franziska Kiele, Mareike Meyer, Stefanie Barbirz

University of Potsdam, Potsdam, Germany

Tailed bacteriophages use a multitude of bacterial cell surface receptors for infection. For phages specific for Gram-negative hosts, the lipopolysaccharide (LPS) outer membrane not only opposes the main obstacle against genome entry but also contains molecular structures exploited by the phage to initiate the first steps of the infection cycle. O-antigen specific phages exclusively grow on smooth strains and cannot infect rough bacteria lacking the O-polysaccharide part of the LPS [1]. We want to elucidate the first infection steps of O-antigen specific phages that can solely be initiated by contact with LPS. We have characterized a set of Salmonella phages that grow on the same host and use the same O-antigen receptor. All three phages ejected their DNA upon in vitro incubation with highly purified, protein-free LPS preparations; however the velocities of these processes differed [2, 3]. Siphovirus 9NA ejected its DNA within 500 s, whereas podovirus P22 and myovirus Det7 took about ten times longer. These kinetics are independent of the host's O-antigen composition as confirmed with the multiple host-specific phage Det7 and the E. coli phage HK620. The individual kinetic profiles of DNA release can be related to the different tail morphologies with the largest number of steps found for the contractile tail system. We show that cleavage of the long O-polysaccharide chains of LPS by bacteriophage tailspike proteins (TSP) is a prerequisite to initiate particle opening only at permissive temperatures. This illustrates that enzymatic degradation via TSP is not an independent clearing step to approach the membrane but an inseparable part of the infection process in O-antigen specific phages.

[1] Broecker, N. K et al., Mol. Microbiol. (2017) 105, 353.

[2] Andres, D. et al., Mol. Microbiol. (2012), 83, 1244.

[3] Andres, D. et al., J. Biol. Chem. (2010), 285, 36768.

[ID: 294] Structure and function of haloarchaeal pleomorphic virus spikes

Elina Roine, et al.

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Virions of the haloarchaeal pleomorphic viruses (HRPVs) are membrane vesicles carrying either single stranded or double stranded DNA genomes (Sencilo et al. 2012). The surface is decorated by proteinaceous spikes protruding from the membrane envelope and anchored to it by C-terminal transmembrane domain while the other major structural protein is membrane associated and mostly facing towards the interior of the particle (Pietilä et al. 2010, 2012). Viral spike structures are often responsible for host recognition and it has been suggested that the spikes of the pleomorphic viruses have dual function in receptor recognition as well as in fusion (Pietilä et al. 2009). In order to elucidate the function of the spikes, we determined the atomic structures of the spike protein from two different haloarchaeal viruses. We determined the crystal structures of the two spike proteins at 2.5 and 2.7 Å resolution, and by cryo-EM we could show that the spikes are monomeric on the virion surface. A model for the initial stages of infection is presented.

Pietilä MK, Atanasova NS, Manole V, Liljeroos L, et al. (2012). Virion architecture unifies globally distributed pleolipoviruses infecting halophilic archaea. *J Virol* 86, 5067-79.

Pietilä MK, Laurinavičius S, Sund J, Roine E, Bamford DH (2010) The single-stranded DNA genome of novel archaeal virus Halorubrum pleomorphic virus 1 is enclosed in the envelope decorated with glycoprotein spikes. *J Virol* 84, 788-98.

Pietilä MK, Roine E, Paulin L, Kalkkinen N, Bamford DH (2009) An ssDNA virus infecting archaea: a new lineage of viruses with a membrane envelope. *Mol Microbiol* 72, 307-19.

Senčilo A, Paulin L, Kellner S, Helm M, Roine E (2012). Related haloarchaeal pleomorphic viruses contain different genome types. *Nucleic Acids Res* 40, 5523-34

[ID: 163] 7-deazapurines, novel modifications of bacterial and phage DNA stolen from the RNA world

Valérie de Crécy-Lagard

University of Florida, Gainesville, United States

The 7-cyanodeazaguanine (preQ0) base is an intermediate in the synthesis of two long-known tRNA modifications Queuosine (Q) and Archaeosine (G+). As soon as we discovered the missing pathway genes for preQ0 synthesis over 15 years ago, we identified preQ0 pathway operons in phages. However, it is only recently that we found that these were not involved in tRNA modifications but in novel DNA modification pathway that inserts deazapurine in DNA of both phages and bacteria. I will discuss, the distribution of this pathway, its potential roles restriction-modification systems or as anti-restriction and summarize our progress in elucidating the mechanisms of insertion in DNA.

[ID: 322] Genome replication and transcription by double-stranded RNA bacteriophages

Minna Poranen

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Double-stranded RNA (dsRNA) bacteriophages of the family Cystoviridae replicate and transcribe their genome within a proteinaceous capsid, called the polymerase complex. This icosahedral protein shell forms the innermost structural layer in the cystoviral virions. It has a unique structure that is highly conserved among bacterial and eukaryotic dsRNA viruses but not found in any other virus group. A key component of the polymerase complex is the viral RNA-dependent RNA polymerase. During cystoviral replication cycle, single-stranded genomic precursor molecules are packaged into the polymerase complex particle where the polymerase subunits synthesize the complementary RNA strands to produce the dsRNA genome. The produced dsRNA genome then serves as a template for transcription and the newly synthesized single-stranded RNAs are extruded from the polymerase complex particle. The self-assembly of the polymerase complex is a key process in the lifecycles of dsRNA viruses. We have used the in vitro self-assembly system of Pseudomonas phage phi6, the type organisms of the Cystoviridae family, to study the process of polymerase complex assembly and to analyze the activities of the polymerase complex particle. Our recent results provide novel insights into the processes that lead to the formation of functional polymerase complexes and secure the incorporation of the polymerase subunits into the phi6 virions.

[ID: 27] A single phage protein efficiently disrupts the host physiology by targeting sigma factors.

Chevallereau Anne ¹, Mathieu De Jode ^{1,2}, Monot Marc ³, Elisa Brambilla ⁴, Gouzel Karimova ⁵, Laurent Debarbieux ¹

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² *Sorbonne Universités, UPMC Univ Paris06, IFD, Paris, France*

³ *Pathogenesis of Bacterial Anaerobes, Microbiology Department, Institut Pasteur, Paris, France*

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⁵ *Group Biochemistry of Macromolecular Interactions, Structural Biology and Chemistry Department, Institut Pasteur, Paris, France*

A bacterial cell infected by a phage becomes a viral factory as most of its resources are dedicated to virions production. To investigate this transformation of the bacteria we are studying the infection of the opportunistic pathogen *Pseudomonas aeruginosa* by the phage PAK_P3. Using a global transcriptomic approach, we found that PAK_P3 alters the transcription of over a thousand host genes and temporally regulates the expression of its own genes¹. Amongst the early expressed genes, we are now focusing our attention on gp92, which is one of the few non-structural genes conserved between Kpp10virus and Pakpunavirus genera.

We discovered that ectopic expression of gp92 in *P. aeruginosa* alters cells morphology: they become spherical instead of rod shaped. This change in morphology does not affect cell growth rate nor cell viability. Additional molecular studies revealed that Gp92 possesses an unusual membrane anchoring amino-terminal sequence that is absolutely required for this phenotype. Next, using a bacterial two-hybrid assay we identified the anti-sigma factor MucA and its target the sigma factor AlgU as putative partners of Gp92. Several assays confirmed that expression of gp92 disrupts the AlgU mediated membrane stress response. Moreover, a mass spectrometry analysis revealed that expression of gp92 leads to a large modification of the cell proteome, including the overexpression of RpoH, another sigma factor. Therefore, the expression of this single phage gene, targeting two sigma factors, alters the regulation of expression of more than 200 host's genes.

We propose that early expressed proteins like Gp92 allow phages to efficiently disrupt the host cell physiology, and transform a bacterial cell into a viral factory.

Reference: Chevallereau, Anne, et al. "Next-generation "-omics" approaches reveal a massive alteration of host RNA metabolism during bacteriophage infection of *Pseudomonas aeruginosa*." *PLoS genetics* 12.7 (2016): e1006134.

[ID: 318] Phage genes inactivate CRISPR-Cas via diverse mechanisms

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The ancient arms race between bacteria and phages has led to the coevolution of bacterial defense systems and phage anti-defense strategies. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) and CRISPR-associated (Cas) proteins constitute a diverse class of bacterial adaptive immune systems. These systems maintain DNA remnants of previous phage encounters in the CRISPR array, which are transcribed into guide RNAs that complex with Cas nucleases to bind and cleave invading nucleic acids in a sequence-specific manner. We previously reported four anti-CRISPR (Acr) proteins (AcrIIA1-4) encoded by *Listeria monocytogenes* prophages in a single “anti-CRISPR locus”. [1] In A118-like prophages, this locus is characterized by the *acrIIA1* signature gene within a small operon containing two to five mystery genes between the “left” integration site and the genes involved in cell lysis. We have now made the surprising discovery that the presence of an A118-like prophage in *L. monocytogenes* causes Cas9 protein loss through a post-transcriptional mechanism. This inactivation mechanism may be adaptive for prophage-encoded anti-CRISPRs to completely silence CRISPR-Cas function. We are now identifying the gene(s) that cause Cas9 protein loss and determining the function of the remaining genes in this locus, which do not inactivate CRISPR-Cas9. Interestingly, we have also identified a new, plasmid-borne anti-CRISPR locus by searching for *acrIIA1* homologs. We will next determine whether this plasmid also causes Cas9 loss, and whether new mechanisms of CRISPR-Cas9 inactivation can be revealed. By mining anti-CRISPR loci, we ultimately hope to uncover novel mechanisms that phages employ to persist as the most abundant biological entity on Earth, and gain insight into the bacteria-phage interactions that drive CRISPR-Cas evolution in many microbes of the Firmicutes phylum.

1. Rauch, B. J. et al. Inhibition of CRISPR-Cas9 with Bacteriophage Proteins. *Cell* 168, 150–158.e10 (2017).

[ID: 134] The E. coli global regulator DksA attenuates transcription during T4 infection

Deborah Hinton¹, Jennifer Patterson-West¹, Tamara James², Llorenç Fernández-Coll², James Iben³, Kyung Moon¹, Leslie Knipling¹, Michael Cashel²

¹ *Gene Expression and Regulation Section, Laboratory of Molecular and Cellular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States*

² *Section on Microbial Regulation, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, United States*

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Faithful gene expression is essential for proper cellular function and development. Cells have evolved regulatory mechanisms to ensure that genes are expressed under appropriate conditions. Viruses have simultaneously evolved mechanisms for altering host gene expression to allow expression of genes required for viral proliferation.

Bacteriophage T4, which infects *E. coli*, relies on host RNA polymerase to transcribe three promoter classes: early (Pe), middle (Pm), and late (Pl). The T4 transcription factor MotA is needed for Pm activation, and T4motAam, a knock-down of motA, significantly impairs middle gene transcription and delays viral DNA replication. Middle genes are also expressed through the extension of Pe transcripts into downstream middle genes. The *E. coli* global transcription regulator DksA, often concomitant with the small molecule ppGpp, functions in *E. coli* to activate or repress multiple host genes. How or if DksA/ppGpp affects T4 gene expression has not been previously examined.

Surprisingly, deletion of DksA or ppGpp significantly increases plaque size of both T4 and T4motAam. To investigate this phenotype, we assessed phage fitness, determined the global transcriptome using RNA-seq/RT-qPCR, performed primer extensions to identify specific regulated promoters, and performed in vitro transcriptions. Despite increasing plaque size, ppGpp0 does not alter burst size or latent period and only modestly affects T4 transcript abundance. In contrast, Δ dskA increases burst size of wt T4 and shortens the latent period of T4motAam. In both cases, DksA deletion significantly increases the levels of specific middle RNAs needed for replication, recombination, and transcription, not by increasing Pm transcription but by increasing transcription from certain Pe's that extend into middle genes. We conclude that DksA attenuates T4 early gene expression, suggesting a new anti-viral role for this host protein. As DksA does not inhibit Pe transcription in vitro, we conclude that attenuation is indirect or perhaps requires additional factors.

[ID: 422] Tinkers, tailors, soldiers, spies? Elucidating phage ORFan gene functions in Pseudomonas

Rob Lavigne

Laboratory of Gene Technology, Division of Animal and Human Health Engineering, KU Leuven, Leuven, Belgium

The *Pseudomonas* genus is one of the most ecologically and medically important group of bacteria, including species with diverse roles as plant commensals, in bioremediation and as both human and agricultural pathogens. The evolutionary arms race between bacterial hosts and their viral parasites has endowed phages with fine-tuned molecular tools to interfere with or even reshape microbial physiology.

Research presented here will focus on our latest data concerning functionally unknown open reading frames (ORFans) present in hypervariable regions that are largely involved in adaptation to host-specific conditions. Understanding the role of this so-called “viral dark matter” using systems biology approaches reveals new methods by which the phage impact the bacterial metabolism. Our data show that these genes have roles in evading host defense systems, as well as acting to co-opt host functions to construct a cell environment conducive to phage infection. In this manner, *Pseudomonas* phages evade bacterial silencing of their xenogeneic DNA, subvert host transcription, adapt post-translation modification systems, impact replication & cell division, and modulate both the central and peripheral host metabolisms.

[ID: 296] Host-bacteria-virus interactions: our experience with phage-derived enzymes degrading carbohydrate barriers

Barbara Maciejewska, Zuzanna Drulis-Kawa, et al.

Department of Pathogen Biology and Immunology, Institute of Genetics and Microbiology, University of Wrocław, Wrocław, Poland

Bacterial carbohydrate barriers such as capsule polysaccharides (CPS), O-polysaccharide chains of lipopolysaccharide (LPS) molecules, extracellular polysaccharides (EPS) forming biofilm matrix and peptidoglycan (PG) layers have to be overcome during phage propagation. Bacteriophages use specific degrading enzymes (lysins and depolymerases) for a successful attack of the host as well as for the release of their progeny. Due to its activity, those enzymes have been investigated as novel and attractive antibacterial agents. Here, we present our lab results in the field of antibacterial properties of phages and their enzymes in biofilm eradication, in vivo treatment as well as biochemical parameters and crystal structure. Depolymerases encoded by *Klebsiella* and *Pseudomonas* - specific phages that we investigated, were proved to reduce bacterial virulence, act in synergy with antibiotics, disrupt biofilm and expand the activity spectrum of phages by reducing bacterial capsule polysaccharides and receptors exposing. In addition, the crystallization studies combined with point mutagenesis conducted to determine the unique catalytic centre of modular endolysin encoded by *Burkholderia*-specific phage AP3 will be also presented.

[ID: 308] Temperate Stx-bacteriophage 24B subverts fatty acid synthesis pathways that promote a bacterial response to antimicrobials

Darren Smith¹, Giles Holt¹, Simon Bridge¹, Muhammad Adnan Tariq¹, Alan McCarthy², Marta Veses-Garcia², heather allison²

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Shigatoxigenic *Escherichia coli* (STEC) infection is a global health concern. A key virulence determinant of STEC infection is the carriage of an AB-holotoxin that is encoded and disseminated by dsDNA, lambda-like bacteriophages. This toxin is responsible for the severe pathophysiology of infection including; haemolytic uraemic syndrome (HUS) and haemorrhagic colitis (HC). Shigatoxin encoding bacteriophages or Stx-phages carry an accessory genome with many small genes, with no associated function yet they are commonly conserved between phages of that type.

Bacteriophage 24B a well-studied stx-phage is no different where current genome annotation only associates function to approx. 35 % of the gene complement and this generally relates to the lifecycle of the bacteriophage. Importantly, this is a relevant model Stx-phage as it was isolated from a clinical outbreak of STEC infection and shares genomic similarity to other environmentally and clinically relevant phage's e.g 933W and Sakai phages. The accessory genome of the 24B is conserved when compared to other reported stx-phages from clinical STEC infection and therefore must be important in the biology of the bacteriophage or bacterium or at least offer a selective advantage. Using metabolomics in comparison to transcriptomics data we illustrate that 24B subverts bacterial cell metabolism during growth and when challenged with antimicrobials chloroxylenol and 8-hydroxyquinilene offering a selective advantage to the lysogen. We here focus on phage subversion of both the biotin and fatty acid synthesis pathways in the lysogen's cellular metabolism and how this compares to alteration of fatty acids presented at the wall that may play a role in antimicrobial resistance (AMR). Importantly this alteration in AMR is not associated with a previously reported gene carried within the phage genome and therefore we hypothesise that this selection is based upon subversion of the bacterial cell physiology.

[ID: 84] Host metabolic reprogramming of *P. aeruginosa* by Phage-based Quorum Sensing modulation

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One of the main challenges in phage biology is the functional elucidation of the in silico annotated open reading frames that lack any similarity to known genes, the so-called viral ‘dark matter’. A large portion of this consists of phage genes expressed early in infection, that are hypothesized to play a major role in the host metabolism take-over to ensure efficient production of new phage particles [1]. By focusing on growth inhibitory proteins, functional genomics research will yield new insights into phage biology and provide a basis for innovative phage-derived antibacterials.

We here show that the early-expressed protein Qst (referring to ‘quorum sensing-associated acetyltransferase’) of the podovirus LUZ19 (a phiKMV isolate) influences the *P. aeruginosa*-specific quorum sensing system PQS, resulting in toxicity and metabolic reprogramming. This function is associated with acetyl transferase activity and is achieved by a complex interaction network, in which Qst interacts with enzymes of both a well-studied (PQS) and a predicted (the PA1221 cluster) [2] signaling molecule biosynthesis pathway. The interacting protein of the latter pathway is also able to neutralize the antibacterial effect of Qst, hinting a phage-mediated effect on cell signaling. Since QS interference is generally believed to less likely select for resistance, these insights may lead to the development of a new antivirulence therapy.

[1] De Smet, Hendrix, Blasdel, Danis-Wlodarczyk, Lavigne (2017). *Nat. Rev. Microbiol.*, 15, 517-530

[2] Gulick. (2017) *Nat. Prod. Rep.*, 34, 981

[ID: 13] CRISPR-Cas-mediated phage resistance enhances horizontal gene transfer by transductionBridget Watson ¹, Raymond Staals ^{1,2}, Peter Fineran ^{1,2,3}¹ *Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand*² *Laboratory of Microbiology, Wageningen University, Wageningen, Netherlands*³ *Bio-Protection Research Centre, University of Otago, Dunedin, New Zealand*

A powerful contributor to prokaryotic evolution is horizontal gene transfer (HGT) through transformation, conjugation and transduction, which can be advantageous, neutral or detrimental to fitness. Bacteria and archaea control HGT and phage infection through CRISPR-Cas adaptive immunity. Although the benefits of resisting phage infection are evident, this can come at a cost of inhibiting the acquisition of other beneficial genes through HGT. Despite the ability of CRISPR-Cas to limit HGT through conjugation and transformation, its role in transduction is largely overlooked. Transduction is the phage-mediated transfer of bacterial DNA between cells and arguably has the greatest impact on HGT. We demonstrate that in *Pectobacterium atrosepticum*, CRISPR-Cas can inhibit the transduction of plasmids and chromosomal loci. In addition, we detect phage-mediated transfer of a large plant pathogenicity genomic island, and show that CRISPR-Cas can inhibit its transduction. Despite these inhibitory effects of CRISPR-Cas on transduction, its more common role in phage resistance, promotes, rather than diminishes, HGT via transduction by protecting bacteria from phage infection. This protective effect can also increase transduction of phage-sensitive members of mixed populations. CRISPR-Cas systems themselves display evidence of HGT, but little is known about their lateral dissemination between bacteria and whether transduction can contribute. We show that, through transduction, bacteria can acquire an entire chromosomal CRISPR-Cas system, including *cas* genes and phage-targeting spacers. We propose that the positive effect of CRISPR-Cas phage immunity on enhancing transduction surpasses the rarer cases where gene flow by transduction is restricted.

[ID: 270] Multiple families of anti-CRISPR proteins encoded by a single archaeal virus SIRV2

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Viruses employ a range of strategies to counteract the prokaryotic adaptive immune system, CRISPR-Cas, including the recently discovered anti-CRISPR proteins (Acrs). Acrs have been found in many bacteriophages that inhibit the CRISPR-Cas subtypes I-E, I-F, II-A and II-C [1]. Recently we identified and characterized the first Acr, AcrID1, from archaeal viruses that inhibits CRISPR-Cas subtype I-D [2]. Interestingly, *Sulfolobus islandicus* rod-shaped virus 2 (SIRV2), one of the viruses encoding AcrID1, propagates efficiently in the host carrying active CRISPR-Cas subtypes I-A, I-D and III-B. This suggests that in addition to AcrID1, SIRV2 also encodes AcrIA and AcrIIB. Here we present the latest results in our search for such Acrs.

[1] Pawluk, A., Davidson, A. R. & Maxwell, K. L. Anti-CRISPR: discovery, mechanism and function. *Nat Rev Microbiol* 16, 12-17, doi:10.1038/nrmicro.2017.120 (2018).

[2] He et al. Anti-CRISPR proteins encoded by archaeal lytic viruses inhibit subtype I-D immunity. *Nat Microbiol*, doi: 10.1038/s41564-018-0120-z (2018).

[ID: 327] Phage ΦKZ's nucleus-like structure confers pan-resistance to bacterial immune systems

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Bacterial immune systems such as CRISPR/Cas and Restriction-Modification (R-M) can end a viral infection by disrupting bacteriophage (phage) replication via genome cleavage. These processes depend on the ability to specifically identify and cleave foreign nucleic acids, without targeting their own genome. In order to resist immune activity by their hosts, phage have evolved varied immunity inhibitors, such as anti-CRISPR and anti-restriction proteins. Though an effective way to avoid specific immune systems, these inhibitors have specific molecular targets and are generally unable to protect against multiple immune systems, a common occurrence in bacterial genomes. Here we report that a recently discovered nucleus-like shell assembled by the jumbo phage ΦKZ protects the phage genome from detection and cleavage by host nucleases. This virus efficiently infects *Pseudomonas aeruginosa* strain PAO1 and avoids targeting by CRISPR-Cas3 (Type I-C), CRISPR-Cas9 (Type II-A), CRISPR-Cas12 (Type V-A), and Type I R-M. This level of pan-resistance to DNA-targeting immune systems has not been previously observed. By treating purified ΦKZ genomic DNA in vitro with a panel of restriction enzymes and CRISPR-Cas9, we ruled out DNA modifications as the resistance mechanism. We find that Cas9 is excluded from the nucleus-like “shell”, likely explaining its lack of function, but fusion of a shell-localized phage protein to Cas9 can reconstitute immune activity. Furthermore, we find that ΦKZ is susceptible to the RNA-targeting CRISPR-Cas13a nuclease from *Listeria seeligeri* (Type VI-A), because phage mRNA is exported into the cytoplasm and is not protected by the shell. These results demonstrate the causal relationship between the phage shell and pan-resistance to bacterial systems.

[ID: 382] Functional and structural characterization of phage enzymes involved in combating the Restriction-Modification defense system

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In a study aiming to discover novel enzymatic functions by expressing foreign DNA in auxotrophic *E. coli* strains, three proteins originating from environmental phage DNA samples provided the ability to rescue $\Delta ilvA$ strains. The *ilvA* gene encodes the enzyme threonine ammonia-lyase, responsible for producing the essential metabolite α -ketobutyrate from L-threonine. However, the enzyme encoded by the *metB* gene, Cystathionine γ -Synthase, has a promiscuous activity of producing α -ketobutyrate from O-succinylhomoserine. It was discovered that the phage genes causing rescue of the $\Delta ilvA$ phenotype encoded S-adenosylmethionine (SAM) hydrolases, leading to depletion of SAM and thereby de-repression of the SAM-regulated met biosynthesis genes, including *metB*. Due to upregulation of *MetB*, sufficient amounts of α -ketobutyrate could be produced to make the cells viable.

SAM hydrolases have been proposed to play a role in combating the Type I RM defense system of bacteria. The only previously known enzyme from this family comes from the T3 phage and was discovered in the 1960's. Until now, there has been no structural and little functional or mechanistic knowledge of this type of enzyme. We have started to characterize the SAM hydrolases discovered in the enzyme screening as well as the T3 SAM hydrolase. We have determined the first structures of a SAM hydrolase by X-ray crystallography and we have conducted various kinds of biophysical studies. Furthermore, we have developed an enzymatic assay to study the kinetics of the enzymes. Based on the kinetics data we can conclude that T3 SAM hydrolase is a more active enzyme compared to the SAM hydrolases discovered in the enzyme screening. Based on structure and activity data as well as QM calculations we also attempt to get mechanistic insights.

[ID: 282] Bacteriophage polysaccharide depolymerases, their role and biotechnological applications

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Several virulence factors have been identified in Gram-negative bacilli, among which the capsular structures which are suggested to be involved in the evasion of microbial defences. Although the capsule is commonly associated with bacteriophage resistance, some bacteriophages can recognize this structure as a receptor with the aid of structural polysaccharide depolymerases. Bacteriophages have then evolved along with the bacteria to recognize different types of capsules. This feature is well illustrated in phages infecting *Acinetobacter* spp. known to display at least 106 capsular types. We have isolated and characterized 5 novel podoviruses infecting the *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* (ACB) complex currently formed by six closely related species. Microbiologically, all phages produce plaques with opaque halos and have distinct narrow host ranges. Their genomes are closed related among them and to all other *Acinetobacter* podoviruses present in Genbank. There is only a very specific genomic pattern variation present located at the C-terminal tail fiber - depolymerase domain - that functions as a capsular depolymerase. We demonstrate that the depolymerase proteins exhibiting pectate lyase domains, specifically recognize bacterial capsular types as ligands for phage adsorption. We further demonstrate that the depolymerases act in a wide range of environmental conditions and that are high stable, probably related with their structural nature designed during evolution to endure harsh external conditions to maintain the phage infectivity. Moreover, we have characterized the anti-virulent effect of those depolymerases and proved that enzyme treated *A. baumannii* were no longer virulent to human lung epithelium. Overall this work demonstrates the great diversity of bacteriophage depolymerases, their role in phage infection and evolution and their possible biotechnological applications in bacteria typing and virulence reduction presenting thus a great contribution for diagnostic and treatment of infectious diseases caused by capsulated bacteria.

[ID: 292] Structure-guided mutagenesis of the bacteriophage endolysin PlyC cell binding domain

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Bacteriophage endolysins are murein hydrolases produced at the end of the phage life cycle and have been studied as novel antibacterial therapeutic agents. PlyC, an endolysin from the C1 streptococcal bacteriophage, possesses potent activity towards specific streptococcal hosts. Structural and biochemical studies reveal the molecular basis behind this potency: PlyC is a nine-protein holoenzyme consisting of one PlyCA subunit, which contains dual catalytic domains, and eight identical PlyCB subunits, which are responsible for binding to the streptococcal surface. To date, no other endolysin possesses such a complex holoenzyme structure. Questions remain on the binding mechanism of the PlyCB octamer and how it affects lytic activity. Likewise, chimeragenesis approaches employed for binding domains of other endolysins are not amenable to PlyCB due to the octameric structure. Here, we demonstrate that the native PlyCB octamer can be engineered to a PlyCB monomer (PlyCBm) through structure-guided mutagenesis that decreases hydrogen bonds between PlyCB octamer subunits. A PlyCB dimer (PlyCBd) is then created via the tandem duplication of the monomers. Gel-filtration and protein cross-linking confirm the size of PlyCBm and PlyCBd, and fluorescence microscopy validates that they retain the same binding capability to streptococci as the PlyCB octamer. In addition, the comparison of EC50 binding studies among PlyCBm, PlyCBd, and the PlyCB octamer suggests a concurrent binding model. The PlyCBm/PlyCBd-derived chimeras provide a rationale to further study the relations between binding affinities and lytic activities. Finally, the observations that PlyCBm translocates epithelial membranes and, with a catalytic domain, kills intracellular *S. pyogenes*, has implications for future bioengineering studies and as a drug delivery platform beyond its role as an endolysin.

[ID: 239] Bacteriophage receptor binding proteins can deliver endolysins across the Gram-negative outer membrane

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Antibiotic resistance is a global and serious threat that requires development of alternative antimicrobials. Bacteriophage encoded endolysins are promising antimicrobials due to their rapid muralytic activity towards the essential peptidoglycan. However, in Gram-negative bacteria the applicability of endolysins is restricted due to the hydrophobic outer membrane that limits their access to the peptidoglycan. Here, we sought to exploit the specificity of phage receptor binding proteins to guide endolysins to the peptidoglycan. As a proof of concept, we fused the endolysin and the receptor binding protein (Pb5) of phage T5, to specifically target such hybrid endolysins to the ferrichrome transporter receptor (FhuA). In total, 12 hybrid variants were constructed and screened for muralytic and antimicrobial activity against *E. coli*. Although the majority of the hybrid endolysins were muralytically active, only one was able to overcome the outer membrane barrier and show bactericidal activity to *E. coli*. Using a *fhuA* mutant, we show that the presence of the FhuA is required for the bactericidal activity, confirming the specificity provided by Pb5. In addition, we found that the bactericidal spectrum of the hybrid endolysin was dependent on the conservation of FhuA. This allows the hybrid endolysin to target other bacterial species carrying FhuA homologs or orthologs such as *Shigella sonnei* and *Pseudomonas aeruginosa* and subsequently kill them. This study provides a novel concept for engineering of phage-derived proteins, allowing them to pass through the outer membrane barrier and kill Gram-negative bacteria. Given the rich diversity of phage receptor binding proteins and their different binding specificities, our proof-of-concept can pave the route for creating an arsenal of pathogen specific alternative antimicrobials.

[ID: 241] Engineering of phage endolysin-based proteins for improved serum circulation half-life and efficient killing of *Staphylococcus aureus*

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Multi-drug resistant (MDR) bacteria are becoming a major concern worldwide. Especially alarming is the manifestation of MDR *Staphylococcus aureus* in hospital and community settings. Consequently, WHO has classified *S. aureus* as a pathogen of high priority for antimicrobial research. Bacteriophage endolysins can constitute such novel antimicrobials. These peptidoglycan hydrolases (PGHs) lyse bacteria from within to release the bacteriophage progeny. PGHs can also lyse Gram-positive bacteria, including MDR strains, from the outside. Their specificity and rapid action offer high therapeutic potential, which has been demonstrated in numerous *in vivo* studies. However, short serum circulation half-life can be a limitation to systemic application of PGHs. Due to their proteinaceous nature and small size, PGHs are susceptible to lysosomal degradation and rapid clearance via glomerular filtration. Fusion to an albumin binding domain (ABD) is a potential strategy for half-life extension of protein therapeutics. Proteins fused to ABD bind serum albumin, forming a complex with large hydrodynamic volume, thus avoiding renal clearance. Lysosomal degradation is reduced due to albumin recycling by neonatal Fc receptor pathway. To deliver proof-of-concept, we fused an engineered variant of the endolysin LysK to ABD. The fusion protein retained staphylolytic activity *in vitro* and showed improved serum circulation half-life and biodistribution in mice. We then applied the same strategy to a set of PGHs with optimal activity in human serum, which we identified by systematically screening a large library of parental and chimeric enzymes. Quantitative comparison of the 25 most promising candidates identified four enzymes that reduced *S. aureus* concentrations in human serum by 3-6 logs during 1 h at 20 nM concentration. When fused to ABD, these enzymes retained high activity under the same conditions. We expect that this comprehensive approach will deliver a set of enzymes with enhanced therapeutic properties for treatment of MDR *S. aureus* infections.

[ID: 165] The phage encoded depolymerase DpoL1 causes a strong synergistic inhibitory effect on *Erwinia amylovora* if applied with capsule independent phages

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Erwinia amylovora is a member of the Enterobacteriaceae and the causative agent of fire blight, a severe disease of Rosaceae plants. To prevent infection, streptomycin is usually applied during the flowering period. However, in some countries, use of streptomycin was banned recently. As an alternative to antibiotics, the application of bacteriophages for fire blight control seems to be a promising alternative. *E. amylovora* usually infects host blossoms via the stigma and invades the ovary. Later it spreads through the xylem vessels of an infected plant. In the xylem vessels *E. amylovora* produces high amounts of exopolysaccharides (EPS), e.g. a capsule, which leads to ooze formation and canker development. The capsule is mainly composed of two carbohydrates, amylovoran and levan, respectively. Transposon mutagenesis of *E. amylovora* revealed that adsorption of the T7-like phage L1 and the SP6-like phage S2 is dependent on the amylovoran synthesis (*ams*) operon. Accordingly, both phages exhibit a Depolymerase (Dpo) with 59 % amino acid identity. The enzyme is a structural component of the virion. DpoL1 binds specifically to amylovoran and cuts the galactose backbone. In vitro treatment of a growing *E. amylovora* culture with DpoL1 did not inhibit growth. However, application of the enzyme together with amylovoran-independent phages revealed a strong synergistic inhibitory effect and caused a 4 log reduction of viable cell counts. In addition, we also identified a *dpo* gene in phage Bue1, a novel member of the VII-like phages, which is clearly different from the podoviral depolymerases (24 % amino acid identity only). However, Bue1 does also infect non-capsulated strains of *E. amylovora*. Transposon mutagenesis indicated that Bue1 adsorbs to LPS. Here we discuss the impact of capsule removal on adsorption and infectivity of different Erwinia phages.

[ID: 12] Engineering the bacteriophage T4 DNA packaging machine for delivery of genes and proteins into mammalian cells

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Bacteriophages have provided powerful tools for various biomedical applications. In tailed phages, a DNA packaging machine is assembled by the attachment of an ATP-powered molecular motor at the special portal vertex of an icosahedral head. Phage T4 employs the fastest and most powerful motor to package its 170 kb viral genome into a 120 x 86 nm head. We engineered the T4 packaging machine to deliver genes and proteins into mammalian cells. DNA molecules were translocated into the empty phage head produced from tail-less mutants and its outer surface was decorated with proteins fused to outer capsid proteins Hoc and Soc. T4 nanoparticles carrying reporter genes, vaccine candidates, functional enzymes, and targeting ligands were efficiently delivered into mammalian cells, or targeted to antigen-presenting dendritic cells, and the delivered genes were expressed both in vitro and in vivo. Mice delivered with a single dose of F1-V plague vaccine containing both the gene and the protein elicited robust humoral and cellular immune responses. This “pro-gene delivery” approach might lead to novel prime-boost vaccines and new types of genetic and cellular therapies against complex infectious and genetic diseases.

[ID: 121] Synthetic biology and CRISPR-Cas: New tools to engineer phages that infect Gram-positive pathogenic bacteria

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The use of native phages for pathogen control and therapy is often restricted by poor killing efficiencies, narrow host ranges, the presence of bacterial defense mechanisms, rapid selection of resistant escape mutants, or low phage stability. In addition, temperate phages can integrate into the host genome without inducing cell lysis, may contribute to the spread of antibiotic resistance by transduction, or increase bacterial virulence through lysogenic conversion, effectively excluding their use as biocontrol agents. Many of these limitations may be overcome by custom-designing or modifying phage genomes. Here, we present two broadly applicable approaches that allow for rapid and marker-free construction of engineered phages that target Gram-positive pathogenic bacteria. These methods include (a) *in vitro* assembly and subsequent reactivation of synthetic phage genomes in cell wall-deficient bacteria [1] and (b) the use of a novel typeII CRISPR-Cas system from *Listeria* to modify genomes of very large, strictly lytic phages (unpublished). We present several concepts to increase the antimicrobial efficiency of engineered phages including the conversion of temperate phages to create strictly lytic derivatives, phage-mediated delivery of antimicrobial effectors as genetic payload, and the modification of host-range by directed evolution of receptor binding proteins. Finally, we show how custom-designed phages could be used in the future to modify bacterial communities beyond simply controlling the target species.

1. Kilcher, S., et al., Cross-genus rebooting of custom-made, synthetic bacteriophage genomes in L-form bacteria. *Proc Natl Acad Sci U S A*, 2018. 115(3): p. 567-572.

[ID: 71] High-throughput design and analysis of modular endolysins to develop lead candidates using VersaTile Shuffling

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Endolysins have emerged as a novel class of antibacterials and are currently under evaluation in clinical trials. A feature of endolysins is their modular structure and the opportunities to customize endolysins towards their specific application. A major expansion of this modularity principle is the fusion of endolysins to outer membrane permeabilizing peptides (Artilysin®s), facilitating efficient transfer across the outer membrane of Gram-negative bacteria, followed by rapid cell lysis.

The scope of this modularity principle is exciting, as it allows engineering of endolysins against any pathogen and customizing antibacterial properties. However, this progress is empirical and hampered by the tedious cloning procedures. To address this technical barrier, we developed a novel DNA shuffling method, coined VersaTile Shuffling. Any modular variant can now be constructed with short hands-on time in a combinatorial manner. VersaTile Shuffling relies on the creation of a repository of Tiles (=modules) that can subsequently be shuffled in a versatile way. Our current Tile repository allows the generation of over 8 million modular variants in a single reaction.

Using the VersaTile Shuffling method, we have implemented a hit-to-lead development process of endolysins, similar to the development of pharmaceutical drugs. Specifically, we have engineered a lead endolysin against *A. baumannii*. In the initial screening experiment, ~400 random variants from a library with >10,000 variants were analysed against four epidemiological multidrug-resistant *A. baumannii* strains. Seven hits (~2%) were identified. Based on the observed structure-activity-relationships, enriched libraries were iteratively constructed, increasing the ratio of active variants stepwise to 45%. The final selection round was performed in human serum, better mimicking in vivo conditions. This resulted in a lead modular variant with low MIC-values against all strains and a high bactericidal effect in human serum.

The scope and potential of this approach represents a key breakthrough in the design of new engineered endolysins.

[ID: 15] A phage-inspired antibacterial peptide targeting bacterial motility

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Phage-inspired antibacterial development is a new approach that recruits phages in the search for antibacterials with new modes of action, in that phages are the biological entities well adapted to perturb host bacterial physiology in favor of their own thrive. We previously observed that phage-mediated twitching motility inhibition was effective to control the infections caused by one of the ESKAPE pathogens, *Pseudomonas aeruginosa* and that the motility inhibition was due to the delocalization of PilB, the type IV pilus (TFP) assembly ATPase by the interaction with the 136-aa phage protein, Tip. These studies inspire a new antipathogenic target based on the ATPases ubiquitously conserved in the motility machineries important in bacterial pathogenesis. As an initial proof-of-concept study, we have hoped to generate an antipathogenic peptide that can target PilB of *P. aeruginosa*. First, a series of truncated and point-mutant Tip proteins to identify the critical residues in the Tip bioactivity were created to generate a fragment peptide derived from Tip. Topics discussed will include our endeavor to characterize the molecular interaction between Tip and PilB, leading to the design of a 15-aa synthetic peptide (P1) that is capable of penetration into *P. aeruginosa* cytoplasm and fully competent in twitching inhibition by exogenous administration *in vitro*. The on-going research to verify its efficacy *in vivo* using P1-expressing transgenic animals and some maneuver to improve its druggability will be covered as well.

[ID: 55] Pulmonary phage therapy and host immune response

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Phage therapy, the use of bacteriophages (phages) to kill pathogenic bacteria is now being accepted to treat patients infected by multidrug resistant bacteria. So far, most of the focus has been on the microbiological characteristics of candidate phages and clinical bacterial isolates. Very few studies have looked at the biological parameters related to patient's underlying health status or side effect of the phages's bacteriolytic mode of action beyond reducing bacterial load.

Using a mouse model of acute pulmonary infection, we found that inhaled phage therapy success was dependent on the immunological status of the host. We found that efficacy was independent of innate and adaptive lymphocytes but relies on neutrophils to clear both phage-sensitive and phage-resistant bacterial variants. By coupling in vivo data and mathematical modeling, we show that 'immunophage synergy' highlights the important tripartite interactions of the phage, the bacterial pathogen and the host for recovery of an acute respiratory infection (1).

Moreover, a consequence of phage therapy that remains unclear is the release of bacterial endotoxin during the phages lytic activity. We found that in vitro endotoxin levels after bacterial lysis by virulent phages (a Podoviridae and a Myoviridae) or an aminoglycoside (amikacin) were similar. By contrast, we showed that bacteria lysed by beta-lactams (ceftriaxone, ceftazidime or imipenem) released a greater amount of endotoxin than phages (2).

Until now there has been little evidence that phages might worsened a patient's condition when killing bacteria nevertheless, the role of immune cells during immunophage synergy requires deeper investigation beyond the management of phage resistant bacteria.

1: Roach DR et al. Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen (2017) *Cell Host Microbes* 22(1):38-47

2: Dufour N et al. The lysis of pathogenic *Escherichia coli* by bacteriophages releases less endotoxin than by beta-lactams (2017) *Clin Infect Dis* 64(11): 1582-1588

[ID: 380] Modulation of LPS effect on innate immunity reaction by T4 phage tail adhesin in vivo

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Bacteriophages that infect Gram-negative bacteria have the intrinsic ability to interact with bacterial products such as lipopolysaccharides (LPS) and surface proteins. This ability is mediated by specific phage proteins. T4-like bacteriophages adsorb to *Escherichia coli* using short tail fiber proteins (tail adhesin gp12) that bind to LPS. LPS is a very potent stimulator of the immune system in animals and in humans. This microbial product is one of pathogen-associated molecular patterns (PAMPs) recognized in a living system by specific receptors that activate inflammatory signalling pathways. Since recombinant protein gp12 can bind LPS, we have tested gp12 potential modulatory effect on LPS-induced immune response. We have produced tail adhesin gp12 in a bacterial expression system and confirmed its ability to form trimers and to bind lipopolysaccharide in vitro by dynamic light scattering. Recombinant gp12 had no toxic or antiproliferative effects on mammalian cells in vitro. Moreover, no harmful effects of this protein were observed in mice. Thus, gp12 was used in combination with LPS in a murine model. We observed decreased inflammatory response to LPS in vivo, as assessed by serum levels of cytokines IL-1 alpha and IL-6 and by histopathological analysis of spleen, liver, kidney and lungs. We propose interaction of the phage tail adhesin gp12 with LPS as a potential modulator of LPS-induced inflammatory effects. In future studies gp12 may be considered as a potential tool for modulation and specifically for counteracting LPS-related physiological effects in vivo.

[ID: 298] Frequent administration of bacteriophage does not adversely affect therapeutic efficacy in an *A. baumannii* mouse wound infection model

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The spread of multidrug antibiotic resistance (MDR) is a widely recognized crisis in the treatment of bacterial infections, including those occurring in military communities. Recently, the World Health Organization published its first ever list of antibiotic-resistant "priority pathogens" – a catalogue of 12 families of bacteria that pose the greatest threat to human health with *A. baumannii* listed in the "Priority 1: Critical" category of pathogens. Novel approaches to develop alternative and more effective antimicrobial therapy are needed, such as lytic bacteriophage specifically targeted against each of the high priority bacterial infections.

Balb/c mice were pre-immunized with PBS or bacteriophage selected to against *A. baumannii* strain AB5075. After 3 weeks, mice were anesthetized, wounded (dorsal) and challenged topically with AB5075. Following infection, mice were subsequently treated with PBS or bacteriophage and evaluated for 3 weeks to assess mortality, bacterial burden, time to wound closure, systemic and local cytokine profiles, alterations in host cellular immunity, and finally presence of neutralizing antibodies to the phage cocktail.

In our study, we found that pre-immunization of bacteriophage led to a significant reduction in monocyte-related cytokines, but a marked increase in total immunoglobulins in serum, particularly IgG2a and IgG2b. Also, we detected no significant changes to circulating blood populations or immune cell populations of secondary lymphoid organs compared to PBS-treated mice. In regards to their therapeutic efficacy, administration of bacteriophage treatment effectively decreased wound size of mice infected with AB5075 without adverse effects.

In conclusion, our data demonstrate that bacteriophages can serve as a safe and effective novel therapeutic agent against *A. baumannii* without adverse reactions to the host and pre-exposure to phage did not adversely affect therapeutic efficacy in a topical wound model. This study is an important proof of concept to support the DoD efforts to develop phage as a novel therapeutic product for treatment of complex infections in military beneficiaries.

[ID: 37] Use of a regression model to study host-genomic determinants of phage susceptibility in MRSA

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Staphylococcus aureus is a major agent of nosocomial infections. Especially in methicillin-resistant strains, conventional treatment options are limited and expensive, which has fueled a growing interest in phage therapy approaches. We have tested the susceptibility of 207 clinical *S. aureus* strains to 12 (nine monovalent) different therapeutic phage preparations and subsequently employed linear regression models to estimate the influence of individual host gene families on resistance to phages. Specifically, we used a two-step regression model setup with a preselection step based on gene family enrichment. We show that our models are robust and capture the data's underlying signal by comparing their performance to that of models build on randomized data. In doing so, we have identified 167 gene families that govern phage resistance in our strain set and performed functional analysis on them. This revealed genes of possible prophage or mobile genetic element origin, along with genes involved in restriction-modification and transcription regulators, though the majority were genes of unknown function. This study is a step in the direction of understanding the intricate host-phage relationship in this important pathogen with the outlook to targeted phage therapy applications.

[ID: 91] Prophages of *Roseburia intestinalis*: the enemy from within

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Bacteriophages are present in all bacterial communities and have profound impact on bacteria, by killing them or bringing new genes through lysogeny. Most of intestinal bacteria are lysogenized by prophages but very few data exist concerning the impact of prophages on their hosts in the intestinal environment (review, De Paepe et al., 2014). Several studies have proposed a role of phages in dysbioses associated to intestinal bowel disease (IBD). We hypothesize that prophages of key species of the human gut microbiota might have a role for the population depletion of their host.

Roseburia intestinalis is a dominant member of the human gut microbiota known for its butyrate production. This species is regularly found depleted in IBD patients. We found two active prophages in the type strain L1-82, named Jekyll and Shimadzu. In vitro, spontaneous induction of these phages results in high phage populations in overnight growth cultures, respectively 8.0×10^8 /ml and 3.6×10^8 /ml. Mitomycin C, that creates DNA Damage, induces Jekyll but not Shimadzu.

To evaluate the activity of these prophages in a gut environment, we colonized germ free mice with *E. coli* and *R. intestinalis*, and followed bacterial and phage populations in faeces for 30 days. We observed that after 10-20 days of colonization, a virulent mutant of Shimadzu emerges and multiplies on its host, its titer increasing until reaching a population level close to 1.10^{12} /ml. As Shimadzu reaches its maximum population, we observe a collapse of its host population. Following an arms race dynamic, bacteria resistant to infection by Shimadzu rapidly emerge and become dominant.

Here we show that a prophage is able to create a transient dysbiosis in a gut, with no external perturbation of the environment. Our results illustrate the impact that temperate phages can have on the bacterial populations in the gut microbiota.

[ID: 326] Phage therapy: beyond the antibacterial action

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In the past years our current understanding of the role of phages in nature and their potential in medicine has shifted from mere “viruses of bacteria” that influence the number and functions of bacteria and could be used to combat bacterial infections to the notion of an important component of our organisms abundantly present in the intestines, from where phages can migrate to blood and tissues. Thus, it is becoming evident that phages can interact not only with bacteria but also with eukaryotic cells. Research on the significance of such phage-eukaryotic cell interactions is at a very early stage. However, data obtained so far strongly suggest that phages may induce immunomodulatory effects which can be used in the clinic in phage therapy (PT). Moreover, these novel findings highlight the potential protective role of “endogenous” phages present in the human body (“natural phage therapy”). In this sense, a better understanding of mechanisms responsible for prophage induction in vivo and the significance of such phages may yield new and exciting data, including the possible role of hydrogen peroxide in regulating this process in health and disease. The doors to further research on phage–eukaryotic cell interactions are open wide. We do not suggest that phage therapy will be a panacea. However, the potential for broader application of PT is evident and it is certainly worthy of further studies (1).

1. Górski A. et al. *Frontiers in Medicine* 2018 (in press).

[ID: 433] Phage therapy – research and application at the Eliava Institute

Mzia Kutateladze

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G. Eliava Institute of Bacteriophages, Microbiology and Virology, Tbilisi, Georgia is a well-known center in the world for bacteriophage research and application. Study of causative bacterial agents of infection and elaboration of phage-based preparations against infectious diseases are the main focus of the Eliava Institute.

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

Main application of the Eliava phages is directed for treatment and prophylaxis of human bacterial diseases. Phages are successfully used to treat acute, as well chronic infections caused by antibiotic-resistant bacterial strains. The author will present several case reports after application of bacteriophages for treatment of various infectious complications.

[ID: 217] Phage therapy research in the Brussels military hospital

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The worldwide emergence of “superbugs” and a dry pipeline for new antibiotics threaten modern medicine with a return to the pre antibiotic era. Phages - the viruses of bacteria - could help fight antimicrobial resistance. In 2002, a first phage therapy related study proposal was submitted to the R&D department of Belgian Defense. It was dismissed as mere “science fiction” with a score of 4/20. Today, phage therapy research has become commonplace in the Queen Astrid military hospital and encompasses different aspects:

i) The isolation, selection and characterization of candidate therapeutic phages active against clinically important pathogens such as *Acinetobacter baumannii*, which is often associated with military operations in the Middle East (PMID: 25111143), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*, including the O104:H4 strain from the 2011 foodborne EAHEC outbreak in Germany (PMID: 23285164).

ii) Clinical trials:

- A small clinical safety study (PMID: 25356373): 10 applications of phage cocktail BFC 1 (PMID: 19300511), active against *P. aeruginosa* and *S. aureus*, in burn wound infections.
- PhagoBurn (www.phagoburn.eu), funded by the European Commission: Evaluating phage therapy for the treatment of burn wounds, infected with *E. coli* and *P. aeruginosa*, through a randomized controlled trial (results pending).

iii) Study of the bacterium-phage (host-parasite) relationship, with an emphasis on bacterial phage resistance evolution and the development of adequate treatment protocols (PMID: 22660719, PMID: 26476097).

iv) Elaboration of a dedicated regulatory framework for phage therapy (www.P-H-A-G-E.org), including realistic production and QC/QA regimens for therapeutic phage products (PMID: 21063753, PMID: 25585954, PMID: 29415431).

In the margin of these studies, and under the umbrella of article 37 (unproven interventions) of “The Declaration of Helsinki,” a small number of patients with multidrug resistant infections were treated with phages in the Brussels military hospital (PMID: 28583189).

[ID: 150] From a phage therapy trial to microbiome analysis in childhood diarrhea

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In a randomized controlled clinical trial T4-like coliphages or a commercial Russian coliphage product or placebo was orally given to Bangladeshi children hospitalized with acute bacterial diarrhea. No safety issues were revealed clinically and by functional tests. 60% of the children suffered from a microbiologically proven *Escherichia coli* diarrhea; the most frequent diagnosis was enterotoxigenic *E. coli* (ETEC) infections. Bacterial co-pathogens were also detected. Half of the patients contained phage-susceptible *E. coli* colonies in the stool, but *E. coli* represented less than 5% of fecal bacteria. Stool ETEC titers showed only a short-lived peak and were otherwise close to the replication threshold determined for T4 phage in vitro. Fecal coliphage was increased in treated over control children, but the titers did not show substantial intestinal phage replication. An interim analysis after the enrollment of 120 patients showed no amelioration in quantitative diarrhea parameter (stool volume, stool frequency) of phage-treated patients compared to standard care recipients (oral rehydration solution supplemented with zinc). The stool microbiota was studied by 16S rRNA gene sequencing. The microbiota was characterized by an overgrowth with *Streptococcus* belonging to the *Streptococcus gallolyticus* and *Streptococcus salivarius* species groups. Their abundance correlated with stool volume production, but genome sequencing of streptococcal stool isolates did not identify virulence genes. In a follow-up study fecal streptococcal dysbiosis was observed in all acute diarrhea pediatric patients from Bangladesh irrespective of diarrhea etiology (viral, bacterial, mixed infections). Only malnourished diarrhea children treated by WHO recommendation with antibiotics showed a dysbiosis with *Escherichia*. A subgroup of these patients displayed a massive endogenous coliphage production, which correlated with low fecal *E. coli* abundance.

[ID: 211] PhagoBurn: efficacy and tolerability of a phage mix produced according to Good Manufacturing Practices to treat *Pseudomonas aeruginosa* infected burn wounds

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PHAGOBURN was a multicenter randomized, double blinded, phase I-II, clinical trial to assess the efficacy and tolerability of a cocktail of 12 lytic phages against *P. aeruginosa* on severe burn wounds. PP1131, was produced according to good manufacturing practices and compared to the standard of care Silver SulfaDiazine (SSD). Patients with documented burn infections were randomized according to treatments and to potential antibiotic intake upon inclusion. PP1131 was applied onto the wounds for 7 consecutive days via an alginate interface (Algosteril™, Les Laboratoires Brothier). Bacterial burden was blindly evaluated daily by hospital microbiological via the “4 quadrants” semi-quantitative method.

The primary endpoint was the median time required to observe a sustainable reduction in bacterial burden of at least two quadrants. Twenty-seven patients were recruited from six European centers (July 2015–January 2017, including a six-month interruption): 25 were eligible and analyzed: 12 received PP1131 and 13 SSD.

Results showed that 144.3 hours (median time) was required with PP1131 to reach the primary endpoint versus 46.9 with SSD. However, patients received much lesser phages than expected (102 instead of 106 PFU/mL) because PP1131 titer dropped significantly after manufacturing but stabilized during the whole trial. An ancillary study showed that the bacteria isolated from patients with failed PP1131 treatment were resistant to such low phage doses. In addition, the initial bacterial load was higher in PP1131 versus SSD group (50% with very high load versus 15%). Supportive antibiotics to control other bacterial species and/or *P. aeruginosa* had no impact on each topical treatment efficacy.

PP1131 was very well tolerated and less often associated to serious adverse events: only one was reported with PP1131 versus 4 with SSD.

Despite a suboptimal MOI due to the low phage/bacteria ratio, PP1131 did reduce *P. aeruginosa* infection on severe burn wounds, however slower than SSD. Strain resistance to low concentrations of PP1131 at D0 correlated with phage treatment failure. Because of PP1131 stability issues and a poor inclusion rate, the trial had to be prematurely stopped. Next phage therapy evaluations should include a phagogram before patient inclusion, ensure the stability overtime of a cocktail and provide a sufficient titer to reach an ideal MOI.

Phagoburn was granted 3.85 M€ by the FP7 of the European Commission (grant #601857)

[ID: 398] The quest for phage genomic signatures

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The virome has been called the dark matter of the genomic sciences. As we continue to develop methods to access and characterize the genomic features of diverse environments we begin to acknowledge that the sequence diversity of viral sequences is extremely large, with over 70% of the sequences generated per project presenting no similarity to any data available on public databases. Recently, complete and partial viral genomes have been assembled from metagenomic studies, and efforts to create guidelines for such assemblies are currently being generated. However, even when we have partial or complete viral genomes, no much information can be extracted with currently available tools. This is partially due to the pace at which new available full genome sequences become available, which has been largely overpassed by the speed at which we generate new sequencing data. We are aiming at extracting genomic information from such assemblies that could give us insights into the biology and taxonomic characteristics of large sets of uncharacterized viral contigs derived from metagenomes. Our strategies are multiple, first, starting from the identification of conserved proteins and protein domains that could be used as markers and classifiers of specific viral taxons or viral groups, we use those domains to generate optimal HMMs that could be used to assemble and characterize previously unknown contigs. A second strategy targets the characterization of genomic composition and biases in viral and bacterial genomes, such strategy can be used to identify potential prophages within bacterial genomes, information that can be further used to characterize viral-host signature and thus be used to identify potential hosts from metagenomic assemblies. In summary, the development of tools and strategies that are independent of high sequence similarity will likely provide a framework allowing the characterization of large viral metagenomic datasets.

[ID: 191] What is ICTV doing to the classification of my phages?

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While we may not like change, philosophers, writers and scientists recognize that “There is nothing permanent except change” (Heraclitus of Ephesus, c. 535 – c. 475 BCE), “Progress is impossible without change” (George Bernard Shaw, 1856 – 1950), and “Intelligence is the ability to adapt to change” (Stephen Hawking, 1942 – 2018). The Bacterial and Archaeal Viruses Subcommittee of ICTV has seen major changes in the classification of phages with the introduction first of protein homology, and then DNA homology, to define taxa resulting in the 18 caudoviral genera in 2008 blossoming into >250 in 2018. This is in spite of the persistent belief that horizontal gene transfer blurs taxonomic boundaries. New techniques such as the GRAViTy pipeline, vConTACT 2.0 network-based viral taxonomy, GGDC (Genome-To-Genome Distance Calculator), VICTOR (Virus Classification and Tree Building Online Resource) and OrthoANI, together with the nucleotide homology analysis of all viruses by NCBI, will greatly assist in defining taxa. But, the official ICTV proposal system remains woefully slow. As a result, there are almost 6000 unclassified phage genomes in NCBI. Major effort has been expended to re-examine the classification of the N4-related phages, transposable viruses and phages currently part of the subfamilies Autographivirinae, Spounavirinae, and Picovirinae. These completed and ongoing studies have resulted in the realization that relationships extend beyond the morphology-based classification with the introduction of a new family – Saltoviridae – containing transposable siphovirus and myovirus representatives.

[ID: 52] The human virome: toward better diversity and functional annotation

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Human virome, including bacteriophages have received an increasing attention recently, owing to the rapid developments in human microbiome research and the awareness of the far-reaching influence of microbiomes on health and disease. Nevertheless, human viromes are still underrepresented in literature making viruses a virtually untapped resource of diversity, functional and physiological information. Here we present the human virome protein cluster database as an effort to improve functional annotation and characterization of human viromes. The database was built out of hundreds of virome datasets from six different body sites. We also show the utility of this database through its use for the characterization of three bronchoalveolar lavage (BAL) viromes from one healthy control in addition to one moderate and one severe chronic obstructive pulmonary disease (COPD) patients. The use of the database allowed for a better functional annotation, which were otherwise poorly characterized when limited to annotation using sequences from full-length viral genomes. In addition, our BAL samples gave a first insight into viral communities of COPD patients and confirm a state of dysbiosis for viruses that increases with disease progression. Moreover, they shed light on the potential role of phages in the horizontal gene transfer of bacterial virulence factors, a phenomenon that highlights a possible contribution of phages to etiopathology.

[ID: 46] K-mer approaches provide valuable insight into mobilome evolution in the domain Archaea

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Kmer approaches have greatly developed in recent years, largely driven by the advent of next-generation sequencing. Their speed and automatism (annotation independent) are major advantages. The interest of applying k-mer approaches to study the mobilome is only starting to be explored, with many efforts oriented towards viral metagenomics [1, 2]. We evaluated the potential of applying simple k-mer approaches to understand the evolutionary history of mobile elements by focusing on viruses and plasmids from the domain Archaea, which includes specific and well-defined extrachromosomal element families [3].

Selecting more than 470 cell, virus and plasmid genomes, we explored the dataset structure by multivariate and statistical analyses of genome tetramer profiles. Basic genome network analyses served as reference. Mobile element families, genome GC contents and host orders were identified as major explanatory factors of the tetramer profile distribution patterns. Genomes overall grouped according to the host order, except for haloarchaea who formed a single supergroup. Within each group, cells tended to cluster together while viruses and plasmids tended to cluster according to their own taxonomic family. This pattern likely results from the combined influence of co-evolution and environmental constraints, highly contrasted among those archaea which comprise methanogens and diverse extremophiles.

It confirms the potential of k-mer signal for extrachromosomal contig analysis [1, 2, 4], especially for their taxonomic assignment. Another application could be the detection of singular evolutionary trajectories by focusing on outliers. Indeed, previously-known and one new case of recent host transfers were efficiently detected. This approach relies on a distinct informational content than the more elaborate gene homology networks and appears as complementary.

References

1. Galiez C et al, *Bioinformatics* 2017, 33:3113-3114.
2. Iranzo J et al, *Journal of Virology* 2016, 90:11043-11055.
3. Ren J et al, *Microbiome* 2017, 5(1):69.
4. Krawczyk PS et al, *Nucleic Acids Research* 2018:gkx1321-gkx1321.

[ID: 98] Expanding knowledge of gene order conservation in viral genomes

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Viral genomes comprise a vastly diverse repository of genetic information. This diversity is achieved by high mutational rates and frequent genome rearrangements including horizontal gene transfer. As a result, viruses can share regions of high sequence similarity, while at the same time encompassing regions of distinct genetic content. This phenomenon is known as a mosaicism. Investigations of genome collinearity has revealed contrasting patterns, where phages showed preservation of the order of distantly-related genes and/or gene functions, while they demonstrated high mosaicism.

Here, we explore interrelationships between two processes: mosaic reorganisations (gene fluxes, deletions and exchange) and preservation of genome collinearity and gene order in naturally occurring viruses. We collected a relatively unbiased dataset of ~334 thousand viral genomes and genome fragments from metagenomes, mostly phages. We used gene content to group the sequences into ~24 thousand clusters, approximately representing different taxonomic genera. We identified sets of “core” and “accessory” genes for individual clusters and used the core genes as anchoring points to assess gene order conservation within each group.

We found conservation of global gene order in many groups. On several occasions, it was possible to assess conservation of gene order between distantly related clusters of phages. To investigate the possibility of gene flux in viral genomes with global gene order conservation, we also explored the consistency of individual gene phylogenies in comparison to the core gene super-alignment phylogeny. This revealed cryptic genome reshuffling, that was not detected in the gene-content analysis. Together, our results reveal gene order conservation across genus-level groups in metagenomes, sometimes masking dynamic genome evolution at the individual gene level.

[ID: 312] Exploring fungal DNA virus to control stem rot of oilseed rape caused by *Sclerotinia sclerotiorum*

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Sclerotinia sclerotiorum is a notorious fungal crop pathogen with wide host range. It secretes oxalic acid and plant cell wall-degrading enzymes to kill host cells and tissues. Recent researches revealed that *S. sclerotiorum* secretes small proteins as pathogenicity factors at the very early stage of infection; these comprehensive mechanisms of pathogenicity are likely to explain the powerful destructivity of this pathogen and the non-available of resistant cultivars. We try to explore hypovirulence-associated mycoviruses to control stem rot of oilseed rape caused *S. sclerotiorum*. Previously, we isolated a fungal DNA virus, SsHADV-1, from hypovirulent strain DT-8, and found this virus has strong infectivity since particles could directly infect hypha of *S. sclerotiorum*, and could spread horizontally among vegetative incompatible individuals; SsHADV-1 could mutualistically interact with a mycophagous insect, *Lycoriella ingénue* and use it as a transmission vector. These properties suggest SsHADV-1 has a great potential to control stem rot of oilseed rape. The present work was conducted to investigate the survival of SsHADV-1-infected *S. sclerotiorum* strain on plants in oilseed rape field after spraying of hyphal fragments and efficacy to control stem rot. The results suggested that SsHADV-1-infected strain could survive on plants well and control stem rot efficiently.

[ID: 9] Enthusiastic algal-virus-hunting days and afterwards - What is the raison de'tre of viruses?

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Studies on algal viruses had started thanks to some historical researchers' enthusiastic interests especially in drastic death or growth inhibition of host algae caused by viral infection; hence, the main research focus was located in "antagonistic relationship" between hosts and viruses; lytic activity, host specificity, genomic information, infection mechanism, ecological implication, etc. Consequently, to date, more than fifty viruses infecting various algal members (eukaryotic microorganisms) have been isolated and characterized to different extent, which have either ds- or ss- RNA or RNA genome. (Actually, I have no opinion but accept that they are so fascinating by their appearance alone!) On the other hand, molecular techniques and apparatus have recently showed significant progress, and in 2016, Urayama et al. improved a new technique (FLDS: Fragmented and loop primer Ligated DsRNA Sequencing) detecting both dsRNA and ssRNA viruses effectively. This highly functional technique provided researchers with chances to effectively discover "symbiotic relationship" between hosts and viruses in natural environments. These observations suggest a possibility that viruses are more "generous" to their hosts than expected; i.e., the typical scenario of viral infection "attachment - entry - replication - host cell death" might be just one aspect of functions driven by viruses. Then, what is the "real" raison de'tre of viruses? What functions do viruses have, and what roles do they play in nature? How can viruses coexist with their hosts in nature in the Earth's history? In the history of evolution, why each of them have allowed and accepted each other? We do not have much time; now it's already time to sincerely tackle these big issues together.

[Reference] Urayama et al. *Microbes Environ.* 2016;31(1):33-40. doi: 10.1264/j sme2.ME15171.

[ID: 399] Exploring cyanophage genomes by genetic engineering

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Marine cyanobacteria are highly abundant primary producers that have major ecological importance. Their viruses, cyanophages, influence largely on cyanobacterial ecology and evolution. Marine cyanophages have been a research subject for decades, including the isolation and sequencing of dozens of strains. Despite this, the lack of a genetic engineering method for these phages has limited our ability to explore the functions of their genes, and to gain understanding of their interactions with their bacterial hosts. Here we describe a simple, low-requirement method for genetic engineering of cyanophages, which can be adapted for other types of phages. Using this method we investigate lysogeny in T7-like cyanophages, an ecologically significant process of which little is known. Although members of the T7-like phages are typically lytic, many members of this group contain integrase genes as well as putative integration sites. So far, however, evidence for cyanophage integration into the host chromosome is scarce and no cyanobacterial lysogen has ever been isolated. We present first evidence that the T7-like cyanophage, S-TIP37, transiently integrates into the genome of its host during the infection cycle, despite behaving like a lytic phage. In addition, abolishing this process by deleting the integrase gene reduces the fitness of this phage. Taken together, these data suggest a non-canonical function of a phage integrase gene.

[ID: 60] Novel archaeal viruses from different anaerobic environments – isolation, characterization and environmental abundance

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In the last decade, the microbiome in anaerobic fermenters and biogas digesters has been in the focus of several studies. The research on the viral domain within these complex habitats is still lacking behind, as in many other environments. The major role of viruses for example in the marine system underlines the importance of viruses for complex environments (1). Their contribution to the turnover of metabolic intermediates, nutrients and trace elements by lysing microorganisms is currently not taken into account when analyzing the anaerobic degradation process.

Using isolation based and next-generation sequencing approaches, we aim to obtain an overall better understanding of the viral community, its diversity and potential virus-host relations. Isolated viruses infecting methanogenic archaea were evaluated, compared and put into context to the overall diversity observed in a metagenomic dataset obtained for the viral fraction of a biogas reactor.

The discussed viruses were isolated from anaerobic habitats rich in methanogenic host organisms (biogas and wastewater treatment plant). Isolation of single viral strains was achieved using liquid and plate based lysis assays depending on the growth morphology of the corresponding host organism. TEM was used for the morphological characterization of the viruses. The genome sequences of the viral isolates were generated using Illumina sequencing and subsequently assembled.

Until today only a few viruses infecting methanoarchaea have been isolated (2). Here we present a comprehensive investigation on a subset of viruses infecting methanogens from three different archaeal families. Our results also show that a large diversity of unknown viruses can be found in biogas digesters, which are therefore an excellent source for novel viruses.

1. Suttle, C.A. Marine viruses--major players in the global ecosystem. *Nature reviews. Microbiology* 5, 801–812 (2007).

2. Pina, M., Bize, A., Forterre, P. & Prangishvili, D. The archeoviruses. *FEMS microbiology reviews* 35, 1035–1054 (2011).

[ID: 157] Diversity of the most abundant human gut virus, crAssphage

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CrAssphage is the most abundant virus in the human gut microbiota of humans. Recently, taxonomic classification was performed on distantly related crAss-like phages from multiple environments leading to the proposal of a familial level taxonomic group (Yutin et al., 2017). Here, we assembled the metagenomic sequencing reads from 702 human faecal virome/phageome samples and obtained 98 complete circular crAss-like phage genomes and 145 contigs ≥ 70 kb. In silico comparative genomics and taxonomic analysis of 244 crAss-like phages was performed, resulting in the identification of 4 candidate subfamilies composed of 10 candidate genera. The detection and abundance of the different crAss-like phage genera varied by geographical location, but not between healthy and diseased cohorts within a country. CrAss-like phages of the proposed 'Candidate Genus I', containing crAssphage *sensu stricto*, form a homogeneous group of viruses. However, variation is observed in their predicted receptor binding proteins, which is expected to target individual crAss-like phages to their specific hosts. Work is underway to isolate bacterial hosts for crAss-like phages from the human gut microbiota. Subsequent characterisation of crAss-like phages will help determine the biological significance of this diverse and abundant gastrointestinal virus on human health.

[ID: 164] How to package DNA: lessons from archaeal viruses

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One of the most intriguing results of the research on viral diversity on our planet is the revelation of the special nature of DNA viruses infecting Archaea, particularly those thriving in extreme thermal environments at temperatures above 80°C [1, 2]. Unusual features of this group of viruses include incredible diversity of elaborate virion architectures — many of which have never been observed among DNA viruses of Bacteria or Eukaryotes— as well as their genetic content, which in some cases is literally terra incognita, without a single gene with homologs in extant databases.

Hyperthermophilic archaeal viruses are remarkable due to their capacity to withstand extreme conditions that are usually destructive for nucleic acids. Reconstruction of virion structures at high resolution helps to understand the molecular bases of such stability [3-5]. Moreover, the results shed light on the diversity of mechanisms of DNA packaging and virion morphogenesis, and contribute to the development of new materials for bionanotechnological applications.

References

1. Prangishvili D. 2013. The wonderful world of archaeal viruses. *Ann. Rev. Microbiol.* 67: 565-585.
2. Prangishvili D*, DH Bamford, P Forterre, J Iranzo, EV Koonin, and M Krupovic*. 2017. The enigmatic archaeal virosphere. *Nat. Rev. Microbiol.* 15: 724–739.
3. Ptchelkine D, S Gillum, T Mochizuki, S Lucas-Staat, Y Liu, M Krupovic, SEV Phillips, D. Prangishvili*, and JT Huiskonen*. 2017. Unique architecture of thermophilic archaeal virus APBV1 and its genome packaging. *Nat. Commun.* 8: 1436.
4. Kasson P, F DiMaio, X Yu, S Lucas-Staat, M Krupovic, S Schouten, D Prangishvili*, and EH Egelman*. 2017. An envelope of a filamentous virus carries lipids in a horseshoe conformation. *Elife* 6: e26268.
5. DiMaio F, X Yu, E Rensen, M Krupovic, D Prangishvili*, and EH Egelman*. 2015. A virus that infects a hyperthermophile encapsidates A-form DNA. *Science* 348: 914-917.

[ID: 351] How horses help us to ride phages: bacteriophage diversity, evolution and phage-host interactions in the equine intestinal community

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Our work was focused on the intestinal viromes of both domestic and feral horses (*Equus ferus caballus*). The digestive physiology and lifestyle of these animals make them a valuable model for research into bacteriophage ecology, evolution and diversity. The sequencing of the intestinal dsDNA viromes of horses indicates the presence of hundreds of viral genotypes that are extensively exchanged between animals making meta-viromes of local horse populations distinct from other geographically distant populations. This finding is in agreement with the results of our culture-based work on horse coliphages.

The most striking feature of the intestinal coliphage population within stabled horses is a dominance of virulent phages. Some of these phages are found to persist in the local population viromes for years and undergo extensive evolutionary adaptation, while at the same time the *E. coli* community of horse faeces features an extreme diversity at strain level. The mechanisms of long term persistence of virulent phages, many of which feature very narrow host ranges, remain largely enigmatic.

Comparative genomic analysis of ecologically linked coliphage strains, endemic for selected horse population, revealed that recombination and modular exchange are the major driving forces of bacteriophage genome microevolution in this natural, high density, microbial community. This is in striking contrast to the evolution via mutation accumulation that was observed in laboratory microcosms. The detailed characterization of multiple bacteriophages during this long-term project extended our understanding of such paradigmatic models as N4-like and T5-like bacteriophages. For both groups, we were able to describe novel organizations of adsorption apparatus, and novel strategies of cell recognition. These data create novel possibilities to develop these viruses into advanced tools for the biological control of bacterial populations (e.g. phage therapy) as well as for other applications.

The work was partially supported by RSF grant #15-15-00134-P

[ID: 381] Differential phage infection dynamics in the mammalian gut

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While exploring phage infections in the gastrointestinal tract (GIT) of mice our laboratory has shown on multiple occasions that a single dose of phages was sufficient to start a coevolution system that is ongoing for several weeks. Interestingly, we could not isolate phage resistant bacteria. Furthermore, by finding that phages display differential efficiencies in distinct sections of the GIT(2) we confirmed that some bacteria are not infected by the phages. Here we investigated how ecological or/and physiological bacterial host factors could modulate the efficacy of phage infections *in vivo*.

Taking advantage of a murine model of controlled microbiota composed of 12 strains (1), we evaluated the efficacy of a cocktail of three virulent bacteriophages to target a murine *Escherichia coli* strain. We found that this cocktail decreases *E. coli* numbers throughout the GIT. We also found an intriguing low abundance of phages in the adherent part of the ileum while the number of *E. coli* cells remained high. This population could act as a reservoir of bacteria from which *E. coli* could repopulate the GIT through source-sink dynamics, allowing both populations to co-exist at high densities.

Paralelly, we addressed the role of the physiologic state of the phage-targeted bacterial population by performing a comparative genome-wide RNA-sequencing analysis of a pathogenic strain of *E. coli*, both *in vitro* and *in vivo*, across different GIT sections. This analysis highlighted 147 candidate genes that were differentially expressed, amongst which some were previously reported as interfering with phage efficiency. These results confirm that the physiological state of bacteria within the GIT can affect the outcome of phage infection.

Therefore, our work shows that deeper comprehensive studies of phage infection dynamics in relevant environments are key to successfully design phage therapy treatments, or to design microbiota engineering strategies.

1. Brugiroux S. et al 2016

2. Maura D. et al. 2012

[ID: 65] Co-regulation of infective and defective prophages promotes *Listeria monocytogenes* virulence

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Most bacterial pathogens are lysogens, namely carry DNA of phages within their genome, referred to as prophages. While these prophages have the potential to turn under stress into infective viruses which kill their host bacterium in a matter of minutes, it is unclear how pathogens manage to survive this internal threat under the stresses imposed by their invasion into mammalian cells. Several years ago, we uncovered a novel pathogen-phage interaction, in which an infective prophage promotes the virulence of its host, the bacterial pathogen *Listeria monocytogenes* (Lm), via adaptive behaviour. More recently, we discovered that the prophage, though fully infective, is non-autonomous- completely dependent on regulatory factors derived from inactive prophage remnants that reside within the Lm chromosome. These findings lead us to expose intimate cross-regulatory interactions between different phage elements that promote bacteria-phage cooperative interactions, which are specific to conditions of mammalian infection. In this talk we will present our recent unpublished data.

Previous 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*, August; 150(4):792-802, 2012.
2. Feiner R., Argov T., Rabinovich L., Sigal N., Borovok I., and Herskovits A.A. A new perspective on lysogeny: prophages as active regulatory switches of bacteria. *Nature Reviews Microbiology*, 2015, Oct;13(10):641-50.
3. 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.

[ID: 261] Endogenous virophages in protists: indicators of a defense system against giant viruses?

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One of the major classes of viruses infecting protists are the so-called giant DNA viruses, with particle and genome sizes that overlap with those of bacteria. Giant viruses of the family Mimiviridae replicate in the host cytoplasm in a replication compartment termed the viral factory, which provides enzymatic functions usually restricted to the nucleus. This feature may have led to the evolutionary adaptation of a smaller class of DNA viruses called virophages (family Lavidaviridae), which use the cytoplasmic transcription machinery of the giant virus instead of the host machinery. Virophages thus strictly depend on a coinfecting giant virus, and they can inhibit the replication of giant viruses during a coinfection. This, in turn, benefits the giant virus-infected host cell population with considerable ecological consequences.

The virophage mavirus infects the marine phagotrophic flagellate *Cafeteria roenbergensis* and protects it against infection by the giant virus CroV. Although mavirus does not replicate in the absence of CroV, it can integrate its genome into the host genome, resulting in an endogenous virophage (or provirophage) that is transcriptionally silent and maintained by the host. Upon CroV infection, the endogenous mavirus becomes active and newly synthesized virophage particles inhibit CroV in subsequent coinfections. If the spread of CroV can be stopped before all flagellates are infected, the host population survives and new virophage endogenization occurs.

In order to gauge how widespread this virophage-mediated defense system is among natural host populations, we sequenced and assembled four *C. roenbergensis* genomes from the Atlantic and Pacific Oceans and found that all of them contained provirophages that were closely or distantly related to mavirus. We hypothesize that these provirophages are specific for and may provide defense against different strains of CroV-related giant viruses.

These findings suggest that virophages may provide long-term protection of marine phagotrophic flagellates against lytic giant viruses.

[ID: 171] Diversity of marine prymnesioviruses and their strategies for stable co-existence with their phytoplankton hosts

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Photosynthetic microorganisms form the basis of the marine food web, accounting for more than half of the primary production on Earth. Haptophytes, being key components within this phytoplankton community, play important roles both as primary producers and as mixotrophs that graze on bacteria and protists. Most haptophytes, such as species belonging to the Prymnesiales, are part of highly diverse communities and occur at low densities, which decreases their chance of being infected by viruses with high host-specificity. Nevertheless, viruses infecting both Prymnesium and Haptolina species (prymnesioviruses) have been isolated (Johannessen et al 2015), and several characteristics distinguish them from viruses infecting bloom-forming haptophytes like *E. huxleyi*. Phylogenetically, most of these prymnesioviruses fall into a distinct clade within the family Mimiviridae, which is a family consisting of giant viruses infecting heterotrophic and autotrophic protists. The diversity within the prymnesioviruses is huge with respect to genome size, gene variety, and infectious behavior, with different strategies employed for successful co-existence with their hosts in the ocean.

References:

Johannessen, T., G. Bratbak, A. Larsen, H. Ogata, E. Egge, B. Edvardsen, W. Eikrem, R.-A. Sandaa. 2015. Characterisation of three novel giant viruses reveal huge diversity among viruses infecting Prymnesiales (Haptophyta). *Virology*, 476:180-188

[ID: 259] Functional characterisation of a cyanophage encoded psbA.

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Our planet is almost three-quarters covered in water, and as such the oceans are an important component of many biogeochemical cycles. Very small single-celled organisms, the photosynthetic picoplankton, are vital for global climate regulation through the production of oxygen and consumption of CO₂. The two dominant marine phototrophs are the cyanobacteria *Synechococcus* and *Prochlorococcus*, which can be responsible for as much as 70% of primary productivity in some regions.

Cyanophages, viruses that specifically infect cyanobacteria, affect host picoplankton assemblage diversity through lysis and in the same way cause the release of organic matter back into the system. More recently, cyanophage have been shown to directly inhibit their hosts ability to fix CO₂, thought to be through the possession of auxiliary metabolic genes (AMGs). These AMGs include key components of the core photosystem II complex e.g. *psbA*, that encodes the rapidly turned over D1 protein. The viral copy of *psbA* is known to be expressed during infection and host photosynthesis is maintained. In addition, the viral version of *psbA* contains a self-splicing intron that is known to be differentially spliced as light conditions change and may act as a regulatory mechanism.

As cyanophages are currently genetically intractable, we have used a heterologous system to study the function of the cyanophage encoded *psbA*. We have successfully expressed cyanophage *psbA* in the cyanobacterium *Synechocystis*, allowing for the characterisation of the viral copy of the gene and its effects on host photosynthesis. Here we present the results from this work, and the implications this could have on global biogeochemical cycles.

[ID: 112] Viral dynamics within three aquifers of differing ages and origins in the Fennoscandian shield terrestrial deep biosphere

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The diversity and functionality of viruses within the deep biosphere as well as the impact they have on their microbial hosts' remains largely unknown. Here, we have investigated the anoxic groundwater contained within three boreholes (depth 181 – 448 meters) in the Åspö Hard Rock Laboratory, southeastern Sweden, containing newly infiltrated (1000 years old (old saline). Duplicate metagenomes from 0.2 µm-filtered water from each borehole were individually assembled and after removing sequences of likely cellular origin, 4051 viral contigs containing >10 ORFs were further analyzed. Of all ORFs, 40% showed similarity to proteins in NCBI nr, although only 3% of the best matches were viral. Comparing the assembled sequences to Refseq viral database, 23% of the ORFs had similarity to known viruses, with 9% similar to Siphoviruse, 7% to Myoviruses, and 4% to Podoviruses. Furthermore, 40% of their genes with previously sequenced viruses within the same viral family, pointing towards novelty within these viral communities. Recruitment of all reads to the contigs showed that 14% of the contigs occurred in all three aquifers. Interestingly, of all contigs occurring in the undefined mix, 98% was shared with the old saline water and only one viral type was unique for the undefined mix. The other two water types, on the other hand, contained 8% (old saline) and 29% (modern marine) of unique contigs. Among interesting auxiliary metabolic genes, the presence of assimilatory sulfate reduction genes and iron scavenging genes occur mainly in the undefined mix and old saline, environments characterized by low organic sulfur and iron content. Overall, these data present unique information regarding the viral diversity, functionality, and spatial variations within the deep biosphere.

[ID: 208] Cool tools made by evolution - how bacteriophage enables rapid diagnostics and efficient control of pathogenic bacteria

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Bacteriophages have been shown to be very useful as biocontrol agents for various pathogens, including *Listeria*, *Salmonella*, *Staphylococcus* and others. We have recently shown that application of carefully selected naturally occurring phage for infection and subsequent killing of undesired target bacteria can be significantly expanded by design and engineering of synthetic bacteriophages, an approach with almost endless possibilities. Another promising strategy is the use of recombinant phage-encoded cell wall hydrolases, which are highly specific and fast-acting antibacterial agents. Recent research from our group is aimed to modify and optimize endolysins for applications in very different environments, from plants disease to animal and human health.

Considering to the need for rapid diagnostic procedures, the use of bacteriophage affinity proteins such as endolysin-derived cell wall binding domains and tail fibers emerged as a superior alternative to the use of antibodies, especially in conjunction with immobilization on solid surfaces such as magnetic beads or sensor fibers. While CBD domains are highly suitable for Gram-positive bacterial cells, the tail fiber adhesins from phages infecting Gram-negative hosts offer the potential to expand this technology platform. Both approaches allow highly specific and very efficient recognition, immobilization, separation and detection of individual bacterial targets. To increase sensitivity and speed, initial capture and immobilization may be followed by a second, sandwich-like application of phage proteins, where the phage-derived affinity carriers are modified to carry fluorescent reporter molecules or enzymes for signal amplification.

In conclusion, phages and their components are not only suitable for biocontrol purposes, but also serve as a perfect toolbox for harnessing the specificity of phage-host cell interactions for rapid diagnostics and detection.

[ID: 332] The impact of horizontal gene transfer on the evolution and function of cellulose-degrading microbial communities

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Horizontal Gene Transfer (HGT) drives the evolution of microbial communities through the dissemination and amplification of DNA. To date most studies have investigated HGT using a comparative genomic approach of individual isolates against previously sequenced genomes to construct phylogenetic networks of HGT events. While these studies have provided invaluable insight into our understanding of the ecology and evolution of HGT, direct experimentation investigating the process and functional impact of HGT on microbial communities is lacking. Here we designed an experimental regime to explore the impact of HGT on the dynamic of molecular and phenotypic evolution in cellulose-degrading microbial communities over the course of one year. Ten founding communities were established by incubating compost with minimal media and providing cellulose paper as the sole carbon source. Following establishment, the founding communities were split into two transfer regimes: vertical and horizontal. In the vertical regime each of the ten communities were homogenized every two weeks and transferred to fresh medium with a new piece of cellulose paper. In the horizontal regime each transfer involved the founding microbial community as well as a mixed cocktail of "phage juice" from all ten bottles, thus providing the opportunity for genes to move between horizontal but not vertical communities. Using comparative metagenomics, we provide evidence for large-scale movement of genetic material between horizontal bottles that involves genes with various predicted functions including iron acquisition, virulence factors, transcription, and individual phage genomes. As a general proxy for community function we also measured the ammonia concentrations during the course of the two-week regime. Surprisingly, we found that the majority of horizontal communities had significantly higher ammonia production compared to their vertical counterparts. To our knowledge these data describe for the first time the emergence of a functional impact of HGT on a complex microbial community through direct experimentation.

[ID: 250] Bacteriophage biodistribution and infectivity– from honeybee to bee larvae

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Bacteriophages have been extensively exploited as biocontrol agents to fight animal and plant bacterial diseases. They offer many advantages compared to antibiotics, in the control of American Foulbrood (AFB). This is a disease caused by *Paenibacillus* larvae, a spore-forming Gram-positive bacteria. It affects honeybee larvae and occurs through the transmission of highly resistant spores that easily spread across apiaries. The appropriated control measure is to burn contaminated hives, which cause serious ecological and economic losses. The use of antibiotics is discarded due to bacterial resistances and to restrictions of European legislation.

Though phages capable of controlling *P. larvae* have already been discovered, their biodistribution in adult bees and bioavailability to young larvae has not yet been determined. The present *in vivo* study investigated the ability of a T7 phage to reach larvae in an infective state after oral administration to honeybees, as this strategy is considered the most feasible toward hive management. Phages were administered in the bee food at 1×10^9 PFU.mL⁻¹ and bees/larvae samples were collected 24 h post-administration. The screening (by direct PFU count) and quantification (by real-time PCR) of T7 phage in bee organs and in larvae after ingestion revealed that phages were successfully uptaken by bee, were transported in their internal organs and reached larvae through the bee-larvae feeding chain. However, considering the total amount of particles detected in larvae (ranging 10^4 phages), a very low quantity was recovered in an infective form, able to reduce *P. larvae* load and to control AFB (in average 32 phages were infective). Their fast inactivation in royal jelly is pointed herein as a potential threat to this therapeutic approach, and therefore, the improvement of the oral delivery effectiveness in the AFB therapeutic might be achieved by the development of phage protection strategies from general hive-derived conditions.

[ID: 20] Mycoviruses of *Monilinia* fungi, causal agent of brown rot in stone fruit

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Monilinia laxa and *M. fructicola*, the causes of brown rot in stone fruit (*Prunus* species) and some pome fruits, were first described in Western Australia in 1997. Since then they have spread to all stone fruit-growing regions in the State where it is mainly controlled by application of the fungicides. The discovery of fungal viruses (mycoviruses) of chestnut blight with hypovirulence and their successful application as biocontrol agents has inspired us to seek the hypovirulence-associated mycoviruses in *Monilinia*.

A hundred and fifty *Monilinia* isolates, both *M. laxa* and *M. fructicola*, was collected from stone fruit orchards around Western Australia. Sixty percent of the isolates analysed contained double-stranded RNA (dsRNA) elements with the various number and size among isolates. When the purified dsRNA samples were deep sequenced by a next generation sequencer, 10 virus-like assembled contigs with the predicted amino acid sequences were found. They were homologous to the RNA dependent RNA polymerase (RdRp) and Coat Protein (CP) of *Sclerotinia sclerotiorum* Hypovirus with 92% identity. This is the first finding of a known mycovirus of *Monilinia* fungi.

The influences of mycoviruses on fungal morphology *in vitro* and on fungal virulence *in planta* will be tested by eliminating (curing) fungal isolates of mycoviruses. We will also study on the transmission of mycoviruses in the fungus on both ascospores (sexual spores) and conidiospores (asexual spores). A major outcome of this research will be an understanding of whether *Monilinia* mycoviruses might be employed as a biological control of brown rot in stone fruit.

[ID: 226] Broad-spectrum antiviral agents for phage infections in industry

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Bacteria-based processes are one of the most important in biotechnology and dominate a number of branches of industry. They exploit natural metabolic capabilities of bacteria to produce drugs, antibiotics, probiotics, vaccines, insecticides, enzymes, fuels and solvents. Possibility of genetic modification broadens the application of bacteria in food industry, agriculture and medicine. Therefore, all factors, which may cause closures of bacteria-based factories, cause millions of dollars in losses. One of most dangerous are bacteriophages. Even a single virus can destroy the whole production line by creating billions of its own copies inside bacteria in very short time. Such mechanism makes fight against phage-based contamination extremely difficult.

We designed antiviral agents that can be used as additives for bioreactors to prevent phage infections. Bacteriophages are prone to changes in ionic strength, i.e. fluctuations of concentration of charges around them. We used gold nanoparticles covered with ligands composed of proper linker and charged chemical groups. Such nanoparticles had virustatic or virucidal properties and at the same time remained harmless for bacteria inside bioreactor. We tested phages T4, T7 and M13, as they have different structures and types of genetic material inside. For comparison we checked that non-charged nanoparticles caused the opposite effect, i.e. stabilized phages.

To understand the nature of interactions between phages and nanoparticles, we used cryo-TEM and analytical ultracentrifuge techniques. There were no visible complexes of phages and non-charged nanoparticles, suggesting that stabilization effect depends rather on weak interactions. We expect the opposite effect in case of virucidal particles that should interact with phages strongly.

[ID: 343] Phage therapy in localized infections

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Since 1930-40, bacteriophages have been extensively used for dysentery and typhoid prophylaxis and treatment in the former Soviet Union. When bacteriophages against major causative agents of suppurative inflammation were identified, they have been also applied in surgical practice to treat purulent wounds and post-operative infectious complications. After the advent of various antibiotics, the use of bacteriophages in surgery was significantly decreased in Russia, but not stopped as antibiotic treatment sometimes failed even with antibiotic-sensitive bacteria. The emergence of multidrug-resistant bacteria revived the interest in phage therapy as a possible alternative to traditional antibiotics. The results of phage therapy and complex therapy including phage and antibiotic treatment in clinical practice in Russia will be presented. Data on application of bacteriophages against localized infections such as wound and postoperative infections, burn infections and infections associated with trophic ulcers, including diabetic foot ulcers, will be described.

[ID: 31] Combatting Lyme disease: phages for diagnosis and treatment

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Lyme disease (LD) is caused by the bacteria *Borrelia burgdorferi sensu lato* and is transmitted to humans through the bite of infected ticks. LD is the most commonly reported tick-borne disease in the United States with estimation of more than 300 000 cases annually by the CDC. In Europe, the number of LD has increased steadily with an approximation of 85 000 cases every year.

The current diagnosis of LD is based on clinical presentation. The FDA-approved laboratory diagnosis is serological test, which cannot detect early LD (ELD) and is too low in sensitivity, missing around 50% of patients. Early diagnosis is vital because ELD can be treated effectively with antibiotics but is harder to treat if the infection is allowed to develop into late LD (LLD). In addition, studies of *Borrelia* infections in animal models revealed the presence of *Borrelia* after antibiotic therapy, which suggests that antibiotics may be unable to eradicate *Borrelia*.

Phages have been investigated for diagnosis and treatment of many types of bacterial infections, except *Borrelia*. We developed and validated a highly sensitive and reliable qPCR assay targeting *Borrelia* phages to diagnose LD. To evaluate the performance of the qPCR relative to the serological test, 222 LLD samples were examined. The qPCR yielded positive results from 200 (90%) samples. In contrast, a total of 56 (25%) samples were positive by serological test, which were also positive by the qPCR. Additionally, 7 (50%) out of 14 serological negative ELD samples showed positive by the qPCR. Further evaluation was conducted against ~1000 ticks collected from ~200 geographical locations throughout the UK. An average of 38% of ticks were positive by the qPCR. To characterise *Borrelia* phages and phage-encoded holins and endolysin, we established methods for phage isolation and in vitro assay to measure lytic activity against *Borrelia*.

[ID: 290] Phages to treat and control cholera in the Democratic Republic of Congo

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Cholera is still a public health issue. Given the antibiotic-crisis and the lack of performant vaccinations, bacteriophages (phages) could be helpful.

Phages (55 Environmental/Clinical, Haiti, 2016) were isolated via plaque purification and spotted against bacterial strains. Titer was normalized to 10⁸, clearings scored based on appearance. Effect of temperature (35°C, 37°C, 40°C), pH (5, 7.5, 8) and salinity (NaCl: 0%, 1%, 3%) on phage adsorption (Φ26P and Φ37B) was determined by performing plaque assay analyses. Two organically extracted genomes were sequenced (Illumina technology). Bacteria (56 Environmental/Clinical, 2014-2017) from diverse DRC-areas were classically isolated- ref: O395 (O1/classical). Species confirmation and strain diversity were assessed through MALDI-TOF-(Bruker) and rep-PCR-fingerprinting (DiversiLabTM-BioMérieux) respectively; antibiogram was determined via (Kirby-Bauer)-diffusion method and agglutination in antisera to type-specific-O-antigens was used for the serotyping.

MALDI-TOF confirmed 77% of isolates as *Vibrio cholerae*. Serotypically they were O1, mostly Inaba. Genotyping revealed high similarity (>97%). Antibiogram (n=19) showed resistance to Cotrimoxazole (95%), Ciprofloxacin (21%), Chloramphenicol (11%), but not to Ampicillin, Cefotaxime and Tetracycline. Phage lytic-spectrum: φ6 lysed 48.8% strains + O395 with medium intensity (score 3: turbid; 4: mostly clear/ halo); φ20S and φ20B lysed 4.7% strains + O395 with medium intensity and 95.3% strains with high intensity (scores 5: clear/ resistant colonies; 6: clear); φ26S and φ26B lysed 2.3% and 97.7% strains + O395 with medium and high intensity, respectively; φ26P and φ37B lysed 4.7% and 95.3% strains + O395 with medium and high intensity, respectively. Host-range analysis: 5 types of phages seem to be present. Genome annotation shows that φ26P and φ37B are highly similar to *Vibrio*-virus phiVC8 and VP2 placing them into the Vp5virus genus; they harbour IG-like domains and no toxin neither AB-R genes were detected. Those results are promising for the potential phage-treatment and biocontrol of *V. cholerae*.

[ID: 92] Getting from bench to bedside: addressing challenges for clinical development of phage products

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A century of reported successful treatment of recalcitrant bacterial infections supports the general belief that bacteriophage therapy can be safe and efficacious. However, the shortage of randomized, blinded, placebo-controlled clinical trials creates healthy skepticism about overall efficacy and whether phage therapy can be implemented in widespread clinical practice. Running such trials involves a number of scientific and practical challenges. In addition to regulatory and logistical requirements for thorough phage characterization and consistent manufacturing to scale, the design of trials themselves can be difficult. Classical challenges for investigating the clinical efficacy of an investigational product include endpoint and comparator selection as well selection of inclusion and exclusion criteria that can affect enrollment rates. Novel challenges for phage therapy include the development and validation of new assays for unique outcome assessments and the identification of optimal dosing regimens without the benefit of the PK/PD methods typically used to bridge animal and human antibiotic dosing.

As of January 2018, seven patients with serious or life-threatening infections not responding to antibiotic therapy had been treated with AB-SA01 (3-phage product targeting *S. aureus*) or AB-PA01 (4-phage product targeting *P. aeruginosa*). Clinical indications included bacteremia, endocarditis, and lung infections. Four patients received a total of 90 IV doses of AB-SA01. Three patients received a total of 402 IV and 92 nebulizer doses of AB-PA01. Among these patients, 28-day all-cause mortality was 14%, versus the 46% predicted by APACHE II scores prior to phage treatment. No treatment-related adverse events were reported and physician assessments were that 86% of patients experienced significant improvement or complete resolution of baseline signs and symptoms. We will present data from individual treatment cases, discuss scientific and CMC (Chemistry, Manufacturing, and Controls) considerations for clinical phage therapy, and consider certain patient-specific investigations that may point to useful assessment methods for future clinical trials.

[ID: 319] Functional characterization of LysinA&B from a New Delhi Mycobacteriophage-PDRPxv, virulent against *M. tuberculosis*.

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Lysins, considered as promising anti-bacterials, are the enzymes used by bacteriophages to release their progeny from the bacterial host. Advantages of lysins over antibiotics include their specificity, ability to kill both dividing and dormant bacteria and remote possibility of occurrence of bacterial resistance. Mycobacteriophages use two lysins: LysinA that destroys the peptidoglycan and LysinB, a mycolylarabinogalactase esterase, which breaks the connection between the mycobacterial outer membrane to the cell wall.

Not many reports on mycobacteriophages from India are available, and none on their lysins. We isolated 17 mycobacteriophages from local environment and found PDRPxv phage, isolated from New Delhi, to be lytic against H37Rv and drug-resistant *M.tuberculosis*. Here, we present characterization of lysins from PDRPxv. LysinA has a modular structure similar to that of D29. However, it shows an unusual propensity to be a secretory protein as evident by its periplasmic expression. Lysin B (shows α/β hydrolase structure and a conserved GXP motif) shares only 32% amino acid sequence identity with D29 lysinB and has a unique long stretch of glycine rich region at the C-terminal (which on analysis, was found to be exclusive to phages B1 sub-cluster phages), resulting in much larger protein (46 kDa) in comparison to D29 (25.4 kDa).

Anti-mycobacterial activity of purified PDRPxv lysinA and lysinB (each at a concentration of 35 $\mu\text{g/ml}$) by turbidimetric reduction assay showed about 49% *M.smegmatis* (OD600 of 0.6) lysis and the specific lipolytic activity of LysinB (p-nitrophenyl butyrate as substrate) was 0.56 U/mg. We believe these findings to be encouraging, highlighting further the importance of exploring the potential of local mycobacteriophages and their derived enzymes for anti-mycobacterial activity.

1. Borysowski, J., et al., Bacteriophage endolysins as a novel class of antibacterial agents. *Exp Biol Med* 2006. 231(4): p. 366-77.
2. Hatfull GF. Mycobacteriophages: Windows into Tuberculosis. *PLoS Pathog* 2014;10:12–4.

7. ABSTRACTS POSTER SESSIONS
– ALPHABETICALLY (FIRST AUTHOR)



[ID: 194] Phage therapy annotated glossary

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Bacteriophages, or phages, are the viruses of bacteria. Bacterial viruses have been used as antibacterial agents, including clinically, approximately since their discovery, now over 100 years ago. In this age of increasing antibiotic resistance, along with concerns over the health impacts of unintentional microbiome modification due to the use of relatively broad spectrum antibiotics, the idea of using comparatively narrow-spectrum, diverse, and abundant bacteriophages as antibacterial agents has come back into fashion. In fact, the use of phages clinically as antibacterial agents never completely went away, and has otherwise been used over the decades by apparently millions, particularly in the former Soviet Union. In the course of these efforts, a certain terminology has developed either in association with phage therapy or as has been coopted from more general phage biology to the use of phages as antibacterial agents. Many of these terms and associated concepts, however, are relatively obscure or, in many cases, seemingly misunderstood. Consequently, I have generated a list of phage-therapy relevant terms and definitions, along with associated discussions of phage therapy from the perspective of its terminology, all as written from a pharmacological perspective. The unpublished manuscript version of this material can be found here:

http://www.phage-therapy.org/postings/Phage_Therapy_Annotated_Glossary.pdf

[ID: 101] Exploring enzymatic and antitubercular activity of novel LysB enzymes

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Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), is considered a health threat due to the highly emerged resistance rates [1]. In 2016, WHO reported 10.6 million new TB cases with 1.3 million mortalities out of which 40% are multidrug resistant TB (MDR-TB) [2].

The critical feature of Mtb is their unique cell wall structure comprising peptidoglycan, arabinogalactan and mycolic acid (MA) layers [3]. MA is a long chain fatty acid (C60–C90) which is important for cell viability, imparting hydrophobicity and antibiotic resistance of Mtb [4].

Mycobacteriophages possess two endolysins, Lysin A (LysA) a peptidoglycan hydrolase [5] and Lysin B (LysB) a lipolytic enzyme that cleaves the ester linkage of mycolic acid to the arabinogalactan layer [6].

In the present study we have mined full sequences of 1142 *mycobacteriophages* for putative lysB genes using the crystallized LysB D29 as a template. The resulting sequences with similarities ranging from 30 to 90% to LysB D29 have been grouped into seven clusters. One gene representing each cluster was selected, cloned and expressed in an *Escherichia coli* host.

The purified enzymes are currently being tested for esterase and lipase activity against Tween 80, different synthetic p-nitrophenyl substrates (C4 – C18), and the mycolyl arabinogalactan layer extracted from *Mycobacterium smegmatis*. Subsequently, their antibacterial activity will be determined against *M. smegmatis* viability.

References

- [1] Muller, B. et al. (2013), *Trends Genet*, 29(3), 160-9.
- [2] W H O. (2016), *Global Tuberculosis Report*.
- [3] Neyrolles and Guillhot. (2011), *Tuberculosis (Edinb)*, 91(3), 187-95.
- [4] Jarlier and Nikaido. (1990), *J Bacteriol*, 172(3), 1418-23.
- [5] Hatfull, G.F. et al. (2006), *Plos Genetics*, 2(6), 835-847.
- [6] Gil, F. et al. (2010), *Microbiology-Sgm*, 156, 1497-1504.

[ID: 405] The use of microencapsulation to increase the resistance of phages to physiological conditions

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One of the most critical problems in health today is the increasing difficulty in treating bacterial infections due to antibiotic resistance in pathogenic bacteria. This led researchers to seek alternative treatment methods for antibiotics. Among these alternatives phage therapy using bacteriophages specifically infecting bacteria become prominent. Although phage therapy has been successfully applied both in vitro and in vivo, one of the biggest concerns in this regard is the stability of phages in body environment. Within the scope of this study, microencapsulation technology was used to increase the resistance of phages to physiological conditions, and the resulting microcapsules were tested in environments simulating body conditions. For this purpose, *Bacillus subtilis* and *Salmonella enterica* subsp. *enterica* serovar *enteritidis* phages were isolated from different sources and then microencapsulated with 2% (w/v) sodium alginate by using spray dryer to minimize damage of physiological environment. Stability of microcapsules in simulated gastric fluid and bile salt presence was controlled. As a consequence, it has been observed that this method significantly increased the stability of phages in simulated gastric fluid and they could maintain their viability at high titers after 2-h incubation while free phages have lost their viability even after 15-min incubation. Similarly, microencapsulation was found to increase stability of phages in the bile salt medium and it was determined that the difference between the titers of microencapsulated phages and free phages as a result of 3-h of incubation could reach up to 3-log units. When the results obtained from this study were compared with the results obtained from studies using other microencapsulation techniques, it is found that microencapsulation by spray drying is much more effective in protecting phages from the stomach environment.

[ID: 53] Transplantation of faecal phage in a model fermentation system

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Our understanding of the human microbiome and its importance in health and wellbeing has been in focus for at least a decade. Evidence continually emerges which opens new avenues of research that can help untangle the complex interplay between hosts and their microbial counterparts.

Although microbes can be found in and on almost every surface of the human body, the majority reside in the digestive tract, harboring up to 10¹² cells per gram faecal material in the colon. Bacterial cells are also estimated to have many times more functional genes than human cells. A less studied part of the gut microbiome are the bacterial viruses called bacteriophages (phages). Their importance lies not just in the sheer number of viral particles (perhaps up to ten times more than bacteria), but in their capacity to lyse their hosts, or incorporate their genetic material into the bacterial chromosome.

Numerous human intestinal diseases are strongly associated with disruptions in the gut microbiome. Also, a new line of treatment for *Clostridium difficile* infection is the so-called Faecal Microbiota Transplant (FMT) that uses faecal material from healthy donors to restore a 'healthy' gut composition, thus making this topic more relevant.

In our study, an in vitro faecal fermentation system was used to investigate changes in microbial composition (via 16S NGS amplicon sequencing) and in viral enriched fractions (sequenced on Illumina HiSeq platform) as a result of high-titer phage addition solely or in combination with their host. We successfully demonstrated the capability of externally added faecal phage to change bacterial composition in our reactor setup during the time frame of the experiment.

These results can have a pivotal role in understanding the interactions in the gut microbiota, and eventually to shed more light on the vastly uncharted "viral dark matter" part of the healthy human phageome.

[ID: 8] Prolate capsid phage with lytic activity on extended spectrum beta-lactamase and avian pathogenic Escherichia coli strains from chickens

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Avian pathogenic Escherichia coli (APEC) and human uropathogenic Escherichia coli (UPEC) share similar genetic relatedness. These strains were implicated in causing human and poultry diseases. The two showed great overlap based on their sero-phylogenetic groups and virulence genotypes. The ability of APEC to spread to human beings, and its capability to act as human UPEC or its potential to act as the reservoir of virulence genes for UPEC has been discerned by genomic studies. Recent reports showed that APEC was incriminated in human urinary tract infections (UTI) which is most common bacterial infections causing significant morbidity and mortality and huge economic burden on the healthcare system worldwide. Also, extended-spectrum beta-lactamase (ESBL) producing Escherichia coli that were resistant to third generation cephalosporin has been isolated from poultry products. These pathogens are a great threat to public health and food safety, and poultry (food chain) is believed to be the source of these pathogens causing diseases in human. Our objective was to isolate and characterize bacteriophage for the biocontrol of APEC and ESBL Escherichia coli in chicken from farm to table. A bacteriophage specifically lytic to APEC and extended-spectrum beta-lactamase producing Escherichia coli strains from the chicken was isolated by simple enrichment, soft agar overlay and incubation at 37°C for 24 hours. Morphological characterization by transmission electron microscopy (TEM) revealed the phage as a prolate capsid bacteriophage of the family siphoviridae, It is concluded that this bacteriophage may be useful in the development of biocontrol intervention of APEC and extended-spectrum beta-lactamase producing Escherichia coli strains in the food chain.

[ID: 26] Isolation of novel phages from micromammals intestinal tracts in Abidjan, West Africa

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Phage Therapy is a tool for fighting bacterial infections using lytic bacteriophages. In a global context of bacterial multi-drug resistance emergence, phages become a biological alternative to treat bacterial infections. In West Africa, micromammals are important reservoirs of bacterial and viral zoonosis, making them probable reservoirs of bacteriophages that can be used for phage therapy. The objective of this study is to isolate and to characterize, for the first time, phages from rodent intestinal tracts.

Samples were collected from Shrews, *Crocidura* spp. and *Mastomys* spp. captured in 6 geographical areas of Ivory Coast. These samples have undergone tests for isolation and for amplification of phages. The bacteria strains have been confirmed by using the API 20E gallery to determine the phages hosts. Resistance of bacteria strains was determined by using the antibiogram test. The virulence of the isolated phages was tested on a set of 23 environmental bacterial strains including multi-resistant clones. Finally, the spectrum of phage and antibiogram test were determined for isolated phages.

12 novel phages from micromammals were isolated. The virulence of phages shows lytic activity for bacteria of the genus *Citrobacter*, *Escherichia*, *Enterobacter*, *Klebsiella*, *Proteus* and *Serratia*.

The host range of phages on bacteria strains shows a spectra that ranged from 13.04 % for the lowest to 95.65 % for the broadest. For multi-drug resistant bacteria, phage activity is effective from 41.67 % to 100 %. This study has first demonstrated novel phages from rodents that can be potential candidates for phage therapy.

Keywords: Phage Therapy, Multi-drug resistance, Micromammals, Rodents, West Africa.

[ID: 231] The diverse ECOR collection promotes isolation of novel E. coli phages belonging to 8 different genera

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E. coli is a highly diverse bacterial species comprising both commensal and pathogenic strains, yet this diversity is usually not taken into account when isolating *E. coli* phages. The ECOR collection consists of 72 wild-type strains originating from humans and other mammals, and was previously shown to cover the genetic diversity of *E. coli*. Here we aimed to capture the diversity of *E. coli* phages in the environment by using the ECOR collection for phage isolation.

Using all 72 strains of the ECOR collection, we screened samples from surface water, wastewater, and animal sources for plaque formation, leading to isolation of 64 phages. Phage genome sequencing and comparative genomics showed that the phages belong to eight genera. Phages within the same genus showed high similarity, still with differences in the tail fiber and tape measure protein genes. Additionally, many phages with only minor SNPs were repeatedly isolated from different environments, suggesting their widespread presence in nature. To identify receptors, we selected phage resistant colonies using a transposon library and tested for phage infectivity using deletions mutants of the receptor genes. Interestingly, phage genus and receptor correlated: T1virus (FhuA), T4virus (OmpC), T5virus (FhuA), FelixO1virus (OmpF), Sp6virus (O-antigen), Seuratvirus (O-antigen), HK578virus (FhuA) and PBunavirus (could not be determined). Plaque assays revealed that 28 ECOR strains were sensitive to phages, but most served as hosts for few phage genera, while only a few were sensitive to the majority of phages. Within a phage genus we observed minor differences in host range, indicating that internal phage resistance mechanism may influence phage infectivity. In summary, our approach led to isolation of several new phages belonging to eight different genera and demonstrated large variation in phage sensitivity within the ECOR collection.

[ID: 383] Synergetic effect between phage and antibiotics as an alternative strategy to control *Pseudomonas aeruginosa* biofilm associated infections

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Bacterial biofilms are sessile microbial aggregates with unique community properties, showing a high degree of tolerance/resistance to disinfection by chemicals, antibiotics, and to the human immune system. The opportunistic pathogen *Pseudomonas aeruginosa* is one of the most frequent causes of biofilm-associated infections, causing infections extremely difficult to treat. Currently, bacteriophages (phages) are becoming a potential solution for the treatment of such infections. In this study, we aimed at assessing the combined effect of a *P. aeruginosa* phage vB_PaM_EPA1 (myoviridae) and antibiotics to control *P. aeruginosa* biofilms. Phage and antibiotics were simultaneously or sequentially (antibiotics were added after of 6 hours of phage action) added to biofilms. After 24-hour treatment, bacterial survival was measured. Results showed that some phage-drug combinations greatly reduced bacterial densities, ranging from 2.6 to 3.7 orders of magnitude. Furthermore, we observed a biofilm eradication with sequential treatment by phage and gentamicin. Overall, our results show that combination of phages and antibiotics are very effective against *P. aeruginosa* biofilms when applied sequentially, and this constitutes a good strategy to control biofilm-associated infections.

1. Chaudhry, W. N., Concepción-Acevedo, J., Park, T., Andleeb, S., Bull, J. J., Levin, B. R., ... Gupta, S. (2017). Synergy and Order Effects of Antibiotics and Phages in Killing *Pseudomonas aeruginosa* Biofilms. *Plos One*, 12(1), e0168615.

2. Pires, D. P., Vilas Boas, D., Sillankorva, S., & Azeredo, J. (2015). Phage Therapy: a Step Forward in the Treatment of *Pseudomonas aeruginosa* Infections. *Journal of Virology*, 89(15), 7449–7456.

[ID: 426] Personalized phage therapy for intensive care unit patients suffering from healthcare-associated infections

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Effectiveness of serially produced bacteriophages is high in case of intestinal and respiratory infections caused by community-acquired antimicrobial resistant pathogens. However, mass-produced phages don't consider the rapid changes in circulating strains causing healthcare-associated infections (HAIs), the formation of anti-phage immunity, phage pharmacokinetics, etc. We have developed an algorithm of personalized phage therapy for intensive care unit patients suffering from HAIs which increases the efficiency of phage therapy by 40 percent. It consists of three consecutive stages: 1. Determination of sensitivity of target bacterium to bacteriophage preparation; 2. Determination by enzyme-linked immunosorbent assay of IgG-antibodies in the patients' sera active against the phages used; 3. Selection of pharmaceutical form and administration route based on the preliminary phage pharmacokinetics study. Sound and safe update of phage compositions, used against HAI-pathogens, requires the creation of an up-to-date collection of pheno- and genotypically characterized bacteriophages that expect to be included into ready-to-use pharmaceutical forms on the first demand of the hospital.

[ID: 118] Flavobacterium columnare and phages interaction on a mucosal environment

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Mucosal surfaces are an important interface between metazoans and the environment. Pathogens which use these surfaces as point of entry are responsible for mortality and morbidity worldwide, and also for significant economic losses when affecting farm animals. Infection by bacteriophages is usually an overlooked variable when studying host-bacterium interaction during bacterial diseases in animals. Recently it has been proposed that phages may protect metazoans from invading bacteria by forming a non-host derived protective layer on mucosal surfaces, but so far a natural model for empirical studies is missing. Given the complexity of the host-bacterium-phage interactions, well-designed models are needed for improving research on mucosal infections. *Flavobacterium columnare*, the causative agent of columnaris disease, is a major aquaculture pathogen responsible for huge economic losses worldwide. *F. columnare* has chemotaxis towards fish mucus and cause epidermal infections on fish skin and gills. Phages capable of infecting *F. columnare* are known and shown to be efficient as phage therapy agents in experimental conditions. By using a simulated mucus environment we became able to modulate *F. columnare* virulence and discovered that this directly correlated to phage susceptibility, suggesting that the trade-off between activation of virulence and being susceptible for phage infections has a major, yet overlooked, role in columnaris disease. Our results allowed the development of optimized strategies for *F. columnare* infecting phages isolation and production, and also revealed important features that have direct impact for host-pathogen interactions, evolutionary biology and phage therapy design and use. In conclusion, our model has potential to be used to further comprehend mucosal bacterial infections and how to develop optimized phage therapy approaches.

[ID: 147] The impact of microbial biodiversity on the evolution of phage immunity and virulence in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is an opportunistic pathogen known for its ability to rapidly evolve antibiotics resistance, and is as such increasingly becoming a target of clinical phage therapy trials. However, the rapid evolution of phage resistance mechanisms remains an issue, with approximately half of all *P. aeruginosa* clinical isolates possessing adaptive CRISPR-Cas immune systems to combat viral infection. While the widespread nature of CRISPR systems suggest they are important in clinical settings, lab-based studies often find that *P. aeruginosa* almost exclusively evolves surface-based resistance. This discrepancy may be explained by differences in the biotic and abiotic environment between *in vitro* and *in vivo* environments. In particular, while *P. aeruginosa* is typically examined in isolation when grown in the lab, during clinical infections *P. aeruginosa* usually coexists with a polymicrobial community of other pathogens. Here, we report how an artificial cystic fibrosis microbial community, consisting of *Staphylococcus aureus*, *Burkholderia cepacia* complex and *Acinetobacter baumannii*, drives the evolution of CRISPR-based phage resistance in *P. aeruginosa* PA14. Using a *Galleria mellonella* infection model, we also show that the evolution of CRISPR-based resistance results in the maintenance of virulence *in vitro* with the ancestral, while the evolution of surface-based resistance leads to reduced virulence *in vivo*. Collectively, our analyses demonstrate how intra-host biodiversity might propagate the evolution of CRISPR resistance, and that the type of resistance mechanism evolved has important implications for *P. aeruginosa* virulence.

[ID: 375] Thermophilic composting virome assessed by metagenomics and by isolation of phages targeting *Pseudomonas aeruginosa*

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Thermophilic composting harbors an impressive microbial richness and has proved a valuable source of information on novel bacteria [1] and bacteriophages [2]. Using *Pseudomonas aeruginosa* PA14 as host, we have screened São Paulo Zoo Park composting samples for phages. One of the six phages isolated (ZC01) was shown to be from the Siphoviridae Yu-A like genus, and the other two (ZC03 and ZC08) were similar to each other and shown to be novel Podoviridae phages [2]. These phages were effective on biofilm degradation, suggesting them as promising antimicrobial agents against *P. aeruginosa*. The three remaining phages (ZC04, ZC06 and ZC07) were classified as Podoviridae and are phylogenetically close to ZC03 and ZC08. Infection assays of 70 different clinical and environmental *P. aeruginosa* isolates revealed a narrow host range for these phages. Phage susceptibility assays in *P. aeruginosa* PA14 mutants point to the type-IV pilus pilin (PilA) as the primary determinant for host spectrum. Moreover, the expression of pilin from PAO1, a *P. aeruginosa* strain resistant to the isolated phages, in a PA14 pilA null mutant, restored the twitching motility but not the phage-susceptibility. Additional determinants of phage susceptibility may result from ongoing comparisons between selected *P. aeruginosa* isolate genomes. The shotgun metagenomic datasets of composting samples were further screened with the MARVEL phage discovery tool (<https://github.com/laboratoriobioinformatica/MARVEL>). Hundreds of phage genomes were identified, most of them having low or no similarity to viral genomes in public databases. Our studies have thus allowed the isolation of new phages and the discovery of new phage genomes from thermophilic composting, contributing to the understanding of tailed bacteriophage diversity and their role in the complex composting environment, and uncovering potential candidates for phage therapy. Funded by FAPESP, CAPES and CNPq.

[1] Antunes et al. *Sci Rep* 2016, 6:38915

[2] Amgarten et al. *BMC Genomics* 2017,18:346

[ID: 106] Abundance, distribution and, genetic and functional diversity of viruses from a tropical estuary (Cochin estuary, India)

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Tropical estuaries are interesting sites to examine virus–bacteria interactions since they may vary gradually or abruptly in salinity, which presumably requires physiological, genetic and ecological adaptations. In such systems, viruses can form an integral component of the microbial food web and play crucial roles in driving nutrient fluxes, food web dynamics and bacterial diversity. However, our knowledge and understanding of their dynamics, activity and interactions with their hosts mainly bacteria in such transient estuarine systems especially from the tropics are limited. This study examines the spatio-temporal variations in viral abundance and their activity, bacteria-virus interactions and their morphological, genetic diversity across the salinity gradient in a monsoon-driven tropical estuarine system (Cochin estuary, India). A recent report from this estuarine system has suggested substantial release of bacterial carbon (ca. $72.9 \pm 58.5 \mu\text{g C l}^{-1} \text{d}^{-1}$) due to lytic viral lysis. In this estuary high viral abundance, lytic infection and viral production rates were accompanied by low bacterial growth efficiency and high bacterial respiration during non-monsoonal months. The contribution of the viral shunt to the dissolved organic carbon (DOC) pool was higher during the dry season (premonsoon) when compared to wet season (monsoon). Additionally, a detailed examination of viral diversity using Illumina HiSeq metagenome approach gave deep insights into the genetic diversity and functional roles of viruses in this estuarine system. Our study indicates that the changes in bacterial community were coherent with changes in viral communities suggesting a tight coupling between viruses and bacteria in this estuary.

[ID: 128] Bacteriophage genome recombination enables host switching and causes genome instability

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Bacteriophage genomes often display a high degree of modularity and mosaicism, suggesting exchange of genetic information between phages. Here, we have mimicked genetic exchange between phages and assessed its success rate using an in vitro genome shuffling method. Using genome fragments of *Escherichia coli*-infecting Myoviridae, Siphoviridae, and Podoviridae viruses, we created novel chimeric phages, whose genomes contain elements recombined from the parental phages. The chimeric virions displayed a distinct host range compared to their parental phages. In ØChi3 about 65% of the phage genes were derived from the parental myophage (mostly contributing to the host-recognition structures), 29% from the siphophage (tail-structure), and 7% from the podophage (capsid elements). Several chimeric phages were studied regarding their genomic stability, where we followed several different lineages for up to ten generations. We observed evolutionary dead-ends where the recombination of foreign elements led to unstable copies of the phage, driving them to extinction in fitness experiments (EOP and burst size decreasing over time). Together, our findings reveal the potential flexibility and adaptability of phage genomes to accommodate and re-arrange modules of novel genetic information, even between distantly related phages.

[ID: 131] Molecular understanding of the DISARM phage defense system from *Serratia*

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Bacteria have been exposed to the pressure of bacteriophage infections for millions of years, driving the evolution of effective defense systems against these viral predators. After successful DNA injection, most bacteria rely on molecular mechanisms such as the restriction-modification (RM) system for phage defense, in which an endonuclease-based mechanism attacks specific sequences on the invading DNA. Recently, a new defense system was described associated with RM systems, that was named DISARM (Defense Island System Associated with Restriction-Modification). This system can be classified in 2 classes based on gene composition, containing three core genes (with helicases and phospholipase/nucleases domains). Here, we focus on *Serratia* sp. strain SCBI, with a class 1 system comprised of an adenine methylase and helicase domains that include an adjacent cytosine-methyltransferase gene located upstream the 5-core gene cluster. We found after challenging the system to a collection of Enterobacteria tailed viruses, a 90% reduction of the viral population for specific Myophages. Preliminary results show that this protection is mainly due to the protein encoded by the cytosine-methyltransferase gene, whose molecular mechanism is discussed here.

[ID: 313] New strategies for genetic modification of bacteriophage properties

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The prospects of modifying bacteriophages to expand, change or control their ability to recognize and kill a given bacterial host or range of hosts are extremely promising for a wide range of applications, from phage therapy, to pathogen detection and biocontrol. Accordingly, significant efforts have been made to develop new strategies for phage genetic modifications and increase the versatility and ease of current practices. In too many cases, phage modification involves tedious procedures which are costly, time-consuming and have a relatively low success rate. The development and use of methods based on yeast artificial chromosomes, CRISPR-cas systems and L-form bacteria help alleviate many of these issues. Our group has developed a method (the ac method) that takes advantage of the acriflavine resistance gene present in many T4-like phages to easily introduce foreign DNA in the phage genome and rapidly select for recombinants.

The present work highlights some of the advantages (not reporter gene necessary, single step recombinant selection, development of a rapid insertion vector) and limitations (maximum insert size, insert composition) of the method. We also demonstrate how different expression cassettes can be designed to modulate the expression of a gene of interest. The ac method is a useful addition to the genetic toolkit for phage modification which can help accelerate the development of technologies for therapy, detection, biocontrol, materials, etc.

[ID: 229] From unique killers to a jumbo genome – morphological and genomic diversity of *Agrobacterium* phages

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Bacteriophages and their lytic peptides can protect plants from phytopathogens such as *Agrobacterium tumefaciens*. To better understand mechanisms of phage mediated host killing, we characterize five lytic bacteriophages with activity against *A. tumefaciens* C58. These phages come in different shapes and sizes—from phages with podoviral morphology and isometric heads to a jumbo phage with a myoviral morphology and a contractile tail— and exhibit varying host ranges and killing efficiencies. The smallest phages are phiKMV-like phages that are efficient at killing their hosts. Their lethality can be attributed to their expression of a unique endolysin, called Phage Peptidoglycan Hydrolase (PPH)1. The atypical domain structure of PPH, along with the absence of obvious accessory proteins, suggest PPH may function independently to mediate host cell lysis. Contrary to the narrow host range of the phage, expression of PPH from an inducible promoter inhibits cell growth and blocks cell division in a broad range of bacteria including *Agrobacterium*, *Sinorhizobium*, and *Escherichia* strains. Unlike the smallest phages, the largest phage in our collection is not a potent killer. *Agrobacterium* phage *Atu_ph07*, has a head diameter of 146 nm, an extended tail length of 136 nm, and a genome of 435 kb. We use *in silico* methods for functional protein predictions as well as experimental mass spectrometry (ESI-MS/MS) to identify structural proteins encoded in the genome. Although we have certainly under-sampled the phages of *Agrobacterium tumefaciens*, our results indicate a high degree of morphological and genomic diversity and also suggest novel mechanisms of host cell killing remain to be uncovered.

Reference:

1. Attai, H., Rimbey, J., Smith, G. P., and Brown, P. J. B. (2017). Expression of a Peptidoglycan Hydrolase from Lytic Bacteriophages *Atu_ph02* and *Atu_ph03* Triggers Lysis of *Agrobacterium tumefaciens*. *Appl. Environ. Microbiol.* 83, e01498-17. doi:10.1128/AEM.01498-17.

[ID: 371] Identification of the first endolysin Cell Binding Domain (CBD) targeting *Paenibacillus larvae*

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Cell binding domains (CBD) from bacteriophage endolysins are recognized by their high specificity and affinity, characteristics that make them promising in designing accurate diagnostic tools. To date, no CBD has been identified targeting *Paenibacillus larvae*, a Gram-positive bacterium that causes the highly contagious American foulbrood that compromises honeybees, with negative impact on pollination and the beekeeping industry.

Previously we have identified the first endolysin (PlyPI23) against *P. larvae* and here, we further explored the endolysin, which does not present an *in silico* identifiable CBD.

The predicted protein 3D structure shows two domains, the first domain enclosing the Amidase_2 domain. By fusing the endolysin C-terminus (after the amidase_2) with a GFP we observed green decorated *P. larvae* cells at the fluorescence microscope and prove the existence of a CBD. Sequential deletions on this peptide stretch enabled the identification of the core CBD with 63 residues, the smallest peptide sequence that retains the binding ability.

Specificity tests shows that the CBD binds to all tested *P. larvae* cells, even those uninfected by the phage. No binding was observed to other related and unrelated bacteria tested, namely *Lactobacillus kunkeei* and *Parasaccharibacter apium* usually present in the larvae microbiota, assuring its specificity. Also, results revealed no interference of homogenized bee larvae in binding guaranteeing its use on field samples.

A total of 15 different endolysins from 25 phages infecting the *Paenibacillus* genus were found at the NCBI. Multiple sequence alignment revealed a well conserved group composed of 9 endolysins, even at the usual non-conserved CBD region, showing that many CBDs targeting *P. larvae* are quite conserved. This allows the identification of the CBD from these endolysins. We have thus identified the first CBD targeting *P. larvae* which presents high potential as a diagnostic tool and also as a GPS for unspecific protein antimicrobials.

Keywords: Endolysin, Cell wall Binding Domain (CBD); detection, *Paenibacillus larvae*; American foulbrood (AFB).

[ID: 423] Use of phages against Enterotoxigenic Bacteroides fragilis ETBF

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Bacteroides fragilis is an irreplaceable commensal of the human gut microbiome and is responsible for normal maturation of immune system. However, DNA of *B. fragilis* may contain bft gene coding metalloprotease toxin causing acute diarrhea in children and increases the risk of colon cancer. The mucosal bft exposure is common and may be a risk factor for developing colorectal carcinoma CRC.

Our study aimed to evaluate the potential of *B. fragilis* phage Φ VA-7 to decrease the risk of development of CRC.

The Φ VA-7 phage previously isolated from the urban wastewater sample, Φ VA-7 susceptible clinical strain of ETBF E3 and a colon epithelial cell line HTC116 was used in the cell culture experiment.

A cell adhesion assay was specifically adjusted for this experiment. 2-3 ml of bacterial suspension 3×10^8 cfu/ml was added to the cell culture and incubated for 3 h anaerobically to allow bacterial adhesion. The cell culture with the attached bacteria was inoculated with the same volume of phage lysate $3-5 \times 10^7$ pfu/ml, i.e. MOI 0.1 and incubated for 3 h. The bacterial cells were then removed using 0.1% Triton solution and plated for bacterial cell counts. Three types of controls were used: 1. Blank control of the cell culture; 2. Cell culture inoculated only with bacteria; and, 3. Cell culture inoculated with bacteria and phage. The experiment was repeated three times.

The results showed 2 log decrease of bacterial counts after 3 h exposition of infected cell culture to phage solution.

The small study gave promising results for the phage therapy to be referred as the solution for the treatment and prevention of infectious diseases caused by *B. fragilis* and decreasing the risk of developing CRC.

Further studies are required for detailed assessment of the potential of phage therapy and prophylaxis in case of infectious and malignant diseases.

[ID: 120] Sequence based classification of bacteriophages

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Introduction: Bacteriophages are viral particles that infect and replicate inside bacterial organisms. Since they are specific to a particular strain of bacteria, advances in their research could lead to novel means of targeted treatment without adverse effects on natural microbiome in patient's body. Moreover, this treatment could be effective against bacterial strains with antibiotic resistance. We consider as one of the main bottlenecks of bacteriophage research the inability to cultivate some of the phages due to missing information about their hosts and insufficient description of their genes. We aim to address this issue with our bioinformatics pipeline which can predict bacteriophage hosts from genomic sequence and which can give us additional information about gene importance for their function.

Materials and methods: We downloaded phage genomic records from NCBI, Viralzone and Phagesdb databases. A total of 6277 genomic FASTA records with information about bacterial hosts were obtained. Genes from each genomic record were extracted using Prokka software. We selected records with 8 most common hosts across our dataset and split this new reduced dataset into training set and testing set. Then we created clusters of similar genes using Markov clustering method. Later we created matrix of each phage against each cluster. Tree classifier algorithm was used to generate predictive model from the matrix.

Results: We created pipeline producing models for predicting phage hosts from genomic sequences. Moreover this model allows us to examine importance of traits for predictions. In results we present breakdown of model and the statistical measures of its specificity and sensitivity.

Conclusions: We hope that our pipeline will assist in discovery of novel phages and underlying mechanisms behind their functionality.

[ID: 154] Grand theft among bacteria and phages: ancient structure of the gene transfer agent

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Rhodobacter capsulatus gene transfer agent (GTA) represents the most sophisticated system of horizontal gene transfer in prokaryotes discovered to date. The particles resemble tailed bacteriophages. In spite of the role of GTAs in bacterial evolution, no structural studies of the GTA were yet reported. Here, we present the near-atomic structure of *R. capsulatus* GTA determined by cryo-EM. GTA particles possess an oblate icosahedral capsid, reducing its DNA packaging capacity by 43 % compared to regular icosahedron. This unique head is decorated with 12 nm-long spikes, which protrude from the capsid pentons and serve for the initial attachment. The neck complex and the major tail protein are similar in structure to Siphoviridae proteins, while the internal tail protein gp11 has homologs in the Podoviridae DNA-associated tail needles. Gp11 thus performs the function of both tape-measure and DNA-transfer proteins. The baseplate of the GTA has three-fold symmetry and contains six types of proteins. The baseplate tail fibers mediate the attachment of GTA to *R. capsulatus* capsule and are encoded outside of the GTA structural region. Baseplate component gp15 probably binds to a secondary receptor, triggering the genome ejection. Subunits with peptidase activity, unlike their phage homologs, are not released from the baseplate during genome ejection and may facilitate penetration of GTA through *R. capsulatus* capsule. Our results describe the mechanism how GTA particles transfer DNA and provide a link in evolution of tails of Siphoviridae and Podoviridae phages.

CIISB research infrastructure project – LM2015043, IT4Innovations National Supercomputing Center – LM2015070, and the National Grid Infrastructure MetaCentrum – LM2015042 funded by The Ministry of Education, Youth and Sports of the Czech Republic are gratefully acknowledged. This work was supported by Grant Agency of the Masaryk University (MUNI/A/0824/2017) and The Czech Science Foundation (GA18-17810S, GA18-13064S).

[ID: 207] Infection Responsive Biomaterials: bacteriophage tethered wound dressings for a targeted therapy

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Chronically infected wounds can exhibit antibiotic resistance, increasing patient morbidity and the associated financial costs of treatment and prevention of nosocomial outbreaks. Evidence of antibacterial efficacy from in vitro, in vivo and clinical trials has amassed over recent years, making bacteriophage therapy of prime interest in the face of the antibiotic resistance crisis. Delivery of bacteriophage to the target site has become an integral component of current bacteriophage therapy research. To facilitate bacteriophage delivery, various formulations and administration methods have been developed including aqueous formulations, impregnated hydrogels, encapsulation technologies, and surface immobilisation.

The objective of this study was to develop dressing materials to treat chronically infected wounds by immobilising bacteriophages, with efficacy against relevant wound bacteria, onto a range of biodegradable and biocompatible films. These materials are intended for the management and repair of skin injury, particularly burns and ulcers, where the risk of infection is high.

The physical and chemical characterisation of a candidate biodegradable film material has been conducted, and a surface coating technique developed for the orientated immobilisation of bacteriophage by exploitation of the reported charge difference between the capsid and tail fibres. A range of *Staphylococcus aureus* and *Escherichia coli* bacteriophages were selected for this study, including both Myoviridae and Podoviridae to assess the capture efficacy of morphologically distinct families. Of the two bacteriophage families, an increase in viable Podoviridae was demonstrated at the modified material interface; however no increase in infective capacity was demonstrated with bacteriophages from the Myoviridae family following the coating procedure.

The coating technique employed has been shown to increase the antibacterial efficacy of the material by increasing the numbers of available lytic Podoviridae on the film surface, hypothesised as due to surface charge interactions.

[ID: 48] Effect of phage JG024 infections dynamics of *Pseudomonas aeruginosa* Strain PA14 in different bacterial life cycle status and life styles

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Pseudomonas aeruginosa is an opportunistic pathogen and the main cause of death of Cystic Fibrosis patients. Furthermore, *P. aeruginosa* possesses multiple antibiotic resistance mechanisms that hinder the successful control with a broad spectrum of antibiotics. As a consequence, in the last decade the interest for alternative treatments, including phage therapy, has increased. To date, many *P. aeruginosa* phages have been isolated, characterised and proposed for therapeutic purposes. However, little is known about the effect of the phage-host dynamics on heterogeneous populations, where bacteria of various life stages and lifestyles cohabit. In pulmonary infections, the populations of *P. aeruginosa* are very heterogeneous. Moreover, *P. aeruginosa* in the lung mucus exhibits a biofilm lifestyle, growing slower and increasing the production of alginate compared to their planktonic counterparts. The impact of this heterogeneity on the success of phage therapy is still unknown. Therefore, the purpose of our study is to evaluate the effect of different bacterial metabolic states and lifestyles on the phage-host dynamics under diverse growing conditions. To do so, we compared the phage-host dynamics of *Pseudomonas aeruginosa* Strain PA14 – Phage JG024 grown in media with different nutrient compositions, ranging from nutrient rich (LB) to starvation (MM9). We also tested the killing capabilities of phages under conditions relevant to lung infections, including different available iron and oxygen concentrations and biofilm formations. The preliminary results obtained from this research will serve as the basis for establishing a framework for analysing of the effects of phage therapy that includes the impact of heterogeneous pathogenic microbial populations as well as setting phage-host dynamics to environmental conditions more alike to the lung.

[ID: 419] Complexity of P1 lytic system – does it make a biological sense?

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P1 is a model temperate bacteriophage of Myoviridae family of about 94-kb genome [1]. It can infect cells of different Enterobacteriaceae family representatives, in which it can develop lytically or form lysogens. Only some strategies of P1 adaptations to propagate in different hosts are known. An atypical feature of P1 is an unusually large number of lytic genes. In addition to an endolysin, Lys, which contains a membrane docking domain (SAR) and hence does not require a classical holin to pass through a cytoplasmic membrane [2] it encodes three proteins of holin properties, LydA, LydC and LydD. Only the holin function of LydA was demonstrated previously. However, while the *lydD* gene is in the same operon as *lyz*, which is a typical arrangement of holin and endolysin genes in phage genomes, the *lydA* and *lydC* genes are in unlinked operons [1]. Additionally, *lydA* gene is linked to the *lydB* gene, which encodes the only known antiholin of P1. In a search for the biological sense of P1 lytic system complexity we have constructed single, double or triple P1 mutants depleted of various lytic functions, and used them to lysogenize different P1 hosts. Surprisingly, the influence of inactivation of particular holin genes on the lysis and lysis kinetics of various P1 hosts upon induction of P1 lytic development varied. While LydA appeared to be essential for lysis of one host it appeared to contribute to lysis only partly in a different host. Additionally the involvement of various holins to the timing of lysis appeared to be host-dependent. Clearly, the complexity of P1 lytic system facilitates the adaptation of P1 to various hosts.

1. Łobocka et al., 2004. *J. Bacteriol.* 186, 7032-7068

2. Xu et al., 2004. *PNAS USA.* 101:6415-6420

[ID: 325] Detailed characterization of SH3b binding domain from staphylococcal phage endolysin LysF1

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Bacteriophage endolysins could be useful tool for combat bacterial infections. Some of these enzymes have modular structure consisting of two or more domains. Catalytic domains are necessary for degradation of bacterial peptidoglycan and can be used as antimicrobials. But cell-wall binding domains (CBD) could mediate interaction of endolysin with bacterial peptidoglycan and could influence catalytic activity of whole enzyme. CBDs can also be used alone for the rapid detection of bacteria in combination with a fluorescent tag or for concentrating bacteria from environmental or food samples

In this work we focused on SH3b binding domain of phage endolysin LysF1 which is natural deletion mutant of well known endolysin LysK. Endolysin LysF1 is consisting of an N-terminal cysteine-histidine-dependent aminohydrolase/peptidase domain (CHAP), C-terminal SH3b CBD and it has deleted middle amidase domain compared to wild-type endolysin. The results of newly designed co-sedimentation assay of CBD showed that it was able to bind to three types of purified staphylococcal peptidoglycan 11.2, 11.3 and 11.8 that differs in their peptide bridge, but also to the peptidoglycan type 11.5 of *Streptococcus uberis*. This binding capability was verified in vivo using the fusion protein with green fluorescent protein and fluorescence microscopy. Using several different approaches, including NMR structure determination, we have not confirmed the previously proposed interaction of the SH3b domain with the pentaglycine-bridge in the bacterial cell-wall. Our results showed that the tested CBD can cover the whole *Staphylococcus* genus, but it also binds to a *Streptococcus* species, and its use in genus-specific diagnostic applications must be carefully considered.

Acknowledgements:

This work was supported by the Ministry of Health of the ČR (Grant No. NT16-29916A) and the Ministry of Agriculture of the ČR (Grant No. QJ1510216)

[ID: 370] Setting up molecular biology tools to study the interactions between the protist *Cafeteria roenbergensis*, the giant virus CroV and the virophage mavirus

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The steadily increasing rate of virus discoveries, driven by metagenomics, greatly outpaces the experimental characterization of these viruses to elucidate their molecular biology, ecology and evolution. A prominent example are the protists-infecting giant viruses and their associated virophages. Despite the vast diversity of giant viruses and their hosts, most lab-based studies have focused on the *Acanthamoeba* system. Our purpose is to establish, for the first time, the basis and the protocols necessary to carry out molecular biology studies with a non-amoebal tripartite host-giant virus-virophage system. We work with one of the most abundant heterotrophic protists in the sea, *Cafeteria roenbergensis*, its giant virus CroV and its virophage mavirus. When only CroV infects a *Cafeteria* culture, the host population is killed; however if mavirus is added to the equation, *Cafeteria* is protected from CroV infection. Interestingly, mavirus is found as provirophage in *Cafeteria* genomes and it is only activated during CroV infection. Two main questions arise: how does CroV activate mavirus and how does mavirus protect the *Cafeteria* population? Here we present first steps to answer these questions, by setting up a protein expression system in this marine flagellate, by pull-down assays to enrich for specific CroV DNA-binding proteins, and by click chemistry-based amino acid labeling to detect CroV proteins with increased sensitivity. These tools will allow us to address central questions in this fascinating tripartite microbial system to better understand the interactions between giant viruses and their virophages.

[ID: 43] Bam35 tectivirus protein interactome as an efficient tool to disclose new functions of viral ORFans

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The best-characterized tectiviruses are classified into two genera, alphatectivirus and betatectivirus. While the first group includes a highly similar group of lytic viruses preying Gram-negative bacteria, typified by PRD1, the second group spans a diverse group of temperate viruses infecting Gram-positive bacteria, whose main model is *Bacillus thuringiensis* virus Bam35. These viruses have been pointed at the origin of several major groups of DNA viruses, including Adenovirus or Nucleocytoplasmic large DNA viruses (NCLDVs). This, as well as their host specificity towards commensal and pathogenic bacteria, recently raised the interest on these viruses. However, nearly half of the betatectivirus viral proteins are still ORFans of unknown function and others have only a putative biological role, often based merely on gene synteny or weak sequence similarity.

We determined the first tectivirus intraviral interactome, derived from a comprehensive yeast two-hybrid analysis among all the putative proteins codified by the Bam35 genome. The protein-protein interaction (PPI) network obtained comprises 76 unique interactions among 24 proteins, many of them are putative structural components of the viral capsid. Among others, these results allowed us to infer that proteins P17 and P24 are the minor capsid or tape-measure protein and the penton component, respectively. Furthermore, we could detect interactions involving non-structural proteins, as the DNA binding protein P1 and the genome terminal protein (P4), which was confirmed by co-immunoprecipitation of recombinant proteins. Altogether, these results provide a renovated overview of the Bam35 viral proteome, with focus on the composition and organization of the viral particle that will be helpful for understanding the biology of betatectivirus.

[ID: 180] Cobaviruses - a phage group infecting marine Rhodobacteraceae is found in highly productive marine regions

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A novel phage group infecting marine Rhodobacteraceae – the Cobaviruses – was investigated using a combination of phage cultivation, functional genomics and database mining. Our aim was to determine genomic characteristics as well as phylogeny, host range, environmental distribution and potential habitats. Cobaviruses include three new, related phages specific for *Lentibacter* sp. SH36 (vB_LenP_VB1, vB_LenP_VB2 and vB_LenP_VB3), further phage genomes from environmental data sets and the isolates *Roseobacter* virus SIO1 and *Celeribacter* phage P12053L. Phylogenetic analysis places them within the new subfamily Riovirinae, genus *Siovirus*.

Gene composition and presence of direct terminal repeats (DTRs) indicate a genome replication and packaging strategy similar to that of T7 phage. Several auxiliary metabolic genes (AMGs) present in the genome are involved in nucleotide metabolism and elimination of the stress response of the host. Lysis proceeds via the canonical holin-endolysin pathway. Genetic analysis indicates that the Cobavirus virion has a classical podoviral structure, with exception of the tail fibers, which have similarities with those from myo- and siphoviruses. Cobaviruses and their hosts are distributed worldwide in highly productive marine areas. The known host range of Cobaviruses as a group includes members of the genera *Lentibacter*, *Sulfitobacter* and *Celeribacter* and it potentially extends to other members of the Rhodobacteraceae. Phylogenetic analysis of the glutaredoxin and ribonucleotide reductase (RNR) genes indicate that cobaviruses potentially infect bacteria associated with phototrophic and grazing protists, potentially resisting lysis in food vacuoles and thus being “protist resistant bacteria”.

[ID: 395] Ascunsovirinae, a new Microviridae subfamily, lysogenizes alphaproteobacteria from terrestrial and marine environments

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Cultivated phages belonging to Microviridae, one of the two ssDNA virus families infecting bacteria, are characterized by a lytic life style. A first evidence that microviruses can undergo a temperate life style came from bioinformatics prediction of Microviridae-related prophages in the genomes of Bacteroidetes. Here, we isolated and genome sequenced vB_SulM_ICBM5, a temperate ssDNA phage infecting *Sulfitobacter* sp. SH24-1b, a marine Rhodobacteraceae. Using the vB_SulM_ICBM5 phage proteins as bait, we identified 23 similar prophages in genomes of Rhodobacteraceae and Rhizobiales. Phylogenetic analysis of the major capsid protein placed the vB_SulM_ICBM5 phage and the prophages in a clade distantly related with the Bullavirinae. We tentatively named the new clade the Ascunsovirinae subfamily. In all ascunsovirus genomes we identified the main proteins characterizing the Microviridae, that is the pilot protein, the major capsid protein and the replication protein, plus lysis proteins. Ascunsoviruses are present both in marine and terrestrial Rhodobacteraceae, and in terrestrial Rhizobiales. Because Rhodobacteraceae have been shown to cross several times from the terrestrial to the marine environment, we propose that they are the transport vehicle for ascunsoviruses in between the terrestrial and the marine environments. With this work we expand the diversity of Microviridae, of their hosts and their habitats. Furthermore, we bring the first laboratory based evidence supporting a temperate lifestyle for this ssDNA phage family.

[ID: 418] Applications of phage therapy currently reaching human patients

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Returning to the use of bacteriophages to treat bacterial disease in humans in the context of Western medical systems is no longer the dream it once was, but is now a reality. Indeed, though it is often forgotten that the previous generation of Western phage therapy only ended in France in the early 90s, there is has since been a clear resurgence of human applications. Here we review, quantify, and discuss published efforts across the world since that time to treat human patients in both emergency and compassionate use contexts, with an exponentially growing number of papers reporting the results of human applications. Indeed, phage therapy is now able to be routinely used in Belgium as part of the standard of care through the application of ‘magistral preparations’ including bacteriophages as their active pharmaceutical ingredient.

[ID: 88] Characterization of gp15, a multifunctional tail protein from bacteriophage BFK20

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Bacteriophage tails are molecular machines created to recognize the host cells, penetrate the cell envelope barrier and deliver DNA into the cytoplasm. The Caudovirales tails have very different size (from ~100 Å to ~8000 Å) and morphology. The tail structural proteins often include enzymatic components (virion-associated peptidoglycan hydrolases), which locally degrade the peptidoglycan in the bacterial cell wall. In the Siphoviridae and Myoviridae phages a minor tail protein or the tape measure protein (TMP) determines the length of the tail tube. The TMP gene is usually the largest gene in the phage genome. In Siphoviridae phages the tape measure protein may be also involved in the creation of a channel for DNA translocation through the cell wall. The coryneophage BFK20 is lytic phage of *Brevibacterium flavum* CCM 251, the producer of L-lysine. Protein gp15 was predicted as a minor tail protein of the BFK20 virion. The protein gp15 is multifunctional protein with TMP domain detected at the N-terminus, a transglycosylase SLT (soluble lytic transglycosylase) domain and lysozyme like domain. Our previous results indicated that gp15 of 1604 AAs is split into a mature gp15a of 1298 AAs and gp15b of 306 AAs [1]. The SLT domain is localized at the C-terminus of gp15a. We have cloned the fragments containing entire gene of gp15 protein (4815 bp), gp15a (3894 bp) and gp15b (921 bp) into the *E.coli* expression vector. The conditions for expression of individual proteins were optimised. The recombinant proteins gp15-NHis, gp15a-NHis a gp15b-CHis were purified by IMAC chromatography and characterized. The lytic activities of gp15 recombinant proteins were tested on cell wall substrates from corynebacteria and *E. coli* using diffuse and a turbidity reduction assays.

Acknowledgement:

This work was supported by the VEGA Grant 2/0139/18 and APVV 16-0168.

Reference:

[1]. Bukovska et al. (2006) *Virology*. 348: 57-71

[ID: 215] Deciphering phage trajectories with interferometric optical microscope

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We recently developed a very simple interferometric optical microscope, which detects nanoparticles including viruses¹. The measurement is based on the determination of the scattering signal level of each particle and its Brownian motion trajectory. For this purpose the microscope records a movie of 200 frames over a few seconds (typically around 2 seconds with the 150Hz New Focus CMOS camera). With these two measurements (scattering signal and particle tracking) we compute the particle diameters and refractive index.

Thanks to the fast acquisition rate of our camera, we were able to observe that T4 bacteriophage instead of classical Brownian walk exhibits anomalous diffusion. We characterize the T4 displacements as well as trajectories from other caudoviruses by two methods: one based on the positions of the particles along given trajectories and the other, which takes in account the time dependence. We will present these recent results and discuss on the advantage of anomalous nanoparticle displacements in different environments².

1. Boccara M, et al. (2016). Full-field interferometry for counting and differentiating aquatic biotic nanoparticles: from laboratory to Tara Oceans. *Biomed Opt. Express*. 7(9):3736-3746.

2. Barr J J, et al. (2015) Subdiffusive motion of bacteriophage in mucosal surfaces increases the frequency of bacterial encounters. *Proc Natl Acad Sci U S A*. 112(44): 13675–13680.

[ID: 348] Identification of microbial diversity in grape must by whole metagenome sequencing

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Wine is a complex beverage, which consist of hundreds of metabolites through the action of bacteria and yeasts. These microbial communities play an important role during the winemaking process, as they metabolize sugars from grapes and produce secondary metabolites that influence aromatic quality of wine. However, there is not enough knowledge about these microbial communities. Our goal is to accurately identify bacteria, yeasts, and especially bacteriophages, the most abundant organisms in biosphere, in grape must from Small Carpathian wine-producing region through the whole metagenomic sequencing method. Therefore, unveiling the microbial biodiversity of grape must from this region will expand our understanding on microbial composition.

We extracted total DNA from the grape must and performed whole metagenome sequencing approach using Illumina MiSeq platform. Data obtained were analyzed with several bioinformatic methods. We used Meta-spades to assembly reads. The likely taxonomic source of each contig was estimated using BLAST. We performed annotation on each assembled contig using RAST. Software VirFinder was used to identify bacteriophage sequences from unidentified bacterial contigs.

The whole metagenome sequencing approach allowed the identification of complex microbial community. We identified a good amount of microbial diversity and also some potential bacteriophage sequences.

Wine has a very diverse community of microorganisms. We examined this variety of microbial communities in grape must from specific taste of wines from Small Carpathian wine-producing region. This study allowed for a better characterization of grape must microbiome and helped to understand specificity of wine in this region.

[ID: 140] Isolation of broadly active lytic phages against Vancomycin-Resistant *Enterococcus faecalis* and *Enterococcus faecium*

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Enterococcus faecalis and *Enterococcus faecium* are members of the microbiome that can commensally colonize the gastrointestinal tract, oral cavity, and vaginal tract of humans. The acquisition of virulence factors and high-level antibiotic resistance by enterococci are causing these organisms to emerge as a leading source of nosocomial infections. Common diseases caused by enterococcal infections include endocarditis, abdominal abscesses, bacteremia, and urinary tract infections.

The aim of this study was to isolate broad host range phages from sewage to target different vancomycin resistant *E. faecalis* and *E. faecium*. Phages were isolated from sewage following two enrichment steps, whereby the faecal material was mixed with multiple- or individual- *E. faecalis* strains in Tryptic Soy Broth (TSB) supplemented with calcium borogluconate, and incubated shaking for 24 h at 37°C. Following centrifugation and filtration, samples were analysed for the presence of phages using standard spot and double-layer plaque assay techniques.

Genome sequencing revealed the virulent nature of four phages isolated with *E. faecalis* and one phage isolated on *E. faecium*. For host range determination, 86 *Enterococcus* strains there were also tested, including representatives of *E. faecalis*, *E. faecium* and as well as other *Enterococcus* spp. such as *E. durans* and *E. casseliflavus*. The five phages demonstrated broad host range specificity in a spot test, especially against vancomycin resistant *E. faecalis* and *E. faecium*. This finding suggest that phage therapy using these five phages might be efficacious to prevent or treat vancomycin resistant *Enterococcus* infections.

[ID: 143] Elucidation of a ssDNA-binding transcription regulator from N4-like, Pseudomonas virus LUZ7

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N4-like phages are distinguished by a specific transcription pattern which allows these phages to shift transcription from early genes towards middle and eventually late genes. These different stages are each marked by their own RNA polymerase and accompanying single-stranded DNA-binding protein (SSB). Homologues of these proteins can be found among nearly all N4-like viruses. However, homologues of Escherichia virus N4 gp2 are not readily identifiable within most N4-like Pseudomonas phages. In N4, this protein is crucial for activation of middle promoters by binding their ssDNA region and recruiting the N4 RNAPII complex by direct interaction.

Here we describe gp14 from Pseudomonas phage LUZ7 as an SSB that may perform the role of N4 gp2 in these Pseudomonas phages. Our results show sequence-independent binding of gp14 to both dsDNA and ssDNA, with high preference for ssDNA. Using X-ray data, a model of gp14 was built, revealing features of a ssDNA binding fold, which supports the ssDNA binding character of the protein. Furthermore, we show by Mobility Shift Assays that gp14 interacts directly with LUZ7 RNAPII. Based on the ssDNA binding properties of LUZ7 gp14, the interaction with RNAPII and the genomic location, we propose that this protein is a functional analogue of N4 gp2. It indicates that the biological role in shifting transcription from early to middle phase is conserved between these proteins, although they contain no sequence similarities.

From an evolutionary perspective, the conservation of this transcription regulation mechanism is particularly interesting and indicates the robustness of the system across species boundaries. Also, the elucidation of this transcriptional regulator can prove useful towards biotechnological applications in synthetic biology.

[ID: 58] Novel spontaneous mutants of bacteriophage 812 exhibit multiple genomic changes and increased lytic effect on MRSA strains

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The phage therapy, an alternative to fight against methicillin-resistant *Staphylococcus aureus* (MRSA) strains, requires precise genome evaluation of used phages to ensure its efficiency and safety. Bacteriophages of the genus *Kayvirus* are commonly used in phage preparations. However, it is necessary to adapt the phage composition to be effective against emerging phage-resistant strains. The selection under laboratory conditions was used to isolate phages with extended host range. We focused on phage 812, characterized by a high lytic activity and a broad host range towards staphylococcal strains. Five phage 812-derived mutants were selected on strains insusceptible to the wild type. The lytic ability of the phages was tested on the set of 94 human and 92 veterinary MRSA strains. The mutant 812h1 showed the highest lytic activity when lysing 82 % of human and 97 % of veterinary strains. The causes of the phage host-range changes were investigated by comparative genomic analysis of the phage mutants. We found interspersed and tandem direct repeats of various motifs differing in copy number and genome location that served as hot-spots for rearrangements creating multiple genomic variants. Mutations specific for each host-range mutant were characterized. Genes for nucleic acid metabolism, tail tube protein and endolysin were affected. Genome of 812h1 contains regions gained from other strains of *Kayvirus* genus suggesting possible recombination events. Although the selection strains carried prophages and plasmids, no studied mutant contained genes of such origin as well as genes for antibiotic resistance or bacterial virulence. The results of our study demonstrate, that genomic changes observed in spontaneous host-range mutants do not possess any risk for the therapeutic use.

This work was supported by Grant of Czech Ministry of Agriculture (QJ1510216), and Czech Science Foundation (GA18-13064S).

[ID: 107] Bacteriophage T5 tail tube structure suggests a trigger mechanism for Siphoviridae DNA ejection

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The vast majority of phages, bacterial viruses, possess a tail that functions in host recognition, cell wall perforation and safe viral DNA transfer from the capsid to the host cytoplasm. Long flexible tails are formed from the tail tube protein (TTP) polymerised as hexameric rings around and stacked along the tape measure protein (TMP). Here, we report the crystal structure of phage T5 TTP pb6 at 2.2Å resolution. Pb6 is unusual in forming a trimeric ring, although structure analysis reveals homology with all classical TTPs and related tube proteins of bacterial puncturing devices (type VI secretion system and R-pyocin). Structures of T5 tail tubes before and after interaction with the host receptor were determined by cryo electron microscopy at 6Å resolution. Comparison of these two structures reveals that host binding information is not propagated to the capsid through conformational changes in the tail tube, suggesting a role of the TMP in this information-transduction process.

[ID: 56] Viruses and antiviral defense mechanisms in the intestinal microbiota and other ecosystems

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Virus-host interactions involve prokaryotic defense mechanisms against phages like CRISPR-Cas. Similar mechanisms are found in eukaryotes. Comparing antiviral defense in both kingdoms may uncover further immune systems. We show some analogies here. Interestingly, some viral replication machineries and the corresponding host cell's defense share orthologous genes, suggesting that viral components supplied to host genomes can evolve into antiviral defense. We noticed analogies of nucleases involved in viral replication and cellular defense including RNase H, an evolutionarily ancient enzyme that is highly abundant in nature. RNase H is involved in both viral replication and host defense like CRISPR-Cas that destroys phage genomes with an RNase H-like nuclease.

We identified RNase H-like enzymes in giant viruses, bacteria, and ancient animal species. We show here their abundance in marine plankton, as inferred from marine metagenomes. RNases H may also regulate bacteria-host dynamics in the human intestine that harbors a stable core phageome. Phage abundance in the gut is lower than in many other ecosystems, suggesting additional restriction factors or reflecting healthy homeostasis. It is particularly interesting to understand the role of phages in pathologically altered gut microbiota of *Clostridium difficile*, obesity and IBD patients that may be treated by fecal microbiota transplantation (FMT). We have shown that phages transmitted during FMT may contribute to successful treatment of *C. difficile* infection. Interactions of phages with CRISPR-Cas and other immune systems are key to cycle organic matter and nutrients and maintain a healthy homeostasis. The gut microbiome is important for successful immune therapy against cancer, as reported before, and under investigation in the University Hospital Zurich. Efforts for genetically modified therapeutic phages are underway.

To further investigate phage-host relationships, we currently characterize RNases H and other conserved protein structures in various ecosystems. Our studies may also reveal novel defense mechanisms. Preliminary data is shown here.

[ID: 185] Study of the RNA polymerase from two Autographivirinae phages infecting Cronobacter spp.

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Cronobacter spp. belongs to the family Enterobacteriaceae and this opportunistic pathogen is associated with serious newborn infections such as meningitis, necrotizing enterocolitis and sepsis. Powdered infant formula was confirmed to be the source in some cases of Cronobacter infections. Bacteriophages offer a safe means for eliminating this pathogen. In the present study we characterized two new Cronobacter-specific bacteriophages Dev-CD23 and Dev-CT57 isolated from sewage. Both phages belonged to Autographivirinae subfamily with the highest degree of identity to the Cronobacter phage vB-CmaP-GAP227. They possessed broad host specificity in Cronobacter genus, infecting 68% and 65% strains covering seven Cronobacter species. Genomes of both phages were 42 kbp long and showed 82% mutual DNA similarity. In next study, we compared phage RNA polymerases. RNAP from Dev-CD23 and Dev-CT57 phages are localized in the middle of the genome according to FKMV-like phages, whereas in T7-like phages this gene is present among the early genes. Comparison of RNAPs showed a highly conserved catalytic domain and, on the contrary, great variability in the promoter specificity domain even between relative Dev-CD23 and Dev-CT57 phages. Five potential promoter sequences (25 bp) were found in both genomes localized upstream of genes gp1, gp21, gp30 encoding for hypothetical proteins, gp35 (major capsid protein) and gp41 (tail fibre protein). The ability of the promoters to trigger protein expression was verified by plasmid probe vectors and the level of transcription was determined by RT-PCR. We have confirmed up to 10-fold increased reporter gene transcription after phage infection. RNAP were specific for its own promoter, however, slight cross-reactivity between Dev-CD23 and Dev-CT57 phage promoters and polymerases were also observed. Obtained results are an important prerequisite for phage application in food control.

[ID: 278] Novel N4-like bacteriophages of *Pectobacterium atrosepticum*

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Pectobacterium atrosepticum is an economically important phytopathogen responsible for potato blackleg and soft rot, and for which current control strategies are limited. In this study, stem samples of potato crops exhibiting blackleg were taken from three farms in Co. Cork, Ireland, and were found to be infected with *P. atrosepticum*. Three closely related bacteriophages (phages) specific to this phytopathogen were isolated and characterized, namely vB_PatP_CB1, vB_PatP_CB3 and vB_PatP_CB4 (abbreviated as CB1, CB3 and CB4). Both CB1 and CB3 were determined to infect 12 strains and CB4 10 strains of the 19 strains of *P. atrosepticum* tested. Morphology, latent periods, burst sizes and their stability at various temperatures and pHs were also examined. Genome sequencing of the three phages revealed that they shared a minimum nucleotide identity of 93% with each other. Their genomes exhibited an Enquartavirinae genome organization, possessing several conserved proteins associated with phages of this group, like the type species *Escherichia virus N4*. Tandem electrospray ionization-mass spectrometry (ESI-MS/MS) allowed the identification of ten structural proteins that form the virion of CB1, six which are conserved in phage N4. In vitro experiments demonstrated that the phages suppress soft rot formation upon co-inoculation with *P. atrosepticum* on tuber slices and whole tubers. Results of this study indicate that CB1 related phages could be good candidates for phage-based control.

[ID: 321] Characterization and comparative genomics of six strictly virulent bacteriophages of Shiga toxin-producing Escherichia coli O157:H7

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Shiga toxin-producing Escherichia coli (STEC) O157:H7 is one of the prominent foodborne pathogens, commonly associated with contamination of animal products. STEC strains are estimated to cause over 2 million acute illnesses every year worldwide. A significant challenge for the food industry and regulatory agencies is detecting and handling bacterial pathogens such as STEC O157:H7 proactively, and phage-based strategies provide a promising platform for such applications. Healthy cattle are the primary reservoir of STEC O157:H7 and, consequently, of its bacteriophages. In this work, six new bacteriophages - previously isolated from commercial feedlots in Alberta, Canada - able to infect STEC O157:H7 were characterized and assessed as potential biocontrol and pathogen detection agents. Morphological characterization through transmission electron microscopy revealed all six phages belong to the Siphoviridae family. Of the 30 standard phage types (PT) of STEC O157:H7 screened, at least 22 were susceptible to all six phages including PT14a which has been responsible for many cases of human disease in Canada. All six phages consist of double-stranded DNA with genome sizes ranging from 45.7 to 46.7 kb, collinear with the Rogue1virus of the Tunavirinae subfamily. Five out of the six phages – isolated from different pens at the same feedlot at different sampling times - share a high genome similarity (99.9%) but display different host ranges and virulence. Comparative genome analysis enabled the identification of potential genes involved in these variations. This study provides a framework for the identification and selection of bacteriophages as biosanitation agents or biosensors for STEC O157:H7.

[ID: 175] RNA bacteriophage component of the human microbiome

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An extremely diverse and dense microbial consortium populates a healthy human gut. While there is a substantial amount of literature and studies involving the bacterial component of the gut microbial system, there is still relatively little known about the viral division, in particular the bacteriophage. Bacteriophages, commonly known as ‘phages’, are viruses that modulate microbial populations by attacking, infecting and destroying specific strains of bacteria. These viruses are the most abundant biological entities on the planet, reaching number of approximately 10³¹ [Clokier MR, Millard AD, Letarov AV, Heaphy S. Phages in nature. *Bacteriophage*. 2011;1(1):31-45. doi:10.4161/bact.1.1.14942].

Although studies into the bacteriophage component of the microbiome have exploded in recent years, databases are predominated by phage encoded by DNA genomes. According to the latest (2017) report by the International Committee for the Taxonomy of Viruses (ICTV), DNA phage are classified into 134 families. The same report separated RNA phage into only two families; Leviviridae (ssRNA phage) with 4 species and Cystoviridae (dsRNA phage) with 7 species.

Through the development of an RNA-phages-specific isolation technique, this study offers a unique insight into the RNA phage consortium of the human phageome, which has remained somewhat enigmatic. Such a study will focus on improving the overall understanding of phage communities within the human gut by the examination of DNA/RNA phages relationships and the abundance of RNA phages. With emphasis on in-depth characterization and classification of RNA phages, the results will be a massive step in further understanding the human microbiome.

[ID: 384] Determination of prophages and viral community and investigate the CRISPR–Cas systems from uncultivated microbes with Metagenomics data analysis

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Due to increases of antibiotic-resistant bacteria, bacteriophage therapy has become prominent again. The main source of new bacteriophage isolation is wastewater activated sludge. Bacterial adaptive immunity against bacteriophages by CRISPR–Cas systems could be a problem for therapeutic usage of bacteriophages. Activated sludge metagenome data sets of four full-scale domestic wastewater treatment plant samples were downloaded from the NCBI-SRA database under bio-project number PRJNA288131 for mining the virus including prophage diversity and CRISPR mechanisms. Diamond BLAST searches against CRISPR–Cas, prophages, virus non redundant protein database, and MEGAN software were utilized to mine data. The aim of this study is to investigate virus and prophage diversity in activated sludge, and to study potential bacterial CRISPR–Cas system against phages with shotgun metagenome data analysis.

Myoviridae, Siphoviridae and Podoviridae were the dominant prophages affiliated with averages of 55.74%, 35.59% and 8.45% of total prophage reads in metagenome data. Myoviridae, Siphoviridae and Phycodnaviridae affiliated reads were the most dominant virus families with 43.17%, 27.16% and 11.3% respectively, of all virus related metagenome reads. For Mimiviridae which infect protists, and Podoviridae which infect algae, the affiliated reads were about 7.98% and 5.36% respectively, of all virus reads. DNA Metabolism related reads were around 18.9% of total CRISPR–Cas BLASTX results. The occurrence of CRISPR related reads were between 25% to 37%, respectively. CRISPR-associated protein Cas1 was the most common CRISPR protein. Existence of CRISPR-associated protein Cas2 and Csn1 family were around 2.2% and 5.6%, respectively. Other DNA Metabolism reads are related to DNA repair mechanisms.

Interrogation of shotgun metagenome sequences of wastewater activated sludge allows us to better understand viral diversity including prophages and bacterial immunity against bacteriophages by CRISPR–Cas systems. Network analysis will also be used to investigate the wide-spectrum profiles of CRISPR-cas systems and their co-occurrence patterns.

[ID: 415] CF-Pipeline: a comprehensive and fast pipeline for the analysis of viromes and metagenomes in a medically relevant timeframe

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Many pipelines exist today to analyze and annotate metagenomes and/or viromes, but most of them focus on a narrow part of the analysis, be it Taxonomy, functional annotation, virus discovery etc.. Furthermore, speed is usually a second concern, as long as the pipeline runs in reasonable time frame (that can be up to a month for servers like mgRAST) it can be used for most research applications. For medical diagnosis applications, however, timing is critical.

Our CF-Pipeline is a collection of software, some developed explicitly for this pipeline, that performs a comprehensive analysis of viromes and metagenomes (QC, host filtering, Assembly, Taxonomy, antibiotic resistance, virus discovery, etc) in under three hours. CF-Pipeline has been packaged to be easy to install and use, only basic computer knowledge is needed to install on a Linux server and AWS and Docker images are available.

We have used CF-Pipeline to analyze viromes and metagenomes from lung infections in Cystic Fibrosis patients. In all cases, the bacterias we identify as pathogenic agree the bacterial cultures performed at the clinic, which takes several days to grow. We were also able to identify antibiotic resistance genes. From the viromes, we identified a *Stenotrophomonas* phage (phiSHP2) that carries a *Zonula Occludens Toxin* (ZOT). There isn't a clinical test for ZOT, it's detection would be hard using a different method.

The full analysis, from sample collection to written report, took less 48 hours. This includes metagenome and virome library preparation, sequencing and data analysis. We believe that the combination of metagenomes, viromes and fast and efficient pipelines is revolutionizing the clinical diagnosis of microbial infections.

[ID: 200] Single cell genomics for the detection of viral signatures in single amplified genomes of uncultured marine Stramenopiles

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The emergence of single cell genomics (SCG) has revolutionized microbial ecology by retrieving genomic information from microorganisms that are ecologically relevant but still refractory to being cultured. This approach offers also the possibility to untangle putative ecological interactions between uncultured protists and other organisms at a single cell level (i.e. infection, prey capture or symbiosis). Here, we have investigated the presence of viral signal among cells of 11 Stramenopiles protist lineages isolated during the Tara Oceans expedition. On a total of 65 Single Amplified Genomes (SAGs) analyzed, 37 presented viral signatures. The new detected viral contigs, generally fragmented, were possible to assign to known viral genomes thanks to new published bioinformatics tools. We were able to relate one of these viral sequences to a Mavirus virophage, which occurred in 4 SAGs belonging to a pigmented uncultured Chrysophyte (Chrysophyte G1) and in 1 SAG affiliated to the clade A of MAST-3 (MAST-3A), among other viral and phage signals in 4 other different SAGs lineages. The virophages associated to the Chrysophyte G1 and MAST-3A lineages were over 95% similar to the annotated Maverick-related virophage infecting *Cafeteria roenbergensis*. Virophages are DNA viruses readily integrating into an eukaryotic genome (provirophage) where they act as an inducible antiviral defense system against a larger infectious virus (for instance CroV in *Cafeteria roenbergensis*). Moreover, an investigation of their global distribution in open sea environments will be performed using metagenomic and metatranscriptomic data from the Tara Ocean expedition.

[ID: 365] Elucidating phage-bacterium interactions that modulate bacterial composition and functional profile in the gut of older adults

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Changes in the composition of the gut microbiome (GM) or dysbiosis are linked to a number of intestinal and extra-intestinal disorders, including age-related impairments associated with the metabolic syndrome. Several environmental factors may contribute to imbalances in GM composition, including diet, drugs and antibiotics administration and enteric pathogens. Less is known about the impact of the virome on the GM composition, its functionality and interactions with age-related comorbidities in older adults. Here, we investigated the gut virome from older subjects by isolating fecal viral particles and characterizing their complete/partial genomes through hybrid-assembly of Illumina (NextSeq) and Oxford Nanopore (GridION) sequencing reads. Co-abundance analysis performed with sparse generalized canonical correlation analysis (SGCCA) on the viral communities and 16S-rRNA high-throughput sequencing demonstrated a large number of phage-bacterium interactions based on the core members from both microbial components. Remarkably, as a function of these microbial associations, fluctuations in bacterial abundance resulted in dramatic variations in their global metabolic potential, as well as host renal function and cholesterol profiles. The latter conditions may rise from a cluster of risk factors associated with the metabolic syndrome. Together, our data demonstrate that members of the gut virome may be able to trigger dysbiosis, entangling viral implications associated with age-related comorbidities.

[ID: 372] Klebsiella pneumoniae phages isolated against previously phage-insensitive clinical strains

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A growing problem in the medical community is antibiotic-resistant *Klebsiella pneumoniae*. According to the Center for Disease Control, Extended Spectrum (ESBL) *K. pneumoniae* present a serious threat to human health. *Klebsiella* phages from the therapeutic phage collection of the Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław, Poland are used in the experimental phage treatment of the patients with chronic urinary and pulmonary tract infections. This project focused on expanding the number of known *K. pneumoniae* bacteriophages in the collection for use in phage therapy.

Eight clinical strains were selected for phage isolation due to their previous insensitivity to tested phages present in the Institute's collection. Nine environmental water samples were collected from the Odra, Warta and Wisła rivers as well as from Malta reservoir. Samples were enriched with strains over three days and then tested for phage presence.

Thirty-five phage samples positive against *K. pneumoniae* strains were obtained. Host range of these novel phages was tested across a variety of *K. pneumoniae* strains that were also previously insensitive to phages from the Institute's collection. Only three of the thirty strains tested were not sensitive to any of the phage samples. Nine of the phage samples were able to lyse 10 or more of the tested strains, demonstrating a broad host range. Several of the tested phage samples presented high anti-*Klebsiella* activity even at a very diluted concentrations.

Hopefully these isolated phages, after additional biological and genomic characterization, can be used in future treatments at the Phage Therapy Unit.

This work was supported by the Fulbright scholarship for Kathryn Cater granted by the Polish-US Fulbright Commission. The views and opinions expressed in this work are the author's own and do not necessarily reflect those of the U.S. Government or the Fulbright Program.

[ID: 203] Improved P1 bacteriophage development in DksA-devoided Escherichia coli

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P1 bacteriophage, a temperate phage of *Escherichia coli* is both a model bacterial virus and a commonly used tool in molecular biology for transduction-based gene transfer. P1 development requires both phage-encoded and host proteins. We found that deficiency of DksA host protein notably supports phage development. DksA as an RNA polymerase-associated protein acts as transcriptional regulator and is involved in multiple cellular processes, including stringent response. Consequently, the lack of DksA causes pleiotropic defects. We observed that the significantly increased plaques of P1 formed on DksA-deficient strain and increased phage yield is related to the sum of moderately increased phage burst size in every lytic cycle, however this phenomenon is not explained by impaired membrane integrity of the mutant strain. Moreover, DksA-deficiency does not suppress the requirement for host SspA protein for phage propagation. However, the expression of phage-encoded genes is affected by DksA: in the absence of this protein, the transcription from C1 repressor, late promoter activator and lysozyme genes are decreased, allowing more time for phage particles production during the lytic growth. The *dksA* mutant-mediated improved P1 development has an important implication for the laboratory practice for providing high titer lysates for P1 transduction. The host-phage interaction as a complex process remains largely unknown in the global level. Therefore the transcriptome analysis of the changes in gene expression of both bacterial and phage genes during P1 infection and lytic development has been performed. This general approach comprises important step in understanding at the molecular level the superimposing processes taking place upon the redirecting the cellular machinery to phage propagation.

[ID: 242] Study of the activity of bacteriophages to MDR Salmonella strains related to various serotypes

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Salmonella is one of the four main causes of diarrheal diseases worldwide. Most serotypes isolated from patients are characterized by a high degree of antibiotic resistance.

The aim of our work was to elucidate the activity of phages against antibiotic-resistant strains and their specificity to various serotypes of bacteria belonging to the genus Salmonella. The strains previously underwent MALDI-TOF identification, serological typing, molecular serotyping by use of a semi-automatic Rep-PCR system (DiversiLab®System, bioMérieux, Marcy l'Etoile, France). The antibiotic resistance profiles of the strains were determined in accordance with the standards adopted by the National Committee for Clinical Laboratory Standards (NCCLS).

Fourteen phage clones, isolated during the period of 2013-2017 from the waters samples of the river Mtkvari (Kura) in Tbilisi, the Black Sea and sewerage system in Tbilisi. To assert the strictly lytic nature of these phages, high resolution genome maps of 12 of the 14 individual phages were obtained using nanopore sequencing.

The activity of phages was determined to 226 strains of Salmonella, of which 102 were of clinical, 105 - veterinary and 19 - of unknown origin. These isolates belonged to 23 serotypes. Most of the strains showed resistance from four to eight classes of antibiotics. The investigated phage clones showed various levels of lytic activity for almost all Salmonella serotypes.

The phages under investigation did not show any predisposition to specific Salmonella strains. They were equally active to MDR strains, as well as to various serotypes of *S. enterica*.

The studied phages revealed a high activity both to clinical (~ 90%) and veterinary strains (>70%), which suggests that they may be used not only in human medicine, but also in veterinary as a preventive measure, and also an disinfection agent for sanitary treatment of surfaces, premises, etc.

[ID: 27] Evolution of CRISPR-Cas resistance against phages encoding anti-CRISPR proteins

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The adaptive immune system CRISPR-Cas can protect bacteria against bacteriophages (phages). During the Adaptation stage of CRISPR-Cas immunity, bacteria evolve CRISPR-mediated resistance by inserting phage-derived sequences (spacers) into CRISPR loci on the bacterial genome. Upon re-infection, processed CRISPR transcripts guide Cas proteins to detect and destroy the phage genome carrying the cognate sequence (Interference stage). Some phages encode anti-CRISPR (Acr) proteins that have been shown to interfere with both Adaptation and Interference stages by specifically binding the Cas proteins involved in these processes (1,2). However, these studies were carried out using genetic models where *acr* genes were overexpressed from plasmids whereas in their natural context, *acr* genes are expressed after the phage genome is injected into the host cell, resulting in lower Acr effectiveness and frequent failure to block CRISPR Interference (3).

In this work we use *Pseudomonas aeruginosa* and its phage DMS3vir carrying an *acr* gene to investigate whether Acr proteins can block CRISPR Adaptation in the context of phage infection. We find that Acr-phages limit evolution of CRISPR-resistance by strongly, but not completely, inhibiting spacer acquisition. We have indeed detected low frequencies of bacterial clones with newly acquired spacers. This correlates with increased Acr-phage persistence in the face of bacteria that can evolve CRISPR-mediated resistance. In addition, we find that the ability of host bacteria to acquire new spacers is inversely correlated to the strength of the Acr protein encoded by infecting phage. These data provide further understanding of the immunosuppressive mechanisms mediated by Acr proteins and their roles in phage lifecycles.

(1) Vorontsova D. et al., 2015, NAR 43(22) 10848-60

(2) Bondy-Denomy J. et al., 2015, Nature 526, 136–139

(3) Landsberger M. et al., (submitted)

[ID: 267] Characterization of lytic Lactococcus c2-type phages isolated from dairy industrial samples.

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In dairy plants, infections of Lactococcus starter strains by lytic bacteriophages during fermentation processes constitute a major problem in production of cheese, buttermilk, sour cream and kefir (1). Due to the worldwide industrial and financial consequences, phages infecting *L. lactis* are among the best-studied and most commonly isolated phages infecting any bacterial species (2). Among the most frequently encountered are prolate-headed, strictly lytic c2 type phages (3).

The main objective of our work was sequencing and analysis of the genomic content of c2-type phages identified in dairy fermentation environments in Poland. Complete nucleotide sequences of 10 different bacteriophages were obtained and subjected to comparative analyses using bioinformatics tools. Phages were all characterized by 20-22-kbp double-strand DNA genomes encoding two divergently oriented blocks of early and late transcribed genes, separated by approximately a 600 bp noncoding region. Results of our studies show that these region act as an origin of phage DNA replication. Overall, the generally high (>80% identity) sequence homology and conserved gene synteny of the phage genomes was observed. The most divergent regions were mapped to late-expressed genes encoding structural proteins. Their role as host range determinants was confirmed against a set of industrial and laboratory Lactococcus strains, which revealed discriminative infection patterns on sensitive hosts.

- (1) Szczepankowska AK et al., (2013) *InTech*. 2: 23-71.
- (2) Kotsonis SE. et al., (2008) *Appl. Environ. Microbiol* 74: 3453–3460
- (3) Eraclio G. et al., (2015) *Appl. Environ. Microbiol* 81: 8358-8365

[ID: 359] Antibacterial properties of poly(propyleneimine) dendrimers against *Pseudomonas aeruginosa* POA1 mutants in the presence of phage endolysin

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Pseudomonas aeruginosa is a multidrug resistant bacteria responsible for a significant part of serious nosocomial infections. With the dramatic increase of antibiotic resistance in *P. aeruginosa*, alternatives like recombinant phage-borne endolysins are considered as effective antimicrobials. External application of endolysins against Gram-negative bacteria is low effective due to presence of impermeable bacterial outer membrane (OM). However, the application of endolysin in combination with OM permeabilizers such as EDTA, colistin or genetically engineered enzymes may overcome these obstacles. In this study, the application of poly(propyleneimine) dendrimers (PPI) with or without maltose on their surface were tested against smooth and rough LPS mutants of *Pseudomonas aeruginosa* PAO1. In the first stage of experiments, the circular dichroism (CD) spectroscopy was used to explore the influence of dendrimers on conformation and physical stability of recombinant endolysin derived from *Klebsiella*-specific phages KP27 (gp126 with endopeptidase activity). The size of formed complexes was determined using dynamic light scattering (DLS). The antibacterial properties of KP27 endolysin in the presence of poly(propyleneimine) dendrimers were analyzed using spectrophotometry and cultivation microbiological methods (colony-forming units reduction) on PAO1 mutants. The analysis of CD spectra and DLS revealed that both tested PPI dendrimers interact with endolysin forming large aggregates but do not strongly affect the secondary structure of protein. The microbiological studies showed that antibacterial effect of dendrimers was correlated with the length of O-antigen and modification of their surface with maltose. The level of bacterial cells reduction by endolysin was higher in the presence of unmodified dendrimers. In conclusion, the idea of dendrimers application as antimicrobial agent in combination with endolysin is promising but needs further studies.

[ID: 85] The study of novel endolysins antimicrobial activity against corynebacteria and streptococci.

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The increasing occurrence of antibiotic-resistant pathogens is a major problem in current health care. We focused our research on streptococci and corynebacteria. *Streptococcus agalactiae* is a common inhabitant of the gastrointestinal and genital tracts and is the leading cause of bacteremia in adults and invasive neonatal infections. Some corynebacteria species are serious pathogens of humans or animals; e.g. *Corynebacterium urealyticum* has been implicated in urinary tract infections and stone formation. These diseases are treated with beta-lactams or macrolides, but the emergence of less susceptible and even fully resistant strains is a cause for concern. Bacteriophage-derived enzymes, as endolysins, and other peptidoglycan hydrolases with the ability to disrupt cell walls represent possible alternatives to conventional antibiotics. Previously we have isolated and characterized two phage endolysins: gp24 from corynephage BFK20 and endolysin gp1.2 from phage phiBP of *Paenibacillus polymyxa*. The modular structure of endolysins provides an opportunity to engineer enzymes with altered bacteriolytic activity. We constructed two chimeric lysins BP-H1 and BFK-H2 by combination of their catalytic and binding domains. The lytic activities of chimeric endolysins were tested on cell wall substrates from corynebacteria and paenibacilli. The second strategy it was preparing constructs of new endolysins originated from streptococci. The genomes of five human clinical isolates of *Streptococcus agalactiae* were sequenced and annotated. Using in silico analysis the search for temperate phage in genomes was accomplished and the sequences of phage lytic proteins – endolysins were identified. Purified genomic DNAs of *S. agalactiae* isolates were used as templates for PCR amplification of DNA fragments with corresponding primers. The entire genes of these endolysins were cloned into expression vectors and the corresponding recombinant proteins were expressed in *Escherichia coli* and purified by affinity chromatography. The lytic activities of isolated novel endolysins were tested against streptococci.

Acknowledgement: This work was supported by grant APVV 16-0168.

[ID: 166] Aqueous Two- Phase Systems as an alternative method for the recovery and purification of phages for phage therapy use: A case study with the Salmonella bacteriophage phi San23

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The WHO consider Salmonella one of the most important zoonotic, foodborne pathogens, and bacteriophages are today one of the possible alternatives to antibiotics in animal therapy, which can also contribute to the food safety. Production of phages is achieved by infection of their host bacteria and subsequent purification. This comprise the removal of the host bacteria, and their cell debris through several strategies. So far, the cost of the downstream processing has been an additional barrier for the development of phage therapy therefore it is critical to develop alternatives for recovery/purification of phage that are cost-effective and can be used at large scale. Aqueous Two-Phase Systems (ATPS) have demonstrated to reduce costs when contrasted against conventional unit operations as chromatography (1). ATPS has proved to be successful in the recovery/purification of a wide array of bioproducts (2,3). This study evaluated the application of ATPS for the recovery and purification of Salmonella bacteriophage phiSan23. The work consisted in evaluating different combinations of solutions in terms of the concentration and infectivity of the phages in each of the possible phases where the viruses could be recovered. Then phage samples with different degrees of purity were used to determine the partition of the product of interest and contaminants. Phage samples included: phage suspension centrifuged and 0.22 µm filtrated; phage suspension centrifuged; and the direct crude phage suspension. It was possible to identify a system that was able to partition phages almost 3,000-fold from the centrifuged and filtrated sample, 55-fold when using the centrifuged sample and 100-fold purification for the crude phage suspension. Based on the results, we propose the incorporation of ATPS into a scaled bioprocess.

1. Ceglarek et al (2013). PMID: 24225840

2. Azevedo, A et al (2009). PMID: 19251328

3. Paquet, et al. (1994). PMID: 18618778

[ID: 358] Isolation of phages infecting clinical *Pseudomonas* and *Escherichia* strains

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Multidrug-resistant (MDR) *Pseudomonas aeruginosa* and *Escherichia coli* have been classified by WHO as 'critical' priority, and new antimicrobials are urgently needed. Bacteriophages offer an alternative treatment and the environment provides sources for isolation of phages against MDR strains. The aim of the project is to isolate phages infecting clinical *P. aeruginosa* and *E. coli*.

In this project, a horse manure sample was filtered through a serial of filters with pore sizes from 24µm to 6µm. Screening of phages was done by plaque assay on loans of *P. aeruginosa* and *E. coli*. Phages in the formed plaques were purified and propagated before extracting phage DNA for the whole genome sequencing.

We studied two different novel phages isolated from the horse manure. Phage Mij3 is a jumbo phage belongs to the family Myoviridae (genome size of 288,170bp) and capable of infecting most of *P. aeruginosa* Liverpool Epidemic Strains and many MDR strains.

Phage MIE1 belongs to the family Myoviridae (genome size of 176,867bp) and it is able to infect a variety of *E.coli* clinical strains isolated from urinary tract infection patients.

In conclusion, novel phages infecting clinical strains can be isolated from unexpected sources, for example horse manure. It is recommended to widen the scope of sampling and screening for useful phages.

References:

Yuan, Y. and Gao, M. (2017) 'Jumbo Bacteriophages: An Overview', *Frontiers in Microbiology*, 8.
Clokie, M.R., Millard, A.D., Letarov, A.V. and Heaphy, S. (2011) 'Phages in Nature', *Bacteriophage*, 1.

[ID: 179] Benchmarking of assemblers for virome data

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The continual improvement and advancement in lab techniques and sequencing technologies has allowed for the examination of host microbiota on a large scale. The field of microbiome research gathered momentum after the discovery of links between these microbial communities and diseases such as Inflammatory Bowel Disease, obesity, and cancer. However, this area has largely focused on the bacteria component of the microbiome. Until recently, technological barriers have hampered virome research. The healthy human gut hosts between 35 and 2,800 viruses and can outnumber bacteria by 1:10. It is now possible to sequence the virome and therefore bioinformatic techniques are required to address new challenges.

Assembly is an integral aspect of virome analysis due to a lack of universal marker genes. Sequenced reads are aligned and merged into contigs and scaffolds in an attempt to reconstruct viral genomes. Due to the large quantity of unknown sequences or “dark matter”, de novo techniques are often required and there exists a large array of software programs to carry out this challenging task.

In this study we aim to conduct a comprehensive comparison of modern Illumina short read assemblers, including all assembly methods used on published viral datasets and several methods which have not. To assess accuracy, we utilised simulated virome datasets, mock communities and human faecal samples spiked with a known double stranded DNA virus (Q33). Furthermore we used published data from human studies to compare assembly speed, resulting assembly statistics and RAM usage. This study assess the suitability of various assemblers for virome data.

[ID: 417] Bacteriophages in cystic fibrosis acute pulmonary exacerbations

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Cystic Fibrosis (CF) lung disease is the primary cause of death in CF individuals (Alexander et al. 2016). Viral (Willner et al. 2012) and microbial (LiPuma 2010) communities are present in the CF respiratory system regardless of the patient's health status. Acute pulmonary exacerbation where a patient's pulmonary function drops sharply (Conrad et al. 2017), can be fatal, often this decline is related to a specific microbe taking over the respiratory system (Lim et al. 2014). The role of bacteriophages in CF acute exacerbations is explored in this work. Viromes, metagenomes and viral and microbial counts were obtained from CF sputum samples during acute exacerbations. Viromes are composed of the genetic material contained in viral capsids (Cobián Güemes et al. 2016) (Angly et al. 2006). Metagenomes are the total DNA in the system. Viral and microbial counts are obtained by staining sputum samples with SYBR gold and particles are enumerated by epifluorescence microscopy (Haas et al. 2014). In most of the case studies, the community is dominated by a predominant microbe resistance to multiple antibiotics and bacteriophage signatures are present in all case studies. In case study CF409, *Stenotrophomonas maltophilia* relative abundance was 97% and the *Stenotrophomonas* phage SHP2 encoding zonula occludens toxin was identified. Zonula occludens toxin disrupts the tight junction of epithelial cells (Jiao et al. 2015), and this virulence factor contributed to the patient's lung function decay. In several case studies, bacteriophage signals were identified such as highly abundant viral proteins of unknown function next to transposases and integrases and abundant *Pseudomonas* strains with integrated prophages. We showed that bacteriophages are important players in CF acute pulmonary exacerbations, they remodel the bacterial community and the host ecosystem as previously proposed (Silveira and Rohwer 2016).

[ID: 113] Comparative genomic analysis of *Lactococcus lactis* lytic phages belonging to the c2 species.

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Lactic acid bacteria are industrially-important microorganisms that are used worldwide in the manufacture of various fermented food and beverages. In the dairy industry, starter cultures based on the mesophilic species *Lactococcus lactis* are employed to ferment milk into various palatable products such as cheeses, sour cream, and buttermilk. However, the presence of bacteriophages in milk and milk-derived products may impair the bacterial proliferation, and thus threaten the fermentation process. Phages infecting *L. lactis* have been classified into ten species, and belong to either the Podoviridae or the Siphoviridae families. The three Siphoviridae species named 936, P335, and c2 are the most prevalent ones in the dairy industry, and hundreds of DNA sequences are available for species 936 and P335. In contrast, only two complete genomes of c2 phages, namely c2 and bIL67, have been established to date. In this work, one hundred c2 lytic phages collected worldwide over a 35-year period were selected for genome sequencing using NGS technologies. Comparative genomic analysis showed that c2 phages can be further classified into two major groups -that could be seen as subspecies-, and polymorphic sequences of the phages could be correlated to the genetic diversity of host strains.

[ID: 40] Structure of a polinton-like virus, the missing link between bacteriophage and eukaryotic viruses of the PRD1-like lineage

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Viruses in the PRD1-like lineage infect organisms across the evolutionary tree and build their capsids from proteins with beta-jelly rolls orthogonal to the capsid surface (1). Polintons are large eukaryotic dsDNA transposons encoding a protein-primed DNA polymerase (POL) and a retroviral-like integrase (INT). Most of them also include a DNA-packaging ATPase and a maturation protease similar to those found in PRD1-like viruses. They also encode genes that could translate into orthogonal jelly roll proteins, suggesting that in certain conditions they could form icosahedral capsids. These observations prompted the hypothesis that Polintons may have evolved from a PRD1-like ancestor (encoding capsid proteins, POL, and ATPase), which entered a proto-eukaryotic host with a bacterial endosymbiont and acquired the protease and integrase genes by recombination with a transposon (2)(3). Subsequent evolution would have resulted in the “polintovirus” elements splitting into two different ways of life: the transposable, capsid-less integrating elements, and the bona fide viruses.

Recently, marine metagenome analyses have revealed a group of putative polinton-like viruses (PLVs) in eukaryotes. PLV genomes contain genes for single and double jelly roll proteins and a packaging ATPase, but lack the protease and integrase genes (4). Therefore, PLVs could represent a minimal version, or a first ancestor, of the PRD1-like lineage in eukaryotic hosts. We are analyzing the structure of the only isolated virus belonging to this newly defined group: *Tetraselmis striata* virus N1 (TsV-N1) (5). The cryo-EM capsid structure at 5.2 Å resolution corroborates the placement of TsV-N1 in the PRD1-like lineage.

1. Krupovic et al., *Nat Rev Microbiol* 6, 941 (2008).
2. Fischer et al., *Science* 332, 231 (2011).
3. Krupovic et al., *Nat Rev Microbiol* 13, 105 (2015).
4. Yutin et al., *BMC Biol* 13, 95 (2015).
5. Pagarete et al., *Viruses* 7, 3937 (2015).

[ID: 155] Effect of bacterial metabolism in the bacteriophage infection

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Bacteria have developed various strategies for survival in unfavorable environments. The stationary phase of growth is such an unfavorable condition requiring adaptation. In the model for Gram-positive bacteria *Bacillus subtilis*, a mix of cells in different physiological states, like sporulation, natural competence for DNA transformation or biofilm development can be observed during stationary phase. L-proline is a highly abundant amino acid that can be used by *B. subtilis* as a sole source of carbon or nitrogen. Its catabolism plays a central role in stationary phase states. L-proline catabolism is controlled genetically by the *putBCP* locus. The first step in the catabolism of L-proline is carried out by the proline dehydrogenase *PutB*. It was previously (Cvirkaite-Krupovic et al 2015) shown that *putB* is a nonessential *B. subtilis* gene that widens the window of opportunity for multiplication of bacteriophage SPP1 in stationary phase.

Since phage infection is an energetically expensive process that consumes a significant part of its host's energy budget, we investigated whether the disruption of the *putB* gene affects the ATP levels during the bacterial cell cycle and its impact in SPP1 infection.

We showed that a $\Delta putB$ mutant has decreased ATP levels, a reduction that becomes more drastic in the stationary phase. This bacterial mutant is defective in sporulation, competence and biofilm formation suggesting that proline catabolism provides energetic resources for stationary phase processes. SPP1 infection is also affected. Furthermore, we showed that SPP1 infection induces ATP production. We will present results on the effects of the catabolic route that degrades proline and its intersecting arginine degradation pathway on *B. subtilis* stationary phase processes and how they impact on phage infection during the bacterial cell cycle.

[ID: 38] Temporal and spatial dynamics of viral community composition and viral-host interactions in groundwater systems

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Bacterial xenobiotic degraders are important members of microbial communities, often helping towards contaminant removal in contaminated groundwater sites. Despite the relevance of bacteriophages in the ecology, diversity and adaptation of bacteria, the study of bacteriophages in groundwater and their interactions with bacterial degraders has been widely overlooked, and as far as we know, no study detailing and linking bacteriophages to degrader communities exist. Moreover, no comprehensive metagenomic study has been published yet on the diversity of viral communities in contaminated groundwater environments.

In this study, we characterized temporal and spatial dynamics of viral communities found in groundwater from an old gasworks site in Northern Ireland. Environmental samples were collected for a full year (May 2016 – May 2017) and a total of 14 viromes were shotgun-sequenced using the Illumina® HiSeq 2500/4000 Systems. Bioinformatic analysis was carried out using state of the art tools.

Taxonomic characterization of the viromes revealed discrete lower-level variations within viral communities over the one-year period and across the site (e.g. Bpp1virus, Pamx74virus and Lambdavirus). Nonetheless, most viral sequences were assigned to bacteriophage members of the Myoviridae, Siphoviridae and Podoviridae families. Putative bacteriophage interactions with bacterial degraders were also investigated. To do this, the host's assignment of viral contigs ≥ 2.5 kb was performed using CRISPR spacer sequence homology, tRNA homology, and viral contig homology against the bacterial RefSeq database. Because total metagenomes were also sequenced in this study, contig homology against local microbial sequences was also performed.

Ongoing work aims to further evaluate and describe relationships between bacteriophages and degraders at the site and examine their interactions in further detail. Particularly, we are interested in detailing viral generalists and exploring their functional profile.

[ID: 39] Bacteriophages: genomic reservoirs of hydrocarbon biodegradation capacity

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Metabolization of recalcitrant compounds such as hydrocarbons by local microbial communities is an active area of research with immediate impact in technological fields such as bioremediation. The ability of bacteriophages to carry metabolic genes in their genome, i.e., auxiliary metabolic genes has been described previously. However, as far as we know, the putative presence of genes involved in hydrocarbon biodegradation within phage genomes has not been explored yet.

For this study, 14 previously generated viral datasets were used to assess the role of bacteriophages as genetic reservoirs of biodegradation capacity. The datasets used were generated using Illumina® HiSeq 2500/4000 platforms and they were obtained in the scope of a broad ecological study of viral and bacterial groundwater community diversities. The sequenced viromes were collected over a yearlong sampling period (May 2016 – May 2017) from an old gasworks site in Northern Ireland suffering from hydrocarbon contamination.

First, a functional characterization of all contigs ≥ 2.5 kb was carried out. Second, gene orthologs encoding enzymes acting in biodegradation pathways of various hydrocarbons were annotated and detected in a small number of contigs. The examples of biodegradation genes found in putative viral contigs are a S-(hydroxymethyl) glutathione dehydrogenase / alcohol dehydrogenase (ahdC ortholog) and a propanol-preferring alcohol dehydrogenase (adhP ortholog), both important participants in the catabolic pathways of naphthalene, chloroalkanes and chloroalkenes. Taxonomic assignment of the corresponding contigs and description of their genetic profile was also carried out. Here, a relevance of bacteriophages for bacterial adaptation and evolution was suggested, particularly, in the putative augmentation of the biodegradative capacity of local microbial communities.

Current work aims to integrate these findings with ongoing research on the viral-host dynamics. Furthermore, we hope that these findings may help to advance the tailoring of bioremediation strategies of polluted environments.

[ID: 94] Survey on prophages infecting lactic acid bacteria *Weissella cibaria* and *W. confusa*

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Lactic Acid Bacteria (LAB) infection by bacteriophages could be responsible for impaired food fermentations. However, there are few studies concerning phages in fermented products other than dairy foodstuffs. *Weissella cibaria* and *W. confusa* belonging to Leuconostocaceae family (also including *Leuconostoc*, *Oenococcus* and *Fructobacillus* genera) have been isolated from a wide range of habitats, and are known to be involved in several traditional food fermentations such as starchy, cereal-based, meat and fish products (Fusco et al. 2015). Moreover, strains of these species are recognized to produce copious amounts of oligosaccharides and exopolysaccharides, with special interest as texturizing agents and prebiotics (Bounaix et al. 2010).

No phage has been yet described to infect *W. confusa* and only two phages infecting *W. cibaria* (ϕ YS61 from kimchi and ϕ 22 from Nham) were currently described, both belonging to the family of Podoviridae (Kleppen et al., 2012; Pringsulaka et al., 2011). Besides, several phages infecting *W. cibaria* were isolated from commercial cucumber fermentation (Lu et al., 2012).

The aim of the present study was to investigate the presence of prophages in *W. cibaria* and *W. confusa* strains previously isolated from French sourdoughs (Robert et al., 2009) and from other diverse biotopes. Prophage excision was achieved with mitomycin C induction and led to observe bacterial lysis for all the strains. Unfortunately, no sensitive bacteria could be detected for any of the putative phages excised from the studied strains. Evidence of phage DNA was thus achieved by indirect approach i.e. purification of phage DNA from mitomycin-induced bacterial cultures. Further DNA characterization was achieved by conventional restriction analysis, and repetitive element (Rep)-PCR profile using (GTG)₅ primer as a new phage DNA comparative technique.

Overall, the results of this study clearly demonstrate the prevalence and diversity of prophages in the genome of *W. cibaria* and *W. confusa* LAB strains.

[ID: 111] Interaction of lytic bacteriophages and plant pathogenic bacteria – an example involving lytic phage ϕ D5 and pectinolytic plant pathogenic *Dickeya solani* IPO2222 in potato-associated environment

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Bacteriophages (phages) are viruses able to infect and kill bacteria. Although phage-bacterial interactions have been studied since the beginning of their discovery, still little is known about their global ecological impact on the spread, adaptation and evolution of bacterial hosts.

In this study we aimed to obtain knowledge on the specific ecological interaction between the pectinolytic bacterium *Dickeya solani* and a broad host lytic bacteriophages isolated in our former studies. We also aimed to assess the bacteriophage viability in natural ecosystem (potato plants) as a preliminary step in the possibility to better understand how bacterial viruses affect populations of plant pathogens in situ. For this, as an example ϕ D5 was characterized for stability in solutions containing copper ions, in potato tuber extracts, in rain water and in soil. The phage was also tested for the ability to survive on the surface of (detached) potato leaves and potato tubers in storage at 8 °C. In tissue culture grown potato plants as well as in experiments in which tubers were grown in potting soil, we tested if application of ϕ D5 presiding application of *D. solani* can protect growing plants from development of disease symptoms. ϕ D5 remained infectious in potato tuber extract, soil and rain water in time but was inactivated in solutions containing copper ions. Both, in tissue culture and soil-grown potato plants, ϕ D5 protected plants from *D. solani*, reducing infection caused by the pathogen by more than 50%.

This is a case study describing viability and interaction of lytic bacteriophage with plant pathogen in their natural environment and may serve as a starting point for similar studies involving different crop systems with the overall aim to use the generated knowledge for development of new environmentally friendly control applications.

This study was funded by grant no. LIDER/450/L-6/14/NCBR/2015 “PATBIOCON” from the National Centre for Research and Development, Poland to RC

[ID: 10] Bacteriophage genotyping using repetitive-PCR

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Repetitive-PCR, which has been primarily used for the discrimination of bacterial strains, was investigated for its potential to genotype bacteriophage. The single repetitive primers, BOXA1R and BOXA2R, which target interspersed repetitive elements in noncoding genome regions, were tested using purified phage DNA, phage lysates and phage plaques. The method successfully differentiated the genetic profiles of both closely related phages, unrelated phages and their respective bacterial hosts. DNA fingerprint patterns were shown to be reproducible for the phage of various bacterial species, including *Lactococcus lactis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*; and the algae *Halorubrum*. The findings provide evidence that rep-PCR using BOXA1R or BOXA2R could potentially be used as a simple, rapid, high resolution and inexpensive method for phage genotyping. Application of the techniques could include the monitoring and management of phage contamination in the use of bacterial starter cultures; the detection of emerging phage strains; defining the ecology of established resident phage flora; monitoring the persistence of a particular phage within a certain environment, its discrimination from naturally occurring phage species, and its potential geographic spread. It could also be used as an adjunct to other phage genotyping methods, for the fingerprinting of newly isolated phage strains to be evaluated for use as biocontrol agents in industrial fermentation processes or for therapeutic applications.

[ID: 240] Enzybiotics as a crop protection product against the kiwi pathogen *Pseudomonas syringae* pv. *actinidiae*

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Enzybiotics derived from bacteriophage-encoded endolysins are a novel class of antibacterial modular enzymes that kill both Gram-positive and Gram-negative bacteria through degradation of peptidoglycan in the bacterial cell wall, followed by rapid osmotic lysis and cell death. Unique in the enzybiotics concept is that their antibacterial properties such as activity, specificity, etc. can be customized depending on their modular composition.

Enzybiotics have been successfully developed for human and veterinary pathogens but their application in plant protection has not been assessed. *Pseudomonas syringae* pv *actinidiae* (Psa) is an aggressive pathogen that strongly compromises kiwifruit production all over the world, including Europe. There are currently no curative products for Psa and all existing treatments, including copper-based bactericides, disinfectants, and antibiotics, are preventive measures, some of which are under ecological/political pressure to be removed from the market. Enzybiotics have the potential to offer a novel, safe, biodegradable, fast-acting and sustainable crop protection product active against Psa.

We have constructed a large collection of enzybiotics with a new DNA shuffling technique, coined VersaTile Shuffling. Each variant is composed of an outer membrane permeabilizing peptide, linker, cell wall binding and enzymatically active domain. From a library of >10,000 variants, we analyzed 288 randomly picked variants for their expression levels and inhibitory activity against Psa. The specificity of the thirty best enzybiotics was assessed against diverse phytopathogens including Psa, *Pseudomonas syringae* pv. *syringae*, *Pseudomonas viridiflava*, *Pseudomonas savastanoi* and the biocontrol *Pseudomonas fluorescens* strain. This analysis yielded well-expressed variants with diverse specificity spectra. Further, we report on the development of an in vitro pathogenicity test, assessment of the phytotoxicity of these enzybiotics.

[ID: 295] Genomic and functional analysis of two orthocluster IV *Listeria monocytogenes* phages isolated from soil samples

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Listeria monocytogenes is an important foodborne pathogen and the causative agent of listeriosis, a potentially fatal infection. Many phages infecting *L. monocytogenes* have been isolated and characterized but only a limited number have been sequenced at the genome level (approximately 40 as of April 2018).

The aim of this research was to compare bacteriophage genomes, their morphology, and perform functional analyses to identify the genes responsible for binding of these phages to their bacterial hosts.

Eight *L. monocytogenes* and two *L. innocua* bacterial strains, including representatives of the *L. monocytogenes* serotypes 1/2b, 4b, 1/2a, 4b, 1/2a, 4d, were used in the screening study. Phages were isolated using two enrichment steps, whereby the soil was mixed with *Listeria* strains in TSB broth supplemented with CaCl₂ and MgSO₄, and incubated while shaking for 24 h at 37°C. Samples were analysed for the presence of phages using standard spot and double-layer plaque assay techniques.

Of nine plaques isolated on *L. monocytogenes* RM2218, we found at least two distinct phage types on the bases of RAPD-PCR and DNA enzymatic restriction profile analysis. Genome sequencing revealed the temperate nature of these phages (ΦAPC_Lm2 and ΦAPC_Lm5). OrthoMCL analyses showed these phages belong to a recently defined group of *Listeria* bacteriophages denoted orthocluster IV, along with phages A500, A118, A006, and LP-030-3. Morphologically, ΦAPC_Lm2 and ΦAPC_Lm5, were found to belong to Siphoviridae family. The two phages demonstrated broad host range specificity in a spot test, especially against *L. monocytogenes* serotype 4b.

[ID: 114] Analysis of bacteriophage-host interactions using SMALP-seq

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New appreciation for the ecological impact, potential applications and role in human health of phages has led to a spike in research interest. For example, metagenomic surveys have uncovered a wealth of viral diversity in natural and human-associated samples, whose environmental and clinical relevance remains unclear. After a century of bacteriophage research, the majority of phages and their host interactions remain unstudied. While recent metagenomics studies have provided a wealth of information on the natural genetic diversity of phages, linking this genetic data to the interactions between phage and host remains challenging.

Here we introduce SMALPseq, a new methodology to study the interaction between phages and hosts that exploits host-specific membrane nanoparticles that may be produced in high-throughput. Upon phage-host recognition, ejected phage DNA can be specifically isolated and sequenced. Thus, the identification of phage-host interactions can be directly coupled to the characterisation of novel phage genome sequences. The current proof of concept shows the specificity of SMALPseq for identifying phage-host interactions for model phages including *Escherichia* phages Lambda, T4, and *Bacillus* phage Phi-29. Phage ejection was measured on various bacteria including their original hosts and unrelated species expressing the phage receptor.

Together, we present a novel method for detecting phage-host interactions with the potential for high-throughput application. We expect that this method will complement existing methods, leading to a more comprehensive picture of the phage-host interactions in nature.

[ID: 122] Temperate phages infecting the gut commensal *Faecalibacterium prausnitzii* belong to novel viral genera that help decipher intestinal viromes in relation with IBD

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Recent viral metagenomic studies have hinted at a role of phages in intestinal dysbiosis associated with several human diseases. However, interpretation of viral metagenomic studies is limited by the lack of information concerning phages infecting human commensal bacteria, especially those of the gut microbiota. In particular, no phages have been described that infect *Faecalibacterium prausnitzii*, a bacterial symbiont dominant in the human gastrointestinal tract repeatedly found depleted in Inflammatory Bowel Disease (IBD) patients. This knowledge gap prevents the study of the potential role of phages on *F. prausnitzii* populations.

Here we describe 18 phages identified as prophages in 15 genomes of *F. prausnitzii*. Comparative genomics delineated 8 phage groups, each corresponding to a genera level cluster. We obtained either experimental or in silico evidence of activity for at least one member of each cluster. In addition, we show that four of these phages are either significantly more prevalent or more abundant in IBD patients than in healthy controls.

Our results suggest a link between phages, decreased abundance of *F. prausnitzii* and IBD pathogenesis. More generally, these new phage genera should prove helpful for future comprehensive analysis of intestinal viral metagenomic studies.

[ID: 87] Optimized phage-robust cultures

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Lactic acid bacteria are widely used as starter cultures for the manufacture of cheese and other fermented milk products. However, during the fermentation process the strains can become susceptible to bacteriophage attack, resulting in delay or even failure of the fermentation process and thereby causing significant economic loss.

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

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

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

[ID: 198] Characterization of the virome of *Paracoccus* spp. (*Alphaproteobacteria*)

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Paracoccus spp. (*Alphaproteobacteria*) are metabolically versatile bacteria. They have been isolated from a wide range of environments in various geographical locations, including pristine and extreme environments as well as anthropogenically shaped ecological niches (e.g. contaminated soils and wastewater treatment plants). There is also one *Paracoccus* species (*P. yeeri*) recognized as a possible human opportunistic pathogen. The metabolic flexibility of *Paracoccus* spp. rely mostly on variety of respiratory processes, including denitrification employing several alternative electron acceptors and the ability to use diverse C1 compounds as electron donors to respiratory chains. These properties, combined with the ability to use a broad range of toxic organic compounds as a source of carbon and energy, as well as efficient degradation of pesticides and herbicides in soils show substantial biotechnological potential of *Paracoccus* spp., especially in bioremediation.

Currently 63 sequences of *Paracoccus* spp. genomes are available in public databases, however only three sequences of active phages infecting members of this genus have been reported, including lytic vB_PmaS_IMEP1 and Shpa, and temperate phage ΦPam-6 – all representing *Siphoviridae* family. In this study 5 novel active temperate phages, originating from five different *Paracoccus* species, were identified and characterized (vB_PbeS_Pbe1, vB_PkoS_Pko1, vB_PsuS_Psul1, vB_PthS_Pthio1, vB_PyeM_Pye1), including the first (in the entire genus) representative of the *Myoviridae* family (vB_PyeM_Pye1).

The genomes of all the identified viruses were sequenced, assembled, annotated and analyzed. Functional characterization of two toxin-antitoxin systems, found within vB_PyeM_Pye1 and vB_PkoS_Pko1, was also performed. Moreover, in-depth bioinformatic analyzes of all available genomic sequences of *Paracoccus* spp. were conducted to identify prophage regions. As a result 54 complete prophages were identified, their nucleotide sequences were manually reannotated and used, together with the sequences of active phages of *Paracoccus* spp., for complex comparative analyses, applying similarity network constructions. This provided the first insight into overall diversity of the *Paracoccus* virome.

[ID: 436] Engineered phage therapeutics against multidrug resistant pathogens

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The problem of antibiotic resistance, long recognized by microbiologists as a threat to effective treatment, has recently received widespread attention and highlighted the need for novel therapeutic approaches. Lytic bacteriophages offer an attractive alternative to conventional antibiotics because they can effectively attack drug resistant bacteria, multiply at the site of infection facilitating effective dosing, are innocuous to human cells and have no damaging effects on the microbiome compared to antibiotics. Despite their therapeutic potential, translational and clinical development of natural phages has been hindered mainly by intrinsic limits, including narrow host range that demands large cocktails of phage, poorly defined drug substance and clinical studies that fail to meet rigorous standards required for FDA approval. Recent efforts to develop highly effective therapeutic phages have largely centered on customized cocktails defined by screening single bacterial isolates, or complex combinations of natural phages which make their broad use in infectious disease difficult. Genetic engineering of phage genomes can overcome many of these hurdles. The benefits of a synthetic phage approach have been demonstrated in multidrug resistant *Pseudomonas aeruginosa* with an engineering platform that allows rapid and iterative modifications of viral genomic DNA. We showed that combining comparative genomics with genome editing enables construction of phages with desired therapeutic attributes. We rationally and rapidly designed phages with broader host range, increased lytic activity and demonstrated that engineered phages have improved efficacy compared to natural phages in *P. aeruginosa* models of infection. Further, these phage attributes had positive impacts in downstream processing, yielding high titers in fermentation at scale, facilitating purification which yields phage devoid of contaminants and appropriate for human use. Appropriate test methods and release criteria are being developed to qualify our clinical candidates produced under GMP conditions at C3J Therapeutics, Inc. for use in human clinical trials.

[ID: 234] Nitric-oxide counteracts prophage induction in *E. coli*

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Bacterial genomes contain large amounts of proviruses also called prophages, either functional or defective for infectious propagation. All these prophages contribute to bacterial genome evolution by providing an additional pool of genes that sometimes accommodate new properties to the host, a phenomenon called lysogenic conversion. Prophages usually hijack the host stress response signalisation to resume a lytic cycle when conditions become threatening for the host and therefore for the integrated prophage.

Our recent work shows that nitric oxide serves as a maintenance signal and induces the production of a nitric oxide reductase, which in turn prevents prophage induction independently of its usual activity. Surprisingly, nitric oxide, which is a potent nitrosative agent responsible for many cellular damages, does not promote prophage induction but rather counteracts the SOS-response outcome. These results make particularly sense for enterobacteria and their prophages when exposed to nitric oxide produced in the gut during inflammation or through their own anaerobic respiration.

[ID: 378] Preliminary studies on pharmacokinetics and pharmacodynamics of virulent Myoviridae and Podoviridae infecting *Pseudomonas aeruginosa* and *Escherichia coli*.

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While the compassionate treatment of antibiotic resistant infections by bacteriophages is being authorized in Belgium, France and in the USA, data about the dose and the timing required to ensure a successful treatment remain mostly empirical.

Over the past decade we have isolated several virulent bacteriophages that have been tested in a murine model of acute pulmonary infection following intranasal instillation [1–3]. Here we investigated, in mice, pharmacokinetics (concentration-time courses in the body resulting from administration of a drug dose) and pharmacodynamics (observed effects resulting from a given drug concentration) of several of these bacteriophages.

First, we recorded bacteriophage distribution over time into several organs (lungs, spleen, liver, kidneys) and blood compartment after an intranasal or a systemic administration. Second, using our animal model of acute pneumonia, we observed over time the bio-distribution in the organs and blood compartment of bacteriophages administrated by intranasal or systemic routes. In each organ we compared the relative amount of bacteria and bacteriophages.

Our data provide valuable information on bacteriophages half-life, their elimination route and their ability to reach the bloodstream. In the future, we aim to implement a pharmacokinetics / pharmacodynamics model of pulmonary phage-therapy.

1. Henry M, Lavigne R, Debarbieux L. Predicting in vivo efficacy of therapeutic bacteriophages used to treat pulmonary infections. *Antimicrob Agents Chemother* 2013;57:5961–8. doi:10.1128/AAC.01596-13
2. Dufour N, Debarbieux L, Fromentin M, et al. Treatment of Highly Virulent Extraintestinal Pathogenic *Escherichia coli* Pneumonia With Bacteriophages. *Crit Care Med* 2015;43:e190–8. doi:10.1097/CCM.0000000000000968
3. Dufour N, Clermont O, La Combe B, et al. Bacteriophage LM33_P1, a fast-acting weapon against the pandemic ST131-O25b:H4 *Escherichia coli* clonal complex. *J Antimicrob Chemother* 2016;71:3072–80. doi:10.1093/jac/dkw253

[ID: 195] Isolation and characterization of novel bacteriophage targeting carbapenem-resistant *Pseudomonas aeruginosa* containing blaNDM-1 gene in Nepal.

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Global emergence of carbapenemase-producing *P. aeruginosa* is a major public health burden, which is often extensively multidrug resistant and organisms harboring MBL enzymes may lead to therapeutic dead ends. Thus, because of the difficulty to treat these “superbug” and menace and some term as “apocalypse” of post antibiotics era, an alternative approach to control this pathogen might be bacteriophage. In this study, we aimed to determine the prevalence of MBL genes in *P. aeruginosa* and isolate and characterize novel bacteriophage against these superbugs for potential use in phage therapy.

Sixty-seven MDR isolates of *P. aeruginosa* were tested for the presence of MBL enzymes. Molecular identification of MBL genes blaIMP, blaVIM and blaNDM was done by PCR. Lytic bacteriophages against these isolates were isolated and purified. Biological features, physicochemical characters were determined. One of the most potent phage: Phage TU_PA 43 was characterized by molecular methods and TEM.

Among 67 clinical isolates, 15 strains (37.5%) of carbapenem resistant *P. aeruginosa* contained blaNDM gene. Further sequence analysis showed the presence of blaNDM-1 in majority of sequenced isolates and 3 novel variants. Among 28 lytic bacteriophages against these isolates, the most potent phage TU_PA 43 showed broad host range. The phage was stable at pH ranging from 2 to 11 and showed good thermal tolerance at 10°C to 70°C. Nucleic acid concentration was 3.16 ng/μl and most predominant polypeptide appeared at a size of approx. 32+ 2 kDa. Electron microscopy confirmed that the phage was tailed and belonged to Caudovirales family.

Predominantly, blaNDM-1 type variant was found in the clinical isolates of *P. aeruginosa* in Kathmandu, Nepal, including novel blaNDM variant. MBL producer *P. aeruginosa* could be treated efficiently by phages. Broad host range, pH and thermal tolerance of Phage TU_PA 43 makes the phages an excellent candidate for therapeutics.

[ID: 338] Sb-1 bacteriophage exhibits antibiofilm properties in degrading exopolysaccharide matrix and targeting persister cells of *Staphylococcus aureus*

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Sb-1 is a therapeutically relevant bacteriophage active against *Staphylococcus aureus* (*Sa*). However, the antibiofilm properties of Sb-1 have not been elucidated yet. Here, the capability of Sb-1 to eradicate biofilm, degrade the biofilm matrix and target “persister” cells of *Sa* was evaluated.

Sa ATCC 43300 (MRSA) biofilm was treated for 24h with different Sb-1 titers (10^2 to 10^7 PFU/ml) and analyzed by isothermal-microcalorimetry. The matrix in the biofilm was stained with wheat germ agglutinin conjugate with Alexafluor488, after the biofilm treatment with sub-inhibiting phage titers and visualized by confocal laser scanning microscopy. Persister cells were either induced by exposing stationary phase cells of MRSA to 400 μ g/ml carbonyl cyanide m-chlorophenylhydrazone (CCCP) or isolated by exposing MRSA biofilm to high ciprofloxacin concentrations. The obtained persister cells (10^6 CFU/ml) were treated with 10^7 and 10^4 PFU/ml Sb-1 for 3h in PBS, followed by CFU counting. Alternatively, bacteria pre-treated with phages were washed and incubated in fresh BHI medium and the bacterial growth assessed after further 24 hours.

Sb-1, tested up to 10^7 PFU/ml, was not able to eradicate MRSA biofilm, although a strong killing was observed. Interestingly, Sb-1 determined a dose-dependent reduction of the exopolysaccharide component of the matrix. In the presence of 10^6 PFU/ml Sb-1, no fluorescent signal related to WGA488 was detected, although bacterial viability was not impaired. The highest titer of Sb-1 (10^7 PFU/ml) determined a strong reduction of persister CFU/ml (2-5 Log), while 10^4 PFU/ml Sb-1 had no effect against persisters in PBS. Noteworthy, Sb-1 pre-treated persisters were completely killed when bacteria, after phage treatment, were inoculated in fresh medium, reverting to a normal-growing phenotype. This study provided valuable data regarding the antibiofilm properties of Sb-1. Its ability to degrade the MRSA polysaccharide matrix and target persisters make Sb-1 suitable for the therapy of biofilm-associated infections.

[ID: 392] Novel DNA polymerase from thermophilic bacterium *Thermus thermophilus* MAT72 phage Tt72

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Thermophilic phages are recognized as untapped source of thermostable enzymes relevant in biotechnology, however their biology is poorly explored. This has led us to start a project aimed to investigate thermophilic phages isolated from geothermal areas of Iceland.

Here, we present report on DNA polymerase of phage Tt72 (Myoviridae) that infects *Thermus thermophilus* MAT72. In silico analysis of Tt72 phage genome revealed the presence of a 2112-bp ORF encoding protein homologous to the members of the A family of DNA polymerases. It contains conserved nucleotidyltransferase domain and a 3'→5' exonuclease domain, but lacks the 5'→3' exonuclease domain. The amino acid sequence of Tt72 DNA polymerase shows high identity to two yet uncharacterised DNA polymerases of *T. thermophilus* phages: ϕ YS40 (91%) and ϕ TMA (90%).

The polA gene is composed of 2112 nucleotides. The overall G+C content of this gene is 31,58%, which is lower than the G+C content of *T. thermophilus* genomic DNA (69.49%). The Tt72 polA gene codes for a 703-aa protein with a predicted molecular weight of 80,477. The isoelectric point of this protein is 5.76. The codon usage of Tt72 polA gene reflects its low G+C content, 79.5% of the codons end with either A or T. In order to determine the differences in the codon usage pattern of the Tt72 polA gene and that of *T. thermophilus*, we calculated the value of the codon adaptation index (CAI). Based on the assumption that genes with high CAI (near 1) belong to the class of highly expressed genes, we infer from CAI value for Tt72 polA gene, which is 0.04, its low rate of expression in *T. thermophilus*. The corresponding gene was cloned into pET15b vector and overexpressed in *Escherichia coli*. The recombinant protein was purified and characterized.

[ID: 249] Characterization and the host specificity of Pet-CM3-4, a new phage infecting Cronobacter and Enterobacter strains

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Cronobacter and Enterobacter are considered opportunistic pathogenic bacteria belonging to Enterobacteriaceae family. Cronobacter strains are responsible for rare but serious neonatal infections. Enterobacter cloacae can cause a range of infections in the vulnerable hosts such as the elderly and immunocompromised persons, it is frequent nosocomial pathogen and many strains are antibiotic resistant. Bacteriophages offer a safe approach for pathogen elimination. In the present study Cronobacter infecting bacteriophage Pet-CM3-4 was isolated from sewage. The phage was able to infect 47 from 50 Cronobacter strains covering seven species. The spontaneous mutant phage Pet-CM3-1 was isolated with host specificity lowered only to *C. malonaticus* serotype Cma O:3 strains. According to the genome sequence the Pet-CM3-4 phage has been assigned to the Tevenvirinae subfamily of Myoviridae phage family as the close relative of CC31 and PG7 phages. The comparison of the Pet-CM3-4 phage with the narrowed host specificity mutant Pet-CM3-1 revealed the K163Q substitution in gp263 gene encoding for the long tail fibre adhesin as the causative mutation of the lowered host specificity. The bacterial receptors involved in adsorption of Pet-CM3-1 and Pet-CM3-4 phages were analyzed by using transposon mutagenesis. Based on infection study of a transposon mutants from *C. malonaticus* strain we propose that the O-antigen is the major receptor of Pet-CM3-1 phage, however Pet-CM3-4 phage probably binds to outer core of LPS in this strain. Besides Cronobacter both phages infected high proportion of *E. cloacae*, *E. asburiae* and *E. ludwigii* strains, but the host specificity of both phages was not limited to one particular O-antigen in Enterobacter genus. Obtained results are an important prerequisite for phage application in the therapy and food control.

[ID: 160] Donor-recipient specific transfer and persistence of viruses and bacteriophages following fecal microbial transplantation

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Fecal microbiota transplantation (FMT) is used in the treatment of recurrent *Clostridium difficile* infection. Its success is typically attributed to the restoration of a diverse microbiota. Viruses (including bacteriophages) are the most abundant and diverse members of the microbiota but their fate following FMT has not been as well studied. We studied viral transfer following FMT from 3 donors to 14 patients. Recipient viromes resembled those of their donors for up to 12 months. Tracking individual bacteriophage colonization revealed that engraftment of individual bacteriophages was dependent on specific donor-recipient pairings. Specifically, multiple recipients from a single donor displayed highly individualized virus colonization patterns. The impact of viruses on long-term microbial dynamics is a factor which should be considered when considering FMT as a therapeutic option.

[ID: 355] Comparison of temperate bacteriophages of *Pseudomonas aeruginosa* from the IPCD International Pseudomonas Consortium Database containing over 1600 *Pseudomonas aeruginosa* genomes.

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Chronic respiratory diseases are characterised by irreversible dilation and damage to the lower respiratory tract and include disorders cystic fibrosis (CF) and bronchiectasis (BR). Both diseases are associated with chronic bacterial infection of mucus coating the lung epithelia due to alterations in ion transport (CF) and epithelial damage (BR). This mucus rich environment offers an ideal site for opportunistic bacteria such as *Pseudomonas aeruginosa* (Pa) to colonise. The accessory genome of Pa is frequently overlooked in comparison to the core genome in how it relates to infection and disease in the chronically infected lung. However, the Pa genome isolated from the lung typically contains several prophages, where multiple can be mobilised simultaneously with a signal to induce from their site of integration. These prophages whilst integrated into the chromosome of the bacteria may play a role in adaptation and evolution allowing for selection and longevity in the lung. This is the largest study of prophage carriage in Pa spanning >1600 genomes present in the IPCD International Pseudomonas Consortium with bacteria obtained from 35 countries including; CF, BR, burn wounds and environmental isolates and historical samples dated as far back as the year 1880. This large comparative study focuses on the difference in prophage carriage between isolates. It also allows comparison between the accessory genome of these prophage regions and how different gene carriage relates to the environment and isolate history. Phaster was used to identify the prophages present in the genomic data and data mined through local virulence gene databases and the AMR database CARD.

[ID: 254] Isolation of phages specific to *Pantoea agglomerans* and detection of their activity on pepper plants

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Pantoea agglomerans is one of the most widespread opportunistic human pathogens. Belonging to the family Enterobacteriaceae it is common in the nature as well. Furthermore, this bacterium is reported to be the frequent cause of nosocomial infections. It is necessary to find alternative treatments as antibiotics are losing their efficacy due to the development of bacterial resistance. We consider bacteriophages as suitable candidates for specific and effective therapeutic use. A bacterium was isolated from tomato fruits, which resemble symptoms of bacterial rot. After that we obtained the bacteriophages that infect specific bacteria isolated from the same tomato. We have accumulated a high titre of viruses and obtained phages to breed true. The bacteriophages were examined in the electron microscope. Morphological properties suggest that those phages belong to the Siphoviridae family. Further activity of isolated bacteriophages to suppress the development of a bacterium was investigated on young pepper plants. The pepper plants were divided into 3 groups: 1 - control group; 2 - plants treated with bacteria; 3 - plants treated both with bacteria and virus. The second group presented the symptoms of bacterial rot and leaf rolling while the third group indicated complete inhibition of bacterial growth in plants. Thus, the efficacy of bacteriophages in suppression of bacterial growth in plants has been proved. The development of phage-based products against specific bacterial strains may help in treatment of both vegetable diseases and intrahospital infections caused by this pathogen.

[ID: 209] Gene editing tools to study virophage biology

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Virophages are unique viruses that parasitize giant DNA viruses inside the share protist host. Interestingly, the virophage mavirus protects *Cafeteria roenbergensis*, a unicellular marine phagotrophic flagellate, from infection by the giant virus CroV. The mutualistic virophage-host relationship implies an important ecological role for virophages. Hence, virophages may act as an antiviral defense system in their hosts. As part of this defense mechanism, mavirus integrates its DNA into the genome of *C. roenbergensis* and is re-activated by superinfection with CroV.

For a detailed characterization of the relationship between the three organisms (protist, giant virus and virophage), genetic tools must be implemented to address genotype-phenotype relationships. We implement the CRISPR/Cas9 technique on *C. roenbergensis* cells carrying an integrated mavirus genome in order to study the biology of the virophage. Our approach is to deliver Cas9 along with the sgRNA as a preformed ribonucleoprotein (RNP) complex using electroporation techniques.

The successful implementation of the CRISPR-Cas9 technique in our tripartite host-virus-virophage system will prove to be an invaluable tool, as it allows to introduce specific genetic changes in mavirus and in the host (and eventually, also in CroV) to identify virophage protein functions, and find interaction points between these three microbial players.

[ID: 336] Global phylogeography and ancient evolution of the widespread human gut virus crAssphage

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Microbiomes are vast communities of microbes and viruses that populate all natural ecosystems. Viruses have been considered the most variable component of microbiomes, as supported by virome surveys and examples of high genomic mosaicism. However, recent evidence suggests that the human gut virome is remarkably stable compared to other environments. Here we investigate the origin, evolution, and epidemiology of crAssphage, a widespread human gut virus. Through a global collaborative effort, we obtained DNA sequences of crAssphage from over one-third of the world's countries, showing that its phylogeography is locally clustered within countries, cities, and individuals. We also found colinear crAssphage-like genomes in wild Old-World and New-World primates, challenging rampant viral genomic mosaicism and suggesting that the association of crAssphage with hominids may be millions of years old. We conclude that crAssphage is a benign globetrotter virus that has co-evolved with the human lineage, and an integral part of the healthy human gut virome.

[ID: 197] qPCR as an alternative to conventional method of double agar overlay applied for phage enumeration

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BFC-2 has been developed as a phage therapeutic cocktail active against clinical strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Acinetobacter baumannii*. The cocktail contains five different phages. To enumerate phage titers in a precise and rapid way during the production process of phage therapeutics and clinical trials, we developed qPCR.

Enumeration of bacteriophages by the double agar overlay method is still considered as the golden standard in phage quantification. However, some well-known limitations of the method (laboriousness, reproducibility) led us to develop a qPCR. We started with the *in silico* design and evaluation of primers, followed by the selection of at least one primer pair for each bacteriophage based on efficiency and specificity within the platform conditions. The qPCR platform has been optimized for quantification of five phages. Based on the concentration of phage in saline as determined with the plaque assay, the detection limit of the qPCR system was log 3 pfu/ml. For detection in human serum spiked with phages the limit was log 6 pfu/ml.

Plaque assay and the qPCR platform were also used to determine phage stability. Phage ISP, active against *S. aureus*, was subjected to different stress and storage conditions. We observed that the manner of storage has a major influence on the viability of the phage determined by PA. However, according to qPCR data the DNA titer remained stable. Also exposure to high and low temperatures did not affect the qPCR data. Our results showed that there was no correlation between numbers of active phage particles (plaque assay) and amplifiable phage DNA (qPCR) in phage suspensions after different storage periods. Further research is needed to elucidate the mechanisms behind decreasing viability of phage stocks.

[ID: 260] Orf61 and orf60a as a potential regulators of lambdoid phages development

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Enterohemorrhagic Escherichia coli (EHEC) strains are human pathogens. Their main virulence factors are Shiga toxins, encoded by genes located in genomes of prophages. The genes coding this toxins are silent in lysogenic bacteria, and prophage induction is necessary for toxin production. This process is provoked by mitomycin C, UV-irradiation and hydrogen peroxide. Our previous results present the evidence for the relation between the exo-xis region of the phage genome and lambdoid phage development [2]. We also indicate that orf63 from exo-xis region may have specific functions in the regulation of lambdoid phages development, especially at the stage of the lysis vs. lysogenization decision [1]. Molecular mechanisms of activities of other genes products are still unknown.

In view of this, I decided to determine the influence of the uncharacterized open reading frame orf61 and orf60a from the exo-xis region on lambdoid phages development using recombinant prophages, λ and Stx phage- Φ 24B.

The phage mutants were constructed by homologous recombination. I investigated the induction of recombinant lambdoid prophages treated with H₂O₂. The phage titer is at the similar level relative to wild-type in both tested mutants with use phage λ . The phage titer is lower compare to the control with use Φ 24B, which is confirmed by the number of survival E. coli bacteria during prophage induction. This number is higher compare to the wild type. Also higher number of cells survived after infection of phage Φ 24B bearing the deletion of orf61 and orf60a. The removal of this orf result in more efficient lysogenization of E. coli bacteria by phage λ and ϕ 24B. I also demonstrated the interesting effects on intracellular lambdoid phage lytic development and phage adsorption on E. coli cells.

Reference list

[1] Dydecka A., et al.(2017). *Frontiers in Microbiology*, 8:1618.

[2] Licznarska K., et al.(2015). *Oxidative Medicine and Celular Longevity*, 2016:8453135.

[ID: 22] The therapeutic potential of bacteriophage ZCKP1 to control pathogenic *Klebsiella pneumoniae*

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The predicted growth in infection by multidrug resistant bacteria necessitates prompt efforts towards developing alternatives to antibiotics, such as bacteriophage therapy. Immuno-compromised patients with diabetes mellitus are particularly prone to foot infections by multidrug resistant *Klebsiella pneumoniae*, which may be compounded by chronic osteomyelitis. Bacteriophage ZCKP1 ϕ , isolated from freshwater in Giza, Egypt, was tested in vitro to evaluate its lytic activity against a multidrug resistant *Klebsiella pneumoniae* KP/01, isolated from foot wound of a diabetic patient in Egypt. Characterization of ZCKP1 ϕ indicated that it belonged to the Myoviridae family of bacteriophages with an approximate genome size of 48 kb. ZCKP1 ϕ lysed a range of osteomyelitis pathogenic agents with activity against different *Klebsiella* spp. strains but also against *Proteus* spp. and *E. coli* isolates. The bacteriophage demonstrated its lytic activity and reduced viability of its hosts by $\geq 2 \log_{10}$ CFU/ml at 25°C and remained functional at 4°C, permitting treatment over a range of temperatures. The phage demonstrated a significant reduction in biofilm biomass (more than 50%) and viability (up to 66%) with high multiplicity of infection (50 PFU/CFU), achieving the greatest effect. These characteristics of ZCKP1 ϕ phage indicate potential therapeutic value to treat *Klebsiella pneumoniae* infections and warrant further investigation.

[ID: 126] Mechanism of arbitrium-based lysogeny regulation in phage phi3T

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In every infection, a temperate virus decides whether to replicate and lyse their host or to lysogenize and keep the host viable. We show that phage phi3T, belonging to the spBeta group of phages, uses a small-molecule communication system to coordinate lysis-lysogeny decisions. During infection of its *Bacillus* host cell, the phage produces a 6aa communication peptide that is released to the medium. In subsequent infections, progeny phages measure the concentration of this peptide and lysogenize if the concentration is sufficiently high. We found that different phages encode different versions of the communication peptide, demonstrating a phage-specific peptide communication code for lysogeny decisions. This communication system, termed the “arbitrium” system, is encoded by 3 phage genes: aimP, producing the peptide, aimR, the intracellular peptide receptor, and aimX, a negative regulator of lysogeny.

[ID: 367] Dependence of physiological complexity on the phage genome size for phages of C1-morphotype

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In phages with small genomes the DNAs are tightly packed with genes essential for performing all processes of phage development cycle. On the contrary, phages with large genomes in addition to the genes coding for complex virion structure, carry genes of no obvious advantage for phage. The questions that remain open are: what is the biological relevance of bacterial gene homologs in phage DNA and whether any vertical evolution of phages can be traced.

To reliably answer these questions, phages of the same morphotype were considered for analysis. We have studied three Podoviridae phages: ZeVs (T7virus), E105 (phiKMV-like phage), and TT10-27 (N4-supergroup). Increment of their DNA size from 37.8 to 74.1 kb, correlates with average gene size increase, phage capsid enlargement, and sophistication of DNA delivery machinery (short tail tube, tail with spikes, and complex tail with appendages).

Complexity increase also correlates with virulence enhancement: developing the ability of completely degrading the host nucleoid, and sophistication of nucleotide metabolism system. TT10-27 codes for proteins that make this process most complex (dCTP deaminase, NTP pyrophosphohydrolase, etc).

Interestingly, with the increase of size the DNA coding capacity decreases: ZeVs uses 99.9% of its DNA for coding, E105 – 93.2%, and TT1027 (though ORFs are transcribed from both strands) – only 94.3%, having 0.1%, 6.8% and 5.7% of non-coding DNA, respectively. The amount of proteins with unknown functions increases from 36% to 72%. Genomes of larger phages contain more unique proteins (E105 – 20%, TT10-27– 10%) and tRNAs genes. TT10-27 also carries at least one bacterial gene.

We assume the presence of bacterial genes in phage DNA to be a required measure for providing content for the enlarged DNA, as its physical dimensions (size) exceed its coding capacity (number of basic functions required for phage development). Increase of complexity occurs on the level of phage adaptation to a peculiar host.

[ID: 350] Defining the function of conserved hypothetical gene products encoded by an Stx phage.

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Stx phages convert their bacterial hosts, providing them with shigatoxigenic potential. All *E. coli* carrying Stx phage are known as Shiga toxin-encoding *Escherichia coli* (STEC) have become a global challenge to food safety. Around 74% of the genes carried by the model Stx phage, ϕ 24B (vb_EcoP-24B), are annotated as hypothetical, and we have been ascribing function to many of these genes. The expression of some hypothetical genes has been shown to be uncoupled from viral replication, but function to aid the lysogen in surviving environmental stresses such as antibiotic and acid exposures. Other genes, like gene 21, the target of this study, are expressed only during phage lytic replication. Gene 21 lies immediately upstream of the S, R, Rz and Rz1 genes. The gene product, P21, has been shown to possess an enzymatically active carbohydrate esterase domain (amino terminus), but its preferred biological substrate remains to be determined. We have created a series of isogenic mutants to establish whether P21 impacts upon phage release. New data show that P21 restricts the escape of 90% of progeny phages from their *E. coli* K12 hosts and limits the production of phage in an *E. coli* O157:H7 host, though release happens 10 min earlier. Work is currently ongoing to better understand how the action of P21 controls these phage release phenotypes. Peptidoglycan modification is used by Gram negative bacteria to increase their fitness and is a known phenomenon. Due to the genomic context and phenotype, our current hypothesis is that P21, a protein not encoded by lambda phage, alters the sensitivity of the lysogen's peptidoglycan, altering the efficiency of the lytic burst. Why the phage would do this left to speculation, but could be due to the significant fitness advantage provided to the lysogen when the phage exists as a resident prophage.

[ID: 170] In vitro and in vivo characterization of a *Pseudomonas aeruginosa* bacteriophage panel

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Phages provide a therapeutic option against multidrug-resistant *Pseudomonas aeruginosa* in combination with conventional antibiotics. A panel of 33 candidate therapeutic phages active against *P. aeruginosa* was isolated and characterized. Full genome sequence analysis and electron microscopy allowed classification of these into six phylogenetic groups, within the families Myoviridae, Podoviridae and Siphoviridae. Host ranges on a diverse panel of 172 multidrug-resistant *P. aeruginosa* clinical isolates varied from 11% to 52% coverage. Overall, 144 strains (84%) were susceptible to one or more of the phages. Seven of the phages efficiently degraded *P. aeruginosa* biofilms. A cocktail of five biofilm-degrading phages administered intraperitoneally protected 100% of mice from lethal *P. aeruginosa* infection (vs. 20% survival in the control group treated with saline), indicating this phage cocktail has therapeutic potential.

[ID: 245] Use of RAPD-PCR for typing staphylococcal phages with a potential for phage therapy

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Staphylococcus aureus is an important pathogen in both humans and animals and its resistance to antibiotics complicates treatment of diseases. A possible solution to this is phage therapy. One of the disadvantages of phages is their narrow host spectrum compared to antibiotics. Therefore it is convenient to use polyvalent bacteriophages or phage cocktails. Broad host range bacteriophages used for phage therapy are lytic phages similar to staphylococcal phage K that lack virulence and antibiotic resistance genes. RAPD-PCR (randomly amplified polymorphic DNA) is a possible way of fast phage typing. The method uses random amplification of genome segments with short primers of arbitrary nucleotide sequence to create specific DNA fingerprints. Compared to commonly used methods for phage typing, RAPD requires a minimum amount of DNA, the method is fast and inexpensive and does not require prior knowledge of the phage DNA sequence.

Ten *S. aureus* phages were isolated from waste water in various cities of Czech Republic. Phages were characterised by electron microscopy and classified by PCR into Myoviridae and Podoviridae families, their host range was determined on a large number of clinical isolates including MRSA strains and their DNA sequence was determined as well. Also, gene encoding endolysin was detected and compared among analysed phages. We used RAPD-PCR to distinguish between similar and highly different phages and compared the efficiency of their differentiation with traditional methods. The method appears to be suitable for preliminary typing of staphylococcal phages for the preparation of phage cocktails that can be used in phage therapy.

We kindly acknowledge the financial support CZ.01.1.02/0.0/0.0/15_019/0004353 and a grant of Ministry of Agriculture of the Czech Republic No. QJ1510216.

[ID: 144] Exploring the marine prokaryotic virosphere for novel therapeutics

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Phages are the most abundant and diverse entities in the biosphere with an estimated global population of approximately 10^{31} . Thus, they represent a largely unexplored source of genomic and functional diversity with the potential to be the major factor explaining and influencing bacterial abundance and diversity patterns. Phages are not only able to control bacteria *in situ*, but they can also be used as alternative antibiotics to control bacteria *in vivo*. Exploration of phage diversity in uncharted environments using metagenomics offers the basis for identifying novel phages to ultimately improve phage application against bacterial pathogens. In our project, we aimed to unravel the diversity of phages in selected marine environments, in detail various locations in the Baltic Sea, using next generation sequence-based metagenomics. With this approach, we aimed to analyze and compare complex marine viromes, ultimately expanding the marine prokaryotic virosphere. In addition, those metagenomic samples were functionally screened to enrich lytic phages in selected host bacteria (pathogens from aqua- and agriculture as well as human infections). Identified phages are currently characterized concerning their structure, host range and stability. Additionally, we established nanopore sequencing with the MinION for viral genome sequencing. With this novel long-read sequencing technology, we were able to assemble several phage genomes in one day. Thus, this technology drastically improves the project time line beginning with sampling to the genomic characterization. Promising lytic phages will be selected to test their *in vitro* and *in vivo* efficacy, and to ultimately evaluate their potential for future applications in phage therapy.

[ID: 344] Faecalibacterium prausnitzii bacteriophages in the human gut

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Background: The importance of *Faecalibacterium prausnitzii* to the human health was not realized until the mid 2000's when high throughput sequencing of 16S rRNA libraries and metagenomic analysis of faecal DNA demonstrated *F. prausnitzii* to be one of the most abundant bacteria in the human gut, accounting for 5-15% of the total bacterial population. There is relatively little known about the specific contents of the human gut virome, and due to the importance of *F. prausnitzii*, this would be a good place to start to increase our knowledge of bacteriophage in the gut.

Objectives: The objectives of this study was to increase our understanding of *F. prausnitzii* bacteriophages and how their relationship with *F. prausnitzii* changes in the human gut, along with the possibility of isolating and finding bacteriophage of particular interest.

Methods: The online prophage search tool PHASTER was used to search for prophages within the *F. prausnitzii* genomes. The results were used to generate a non-redundant catalogue of putative prophage and prophage-like element sequences. Prophage with greater than 90% identity and 90% representation were removed from the dataset. Quality filtered and trimmed reads from a number of published and unpublished human faecal virome studies were then aligned to the refined prophage database.

Conclusions: A total of 89 prophage and prophage-like elements in the genomes of 31 *F. prausnitzii* strains were identified (1-6 elements per strain), with sizes ranging from 6.3 to 64.6kb. Hierarchical clustering of prophage sequences based on the percentage of shared orthologous genes with a cut-off level set at 40%, enabled us to identify 24 clusters with 2-5 members per cluster and 15 orphan sequences. Studying the highly abundant prophage in the genomes of *F. prausnitzii*, could further our understanding of the relatively unstudied bacteria and its associated health benefits.

[ID: 182] Effects of Staphylococcus aureus bacteriophage K on expression of cytokines and activation markers by human dendritic cells in vitro

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A potential concern with bacteriophage (phage) therapeutics is a host-versus-phage response in which the immune system may neutralize or destroy phage particles and thus impair therapeutic efficacy. Current literature is discrepant with regard to the nature and magnitude of innate and adaptive immune response to phages. The purpose of this work is to study the potential effects of *Staphylococcus aureus* phage K on activation of human monocyte-derived dendritic cells. Commercially available human CD14⁺ monocytes (ATCC, Manassas, VA) and a Dendritic Cell Differentiation Kit (R&D Systems, Minneapolis, MN) were used. Repeated experiments demonstrated that phage K had little impact on the expression of pro- or anti-inflammatory cytokines, or on MHC-I/II and CD80/CD86 protein expression. Given that dendritic cells are potent antigen-presenting cells and messengers between the innate and the adaptive immune systems, our results may suggest that staphylococcal phage K does not affect cellular immunity or has a very limited impact on it.

[ID: 216] Group B Streptococcus bacteriophages with broad clinical host range

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As a leading cause of sepsis in neonates, *Streptococcus agalactiae* or Group B Streptococcus (GBS) is a significant obstetric pathogen. Numerous screening strategies have been implemented worldwide to identify mothers at risk of transmitting the organism to their newborn. Risk-based strategies and detection of GBS by culture screening all result in antibiotic administration. Although penicillin remains effective, resistance is inevitable and the impact of antibiotic administration on the maternal and neonatal microbiomes is not well-understood. An ideal treatment option would be one in which GBS are targeted in colonized women, with no effect on commensal organisms. Use of bacteriophage (phage) therapy, is one such option. Four phages (LF1 – LF4) were isolated from wastewater using standard enrichment techniques. All phages showed varied and broad ranging activity against clinical GBS isolates collected from pregnant women (colonising) and neonates (disease). Phages displayed lytic activity in vitro against antenatal GBS isolates with 73.3% of GBS isolates (n = 135) susceptible to at least one phage. LF2 and LF4 showed activity against all neonatal disease-causing isolates (n=10), while LF1 was also active against 90% of these isolates. Transmission electron microscopy confirmed all phages as members of the Siphoviridae family. Whole genome sequencing revealed genomes ranging from 32,205 – 44,768 bp. LF1 and LF4 share 99.9% nucleotide identity and are closely related to putative prophage of GBS. LF2 shows homology to a different putative prophage, although its genome organisation differs, while LF3 exhibits genome similarity to a *Streptococcus pyogenes* phage. The presence of genes required for lysogeny including integrase, repressor and regulatory modules suggests all are temperate phages, which are not typically used for phage therapy, however, the in vitro activity of these phages against a broad range of clinically important isolates is extremely promising and they may have therapeutic use as bioengineered phages or purified lysins.

[ID: 424] Application of gram-negative bacterial phagelysates increases the efficacy of chemotherapy in cancer-bearing mice

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Application of bacterial preparations for stimulation of organism's nonspecific immune reactions has been considered as a promising way of immunotherapy. The aim of the study was investigation of antitumor and adjuvant potential of gram-negative bacterial phagelysates. E.coli –Un phage lysate was obtained by propagation of Un phage in semisynthetic medium with subsequent centrifugation and fractionation via membrane filtration and tested for sterility, optical density, content of viable phage particles, total protein concentration, endotoxin content, safety on healthy mice. Laboratory mice (20-25 g albino) with Ehrlich carcinoma were treated with E.coli –Un phagelysate and combined use of phagelysates with chemotherapy using doxorubicin, cyclophosphan and toruracil. Treatment effect was estimated by animal's lifespan, survival percent and cancer growth inhibition. Immunological parameters - IL-12, TGF- β , IFN- γ , CD3CD4, CD3CD8, NK1.1, CD4CD25, CD25Foxp3 were studied in healthy and cancer-bearing control mice, also animals subjected to phagelysate- and combined treatment. The number of Tregs and NK cells were measured by FACS Array Bioanalyzer using WST-8 Kit. IL-12, TGF- β , IFN- γ were studied by ELISA. The application of optimal doses of E.coli phagelysate was well tolerated in mice and delayed cancer growth. After 3-4 vaccinations obvious treatment effect was obtained that coincided with increased secretion of IL-12 and IFN- γ in treated mice compared to untreated controls. At 5-8 vaccinations the difference between the results for experimental and control groups wasn't statistically significant. Polychemotherapy inhibited cancer growth without notable increase in lifespan while combined treatment increased efficacy of chemotherapy significantly. Cancer development was inhibited by 80–90% with complete regression of tumors in 13-19% of mice. Immunological investigations revealed increase in IL-12, IFN- γ , CD3CD8, CD3CD4 and NK cells, and decrease in TGF- β and T-reg's. Based on the anticancer immunomodulatory properties of Eco-Un phagelysate it can be recommended as efficient adjuvant in anticancer therapy with treatment outcome depending on vaccination regimen.

[ID: 176] Two predators competing for their prey: phage-bacteria coevolution in Salmonella

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In the environment, bacteria are exposed to a large and diverse population of lytic and temperate phages, competing for the same prey and possibly affecting bacteria evolution. Yet, population dynamics and bacterial response to multiple phages is barely explored in phage ecology and evolution. Here we elucidated the population dynamic and phage resistance development in *Salmonella Typhimurium* exposed to two different phages isolated from the same ecological niche.

For the experiment, we combined a lytic phage (S118) and a temperate phage (S107) isolated from the same wastewater sample and infecting *S. Typhimurium*. While both phages are dependent on O-antigen of lipopolysaccharide for infection, they show no genetic similarity and belong to different phage genera. During a nine-day coevolution experiment, the phage population showed decreasing infectivity against the bacteria population, suggesting arms-race dynamics. Plaque assay and sequence analysis showed that the initially predominant S118 was partially outcompeted by S107 during the experiment, even though point mutations in phage S118 tail fibers suggested genetic adaptability. Over time, the selective pressure imposed by the two phages resulted in accumulation of cells resistant to both phages, yet without formation of S107 lysogens. Sequence analysis of phage resistant colonies identified mutations in the *rfaJ*, *rfaD* and *rfaH* genes, all important for O-antigen synthesis. Interestingly, resistant strains isolated at the beginning of the coevolution lost the O-antigen, while later in the experiment resistant strains with a short O-antigen dominated the population. Thus, different mutations were selected over time and their temporal appearance may be correlated with changes in the phage population. We are currently investigating how S107 may provide an evolutionary advantage over S118 during coevolution. In summary, competition between the two phages resulted in a complex phage resistant development affecting O-antigen diversity of the *Salmonella* population, suggesting phages to be important drivers of *Salmonella* evolution.

[ID: 232] A new polyvalent phage infecting *Cronobacter* and *Salmonella* species

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Phages are usually species- or even strain-specific, yet polyvalent phages can infect across different bacterial species or even genera. Polyvalent phages are therefore unique tools to study the molecular basis of phage receptor binding apparatus evolution and diversity. Here we characterized a novel polyvalent phage, S144, isolated from wastewater on *Salmonella* Infantis.

Screening a large collection of 194 Enterobacteriaceae strains including *Salmonella*, *Escherichia*, *Enterobacter* and *Yersinia* showed that phage S144 form plaques on *Salmonella* Derby, Enteritidis and Muenster as well as on *Cronobacter sakazakii*. TEM showed that phage S144 belongs to the Myoviridae family carrying a prolate head, short tail fibers and a contractile tail. The S144 genome has a GC content of 45.8 % and is 53628 bp long encoding 77 ORFs and no tRNA genes. Whole genome alignment and BLASTn of the S144 genome revealed only homology to two understudied and unclassified phages in GenBank (79% nucleotide identity to *Salmonella* phages UPF_BP2 and BP63), indicating that a novel Myoviridae genus containing these phages could be proposed. RAST annotation proposed both ORF35 and ORF49 as tail fibers, yet BLASTn and HMMER analysis could not provide further evidence of their potential role in host recognition. To identify the receptor, we selected and sequenced phage resistant mutants of both *Salmonella* and *Cronobacter*. Comparative genomics and SNP analysis of resistant *Salmonella* identified mutations in the oligosaccharide repeat unit polymerase wzy, suggesting that the receptor recognized by S144 is the O-antigen of the lipopolysaccharide. Analogous analysis is in progress to identify the recognized receptor in *Cronobacter*. Finally, we are currently investigating the mechanism by which the two putative tail fibers allow phage S144 to infect both *Salmonella* and *Cronobacter* strains. These results will contribute to the understanding of polyvalency from a molecular point of view.

[ID: 115] Receptor binding proteins of phages specifically infecting *Staphylococcus* species: a bioinformatics study

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Receptors in bacterial cell wall are specifically recognized by phage receptor binding proteins (RBPs), forming a phage-host interaction, followed by phage adsorption on the host cell wall. The recognition of bacterial receptors by phage RBPs represents a key feature not only for purposes of bacterial detection, but also a partial tool for a novel alternative for treatment of infections caused by antibiotic-resistant *Staphylococcus* strains. Present work brings bioinformatics analyses of all phage RBPs recognizing the genus *Staphylococcus*. In the first step, sequence records of *Staphylococcus*-infecting phage-encoded genomes were obtained from GenBank, as well as the individual protein sequences of putative RBPs (March 2018). Together, 111 identified genomes from phages infecting *Staphylococcus* species were examined for putative RBPs. Of that, 158 identified RBPs were further analysed on available characteristics and functional domains, which were also verified against the CDD. Following parameters were subsequently taken into consideration: phage family from which the protein originates, RBP localization across the genome, predicted protein function, host strain range, amino acid sequence of proteins. As a result, seven proteins with predicted function as RBP from Siphoviridae phages, along with six proteins from Myoviridae phages were selected for following phylogenetic analyses. These were performed for each of two specific groups, resulting in phylogenetic trees of proteins, displayed for each phage group using iTOL. In order to find sequence homology across the amino acid sequence record, protein sequences were further compared by ClustalOmega. Subsequently, to predict tertiary structures of selected analysed RBPs, PDB files were prepared by Phyre2, displaying 3D models. Last but not least, by displaying these structures in WebLab, 13 final models were obtained, representing RBPs encoded by phages infecting *Staphylococcus* species selectively. The sequences are yet ready for further bioinformatics studies prior to protein engineering experiments.

Acknowledgement: This work was supported by FPPV-12-2018.

[ID: 401] Shiga toxin-converting bacteriophages: comparative studies and analyses of genomic sequences

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Escherichia coli is a wide-spread, symbiotic bacterium. However, some strains of this bacterium can be a threat to human health. One such a group is the enterohemorrhagic *E. coli* (EHEC), which is the major cause of gastroenteritis, enterocolitis, bloody diarrhea and hemolytic-uremic syndrome (HUS). The major virulence factors of EHEC are Shiga toxins. Genes encoding these toxins, called *stx*, are located in the genomes of Shiga toxin-converting bacteriophages (Stx phages), which are integrated in the *E. coli* chromosome as prophages.

Current knowledge about the Stx phages genomic sequences, their molecular mechanisms and interplays with their bacterial hosts cannot keep up to the number of new isolates, which are discovered every year. The aim of this work was to characterize the stability of three Stx phages (P22, P27 and P32) under various environmental conditions, to determine and analyze their genomic sequences, and to compare them with known lambdoid phages: λ , 933W and Φ 24B.

The genomic sequences were determined using next-generation sequencing (NGS). The contigs assembly was analyzed and corrected with the use of BLAST and Progressive MAUVE programs. All genomic annotations were created using myRAST software and manually corrected using the UGENE program. ClustalW algorithm was used for all pairwise and multiple sequence alignments. HMMER tool was used to search for any potential protein domains. The stability of phage particles was measured at 4°C, with the change of other environmental conditions (e.g. pH, NaCl concentration).

Despite high global similarity in genomic sequence, key regions responsible for the phage development show differences in sequences between phages. Special attention was also paid to fragments of highly conserved sequence but still unknown function, such as the *exo-xis* region of lambdoid phages. Protein and genomic sequences of other lambdoid phages were also used to determine open reading frames that could encode potential structural virion proteins.

[ID: 236] Host range determinants of bacteriophages infecting *Salmonella*

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The host range of a phage defines the breadth of bacteria that a phage is able to infect and kill to propagate. The host range is a result of complex evolutionary mechanisms of phage adaptation to bacteria in its environmental niche. While phages infecting *Salmonella enterica* have been studied at the level of single phages, the lack of holistic approaches hamper the understanding of phage-host interaction in an ecological and evolutionary perspective. Here we combined biological data with genetic and statistical analysis to establish the variables determining the host range of 50 novel phages infecting *Salmonella*, isolated from animal, environmental and wastewater samples. We determined the host range by plaque formation on a large collection of wild type *Salmonella* strains, identified the receptor of each phage and taxonomically assigned them to genera by comparative genomics. We observed that O-antigen-dependent Jerseyvirus and Vi1virus phages had a relatively restricted host range compared to T5virus phages. According to the host range data, we ordinated the phages in a 2D plot using Non-Metric Dimensional Scaling, allowing evaluation of isolation niche, isolation strain, phage receptor and phage genus as variables possibly influencing the host range. Interestingly, statistical analysis applied to the 2D plot revealed that multiple variables influence the host range of these *Salmonella* phages. While the isolation strain did not significantly affect the host range, the sampling site had an impact in which, more generalist *Salmonella* phages were isolated from ecosystems hosting diverse bacteria than from consolidated niches. Yet, phage genus and receptor in combination explained 79% of the ordination, indicating that among the investigated variables, the phage receptor and phage genus had the major influence on the host range of the phages. This study demonstrates that combining statistical approaches with biological and genetic data can reveal novel insights into ecology of phage-host interactions.

[ID: 204] Evolution of a receptor binding protein and its cognate receptor: H-fiber variants of CJIE1 prophages follows PorA lineages of *Campylobacter jejuni*

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Prophages contribute immensely to bacterial diversity and are also found in zoonotic pathogen *Campylobacter jejuni*. One Mu-like prophage (CJIE1) is widespread but show significant diversity between different strains. Unfortunately, isolation of free phage particles has been repeatedly unsuccessful and so far, it is not understood how CJIE1-like prophages recognise their host and circulate in the population. Here, we provide novel insight of phage-host interaction of CJIE1-like prophages by identifying the receptor binding protein (RBP) and the cognate receptor.

Bioinformatic analysis identified a H-fiber gene present in CJIE1-like prophages as a putative RBP. To demonstrate the function, we constructed a chimeric R-type pyocin containing the C-terminal part of the H-fiber and showed that it was able to bind and kill an array of *C. jejuni* strains. Killing was unaffected by the absence of flagella or capsule that were previously shown to be receptors of virulent *C. jejuni* phages, suggesting that the receptor may be a conserved protein. In silico comparison of outer membrane proteins of *C. jejuni* showed that all strains sensitive to the chimeric pyocin carried a specific allele of the essential major outer membrane protein, PorA. To verify that PorA is the receptor, we expressed PorA in *E. coli* and showed that it became sensitive to the chimeric pyocin. Interestingly, detailed analysis of a large number of genome sequences demonstrated that H-fiber variants of CJIE1-like prophages correlate with the PorA allele of the strain. A new chimeric pyocin carrying a different H-fiber variant exclusively killed *C. jejuni* carrying the corresponding PorA allele. Thus, our data suggests that the specific interaction of H-fiber RBP and the PorA receptor may influence circulation of CJIE1-like prophages in the *C. jejuni* population. Finally, our study shows that pyocins are a powerful tool for discovering novel RBP-receptor interactions of phages that cannot be isolated.

[ID: 434] Effect of phage therapy on mouse microbiome

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The main apprehensions concerning bacteriophage therapy are the potential for bacteriophage-induced genome evolution as well as possible perturbations towards the microbiota. There exists, however, a significant gap in knowledge regarding the direct and indirect effect of phage therapeutics on the microbiota inside the mammalian gut. It has been shown, on multiple occasions, that *in vivo* interactions of phage and its host can only to a limited extent be predicted from their *in vitro* growth properties. These gaps in the understanding significantly limit the rational design of phage therapy approaches.

We investigated the hypothesis that phage-resistant mutants will interact/interfere with the microbiota in a different manner than their parental (phage-susceptible) strains. Although the introduction of lytic phage into the body is not believed to disturb the microbiota directly, lytic bacteriophages have been identified as agents that can drive diversification of bacteria due to the strong selective pressure they exert on the bacterial host communities, giving rise to phage-resistant mutants with a different phenotype.

We present the results of fecal community analysis (16S rRNA sequencing and qPCR) during a long-term phage challenge against a non-pathogenic *Escherichia coli* strain in the mouse gut. We further present the phenotypic variations in fitness and motility between resistance variants of *E. coli* evolved *in vivo* with time and compare that to resistant variants evolved *in vitro*. Our results suggest that interaction of highly specific, strictly lytic phage with its host inside the mammalian gut, indirectly affects the microbiota and that resistant mutants evolved *in vivo* vary significantly in terms of fitness from those evolved *in vitro*.

[ID: 206] High-throughput design and analysis of modular endolysins to develop lead candidates using VersaTile Shuffling

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Endolysins have emerged as a novel class of antibacterials and are currently under evaluation in clinical trials. A feature of endolysins is their modular structure and the opportunities to customize endolysins towards their specific application. A major expansion of this modularity principle is the fusion of endolysins to outer membrane permeabilizing peptides (Artilysin®), facilitating efficient transfer across the outer membrane of Gram-negative bacteria, followed by rapid cell lysis.

The scope of this modularity principle is exciting, as it allows engineering of endolysins against any pathogen and customizing antibacterial properties. However, this progress is empirical and hampered by the tedious cloning procedures. To address this technical barrier, we developed a novel DNA shuffling method, coined VersaTile Shuffling. Any modular variant can now be constructed with short hands-on time in a combinatorial manner. VersaTile Shuffling relies on the creation of a repository of Tiles (=modules) that can subsequently be shuffled in a versatile way. Our current Tile repository allows the generation of over 8 million modular variants in a single reaction.

Using the VersaTile Shuffling method, we have implemented a hit-to-lead development process of endolysins, similar to the development of pharmaceutical drugs. Specifically, we have engineered a lead endolysin against *A. baumannii*. In the initial screening experiment, ~400 random variants from a library with >10,000 variants were analysed against four epidemiological multidrug-resistant *A. baumannii* strains. Seven hits (~2%) were identified. Based on the observed structure-activity-relationships, enriched libraries were iteratively constructed, increasing the ratio of active variants stepwise to 45%. The final selection round was performed in human serum, better mimicking in vivo conditions. This resulted in a lead modular variant with low MIC-values against all strains and a high bactericidal effect in human serum.

The scope and potential of this approach represents a key breakthrough in the design of new engineered endolysins.

[ID: 342] Complete genome sequences of bacteriophages Sole and Sato expand the view of the genetic diversity found in the family Tectiviridae

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Bacteriophages are an important repository of genetic diversity and source of unexplored genes. In the particular case of tectiviruses infecting the *Bacillus cereus* group, these bacteriophages 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 (Jalasvuori et al., 2013; Gillis and Mahillon 2014). Among them, partial DNA sequencing of tectiviruses Sole and Sato, isolated from *B. cereus sensu lato* and emetic *B. cereus* strains, respectively, uncovered a highly variable region potentially involved in lysogeny maintenance (Jalasvuori et al., 2013). Here, we analyze the complete genome sequences of bacteriophages Sole and Sato. The genome of Sole spans 14,445 bp with 28 putative CDSs, whereas the one of Sato has 14,852 bp comprising 31 putative CDSs. DNA sequence identity comparisons of Sato and Sole against *Bacillus virus Bam35* (*Betatectivirus* type species) indicated 86 and 89% sequence identity, respectively. Additionally, phylogenetic analyses and genome alignments suggested that both isolates represent novel tectivirus species. Hence, tectiviruses in Gram-positive bacteria have shown to be more diverse than those infecting Gram-negative bacteria, as the latter ones display a very high level of sequence identity. In an effort to assess the host range of Sole and Sato against emetic *B. cereus* and *Bacillus cytotoxicus*, two species involved in food poisoning outbreaks, 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. Overall, genome sequencing and comparative analysis of tectiviruses Sole and Sato have expanded the view of the genomic diversity occurring in plasmidial prophages found in members of the *B. cereus* group.

[ID: 340] Spontaneous phage-resistant mutants in *Bacillus thuringiensis* defy tectiviral infection through different mechanisms and phenotypes

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Phages and bacteria are constantly involved in an evolutionary process in which bacteria develop resistance to phages, and phages counter-evolve to fight back the resistant bacteria. Despite the importance of this dynamic process, phage-resistance in members of the *Bacillus cereus* group and the mechanism(s) involved are still poorly understood. This work aimed to depict bacterial resistance triggered by the presence of tectiviruses, a relative rare group of non-enveloped tail-less phages that are able to replicate as linear plasmids in their *B. cereus* *sensu lato* host. To this end, lytic variants of tectiviruses GIL01 and GIL16 were obtained by selecting clear plaques that emerged among the turbid plaques in lawns of *Bacillus thuringiensis*. The lytic variants were used to generate a collection of resistant *B. thuringiensis* mutants by using an improved method that combined the classical spotting assay (to generate the bacterial mutants) and an inverted spotting assay to evaluate the resistance of the candidate mutants. Twenty fully-resistant mutants were selected and validated to confirm complete resistance to tectiviruses GIL01 and GIL16. These resistant bacteria showed differences in cell and colony morphotypes and displayed distinct adaptation features (i.e. biofilm formation, sporulation rate, swarming motility, differences in metabolic profiles and antibiotic susceptibilities). To unravel the genetic changes responsible for tectivirus-resistance in *B. thuringiensis*, a pooled high-throughput whole genome sequencing was used. Potential genes causing the resistant phenotype were identified and several genes associated with cell-wall metabolism and turn-over, as well as cell-surface proteins, have been pinpointed. Additionally, three phage-resistant mutants were re-sequenced independently and a variant calling mapping was performed, along with differential proteomic analyses (ICPL), as these mutants displayed striking phenotypic changes that resulted in blocking tectiviral infection. SNPs and indels found in several genes, combined with differential expression of proteins, underlay the cost of resistance in the complicated tectivirus-bacteria interplay.

[ID: 429] Alternative survival strategies of a polyvalent obligately lytic staphylococcal podovirus phiAGO1.3

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Phage phiAGO1.3 is a polyvalent staphylococcus lytic podovirus of about 18-kb genome [1]. Surprisingly, we observed that under certain conditions the exposure of *Staphylococcus aureus* cells to phiAGO1.3 can lead to the establishment of two alternative relationships of the phage with its host, namely the emergence of a mixed population in which bacteria and bacteriophages are in a more or less stable equilibrium, and remain in stable ratios even following several serial passages – an equivalent of multiple generations. This interaction is reminiscent of the so called phage carrier state, which is also described as pseudolysogeny. Colonies of cells remaining in such interaction with the phage are small and have a glossy, transparent appearance. Their cells form similar small, glossy, transparent colonies, in which phage lytic development is resumed in some fraction of cells, as well as colonies of normal appearance deprived of any phage DNA. Apparently, phage development may be temporarily blocked in an infected cell enabling this cell to divide to give rise to a phage infected and phage free cell. Such outcome of phage infection enables a continuous production of bacteriophages at the expense of newly formed phage sensitive cells in a population. The continuous presence of phage favours the emergence of phage resistant variants of host cells. Importantly, cells isolated by us as resistant to phiAGO1.3 are sensitive to obligately lytic staphylococcal myoviruses. Identification of host genes involved in the acquisition of resistance to phiAGO1.3 is in progress.

[1] Gozdek et al., Complete genome sequences of two novel *Staphylococcus aureus* podoviruses of potential therapeutic use: vB_SauP_phiAGO1.3 and vB_SauP_phiAGO1.9. Genome Announcements. In press.

[ID: 361] Looking for a novel T5-like phage receptor proteins

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T5-like bacteriophages are considered as good candidates for phage therapy agents due to their ability to infect a wide range of enterobacteria (Sváb et al., 2018). Infection of bacterial cell by T5-like phage requires irreversible binding of phage central tail fiber receptor recognition protein (pb5) to host surface proteins – cyanocobalamine transporter BtuB (phage BF23 group) or ferrichrome transporter FhuA (T5 group). The function of L-shaped tail fibers is not essential on the rough strains and only required for penetration of the surface polysaccharide shield of the cells producing O-antigen and/or capsules (Golomidova et. al 2016). We extracted from GenBank database a.a. sequences of pb5 homologs from all known T5-like bacteriophages. The reconstructed phylogeny of these protein sequences indicates that at least four distinct clusters are present. The host receptors for two such groups are not yet known. We isolated a novel T59g coliphage that belongs to one of two uncharacterized groups. Pb5 aa sequence of this virus is closely related to Salmonella_phage Shivani pb5. Providencia_vB_PreS_PR1 and Proteus_PM135 phages forming the remaining group are not currently available to us.

T59g is able to infect E. coli DH5a, and host mutants resistant to T59g infection were isolated. Interestingly, T59g-resistant mutants gained also resistance to 9g phage, characterized in our lab previously (Kulikov et al., 2014), while the mutants selected for resistance to 9g phage remained sensitive to T59g. This fact indicates that T59g phage recognizes two different host receptors, one of which is shared with phage 9g. A comparative full genome analysis of these host mutants and wild type host will help us to identify a novel type of E. coli T5-like phage receptor(s).

The work was supported by RSF grant #15-15-00134-P

[ID: 130] Optimizing encapsulation of *Staphylococcus aureus* bacteriophage phiIPLA-RODI

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The antimicrobial properties of bacteriophages make them suitable biopreservatives for foods. In this study, we evaluated the encapsulation as a system to delivery bacteriophages and to increase their stability during foodstuffs processing. *Staphylococcus aureus* bacteriophage phiIPLA-RODI was encapsulated in simple and double emulsions, niosomes, liposomes and transfersomes. Out of them, niosomes, liposomes and transfersomes were found to be efficient systems to encapsulate phage phiIPLA-RODI. Niosomes and liposomes formulated with 30 and 50 mg/ml of surfactants showed percentages of encapsulation of 94-100%, while 50 or 70 mg/ml of surfactants were necessary to obtain similar results with transfersomes. Size distribution of emulsions ranged from 0.98 μm to 29 μm , while size of niosomes, liposomes and transfersomes were $0.82 \pm 0.09 \mu\text{m}$, $1.66 \pm 0.21 \mu\text{m}$ and $0.55 \pm 0.06 \mu\text{m}$, respectively. Bacteriophage infectivity was well maintained along the storage time at 4°C for at least 6 months, with the exception of those encapsulated in liposomes with 30 mg/ml of surfactants, which reduced phage titer in 3 log units after four months. Regarding the viability of free and encapsulated phages, niosomes protected phage particles from low pH. Thus, free phiIPLA-RODI was not detectable after 60 min exposure to pH 4.2, while titer of encapsulated phage in niosomes only decreases 2 log units. On the other hand, the treatment of vesicles with 4.5 M NaCl during 60 min resulted in a high instability of the vesicles and the consequent release of the encapsulated phage phiIPLA-RODI. Overall, our results showed that encapsulation represents an appropriate procedure to improve stability and antimicrobial activity of phages even in adverse conditions used in food processing industry.

[ID: 28] Genomics of the phage-host interactome and infection efficiencies. A case study on the marine bacteria *Alteromonas* sp.

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The relationship between phages and bacteria has been the central issue of many studies during the last decades. However, the full understanding of the reciprocal influences leading to the host-parasite coevolution is likely to need further information.

Here, we integrate parasite-host range experiments and genomic analyses in order to envisage the key-mechanisms ruling phage infection efficiency and host resistance. Several phages that infect bacteria belonging to the genus *Alteromonas* were isolated from inshore water samples collected during a five-year campaign conducted in the Mediterranean Sea.

Host-range experiments showed that these phages were able to infect some strains of two species of *Alteromonas* with different infection efficiencies while other strains were resistant to all the phages. Moreover, genomic comparisons between phages revealed that we isolated similar phages from different years belonging to the species AltAD45-P(n) (ANI > 99%) and were classified as AltAD45-P5 to P16. The major differences were several hot-spots of polymorphic sites at the level of specific genes (mostly hypothetical proteins) and genomic rearrangements of two genes encoding a tail fiber and the hypothetical protein CDS-2. In total, we identified 2 types of tail fiber that are different by a 1 Kb deletion and 4 CDS-2 genes characterized by variable number of repetitive elements.

These two genes are candidate to play a key-role in the different infection performances that we observed. Differences in the tail fiber could determine different ability to recognize the primary receptor of the host. At the same time, CDS-2 variations correspond to variable numbers of repetitive Gly-Ser elements that could imply protein conformational changes that might be involved in the secondary receptor interaction. Thus, we identified two variable genomic components in the phage genomes that could represent the identifying tools that these phages use in the coevolutionary process with their hosts.

[ID: 77] Antibiotics or phage cocktails - comparison of the effectiveness in eradication of various *Salmonella enterica* serovars under laboratory conditions

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Salmonella enterica is a human and animal pathogen that causes salmonellosis, a disease which is common throughout the world. Main symptoms such as vomiting, diarrhea and abdominal pain resemble symptoms of digestive intoxication. However, some of *S. enterica* serovars manifest as an enteric fever, which is life-threatening illness requiring prompt antibiotic therapy. The future of antibiotic therapy is questionable as we see a rapid development of drug-resistant bacterial strains is evident. This poses a serious consequences for human health. We assume that bacteriophages may reduce the risk of salmonellosis - associated with consumption of contaminated poultry meat.

In this work we have compared effectiveness of antibiotics and experimental phage cocktails in eradication of various *Salmonella enterica* serovars under laboratory conditions. We have conducted a series of experiments - lysis profiles, using two experimental phage cocktails containing phages isolated from urban sewage and poultry feces. We compared the effectiveness of cocktails with commonly used antibiotics like tetracycline, ampicillin, streptomycin and colistin. We have also studied the development of bacterial resistance against phages and antibiotics. Preliminary results suggest that effectiveness of phage cocktails depended on used *S. enterica* serovars whereas antibiotics tended to be equally effective against all tested *S. enterica* serovars.

Multidrug-resistant bacteria are an increasing problem in medicine. Bacteriophages may be the only available method of treatment of some bacterial diseases in the future, particularly if the misuse of antibiotics is to be continued. Therefore, it is important to study the effectiveness and biology of bacteriophages in regard for future medical applications.

[ID: 366] Elimination of clinical Salmonella Enteritidis via phage therapy in murine in vivo model

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A MDR clinical Salmonella Enteritidis, isolated from AM untreated patient diagnosed salmonellosis was used in this study. Phage susceptibility test was performed with twelve Salmonella specific bacteriophages. Three most active phages (Tr - Podoviridae, Hi - Siphoviridae, BS- Myoviridae) were chosen to develop a mixture applied in In vivo studies. A treatment regimen and infectious doses were determined during preliminary studies.

Twenty male albino Mus musculus weighted 18-20g were distributed in experimental and three control groups with five individual in each. Suspension of S. Enteritidis (109cfu/ml) and the phage mixture (108pfu/ml) were administered orally encapsulated in 800mg gelatine cubes to provide titer stability and resistance to gastric acid. The mice from experimental and positive control groups were fed with gelatine cubes inoculated with bacteria every 12h for two days. After development of infection the mice in experimental group were treated with the phages every 12h for four days. The phage positive control group received phage gelatine cubes. The mice included into negative control group were given sterile gelatine. Development and progress of infection and treatment effect were monitored via faecal sampling and analysis.

The positive control of bacterial infection showed in average 107cfu/g S. Enteritidis in faeces throughout the experiment. The positive control of phage group showed in average 106pfu/g viable phages. The negative control did not show any side effects related to gelatine. In experimental group the phage treatment led to immediate effect within 6 h and elimination of S. Enteritidis. The mice remained free of infection during whole treatment period. Meanwhile the titer of phage mixture remained constant (106pfu/g), the infection symptoms in mice disappeared after the second day of phage therapy.

The tested phages in this in vivo study demonstrated high treatment potential of MDR Salmonella infection.

[ID: 320] Properties of bacteriophage infecting mastitis causing *Staphylococcus aureus* strains

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Staphylococcus aureus is a hazardous etiological agent of bovine mastitis. Additionally, growing antibiotic resistance health of *Staphylococcus aureus* (MRSA) became a great challenge in terms of eradication of these pathogen. Bacteriophage-based therapy can be an effective method for treatment of the *S. aureus* mammary glands infections [1]. Additionally, phage therapy can significantly reduce the cost of standard treatment based on antibiotics [2].

The aim of this study was to isolate a novel *Staphylococcus aureus*-infecting bacteriophage and to evaluate its lytic activity against *S. aureus* strains isolated from bovine mastitis.

Bacteriophage was isolated from bovine milk with *S. aureus* ATCC 6538 used as a host. The morphology of the bacteriophage was observed using transmission electron microscopy. The host range together with the efficiency of plating, adsorption rate, multiplication parameters, pH and thermal stability were evaluated by plaque assay based on the double agar layer method. The lytic activity of bacteriophage as well as the degradation of biofilm formed by *S. aureus* were assessed by spectrophotometric methods. Additionally, the activity of bacteriophage on *Galleria mellonella* in vivo model was evaluated.

The isolated bacteriophage can effectively lyse planktonic cells and the degrade biofilm biomass of numerous *S. aureus* strains (n=87). This suggests that isolated bacteriophage could be applied as a therapeutic/biocontrol agent against *S. aureus* strains of human and animal origin.

1. Gill J.J., Pacan J.C., Carson M.E., Leslie K.E., Sabour P.M.: Efficacy and pharmacokinetics of bacteriophage therapy in treatment of subclinical *Staphylococcus aureus* mastitis in lactating dairy cattle. *Antimicrob. Agents Chemother.*, 50, 2912–8 (2006)
2. Miedzybrodzki R., Fortuna W., Weber-Dabrowska B., Górski A.: Phage therapy of staphylococcal infections (including MRSA) may be less expensive than antibiotic treatment. *Postepy Hig. Med. Dosw. (Online)*, 61, 461–5 (2007)

[ID: 141] CrAss-like phages in the human gut – in vitro analysis

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In 2014, a previously unidentified phage was discovered. crAssphage sensu stricto was originally described as a single entity through bioinformatic inference (Dutilh et al., 2014). This enigmatic phage, previously overlooked in metagenomic studies due to its lack of homology with phage sequences in current databases, was found to occur in the gut of over 50% of the human population. Interestingly, in some cases, crAssphage contributes to up to 90% of reads in the viral fraction of faecal samples. More recent taxonomic analyses of crAssphage found that this phage in fact forms a familial group of crAss-like phages as opposed to occurring as a single entity (Yutin et al., 2017).

The work discussed henceforth reinforces this finding through in vitro analyses. Fermentations were initiated using faeces confirmed, through qPCR analyses, as being rich in crAssphage sensu stricto. Primers designed based on a segment of the crAssphage sensu stricto DNA polymerase gene allowed the propagation of the prototypical phage to be detected in fermentation samples collected over six time points. However, sequencing of the same samples showed that the faeces contained six additional crAss-like phages. These phages could be assigned to proposed crAss-like phage sub-families/genera based on a database of crAss-like phages generated by APC Microbiome Ireland. In addition to demonstrating an effective means of propagating and detecting these phages we provide strong evidence that crAss-like phage are members of the Podoviridae family. This is based on TEMs and mass spectrometry performed on faeces from the same donor as used to initiate the fermentation.

A key aim is to ultimately identify one or most associated hosts. This work will play a significant role in discovering further potential crAss-like phages. Most importantly it will help work towards understanding the role these phages play in the complex community that is our gut.

[ID: 96] The evolution of NCLDV's and their relationships with Eukaryotes

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Since their discovery, giant viruses have regularly drawn attention from the scientific community on their very unusual features for the viral world. They all belong to the NucleoCytoplasmic Large Dna Viruses (NCLDV) group, which also encompasses large eukaryotic DNA viruses from various families, including Poxviridae and Phycodnaviridae. The evolution of this group is much less understood, with the few studies on this topic offering very contrasted results and controversies, notably over their potential relationships with Eukaryotes. In an attempt to get more insights, we performed in-depth phylogenetic analyses of core proteins shared by NCLDV's, and notably of DNA-dependent RNA polymerase (RNAP) large subunits. Our results show that the core proteins have undergone a congruent evolutionary history, likely to represent a species tree. Importantly, our RNAP trees strongly support the Woese tree of life, and suggest that NCLDV's diversification predated that of modern eukaryotes and that some eukaryotic RNAPs could have originated from transfers from viruses.

[ID: 220] Antibiotic effects on phage-bacteria evolution: towards combined therapy

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With escalating resistance to antibiotics there is an urgent need to develop alternative therapies against bacterial pathogens. Phage therapy has been proposed as a promising alternative to antibiotics, but an increasing number of studies suggest that both of these antimicrobial agents in combination are more effective in controlling pathogenic bacteria than either alone [1]. Evolutionary studies have demonstrated that time scale is an important factor in understanding the consequences of antimicrobial strategies, but this perspective is generally overlooked in phage-antibiotic combination studies. Lately, we experimentally evaluated the impacts of single and combined applications of antibiotics and phages on *in vitro* evolving populations of the opportunistic pathogen *Pseudomonas aeruginosa*. We found a strong synergistic effect of combining antibiotics and phages on bacterial population density and in limiting their antibiotic resistance levels [2,3]. Surprisingly, a recent study by our team (manuscript submitted) reveals that antibiotics had a negative effect on phage density and efficacy early on, but not in the later stages of the experiment. From an applied perspective, our results indicate that phages can contribute to managing antibiotic resistance levels and that antibiotics impact phage adaptation. We discuss the relevance of our findings for future research aimed at understanding and treating bacterial infections.

1. Torres-Barceló, C. and Hochberg, M.E. (2016) Evolutionary Rationale for Phages as Complements of Antibiotics. *Trends Microbiol.* 24, 249–256
2. Torres-Barceló, C. et al. (2016) Long-term effects of single and combined introductions of antibiotics and bacteriophages on populations of *Pseudomonas aeruginosa*. *Evol. Appl.* 9, 583–595
3. Torres-Barceló, C. et al. (2014) A window of opportunity to control the bacterial pathogen *Pseudomonas aeruginosa* combining antibiotics and phages. *PLoS One* 9, e106628

[ID: 196] Deciphering molecular biological aspects of *Clostridium perfringens* virulent bacteriophage CPS2 and its thermostable endolysin LysCPS2

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Clostridium perfringens is a spore-forming, Gram-positive, anaerobic bacterium and is one of the most common causes of foodborne illnesses. The increasing prevalence of multidrug-resistant bacteria requires the development of alternatives to typical antimicrobial treatments. Here, we isolated and characterized a *C. perfringens*-specific virulent bacteriophage CPS2 from the chicken feces. CPS2 phage contains 17,961 bp double-stranded DNA genome with twenty-eight predicted open reading frames (ORFs) belonging to the Picovirinae, subfamily of Podoviridae. Bioinformatic analysis of CPS2 genome revealed a putative endolysin, LysCPS2, which is homologous to endolysin of *Clostridium* phage phiZP2 and phiCP7R. The enzyme showed strong lytic activity against *C. perfringens* with optimum condition at pH 7.5-10, 25-65°C and over broad range of NaCl concentrations. Interestingly, LysCPS2 was found to be highly thermostable, with up to 30% of its lytic activity remaining after 10 min of incubation at 95°C. Cell wall binding domain in C-terminal region of LysCPS2 has a specific binding spectrum on *C. perfringens* strains. This is the first report to characterize highly thermostable endolysin isolated from virulent *C. perfringens* bacteriophage. The enzyme can be used as an alternative biocontrol and detection agent against *C. perfringens*.

[ID: 335] Efficacy of a commercial phage cocktail in reducing Salmonella contamination in artificially contaminated food products and in naturally contaminated product in poultry processing plants

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The commercial Salmonella phage product PhageGuard S was used in these experiments. For the artificial contamination, susceptible products (meat and vegetable products) were purchased from local stores. A streptomycin resistant Salmonella Enteritidis strain was used to contaminate all products with ~10⁴ CFU/cm². Phage were applied at 1 and 2 x10⁷ pfu/cm² after 30 min. Bacteria were retrieved from treated and control samples after 24, 48 and 144 hours. In the industrial trials phages were applied by dipping the products in phage solutions containing 1-4% of the phage product resulting in similar numbers of phages as used in the artificial contamination studies. Experiments were performed off-line allowing comparison with regular non-treated product. In short, chicken livers, necks and breasts as well as turkey backs were treated and absence/presence of Salmonella was established according to the establishments preferred testing method and compared to an equal number of untreated control samples (180, 120, 450 and 74 tested samples in total respectively).

Phage application resulted in > 1 log reductions for both phage concentrations used in all food items tested. The reduction in cells did change after 24 hours indicating that phage are active for a short period of time. In the industrial trials the reduction in positive samples was 94%, 58%, 80% and 88% in chicken livers, necks, breast and turkey backs respectively. Analysis using Fischers' exact test shows that the differences are statistically significant.

The results from the artificial contamination experiments show that phage can significantly reduce Salmonella on poultry products. The results from the industry trials show that this translates into meaningful reductions in real life and that phages offer a valuable new tool to enhance food safety.

[ID: 30] Identification of a minimal region with ATPase and helicase activities in replication protein gp43 from bacteriophage BFK20

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Protein gp43 from bacteriophage BFK20 is a multifunctional, 983 residue protein, with primase, polymerase, NTPase and helicase activities. Gp43 comprises an N-terminal prim-pol domain, and a C-terminal domain similar to SF4-type replicative helicases. Recently we detected ssDNA dependent ATPase and helicase activities in recombinant protein gp43-1 comprising both the prim-pol and helicase domain (1). In the present work we identified a minimal region of gp43 with ssDNA dependent ATPase and helicase activities. We prepared four deletion mutants with a missing prim-pol domain and a helicase domain truncated to varying extents. Protein gp43HEL519-983 contained residues 519 - 983 of the BFK20 gp43, gp43HEL557-983 residues 557 - 983, gp43HEL519-855 residues 519 - 855, and gp43HEL519-937 residues 519 - 937. We optimized conditions for expression and isolation of mutant proteins. We tested ATPase and helicase activities of isolated proteins. We detected a strong ssDNA dependent ATPase and a helicase activity in recombinant protein gp43HEL519-983. Removal of next 38 or 46 residues from the N- or C-terminus respectively, resulted in significant modification of both activities. Alterations of ATPase and helicase activities do not correlate strictly. Gp43HEL519-983 is a minimal protein with strong ssDNA dependent ATPase and a helicase activity. The ATPase activity of gp43HEL519-937 was much lower and the helicase activity significantly higher. It is likely that the terminal sequences are responsible for fine tuning of the protein activities.

This work was supported by VEGA grant 2/0139/18 from Slovak Academy of Sciences.

References:

1. Halgasova, N., Soltészová, B., Pevala, V., Košťan, J., Kutejová, E., Bukovska, G., 2015. A RepA-like protein from bacteriophage BFK20 is a multifunctional protein with primase, polymerase, NTPase and helicase activities. *Virus Res.* 210, 178-187.

[ID: 337] Influence of sera and specific response of immune system (mice) on endolysin activity

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Combining strong antimicrobial effect of the drug with low risk/burden for user/patient was always goal of people trying to contain bacterial infections. In both food industry and medicine above mentioned abilities are highly needed and researched by scientists all around the world.

Endolysins are phage proteins that cleave covalent bonds connecting bacterial cell wall what leads to their lysis. They can be strain specific or potent against wide range of hosts. Such proteins could be use in medical treatment but one of unknown issues is their effect on immune systems and effect of blood on their activity.

We have chosen two endolysins – PAL and Cpl-1 (from phages Dp-1 and Cp-1) and performed series of kinetic experiments with two types of sera. One is from mices previously immunized with purified endolysin and confirmed presence of specific IgG prior to the experiment. The second one is from control mice (injection with PBS). We also performed kinetic experiments with different dilution of the sera. Observed results show influence of sera and specific immune response on enzyme activity. This is one of few required steps to understand and exploit mechanism that enable creation and use of potent antimicrobial.

[ID: 145] Phages are a mortality factor of bacterial key groups during a spring bloom

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While factors inducing phytoplankton and succeeding bacterioplankton blooms are intensively studied and fairly well understood, the mortality factors responsible for the subsequent decrease in microbial abundance are not well explored yet. The mortality of a bacterial population can be bottom-up controlled by substrate limitation or top-down controlled by protist grazing and viral lysis. To test the relevance of phage lysis as mortality factor, lytic phages were isolated from the coastal waters off Helgoland in the German Bight during the annual spring bloom using as host bacterial strains of previously identified key groups of the spring bloom. These strains were isolated from the same location in the past. Host strains were incubated in freshly collected 0.2 µm filtered sea water for 5 days to induce and proliferate phages. Phage propagation was determined using a spot test. A plaque assay was applied to isolate and purify phage strains. Both phage and host were genome sequenced (PacBio Sequel) and visualized by transmission electron microscopy. Repeated isolation revealed strain-specific detection patterns over the course of the spring bloom for the different bacterial key species. Using this approach phages for six bacterial hosts, two Flavobacteria, three Gammaproteobacteria, and one Alphaproteobacterium, were isolated. Most of these phages belong to at least two families of the Caudovirales. For one Flavobacterium strain even two different phages were isolated from a single sample. Genomic analysis revealed that the phage strains are non-lysogenic to our host strain due to not significantly overlapping regions between the host and phage genome. Read mapping against the Tara Ocean dataset indicated that their distribution is confined to the waters off Helgoland. Our results provide first evidences that phages from different families co-occur with their bacterial host during spring blooms. Therefore phages are indeed a mortality factor of blooming bacterial hosts.

[ID: 363] Phage biodiversity across a depth profile of the Mediterranean sea

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Marine bacteriophages affect ocean ecosystems not only by killing their hosts but also by mediating gene transfer and by modulating microbial metabolism (Breitbart, 2012)*. Yet little is known regarding how ecological gradients modulate the influence bacteriophage communities across marine habitats. Previous studies have addressed this topic (Mizuno et al., 2013; Brum et al., 2015)* but did not assess changes in viral communities across fine-scale depth gradients within stratified waters or did not assess viral and microbial communities simultaneously. Here we sought to fill this gap by analysing both cellular and viral communities across a depth gradient at the Mediterranean sea. Samples retrieved at depths of 15, 45, 60 and 2000 meters displayed markedly distinct taxonomic and functional profiles for both cellular and viral fractions. Metagenome assembly yielded 71 prokaryote genomes and 7,164 viral genome fragments, for which putative hosts could be predicted through computational approaches. Micro-diversity within phage genomes suggested that viral communities across the depth gradient are subjected to different types of selective pressure that follow the changes in host community composition and metabolism across the depth gradient (Luo et al., 2017)*. Our findings broaden the understanding of marine viral diversity and how it is affected by ecosystem changes and vice-versa.

References:

1. Breitbart, M. (2012) Marine viruses: truth or dare. *Mar. Sci.* 4: 425–448.
2. Brum, J.R., Ignacio-Espinoza, J.C., Roux, S., Doucier, G., Acinas, S.G., Alberti, A., et al. (2015) Patterns and ecological drivers of ocean viral communities. *Science* 348: 1261498.
3. Luo, E., Aylward, F.O., Mende, D.R., and Delong, E.F. (2017) Bacteriophage Distributions and Temporal Variability in the Ocean's Interior. *MBio* 8: 1–13.
4. Mizuno, C.M., Rodriguez-Valera, F., Kimes, N.E., and Ghai, R. (2013) Expanding the marine virosphere using metagenomics. *PLoS Genet.* 9: e1003987.

[ID: 435] Characterization of Acinetobacter podophage Petty reveals a novel lysis mechanism and tail-associated depolymerase activity

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The increased prevalence of drug-resistant, nosocomial *Acinetobacter* infections, particularly from pathogenic members of the *Acinetobacter calcoaceticus-baumannii* complex, necessitates the exploration of novel treatments such as phage therapy. In the present study, we characterize phage Petty, a novel podophage that infects multidrug-resistant *Acinetobacter nosocomialis* and *Acinetobacter baumannii*. To our knowledge, it is the first phage reported able to infect both *Acinetobacter* species. Genome analysis reveals that phage Petty is a 40,431bp ϕ KMV-like phage, with a coding density of 92.2% and a G+C content of 42.3%. Interestingly, the lysis cassette encodes a class I holin and a single subunit endolysin, but lacks canonical spanins to disrupt the outer membrane. Analysis of other ϕ KMV-like genomes revealed that spanin-less lysis cassettes are a feature of phages infecting *Acinetobacter* within this subfamily of bacteriophages. Unlike the rounding of lambda lysogens lacking functional spanin genes, *A. nosocomialis* cells infected with phage Petty lyse by bursting, which suggests phages like Petty employ a different mechanism to disrupt the outer membrane of *Acinetobacter* hosts during lysis.

The observed halo surrounding Petty's large clear plaques indicated the presence of a phage-encoded depolymerase capable of degrading capsular exopolysaccharides (EPS). Gene 39, a putative tail fiber, was hypothesized to possess depolymerase activity based on weak homology to previously reported phage tail fibers. The 101.4 kDa protein gp39 was cloned and expressed, and its activity against *Acinetobacter* EPS in solution was determined. The enzyme degraded purified EPS from its host strain *A. nosocomialis* AU0783, reducing its viscosity, and generated reducing ends in solution, indicative of hydrolase activity. Given that the accessibility to cells within a biofilm is enhanced by degradation of EPS, phages with depolymerases may have enhanced diagnostic and therapeutic potential against drug-resistant *Acinetobacter* strains.

[ID: 192] Evaluation of Salmonella bacteriophage phi San23 life cycle in anaerobic conditions

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For practical reasons, bacteriophage that infect facultative anaerobic bacteria are usually studied under aerobic conditions. However, the niche where the phage therapy is expected to occur is frequently anaerobic. Therefore, it is necessary to evaluate these bacteriophages in anaerobic conditions in order to know if the absence of oxygen affect their behavior.

In the present study, we used the Salmonella bacteriophage phi San23 as a model to evaluate the infection efficiency and life cycle parameters under anaerobic conditions. After anaerobic protocols standardization protocols, aerobic and anaerobic bacteria growth curves and one-step curves were performed. In addition, to estimate changes in bacteria morphology, microscopy and flow cytometry assays were performed. Results revealed that plaque morphology different; plaques in anaerobic conditions are bigger and opaquer than plaques in aerobic conditions. The infection efficiency and burst size changed also. The burst size in anoxic conditions is smaller than the burst size with oxygen. Moreover, the infection efficiency decreases without oxygen.

We postulate that these effects are consequences of changes in the bacteria cell. This hypothesis was confirmed by microscopy and the flow cytometry results. Altogether, these results allow to understand the behavior of bacteriophages that infect facultative bacteria in anoxic environment and its implications in the ecology and control of the bacteria populations.

[ID: 302] Conserved repeat regions promote a variable modular genome organization of Cp220virus phages

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The 180 kb genomes of Cp220virus phages infecting *Campylobacter* contain six unusually long regions of highly similar repeats. The repeat regions range in size from 0.6 to 2 kb consisting of up to 19 copies of a conserved repeat sequence, separating the genomes into six modules of coding sequences that potentially are prone to shuffle. Indeed, two different types of modular genome organization have been observed for Cp220virus phages resulting in a sub-grouping with phages CP220 and CP21 as prototypes. Here, we aim to determine the modular genome organization of ten novel Cp220virus phages.

Genomic DNA from ten phages in our collection was prepared either from a single plaque (Direct Plaque Sequencing) or from a phage stock solution and subsequently sequenced using the Illumina MiSeq platform. To sequence across the repeat regions, the phages were also sequenced using the SMRT (Single Molecule Real-Time) technology. By combining all sequencing assemblies, the genomes were successfully closed. Interestingly, while the sequence of the repeat was almost identical in all phages, the lengths of specific repeat regions were unique to each phage, which was verified by PCR. Furthermore, both direct and inverted repeat sequences were observed in many of the larger repeat regions. To visualize the modular organization, the genomes were aligned using progressive Mauve. The alignment showed considerable sequence homology between the phages, but the genome organizations did not fall into the sub-grouping suggested by the prototype phages Cp220 or Cp21. In contrast, unique modular genome organizations were observed that related to the isolation origin of the phage.

Thus, our work shows that modular genome organization of Cp220viruses is very heterogeneous and promoted by a highly conserved repeat sequence that allows shuffling of coding sequence modules. Furthermore, we found that the modular genome organization was associated with the isolation origin of the phage.

[ID: 17] Introducing next-generation phage therapy in agriculture

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Bacterial infections in agriculture are estimated to cause losses up to 10%. Relevant crops for agriculture in Belgium (Flanders) include *Pseudomonas syringae* pv. *porri* (Pspo) and *Xanthomonas campestris* pv. *campestris* (Xcc). These bacteria are known to cause bacterial blight in leek and black rot in *Brassica* spp., respectively. Until recently, bacterial infections were treated using copper-based chemicals and antibiotics like streptomycin. However, the use of these compounds is not sustainable since they are prone to resistance development and have a detrimental impact on the environment. A valuable alternative is the use of biocontrol agents like bacteriophages to fight bacterial infections.

In this regard, different phages have been isolated to tackle both Pspo and Xcc infections. In the case of Pspo, five different phages, KIL1-KIL5 (KULeuven-ILVO), along with one host range mutant KIL3b, were found that are able to collapse 88% of Pspo strains found in Flanders. For Xcc, we isolated eight different phages that can lyse 71% of the strains relevant for agriculture in Belgium (SoPhi1-7 and Phibonacci). These phages are being investigated both genetically and microbiologically to determine their host range, infection efficiency, biosafety and potential to be used in phage therapy. Preliminary field trials have shown that the KIL cocktail is able to reduce the number of symptomatic plants from 63% to 38,5%, which is a promising result.

Further research is needed to optimize the cocktail and its production to tackle both Pspo and Xcc. Screenings and evolution experiments are being performed to select for phages with expanded host and stability ranges. Also tests to evaluate phage biosafety are performed. In terms of production, progress has been made using a novel system for phage purification based on anion exchange chromatography using CIM® Disk (BIA Separations) FPLC. In the case of the KIL cocktail, a recovery of 95% of the loaded phages was obtained.

[ID: 93] A variety of deazapurine modifications that protect the phage DNA from the restriction system of the hosts

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Escherichia coli siphophage 9g is resistant to many restriction enzymes and we recently discovered its DNA was heavily modified with 2'-deoxyarchaeosine (dG+). Archaeosine (G+) is a 7-deazaguanine-derivative normally found in tRNA of Archaea. G+ is synthesized from GTP through the 7-cyanodeazaguanine (preQ0) base intermediate by several enzymes (FolE, QueD, QueE and QueC), which is then integrated to the tRNA by Tgt, and further modified in G+ by either Gat-QueC, QueF-L or ArcS. Phage 9g genome encodes homologs of the tRNA-G+ modification synthesis pathway genes. Expression of the 9g phage folE, queD and queE genes complemented the Q-phenotype of the *E. coli* mutants lacking the respective homologs. In addition, the expression of 9g tgt and gat-queC in trans in *E. coli* was sufficient to reproduce the modification and restriction resistance phenotypes, independently of *E. coli* QueC.

Phage genomes encoding G+ biosynthesis pathway proteins can be classified as follow: 1) those encoding Tgt and other proteins of the G+ biosynthetic pathways; 2) those who only harbor Tgt; 3) those encoding some G+ biosynthetic pathway proteins but not Tgt. Interestingly, members of the first group could be subdivided into two Tgt sub-groups: the first contains a conserved H histidine, at position 196 (9g Tgt numbering), that is specific to this Tgt subfamily. The second Tgt subgroup is shorter and more divergent from canonical Tgt families. A Mass Spectrometry (MS) analysis of genomic DNA from a subset of these phages showed that phages from the first group harbored dG+ or other 2'-deoxyribose-7-deazaguanine derivatives. This work extends the discovery of G+ in DNA to other 7-deazaguanine derivatives that seems to protect phages from a wide variety of restriction enzymes encoded by the hosts.

[ID: 132] Isolation of phage for phage therapy

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The isolation of novel bacteriophages for phage therapy is necessary to find phages against an increasing number of pathogenic bacteria and to provide phages against bacteria that become resistant to previously used phages. The basic procedure for isolating bacteriophages remains relatively unchanged from the original procedures pioneered by Felix D’Herelle. An environmental sample appropriate for the target bacteria is obtained, processed to remove bacteria and larger material, and the resulting viral suspension is mixed with an isolation host bacterial culture. After incubation, any remaining bacteria are removed and the putative phage culture is screened for the presence of bacteriophages.

A number of researchers have developed alternative versions of this basic procedure. These include omitting the sample clearing before adding the isolation host; use of multiple isolation hosts that are closely related; use of isolation hosts that are distantly related; multiple rounds of isolation with varying hosts for each round. Each of these variations represents a way to deal with particularities of some phages and hosts such as phages that infect bacteria found in soil, phages which are often difficult to remove from soil particles. Sometimes the variation is meant to increase the numbers of phages isolated while other times the change is intended to increase the chances of isolating phages with a specific property, commonly increased host range.

Here we review these variations and discuss the strengths and weaknesses of each compared to the standard protocol. Finally, we make recommendations of when particular variations might be most useful in isolating novel bacteriophages for use in phage therapy.

[ID: 97] Novel temperate phage induced from *Macrococcus caseolyticus* isolate from human clinical material changes virulence of its host by negative and positive lysogenic conversion

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Bacteriophages affect bacterial populations in many ways, from the eradication of susceptible strains by lytic phages, through the lysogenic conversion of bacterial genomes caused by resident prophages to the horizontal gene transfer mediated by transducing phage particles. In this work the role of temperate phages found in macrococci, which are close relatives of well-known human pathogens staphylococci, was investigated. *Macrococci* isolated from human clinical specimens were tested for the presence of prophages. Phage designated ϕ MC1 was successfully induced from a human isolate of *Macrococcus caseolyticus* CCM 7927 by UV-light. Transmission electron microscopy classified the phage ϕ MC1 into the Siphoviridae family. ϕ MC1 genome sequence extracted from the whole genome sequencing of its host strain was examined for the presence of virulence factors. ϕ MC1 encoded genes for putative immunoglobulin-binding regulators IbrA and IbrB, hence it positively converts *M. caseolyticus*. At the same time, the detailed inspection of the attachment site in bacterial genome revealed that ϕ MC1 is integrated into the comGC gene of the comG operon, thus causing its negative conversion. Since com operons play a key role in the uptake of exogenous DNA during the transformation, inactivation of the comG operon would dramatically reduce the ability of the bacterium to exchange DNA and subsequently its genomic variability, which was indeed seen in the host strain genome compared to the other *Macrococcus* sp. strains. Overall, the identification of the ϕ MC1 siphophages and its ability to positively and negatively convert its host provides new insights into the evolution of the Staphylococcaceae family.

This work was supported by grants GA18-13064S, MUNI/A/0824/2017, and NT16-29916A.

[ID: 103] A non-canonical pathway to establish immune diversity in type I CRISPR-Cas systems

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Bacterial viruses (phages) and mobile genetic elements, such as plasmids, have a substantial influence on bacterial evolution. However, ~50% of bacteria protect themselves from these invaders using CRISPR-Cas (clustered regularly interspaced short palindromic repeats and CRISPR associated genes) adaptive immune systems. CRISPR-Cas systems function as sequence-specific nucleases that identify and destroy foreign genetic material. CRISPRs store genetic memories of prior invaders in the form of short DNA segments termed spacers. The sequences of these spacers determine immune specificity. However, phage and MGE variants with genetic mutations can escape CRISPR-Cas recognition. Therefore, CRISPRs must be updated by the addition of new spacers, a process termed CRISPR adaptation. Within cell populations, a high diversity of spacers targeting a specific phage is important for effective individual and community level protection. Currently, we have limited understanding of how CRISPR diversity in populations is established. Several type I CRISPR-Cas systems have been shown to increase their spacer repertoires by a process termed primed CRISPR adaptation (priming). Priming forms a positive feedback loop that reinforces immunity during infections and is also triggered by phage escape mutants or divergent phages. Here, we present a new pathway that promotes priming, caused by imprecision that occurs during initial spacer acquisition, termed slipping. We demonstrate that the CRISPR adaptation pathway initiated by slipping provides a mechanism to increase CRISPR diversity that pre-empts the proliferation of phage mutants that might completely evade host immunity, thereby establishing population-level immune resilience.

[ID: 281] Synergistic phage and antibiotic combinations to target biofilm-mediated infections

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Multidrug resistant (MDR) bacterial infections are a significant threat to the civilian and military populations, specifically those caused by biofilm-producing bacterial species. Surgery and antibiotics are the current standard-of-care for the management of traumatic wound infections. However, treatment failure is common and could result in debilitating amputations or lengthy antibiotic treatments. Our research is focused on developing bacteriophage cocktails that will work in combination with antibiotics to target complex MDR infections, preventing the need for repeated surgeries and therapeutic treatments.

Initial in vitro assessment of antibiotics (e.g., colistin, gentamicin, ciprofloxacin, ceftazidime) against *Pseudomonas aeruginosa* showed that while planktonic bacteria are susceptible to these antibiotics, when grown in biofilm even 1000 times the MIC was not sufficient to disrupt the biofilm and eliminate the bacteria. We assessed a five-member phage cocktail against *P. aeruginosa* biofilms and discovered that alone at higher concentrations (>10⁷ PFU) the cocktail reduced the biofilm mass by 25-50%. Preliminary assays of phage-antibiotic combinations against biofilms suggest that synergistic killing does occur; further testing is necessary to confirm these results.

In a mouse wound infection model, systemic treatment with the five-member phage cocktail alone or with antibiotics alone resulted in a slight reduction in bacterial burden in the wound. This reduction was only seen when using high doses of phage (>10⁸ PFU) or antibiotics (>1000 mg/kg). Using synergistic combinations of antibiotics and phage should reduce the bacterial burden in the wound while also allowing for the concentration of both treatments to be decreased; these studies are ongoing.

[ID: 174] Auresine - how to turn peptidase into antimicrobial weapon

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M23 zinc-dependent metallopeptidase family is a group of enzymes which cleaves cross-bridges in the peptidoglycan chains of bacterial cell walls leading to instant cell lysis. Members of M23 family are found among viral peptidases (e.g. gp13 of *B. subtilis* bacteriophage phi29), bacteriocins (lyostaphin of *S. simulans*) and bacterial autolysins (e.g. LytM of *S. aureus*, LytE of *B. subtilis*). LytM is one of *Staphylococcus aureus* autolysins, enzymes which in a very controlled manner cleave peptidoglycans to allow bacteria to grow and divide. Although LytM is abundant in *S. aureus* cell wall, its native form is inactive. Based on structural studies, the active form, called Auresine, has been engineered¹. Auresine applied from without is extremely efficient, stable and safe non-antibiotic weapon against *Staphylococcus*. The enzyme cleaves only staphylococcal cell walls, leaving natural microflora untouched. Moreover, Auresine is active in a wide range of temperatures (even on ice) and in low conductivity conditions (e.g. water)². Auresine can serve as a selective, non-toxic, biodegradable antibacterial agent. It can be applied as additive to surface disinfectants, food biopreservatives, component of veterinary and human hygiene products for skin infection prevention and treatment, but also as a component of wound dressing*. Due to its high specificity, Auresine may serve as a part of diagnostic test.

Keywords: peptidoglycan hydrolyses, *Staphylococcus*, non-antibiotic treatment

1. Odintsov S et al (2004) *JMB* 335:775
2. Sabala I et al (2012) *BMC Microbiology*, 12:97
3. Grabowska M et al (2015) *Scientific Reports*, 5:14833

* data not published

[ID: 345] Bacteriophages lytic to Burkholderia cepacia, B. thailandensis and B. vietnamensis

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The closely related species of Burkholderia cepacia complex, including B.cepacia and B.vietnamensis, have emerged as multidrug resistant opportunistic pathogens associated with serious hospital infections. The morbidity due to these species is higher in immunocompromised patients and those with cystic fibrosis. B.thailandensis, although closely related to EDP species B. pseudomallei, rarely causes disease in humans. Above mentioned species besides their clinical importance can serve as convenient models/surrogates for studies on highly pathogenic Burkholderias and for development of effective measures for their control. Phage therapy is considered as promising and in some cases the only possible treatment for critically ill patients infected with intrinsically drug resistant bacteria. Creating of collection of Burkholderia –specific lytic phages is crucial while the existing reserve is quite scarce. Especially little is known about B.thailandensis phages. The presented study aimed isolation and characterization bacteriophages lytic to B. cepacia, B. thailandensis and B. vietnamensis. The standard and modified techniques were applied for enrichment of water and soil samples collected in mainly in Georgia. In total 16 phage isolates were obtained, although only 6 of them showed good propagation and concentration capabilities, lysis stability and other properties of lytic phages. BtJG-1 and BtJG106 were active to B.thailandensis and B.oklahomensis strains, BvET phage lysed B. vietnamensis strains and BoD15 phage showed activity to B.oklahomensis. The phage Bcp2/III primarily propagated on environmental isolate B. cepacia EJ2 showed lytic activity also on B.cepacia clinical strains. Virion morphology studied by TEM showed that majority of Burkholderia phages belong to Myoviridae family with some differences in size. The selected 6 phages were characterized by basic biological properties: lytic activity towards Burkholderia spp. and related genera, one-step growth cycle, influence of physical-chemical factors, also DNA restriction profiles. Further studies on selected phages, as potential biocontrol agents, are ongoing, including full genome sequencing.

[ID: 228] Phage-based bioconjugates as a tool for fast bacteria separation and detection

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Nowadays there is a constant and urgent need for faster and more selective methods for bacteria detection. Utilization of bacteriophages seems to be a perfect choice that will be a future of that field. Here we demonstrate a novel multifunctional bioconjugates as specific probes for fast bacteria separation and detection [1]. In our approach magnetic-fluorescent microparticles were coupled with specific bacteriophages. The magnetic properties of the bioconjugates facilitate the separation of captured target bacteria from other components of complex samples and other bacteria species. Fluorescence enables simple analysis. We chose flow cytometry as detection method as it is fast, widely used for bio-tests, and available in many bioanalytical laboratories and hospitals. We aimed to create a method that could be applied easily, even in non-biological laboratories and without additional equipment, i.e. at low initial costs. Thus, prepared probes are cheap, simple to synthesize, versatile, and easily tunable for different bacteria species. Moreover, the whole procedure of bacteria separation and detection takes no more than 15 minutes. Here, a well-studied pair – the T4 bacteriophage and the Escherichia coli bacteria was used. The bacterial capture efficiency of the prepared bioconjugates is close to 100% in the range of bacteria concentrations from tens to around 10^5 CFU/mL. That makes proposed method suitable for fast (15 min) screening tests that are intended to quickly and precisely determine bacterial species in infected samples. The method may be also combined with a preincubation step to detect initial stages of bacterial contamination. The limit of detection and time of analysis can be balanced depending on the specific needs.

[1] Janczuk, M.†, Richter, Ł.†, Hoser, G., Kawiak, J., Łoś, M., Niedziółka-Jönsson, J., Paczesny, J., Hołyst, R., *Bioconjugate Chem.* 2017, 28, 419-425

† These authors contributed equally

[ID: 83] Evaluation of therapeutic potential of *Aeromonas hydrophila* phages in simulated infection using the zebrafish model

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Aeromonas hydrophila is important causes of infections in freshwater fish in Georgia. Chemotherapy is commonly used for treatment of disease outbreaks in fish farms, leading to a dramatic increase in antimicrobial resistance. Phage therapy can be considered as effective tool for control of fish bacterial infections.

The idea of the presented work was to prove the efficacy of phage-based strategy in vivo, on model zebrafish. Naturel mixture of *Aeromonas* phages and *A. hydrophila* virulent strain #3-10 isolated from diseased trout were simultaneously administrated to zebrafish using passive immersion in conjunction with intraperitoneal injection. Briefly, two groups of zebrafish inoculated with *A. hydrophila* #3-10 were set to the aquaria containing the same bacterium. One group was immediately treated with phage mixture. Third group, only exposed to phages was observed to exclude the eventual toxic effect of phages on the fish organism. A fourth group received only saline through intraperitoneal injection (fish control). Each group consisted of 30 individuals of zebrafish equally divided into three parallels.

The presence of bacteriophages in fish was detected immediately after exposure of fish to phage containing aquaria. The concentration of phage particles increased by 2log within 48 hours and persisted in fish throughout the experiment (14 days). The bacterial counts in phage treated fish was gradually reduced during 8 days compared to the fish group not exposed the phage treatment. Bacterial counts in the same aquaria water were also decreased. The survival rate in infected fish without phage treatment was significantly lower compared to the phage treated fish. Mortality in the fish challenged with bacteria only was registered at the 2nd day and in 2 weeks after infection reached 40%, when the mortality in phage treated group was only 10%. The conducted study showed effectiveness of phage therapy for fish disease caused by *A. hydrophila* and can serve as a starting point for its practical application in aquaculture.

[ID: 386] Subtyping of clinical strains of *P. aeruginosa* and *A. baumannii* by phage susceptibility profiles: easy and reliable approach to identify the origin of infection

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Bacterial agents remain the most commonly recognized cause of health care associated infections (HAI) as well as community acquired infections. Understanding of pathogen distribution and relatedness is essential to determine the epidemiology of infections and to elaborate rational pathogen control methods. Subtyping of bacterial isolates based on comparison of phenotypic characteristics such as bio- and serotypes, phage- and antimicrobial susceptibility profiles etc along with molecular typing methods can aid in the identification of the source of organisms and distinguish infectious strains. The studies on grouping of *P. aeruginosa* and *A. baumannii* clinical strains have been done using phage spot test techniques in parallel with determination of antibiotic susceptibility by Kirby-Bauer method and detection of AMR genes. Majority of bacterial strains appeared to be highly resistant to majority of antibiotics commonly used in clinical practice. The specific phage typing sets were composed with 8 phages for 164 Georgian clinical isolates of *P. aeruginosa* while set of 6 phage was used for 66 *A. baumannii* clinical strains of different geographical origin, including 32 isolates collected in Georgia. *P. aeruginosa* hospital isolates (from Children's Hospital, Burn wound center, Multiprofile Military Hospital) were sub-typed into seven phage groups and up to 40 sub-groups, nicely reflecting the particular hospital origin of strains. The subtyping of 58 *P. aeruginosa* isolates (community acquired strains) collected at the Eliava Diagnostic Centre showed much higher diversity. The phage susceptibility profiles obtained for geographically diverse collection of *A. baumannii* clinical isolates also allowed to group them into 10 clusters with concentration of Georgian strains in 2 groups. The studies have shown that phage-based typing methods guaranteed by existence of effective phage set can be considered as easy and reliable approach for subtyping of clinical isolates and can aid in timely identification of infection source.

[ID: 116] The detection of *Staphylococcus aureus* and *Streptococcus agalactiae* by specific binding domain from phage endolysin

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Endolysins are phage-encoded lytic enzymes which are synthesized at the end of phage lytic cycle. Bacterial elimination is not their only unique application how to use them: many studies have led to the knowledge how to possibly target parts of endolysins to the bacterial cell wall. Bacteriophage endolysins targeting Gram-positive bacteria feature a modular structure and so are composed of at least two separated functional domains: the N-terminal catalytic domain; and the C-terminal domain (CBD) with the host cell wall binding activity. CBDs allow to whole molecule to bind directly to the host cell wall and may be responsible for specificity of strain- or species binding. CBDs can recognize and bind to specific receptors in the bacterial cell wall, thereby affinity of enzymes is increased. Therefore, the nucleotide sequence of a specific binding domain (SBD) was specifically *in silico* designed for endolysin, applicable on multiple strains of *Staphylococcus aureus* and *Streptococcus agalactiae*. These bacteria represent pathogens, causing infectious human and veterinary diseases, contaminating foodstuffs. In the bioinformatics part, extended analyses were performed, comprising CBDs of endolysins from phages infecting *S. agalactiae*, *S. aureus*, in which multiple sequence alignments of SH3 binding domains were assessed. Plasmid construct pET28b-sbd1-gfp was prepared, containing the proposed sbd1 in fusion with the gene for green fluorescent protein (GFP). After the verification of cloning, induced heterogeneous expression of SBD1-GFP fusion protein was carried out in *Escherichia coli*. SBD1-GFP expressed fusion protein was partially purified by IMAC. In specific binding assays, SBD1-GFP was incubated either with *S. aureus*, or with *S. agalactiae* cells. SBD binding activity towards the cells was confirmed by fluorescence microscopy. Along with this, the detection of different *S. aureus* or *S. agalactiae* strains was demonstrated, which could also find a potential application in microbiological practise.

Acknowledgement: This work was supported by APVV-16-0173 grant.

[ID: 391] Prevention and treatment of the American and European Foulbrood of honey bee using specific bacteriophage preparation

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The purpose of our studies was to identify a weapon against American and European Foulbrood – serious health problems of honey bees – which leads both to the weakening of the vitality of bee colonies and significant economic losses in agriculture and horticulture. The vast majority of commercial pollination of crop plants in the world has been performed by the honey bee (*Apis mellifera*), therefore it is extremely important to develop an effective bacteriophage preparation for the prevention and treatment of American Foulbrood and European Foulbrood, diseases causing death of honey bees.

For phage isolation 1321 biological and environmental samples (e.g. soil, water, wax, bees, honey) obtained both from infected and mature apiaries – which have not demonstrated pathological symptoms – were used. Phage isolation, lytic spectrum, activity as well as phage amplification were prepared using plate method.

We have obtained a unique collection of phages specific to *Paenibacillus* larvae - the main etiological agent of the American Foulbrood. From tested 627 *P.* larvae filtrates it was obtained 32 phage isolates in titers ranging from 1×10^4 - 10^5 pfu/ml. From the isolated cultures lytic phages with the widest spectra are selected and characterized (the ultrastructure, morphology, biological properties, storage stability, genome sequencing).

We have established conditions to prepare phage preparations that could be used in prophylaxis and treatment of bee families infected with American or European Foulbrood. The phages of widest spectrum with confirmed resistance to physical and chemical factors and good stability under storage conditions were selected for further development. Three prototypes comprising various phages in series of formulations have been prepared to test their efficacy in bees.

[ID: 169] Determination of bacteriophage parameters in chemostat without influencing physiological state of bacteria

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Bacteriophage amplification cycle begins with bacteriophage adsorption onto bacteria, followed by injection of bacteriophage genome, which results in the synthesis of new bacteriophages. After lysis of bacteria new bacteriophages are released [1]. Better understanding of bacteriophage-bacteria interactions enables us more efficient use of bacteriophages in different applications. These interactions can be described with three parameters: adsorption constant, burst size and latent period, which were found to be dependent on bacteria physiological state [2]. Because commonly used techniques to determine bacteriophage parameters can also affect physiological state of bacteria, we developed a specific experimental system using chemostat. The system had a continuous inflow of substrate into a stirred bioreactor, where bacteria were continuously growing and a continuous outflow to achieve steady-state conditions [2]. In this way, different constant bacterial physiological states can be achieved by varying the dilution rate. The experiment of bacteriophage parameters determination started after the steady state was achieved, with a single injection of bacteriophages directly into the bioreactor. Immediately afterward, samples were collected from the bioreactor at different times. Determination of free bacteriophages was performed by plaque assay method. Measured concentration of bacteriophages was fitted by modified mathematical model describing pulse injection in continuous stirred tank reactor [1, 3], in order to determine mentioned parameters.

Such approach enables determination of bacteriophage parameters without affecting bacteria physiological state. Results obtained with described approach are compared with traditional method and differences are discussed. Furthermore, we implemented proposed methodology to investigate changes in bacteriophage parameters due to the potential effect of hibernation [4].

[1] Santos S. B. et al., PLoS ONE, 9(7): e102507, 2014.

[2] Nabergoj D. et al., MicrobiologyOpen: e558, 2017.

[3] Gáspár S. et al., Zeitschrift für Allgemeine Mikrobiologie, 19, 3, 163-169, 1979.

[4] Bryan D. et al, Frontiers in Microbiology, 7:1391, 2016.

[ID: 299] The mapping of the replication region in the newly isolated coliphage vB_EcoS-95

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The aim of this study was to map the region necessary for the DNA replication in a newly identified environmental bacteriophage through in silico and molecular methods. The novel coliphage, named vB_EcoS-95 showing highly lytic activity against various E. coli strains was isolated from urban sewage. The genomic analysis of its sequenced genome indicated that the phage consists of linear, double-strand DNA of 50,899 bp, encoding 89 putative ORFs. The sequence presented 78% identity with the sequence of Shigella phage Spf-1. Genomic comparison analysis enabled the selection of 3 putative regions involved in DNA replication in vB_EcoS-95, containing all the genes and regulatory sequences necessary for their stable maintenance in the host cell. Our attempt was to construct a phage-derived plasmid by ligating these fragments, previously amplified through PCR, with an antibiotic-resistance genetic marker. The method would provide a positive selection, as only plasmid-bearing E. coli cells will grow on medium containing a proper antibiotic. The method was successfully used previously by our team for the construction of plasmids from lambdoid phages. So far we did not succeed in obtaining a replicating construct of vB_EcoS-95, however efforts are being made to optimize the specific cloning method. We hope that the construction of the phage-derived plasmid would facilitate further investigation on the replication regulation mechanism in the novel phage.

[ID: 274] Phage control of potato soft rot using the sets of specific bacteriophages

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Pectobacteriaceae belonging to the genera *Dickeya* and *Pectobacterium* causing soft-rot and black leg of potato are a serious threat to agriculture. Latent infection of the seed tubers resuming an activity with the rise of environmental temperature promote the geographic spread of the disease. The prophylactic treatment of the harvest and seed material with specific bacteriophages is an effective method to maintain the quality of potato.

In the course of the project we have shown that the diversity of pectolytic Pectobacteriaceae is limited. Their phage susceptibility can be divided into groups partially corresponding to their taxonomic demarcation. We have isolated lytic bacteriophages highly infective against the strain groups of *Pectobacterium atrosepticum*, *P. carotovorum* subsp. *carotovorum*, *P. parmentieri* and *Dickeya solani* most widespread in the European part of Russia. Based on a comparison of electrophoretic patterns of bacterial lipopolysaccharides and genomic fingerprinting (BOX-PCR) we assume that the specificity of bacteriophages is governed by the structure of external polysaccharides (O-antigen).

The project is supported by Russian Science Foundation grant #16-16-00073

[ID: 110] Composition and host specificity of five commercial bacteriophage cocktails

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Introduction: Bacterial infections causes many complications. According to WHO, the problem has two main reasons: on one side, there are an ever increasing number of human bacterial pathogens becoming resistant to antibiotics, and on the other side, there are less new antibiotics developed by the pharmaceutical industry. A promising strategy for treatment and prevention of bacterial infections is phage therapy. The use of phages against pathogenic bacteria was intensively studied in former Soviet-union. Eastern scientists prepared phage cocktail products that are distributed as a treatment all over post-soviet countries. Our main objective was to characterize five different phage cocktails from Russia and Georgia. We also would like to determine proportional representation of phage families, which create phage products and the effectivity of infection against clinical strains from Slovak republic.

Material and methods: Plaque assay on double agar plates was used for host specificity determination. Partial genome sequences were obtained using Next-Seq system (Illumina) and assembled by MetaSpades. Annotation of partial genomes was defined by RAST and verified by BLAST.

Results: Two preparations are distributed in Russia as Intestiphage, one as Sextaphage and the last one as Pyophage. Host specificity was determined against set of 64 bacterial strains. Testing group consists mostly of Escherichia, Enterobacter, Cronobacter and Streptococcus species. The broadest host range was specified in case of Pyophage. Product was able to infect 39 tested strains. On the contrary, the narrowest specificity reached cocktail from Georgia that infected 26 strains. According to partial genome analysis, all the preparations are mixtures of phages from different families. For example, Sextafag consists of 31 phages, from Myoviridae, Siphoviridae and Podoviridae family.

Conclusion. All five preparations were able to infect bacterial strains from our collection. Phage cocktails consists of variable number of phages from order Caudovirales.

[ID: 352] Study of the lytic bacteriophages active against clinical and dairy *Enterococcus* isolates

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Enterococcus spp. are known to be common in artisanal food products, frequently contaminating dairy fermentation facilities. Meanwhile vancomycin-resistant *E. faecium* was assigned to high risk pathogen group in the WHO priority list. Thereby precise control and elimination of virulent *Enterococcus* spp. in dairy industry is crucial for food safety and public health.

Ninety eight *Enterococcus* strains were isolated from the artisanal yogurt-like product Matsoni samples and analysed with RAPD-PCR typing, UPGMA clustering, ssPCR and 16S rRNA sequencing of the cluster representative strains. Thirteen groups of strains were identified as clonal by genotyping. Several strains from each of 13 groups had identical RAPD fingerprint profiles. The phages were isolated from various sources using enrichment method. Phage morphology and purity was determined by TEM. Five phages: Φ 1-Dielo, Φ 4-Sazandari and Φ 5-Muxambazi belonging to Siphoviridae family and phages Φ 3-Buba and Φ 2- Elibo of Mioviridae family were studied. In total 98 *Enterococcus* isolates from artisanal Matsoni samples including 21 - *E. durans*, 50 - *E. faecium* and 21 - *E. faecalis*, and 46 clinical *Enterococcus* spp. were screened for phage susceptibility. Assessment of the results was performed by calculation of phage activity index.

The clinical strains showed higher susceptibility than dairy isolates, with highest score of phage Φ 4-Sazandari with index point 1.2 on clinical strains and 0.54 on food isolates. Notably, among Matsoni isolates *E. faecalis* showed highest susceptibility to phages. Three out of thirteen clonal RAPD groups showed identical phage susceptibility patterns. Genotype similarity of the strains does not match with phage susceptibility patterns. Thus, it can be concluded that the studied bacteriophage clones potentially can be used as natural decontamination agents in the manufacturing environments.

[ID: 412] M13 bacteriophages as a scaffold for synthesis and structure transformations of gold nanoparticles

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Despite their small size and simple structure, bacteriophages (phages) have a remarkably high range of applications in medicine and biotechnology. We successfully used phages as a scaffold to bind and synthesize nanoparticles of europium oxide, zinc oxide, and – most recently – gold (AuNPs). Gold nanoparticles have been among the most intensively studied structures in recent years creating the need for effective methods for their modifications and manipulations. They have been tested as promising agents in cancer therapy and investigated as drug carriers, photothermal agents, contrast agents and biosensor components.

In this work, using phage display technique, we constructed phage M13 derivatives that expose peptides effectively binding AuNPs. After the addition of a gold colloid to the phage suspension, aggregation of nanoparticles occurred, as confirmed by absorption spectroscopy measurements. Conversely, when the phage suspension was added to the gold colloid, precipitation of gold nanoparticles was observed. Importantly, the phages were able to not only bind, but also to synthesize AuNPs in the presence of ammonia or trimethylamine, as revealed by scanning electron and transmission electron microscopy and spectroscopic analyses. The novel Au-binding bacteriophages are active in various processes of gold structure transformation, which suggests their potential applicability in diverse AuNPs manipulations.

We are now focusing on testing the synergistic therapeutic effect of phages and nanoparticles. Preliminary results indicated the ability of nanoparticles to augment the effect of phages against biofilm-associated bacterial cells.

[ID: 266] Role of viruses in the evolution of eukaryotic B-family DNA polymerases

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Genome replication is an essential process for all living entities, including viruses. Double-stranded DNA viruses have evolved different strategies to replicate their genetic material. Smaller viruses either rely on host's replication apparatus or use protein-primed DNA replication (Kazlauskas and Venclovas, 2011). By contrast, viruses with large genomes usually encode their own replication proteins, including helicases, primases and DNA polymerases (Kazlauskas et al., 2016). Family B DNA polymerases (PolB) are particularly widespread in DNA viruses and cellular organisms from all three domains of life. In some organisms, polB genes are present in multiple copies. For instance, humans encode four PolB homologs called alpha, delta, zeta and epsilon, with epsilon itself containing two PolB domains (Tahirov et al., 2009). Here, we collected representative bacterial, archaeal, eukaryotic and viral B-family members and performed a comprehensive phylogenetic analysis and structural modeling to determine possible origins of cellular and viral PolBs. Our results revealed a complex evolutionary history of viral PolB, with multiple exchanges between viruses and cells. We show that PolB of large eukaryotic DNA viruses from the orders "Megavirales" and Herpesvirales are polyphyletic. Furthermore, we were able to trace the origins of the two PolB domains of the eukaryotic polymerase epsilon, with one of the two domains apparently being recruited from bacteriophages.

1. Kazlauskas, D., Venclovas, Č. (2011). Computational analysis of DNA replicases in double-stranded DNA viruses: Relationship with the genome size. *NAR*, 39(19), 8291-8305.
2. Kazlauskas, D., Krupovic, M., Venclovas, Č. (2016). The logic of DNA replication in double-stranded DNA viruses: Insights from global analysis of viral genomes. *NAR*, 44(10), 4551-4564
3. Tahirov, T. H., Makarova, K. S., Rogozin, I. B., Pavlov, Y. I., Koonin, E. V. (2009). Evolution of DNA polymerases: An inactivated polymerase-exonuclease module in Pol ϵ and a chimeric origin of eukaryotic polymerases from two classes of archaeal ancestors. *Biology Direct*, 4(1)

[ID: 400] Humoral response to staphylococcal bacteriophages A3R and 676Z and their selected structural proteins in a murine model

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Objectives: Two staphylococcal bacteriophages: A3R and 676Z were assessed regarding their overall ability to induce specific anti-phage antibodies in mice with two different routes of administration: intraperitoneal (i.p.) and per os. Additionally, individual immunogenicity of three gene products previously annotated as structural proteins was estimated.

Results: In the i.p. model a clearly visible, marked increase in serum IgM and IgG levels was observed. IgM peaks were detected on day 5, while IgG levels increased significantly on day 8 for both phages. After day 15 the levels of antibodies did not change in a significant range, with slight increases after boosting injection (day 20). In case of per os administration, anti-phage IgA antibody levels were also assessed. Both plasma and fecal IgA levels started to increase 5-6 weeks after the initiation of phage treatment. A marked increase in plasma IgG was observed 2-3 weeks following the initiation of the treatment and IgM peak was observed on day 15. Decrease of phage titers in feces correlated with an increase of IgA levels, but not IgG.

Structural function of proteins AFN38122.1 (A2), AFN38181.1 (A4), AFN38152.1 (A8) was confirmed using immuno-EM and the immunogenicity of these proteins in the per os model was assessed, revealing A2 (major capsid protein) and A4 (tail protein), but not A8 (baseplate protein) to be strongly immunogenic.

Conclusions: These data show that staphylococcal bacteriophages A3R and 676Z induce production of anti-phage antibodies in vivo when applied both per os or i.p. Major capsid protein (A2) and tail protein (A4) contribute to the induction of humoral response. In the model of per os administration, increase in anti-phage IgA levels correlates with a decrease of phage titers detected in feces.

Acknowledgements: This work was supported by the National Science Centre in Poland grant UMO-2012/05/E/NZ6/03314. Presenting authors (Z.K. and J.M.) contributed equally to this work.

[ID: 33] Removal of staphylococcal enterotoxins from bacteriophage preparations

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Staphylococcus aureus is a commensal and pathogenic bacterium that causes infections in humans and animals. It is a major cause of nosocomial infections worldwide and WHO currently regards antibiotic-resistant *S. aureus* as one of the major concerns for public health. Due to increasing prevalence of multidrug resistance, alternative ways to eradicate the pathogen are necessary. Phage therapy is one potential cure for infections caused by antibiotic resistant *S. aureus*.

S. aureus strains produce a large variety of exoproteins that enhance virulence and cause disease in mammals. Among these proteins are staphylococcal enterotoxins (SEs) that are emetic and cause food poisonings and toxic shock syndrome. SEs are small proteins (20 – 30 kD) and very stable; they are resistant to heat, acid, and gastrointestinal proteases. SEs are very potent, < 1µg can cause disease, and concentrations below 0.5 ng/ml have been associated with outbreaks.

The aim of this work was to study whether there are differences in SE concentrations in phage lysates produced in different *S. aureus* strains, and whether SEs are removed during bacteriophage purification. Our results show that the choice of production strain can greatly influence the amount of SEs in phage lysate. The combination of ultrafiltration and ion exchange chromatography reduced the SE concentration from 320 ng/ml of the raw lysate to < 1 ng/ml (i.e. below the detection limit of our assay).

[ID: 214] Design and Construction of *Vibrio cholerae* Strains that Harbor Various CTX Prophage Arrays

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Toxigenic *Vibrio cholerae* strains arise upon infection and integration of the lysogenic cholera toxin phage, the CTX phage, into bacterial chromosomes. The *V. cholerae* serogroup O1 strains identified to date can be broadly categorized into three main groups: the classical biotype strains, which harbor CTX-cla; the prototype El Tor strains (Wave 1 strains), which harbor CTX-1; and the atypical El Tor strains, which harbor CTX-2 (Wave 2 strains) or CTX-3~6 (Wave 3 strains). The efficiencies of replication and transmission of CTX phages are similar, suggesting the possibility of existence of more diverse bacterial strains harboring various CTX phages and their arrays in nature. In this study, a set of *V. cholerae* strains was constructed by the chromosomal integration of CTX phages into strains that already harbored CTX phages or those that did not harbor any CTX phage or RS1 element.

Strains containing repeats of the same kind of CTX phage, strains containing the same kind of CTX phage in each chromosome, strains containing alternative CTX phages in one chromosome, or containing different CTX phages in each chromosome have been constructed. Thus, strains with any CTX array can be designed and constructed. Moreover, the strains described in this study contained the toxT-139F allele, which enhances the expression of TcpA and cholera toxin. These characteristics are considered to be important for cholera vaccine development. Once their capacity to provoke immunity in human against *V. cholerae* infection is evaluated, some of the generated strains could be developed further to yield cholera vaccine strains.

[ID: 69] A pilin region affecting host range of the *Pseudomonas aeruginosa* leviphage, PP7

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The host range of a phage is determined primarily by phage-receptor interaction. Here, we profiled the host range of an RNA leviphage, PP7 that requires functional type IV pilus (TFP) in order to enter into its host bacterium, *Pseudomonas aeruginosa*. Out of 25 twitching-proficient *P. aeruginosa* strains, 4 with group I pilin and 7 with group III pilin displayed PP7-resistance. The remaining 14 possessed group II pilin, which included 10 PP7-sensitive and 4 PP7-resistant strains, suggesting that only the strains with TFP consisted of a subset of group II (hence, group IIa) pilin were susceptible to PP7. The co-expression of the PAO1 (group IIa) pilin rendered all the strains susceptible to PP7, with the exception of the 4 strains with group I pilin. Moreover, the expression of PA14 (group III) and PAK (group IIb) pilin in the PAO1 pilA mutant restored the twitching motility but not the PP7-susceptibility. Site-directed and random mutation analyses of PAO1 pilin enabled us to identify a pilin mutant (G96S) that is fully functional but resistant to PP7 infection. This is due to the lack of any phage-receptor interactions, suggesting the structural properties of the β 1- β 2 loop in the variable region 2 of the group II pilin might be involved in PP7 infection.

[ID: 105] A cocktail of three virulent bacteriophages prevents *Pectobacterium carotovorum* subsp. *carotovorum* infection in Chinese cabbage

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Pectobacterium carotovorum subsp. *carotovorum* (Pcc) is a broad host range plant pathogen, which causes soft-rot disease on a variety of vegetables. Various cell-wall-degrading enzymes that allow infiltration and maceration of plant tissues are produced by this phytopathogen. Bacteriophages have been proposed as an alternative method to control this soft-rot pathogen to replace the chemotherapy. To prevent agricultural products from Pcc infections, three Pcc-specific virulent phages (POP10, POP15, and POP72) were isolated from environmental samples and their viable stability was evaluated under various stress conditions. The Tn5 random mutant library of Pcc was screened for resistance against POP phage infection to determine the host receptor of each phage. Analysis of POP phage-resistant Pcc mutants revealed that the host receptors of POP10, POP15, and POP72 are lipopolysaccharide, flagella, and colonic acid, respectively. A cocktail comprising three phages that target different host receptors suppressed the emergence of phage-resistant Pcc up to 26 h in vitro. The efficiency of the phage cocktail to prevent soft-rot disease in the plant was tested in Chinese cabbage. Treatment of artificially inoculated Chinese cabbage with phage cocktail at an MOI of 104 resulted in a significant decrease in Pcc population compared with the phage-untreated cabbage ($P < 0.05$). These results implied that POP cocktail would be an efficient alternative antimicrobial reagent for the protection of agricultural products from Pcc infection.

[ID: 334] Characterization of bacteriophages active against Staphylococcus aureus isolated from human saliva

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Implant-associated infections are related to formation of a biofilm, which hinders the eradication with antibiotics. Phage therapy is a promising alternative treatment option for biofilm infections, potentially active also against biofilms. Staphylococcus aureus is one of the main bacterial species associated with periprosthetic joint infection (PJI). (1) Aim of this study was to screen human saliva samples for the presence of lytic bacteriophages active against S. aureus and to characterize the newly isolated phages for their potential antimicrobial activity versus PJI associated strains of S. aureus.

Six bacteriophages specific for S. aureus were isolated from different saliva sample donors. Transmission electron microscopy analysis revealed first insight in the morphology of isolated bacteriophages. Phages are resistant to chloroform and temperature up to 50°C. Calorimetric analysis showed that 10^7 phages completely lyse 10^6 bacteria. Moreover, higher phage titers (10^8) reduce > 50% S. aureus biofilm in vitro, although a complete eradication of S. aureus biofilm was not observed when using this dose. Phages showed a broad host spectrum among clinical isolates of S. aureus and infect 77-85% of clinical strains.

Based on these in vitro analysis, phages used in this study might be suitable for phage cocktail formulation for the treatment of S. aureus infections. Sequencing analysis is needed to exclude the presence of lysogenic genes in the phages nucleic acids. In addition, the ability to strongly reduce the biofilm suggest a possible application in combination with antibiotics for the treatment of implant-associated infection.

(1) Zimmerli, W. (2014). Clinical presentation and treatment of orthopaedic implant-associated infection. J Intern Med, 276(2): 111–19

[ID: 385] Studying DNA packaging motor of bacteriophages infecting thermophilic bacteria

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Viruses are the most abundant biological agents on our planet, able to infect organisms of all domains of life. The packaging of viral genome into a capsid is essential part of viral life cycle and is mediated by specialized molecular machines fuelled by energy derived from macroergic compounds hydrolysis. While there are structural and functional similarities, these machines have evolved to perform different tasks and many of them are still poorly characterised.

Though in recent years progress in understanding the 3D structures of individual components of viral DNA packaging motors was achieved and some functional data generated using innovative "single-molecule" experiments were obtained, most aspects of the mechanism of DNA packaging into viral particles remain unknown. Stoichiometry of subunits in the DNA packaging motor; functional interactions between motor components during packaging; mechanism of DNA recognition; mechanism of dissociation of the complex after the end of packaging, - all these questions remain open.

To address these issues we conduct structural and functional characteristics of the DNA packaging apparatus from bacteriophages infecting thermophilic bacterium *Thermus thermophilus*. We predicted cluster of genes encoding for DNA packaging motor components. Individual proteins were purified to homogeneity and tested for oligomeric state. Increased stability and robustness of DNA packaging apparatus and its individual components from bacteriophages infecting thermophilic bacterium *Thermus thermophilus* open new horizons in studying viral DNA packaging.

[ID: 323] Receptor binding in bacteriophages of Gram-negative and Gram-positive bacteria

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Many uses for bacteriophages in detection and control of bacteria have been developed. However, our knowledge of the initial steps of phage infection, the adsorption to the host cell, is still scarce. We have been studying receptor binding in bacteriophages of *Salmonella* and *Listeria* and were able to develop near-atomic models of the receptor-binding apparatus of these phages, and also apply our results for the development of a *Salmonella* detection assay.

Listeria phage A511 is a model organism for the SPO1-related phages of Gram-positive bacteria. Its tail adsorption apparatus resembles a contractile injection system known from other biological systems. We have obtained structures of the A511 tail in pre- and post-contraction state. We could demonstrate that the A511 baseplate undergoes a massive conformational change during adsorption and switches its symmetry. We could also show, for the first time in native conditions, that the tail contraction starts from the baseplate and propagates through the sheath as a wave.

Salmonella phage S16 is a T4-like phage but features an T2-like organization of the long tail fiber (LTF) the structure of which has been elucidated by protein crystallography. It consists of a single copy of gp38 attached to a trimeric gp37 structure and binds to OmpC and the lipopolysaccharide outer core of the *Salmonella* cell wall. The gp38 adhesin features an unusual “polyglycine sandwich”, which mediates receptor binding.

The S16 LTF protein has also been developed into a highly efficient affinity molecule for the detection of *Salmonella* in liquids and food. Paramagnetic beads coated with S16 gp37-gp38 LTF complexes are able to specifically bind and enrich *Salmonella* cells from almost any matrix in food with 95% recovery rates and sensitivity down to 10 cells/g food. We present data on our colorimetric ELLTA-assay, which allows for the rapid, reliable and cost-efficient identification of *Salmonella* contaminations.

[ID: 273] Phage NgoΦfil as Vaccine against Neisseria gonorrhoeae

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All *Neisseria gonorrhoeae* strains whose DNA sequences have been determined possess filamentous phage sequences. The ubiquitous presence of these phages suggests that purified phage particles might be used as a gonococcal vaccine. To test this hypothesis, we purified filamentous phages NgoΦfil and immunized rabbits subcutaneously. The elicited sera contained large quantities of anti-phage IgG and IgA antibodies that bound to the surface of *N. gonorrhoeae* cells, as shown by ELISA and flow cytometry. The elicited sera bound to structural NgoΦ6 proteins present in phage particles and to *N. gonorrhoeae* cells. The sera did not react with gonococcal outer membrane proteins. The sera also had bactericidal activity and blocked adhesion of gonococci to tissue culture cells. These data demonstrate that Ngofil phage particles can induce antibodies with anti-gonococcal activity and may be a candidate for vaccine development.

[ID: 152] Mutations in the bcs operon of *Erwinia amylovora* abolish infection by bacteriophages S6 and M7

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The plant pathogen *Erwinia amylovora* is the causative agent of fire blight affecting members of the Rosaceae family. One of its major virulence factors is the ability to form biofilms, which leads to clogging of plant vessels and therefore provoke disease symptoms. Although the application of streptomycin can effectively control infection, the antibiotic is banned in an increasing number of countries. Bacteriophages are a promising alternative to conventional antibiotics. The identification of phage receptors on the host surface is crucial for effective phage cocktail formulation. A high throughput screen of a Tn5 transposon mutagenesis library revealed genes of *E. amylovora* CFBP 1430, which are involved in phage adsorption. The Felix O1-like phage M7 and the N4-like phage S6 were no longer able to lyse mutants with defective genes in the bacterial cellulose synthase operon (*bcs*). The constitutively expressed *bcs*-complex is responsible for production and secretion of bacterial cellulose, a compound associated with biofilm formation. Deletion of the entire *bcs* operon or single *bcs* genes verified their importance for infection of *E. amylovora* with M7 and S6. Experiments with the cellulose binding dye Congo red and the fact that both phages harbour a set of putative cellulases and endoglucanases further indicate an interaction between M7 and S6 with cellulose. These findings suggest that M7 and S6 might recognize different patterns either on the cellulose synthase complex, namely its outer membrane protein BcsC, or the cellulose cargo itself.

[ID: 63] Structural biology of the genetic switch from bacteriophage TP901-1 - focusing on the CI repressor

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TP901-1 is a temperate bacteriophage of the P335 species that infects *Lactococcus lactis* bacteria. It has a bi-stable genetic switch making it able to shift between either the lytic or lysogenic life cycle; this is controlled by two regulatory proteins, the CI repressor and the MOR anti-repressor. CI consists of two functional domains connected by a flexible linker, namely an N-terminal domain (NTD, 1-80) responsible for DNA binding through a helix-turn-helix motif (HTH), and a C-terminal domain (CTD, 90-180) responsible for the formation of a hexamer. Previously, we have showed the structure of NTD in complex with DNA revealing an extension in the scaffolding helix of the HTH.

Here we focus on the crystal structure of the CTD1 (90-124) helical hook dimerization motif and the solution structure of CI Δ 58 (1-122) in complex with its operator site.

Numerous closely genetically related pathogenic species, such as *Staphylococcus aureus* phages, are expected to possess a similar genetic switch, so structural studies of TP901-1 may have wider perspectives.

1. Johansen, A. H., Brøndsted, L. & Hammer, K. Identification of operator sites of the CI repressor of phage TP901-1: Evolutionary link to other phages. *Virology* 311, 144–156 (2003).
2. Pedersen, M., Lo Leggio, L., Grossmann, J. G., Larsen, S. & Hammer, K. Identification of Quaternary Structure and Functional Domains of the CI Repressor from Bacteriophage TP901-1. *J. Mol. Biol.* 376, 983–996 (2008).
3. Pedersen, M., Ligowska, M. & Hammer, K. Characterization of the CI repressor protein encoded by the temperate lactococcal phage TP901-1. *J. Bacteriol.* 192, 2102–2110 (2010).
4. Frandsen, K. H. et al. Binding of the N - Terminal Domain of the Lactococcal Bacteriophage TP901 - 1 CI Repressor to Its Target DNA: A Crystallography, Small Angle Scattering, and Nuclear Magnetic Resonance Study. *Biochemistry* 52, 6892–6904 (2013).

[ID: 67] Isolation and characterization of *Weissella cibaria* infecting bacteriophages from Kimchi at the early fermentation

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Weissella cibaria infecting bacteriophages were isolated from Kimchi at the beginning of fermentation. From two Kimchi samples, 96 isolates of lactic acid bacteria were obtained and employed as hosts for isolation of bacteriophages. Host bacteria were identified by the 16s rRNA sequencing and BLAST analysis. Also, two *Weissella cibaria* type strains were employed as hosts. The five bacteriophages, Φ WC51, Φ WC52, Φ WC005, Φ WC130 and Φ WC248, were characterized in this study. The plaques of phages were mostly clear and round-shaped on the MRS agar plates. On the basis of transmission electron microscope, the phage Φ WC51 and ϕ WC52 belongs to the Myoviridae family and Φ WC005, Φ WC130, and Φ WC248 belongs to the Podoviridae family. D value of three phages, Φ WC52, Φ WC130, and Φ WC248, were calculated 75, 32, and 84 seconds at 60°C and two phages, Φ WC51 and Φ WC005, were calculated 71 and 34 seconds at 70 °C by a thermal inactivation, respectively. Therefore, it could be seen that most heat-stable phage is Φ WC005. The pH stability test (pH 2.0-4.0) showed that Φ WC005 is most stable and Φ WC52 is most unstable at low pH. One-step growth curves of phages showed that the latent period of five bacteriophages, Φ WC51, Φ WC52, Φ WC005, Φ WC130, and Φ WC248, was 90, 70, 85, 85, 70 minutes and burst size is 186, 195, 11, 79, 7 particles/infected cell, respectively. Isolated phages DNA were digested with three restriction enzymes, EcoR I , Sal I , and EcoRV. As a result, it is confirmed that five phages have different DNA sequences and the overall size of the phage genome was estimated to be 32-37kb. This study revealed that five isolated bacteriophages divide into two morphological features and have clearly different properties. The presence of *Weissella cibaria* infection bacteriophage may be very interesting in Kimchi fermentation.

[ID: 78] Bacteriophages infecting *Salmonella enterica*: isolation, characterization and potential use as a mean for prevention against *Salmonella* rods

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Salmonellosis is one of most common foodborne diseases. Every year more than 100 million people suffers from infections caused by *Salmonella* rods all around the world, and about 90 thousand cases ends with patient's death. *Salmonella* rods are often present in healthy chicken's gut and the main source of human infection are contaminated chicken meat and eggs. There are over 2500 of different *Salmonella enterica* serotypes and because of such variety the fight against this bacteria is found very difficult and often ineffective. Antibiotics that are commonly added to chicken feed as a mean for prevention against *Salmonella* rods are becoming less effective due to rapid development of bacterial resistance. The use of bacteriophages is seen as an alternative to antibiotics and a way to avoid further spread of antibiotic resistance.

In our work we have isolated bacteriophages infecting various *Salmonella enterica* serotypes, characterized them and tested their effectiveness in eradication of *Salmonella* rods in laboratory conditions. We would like to present the isolation procedures we used and how they affected our results. We have studied capsid morphology, susceptibility to various environmental conditions and growth parameters. We have also tested the ability of isolated phages to eradicate various serotypes of *Salmonella enterica* in laboratory conditions. We hope that our research will arouse the interest in phages as a potential mean of prevention against *Salmonella* outbreaks.

[ID: 139] Detection of deazapurine modifications in phage DNA using nanopore sequencing

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Bacteria, continually exposed to bacteriophages, have developed several defense mechanisms e.g. restriction-modification or CRISPR-Cas systems. On the other hand, bacteriophages have developed several strategies to evade these mechanisms.

Escherichia coli virulent siphophage CAjan, belonging to Seuratvirus genus and the closely related genus nonagvirus, contains a homolog of the tRNA-deazapurine modification synthesis pathway. The evidence is piling up, that CAjan and other similar bacteriophages use these deazapurine modifications in their DNA to evade bacterial restriction-modification systems. In order to investigate this novel DNA modification pathway in detail, we have used several methods including; direct phage plaque sequencing, CRISPR-Cas editing of phage genome and nanopore sequencing of viral DNA. Through generation of specific mutants within the deazapurine modification synthesis pathway followed by nanopore sequencing, we were able to 1) obtain a restriction-sensitive phenotype in the CAjan bacteriophage and 2) detect the modified bases using nanopore sequencing, thus providing new insights on the use of alternative bases by bacteriophages.

[ID: 360] New mechanism of host resistance to E. coli RB49-like phages

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In 2016, we isolated an unusual human uropathogenic E. coli strain UP1. We found that a RB49-like phage PF17.6, infecting E. coli strains K12 C600 (rough, no O-antigen), and F17 (new type of O-antigen structure) is also able to infect UP1 (O155) cells. We obtained mutant variants of UP1 resistant to PF17.6. The SDS-PAGE profiling of the LPS of these mutants revealed an altered LPS profile, with O-antigen molecules shortened in comparison with UP1 wt O-antigen. A NMR study of LPS of these mutants showed that the chemical unit structure of O-antigen was not affected. Genomic sequencing of wt and mutated UP1 indicated a frameshift in wzzB gene of a mutant. The complementation of WzzB protein from a plasmid fully restored the phenotype of UP1 mutants to wild type, including their phage sensitivity and LPS patterns. Thus no other mutations are involved in PF17.6 resistance.

To explain all the data we propose following hypothesis concerning the mechanism of RB49-like phage infection. The long tail fibers' terminal gp38 possesses a narrow extended bundle of receptor binding loops. We speculate that this structure is able to penetrate through O-antigen layer to bind an unknown receptor on the intimate OM surface. This is followed by the baseplate reorganization. The short tail fibers (gp12) then penetrate to their receptors through LPS layer by physical force of their deployment. This strategy explains the fact that T-even related phages often infect a wide range of hosts, despite the fact that their adhesins are likely not able to specifically recognize or degrade host O-polysaccharides. Our observation of an UP1 shortened LPS variant effectively preventing phage infection is a potentially new phage resistance mechanism that alters the spatial relations between phage and host outer membrane.

The work was supported by RSF grant #15-15-00134-P

[ID: 389] DNA helicases from the *Thermus thermophilus* MAT72 phage T72

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Helicases are ubiquitous molecular motor proteins that use energy derived from the ATP hydrolysis to unwind the complementary strands of nucleic acid duplex. As such, they are involved in many aspects of cellular nucleic acid metabolism. Deregulated expression of these proteins or their malfunction in eukaryotic cells have been linked to numerous diseases including cancer, developmental defects, and neurodegenerative diseases. On the other hand, prokaryotic and phage helicases found their potential application in PCR technology due to their ability to increase correct annealing of primers and efficient reduction of nonspecific products.

In the present study we focus on two DNA helicases from the *Thermus thermophilus* MAT72 phage T72, classified to superfamily 2 (SF2) by *in silico* analysis. Helicase I (487 aa, Mr=56,106, isoelectric point=5.8) belongs to DnaB-like helicases, while Helicase II (449 aa, Mr=51,453, isoelectric point=9.3) contains the DEAD domain. The genes coding for both helicases were analyzed to determine the differences in the codon usage pattern with respect to the genetic determinants of the host bacterium. Therefore, we calculated the values of the codon adaptation index (CAI) for both genes. Based on the assumption that genes with high CAI (near 1) belong to the class of highly expressed genes, we infer from CAI values (0.05 either for hel1 or hel2), their low rate of expression in *T. thermophilus*.

Recombinant enzymes were overproduced in *Escherichia coli* as a His-tagged fusion proteins and purified to electrophoretic homogeneity using a metal-affinity chromatography. We tested their unwinding activity using as substrates double-stranded DNAs: forked, 3' overhung, 5' overhung and blunt end. Both helicases exhibit the highest activity in case of forked substrate, and to lesser extent toward DNA with 3' and 5' overhangs. We found no activity toward the blunt end dsDNA.

[ID: 330] An increasing number of non-tailed, membrane-containing, ssDNA phages infecting Bacteroidetes detected

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Recently we described a new virus type, isolated from a boreal lake together with its Flavobacterium host. This was a tailless, membrane-containing ssDNA phage named FLiP, with a 9174 nt long circular genome. Structural analysis of the major capsid protein (MCP) revealed a double beta-barrel fold, linking FLiP e.g. to Pseudoalteromonas phage PM2 and archaeal virus STIV, despite of any genome sequence similarities. Here we present additional ssDNA phages with similar characteristics as FLiP; three marine phages infecting *Cellulophaga baltica* and a temperate phage induced from *Flavobacterium johnsoniae*. Common characteristics for these phages are a tailless capsid with a diameter ranging between ~ 60 to 73 nm containing a relatively small ssDNA genome (9-11 kb). We demonstrate that the phages also have similar structural protein profiles in Tricine-SDS-PAGE and modelling of the predicted MCP of *Cellulophaga* phage phi48:2 showed that it aligns with FLiP MCP. Sudan black staining indicated that the *C. baltica* and *F. johnsoniae* phages possess a lipid membrane, likely occurring inside the capsid like it has been shown for FLiP. In addition to these phage isolates, ten prophage candidates could be detected in the genomes of different bacteria belonging to the phylum Bacteroidetes. Hence, it is suggested that this phage type, with the potential of lysogenic infection cycles, is prevalent among members of the phylum Bacteroidetes. The phage isolates presented here provide the tools for exploring their potential impact to the ecology of this ubiquitous group of aquatic bacteria. In addition, this work not only advances the search and identification of viral sequences but also aids in deciphering the evolution of the virus world.

[ID: 136] Phages infecting bloom-forming cyanobacteria show high sequence and phenotypic variability

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Bloom-forming cyanobacteria are an increasing global phenomenon that negatively impact aquatic environments worldwide. These blooms are comprised of multicellular cyanobacteria that form filaments or colonies. Both phages and eukaryotic grazers play a significant role in the top-down control of cyanobacteria. While there are eukaryotic grazers that have the potential to temporarily prevent cyanobacterial blooms or control their biomass, the role of phages in cyanobacteria bloom dynamics is poorly understood. In order to expand our knowledge of phages infecting bloom-forming cyanobacteria, we have isolated 15 cyanophages from Lake Kinneret (Israel), using *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum* as hosts. The host range of these phages varied from a single host to broad host range. Some phages infected both filamentous and unicellular strains. Electron microscopy imaging of the phages show they belong to siphoviridae and podoviridae families and differ by their tail and head morphologies (even within a family). Sequence analysis of a subset of the phages suggest that these phages are Lambda-like, although the genes shared by the different phages and Lambda vary, and there is no nucleotide or amino acid similarity between the newly isolated strains. Moreover, although approximately 85% of their genes have unknown function, many genes have high similarity to genes found in prophages, suggesting these phages may be lysogenic under different conditions or in other hosts than the ones we analyzed. The phenotypic and genotypic diversity of these phages, that were all isolated at the same time from the same location, suggest that phages infecting bloom-forming cyanobacteria are extremely diverse. Additionally, the various host range patterns and the sequence similarity to prophages suggest that these phages have multifaceted interactions with their hosts.

[ID: 133] A gut bacterium evades lytic phage in the gastrointestinal tract

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Although bacteriophage have been used extensively against pathogenic bacteria in phage therapy, their interactions with resident members of the human microbiome and the mechanisms through which these bacteria evade phages in their natural environment remain poorly understood. We screened municipal wastewater for phage against a collection of 20 representative members of the human gut microbiome, and successfully isolated phage for 5 species that span 3 of the 4 dominant bacterial phyla in the gut: Bacteroidetes, Firmicutes, and Actinobacteria. Of note, this includes the anaerobic Actinobacterium *Eggerthella lenta*, a genetically intractable but highly prevalent member of the human gut. Restriction digest-based fingerprinting and genome sequencing of *E. lenta* phage isolates revealed two novel and nearly identical phages with 45-kb genomes, predicted to be Siphophage, possess direct terminal repeats, and lack genes for lysogeny. Interestingly, the oral delivery of phage to germ-free mice colonized with *E. lenta* did not have a significant impact on bacterial abundance in the distal gut, despite the ability of phage to effectively clear cultures in vitro, phage tolerance to acidity levels characteristic of the stomach, the application of a high dose of phage to mice, and successful transit of phage through the gastrointestinal tract. To address whether phage resistance could be arising rapidly in the gut, we generated resistant *E. lenta* mutants in vitro to determine the mutations able confer resistance, although it is possible that other factors may be contributing to the ability of *E. lenta* to evade phage in the gut. Though these results reaffirm the lesson that phage lysis in vitro may not translate in vivo, characterizing the factors that affect infection and lysis within the gut will aid in understanding how phage and bacteria interact and impact each other in the gut environment.

[ID: 353] Asymmetrical flow field-flow fractionation – a novel virus purification method to meet high purity standards of phage applications

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Viral preparations of high purity and integrity are required for virus research and for different applications such as phage therapy. Toxins derived from the host bacteria can cause uncontrolled inflammation reactions and chemical remnants from the purification process may compromise the safety of the final preparation. The traditional ultracentrifugation based purification methods may induce aggregation and lead to relatively low yields of infective viruses. In addition, traditional methods are laborious and time consuming. Asymmetrical flow field-flow fractionation (AF4) provides an attractive alternative method for large scale virus purification, being a rapid and gentle separation method preserving biological functionality.

We have optimized the AF4 conditions to be used for the purification of prokaryotic viruses with different morphologies, biochemical and –physical properties. Our results show that AF4 is well suited for virus purification as monitored by high recovery of infectious viruses and increased specific infectivity. Short analysis time (~50 min) and high sample loads enabled us to use AF4 for preparative scale purification of prokaryotic viruses. Furthermore, we show that AF4 allows rapid real-time analysis of progeny virus production in infected cells. The data presented here is from the purification of three different prokaryotic viruses: a tailed virus, (*Haloarcula vallismortis* tailed virus 1, HVTV1), an icosahedral virus with an inner lipid envelope (PRD1), and a spherical virus with outer lipid envelope ($\phi 6$).

Direct AF4 purification from cell lysates resulted in high infectious virus yields and specific infectivities of virus containing fractions, whereas host-derived contaminants were efficiently removed to other fractions. Consequently, AF4 provides a rapid, one-step method to produce virus material from crude cell lysates for biochemical and –physical characterization of prokaryotic viruses to be used in applications with strict purity and safety standards including potential therapeutic applications.

[ID: 47] Bacteriophages lytic to Georgian strains of *Ralstonia solanacearum* race 3 biovar 2.

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The phytopathogenic bacterium *Ralstonia solanacearum* has a very wide host range, affecting more than 200 plant species, including important crops such as potato, tomato, eggplant, pepper, banana, tobacco etc. Control of plant infections caused by *R. solanacearum* is a challenging issue. Phage preparations are considered as potential effective tool for prevention of infection spread in the environment and in the seed plant material. During 2012-2017 more than 60 *R. solanacearum* isolates were collected in Georgia from diseased potatoes and tomatoes, majority attributed to race 2 biovar 3. The presented work aimed at isolation and characterization of bacteriophages specific to Georgian strains of *R. solanacearum*. The ultimate goal of the study was to select phages for detection and subtyping of *R. solanacearum*, and for control of related plant infections. Processing of 34 primary lysates resulted in obtaining of 25 individual Rs phages. Initial grouping of phages was done based on negative colony morphology and lytic spectrum. Transmission electron microscopy revealed the prevalence of Myoviridae type virion morphology among Rs phages, although single phages were attributed to Podoviridae and Siphoviridae families. The Rs phages showed diverse host range and allowed to perform preliminary subtyping (phagetyping) of Georgian isolates of *R. solanacearum*. The phage - and antibiotic susceptibility profiles of *R. solanacearum* strains were compared and showed no correlation between these two characteristics. Ten bacteriophages with overlapping spectrum were characterized in more details, including DNA restriction profiles, lysis stability in liquid culture and viability in various conditions. The phage mixture, composed of 4 selected phages, demonstrated promising antibacterial activity in bioassays on potato discs and in small-scale challenge experiments in vivo.

[ID: 230] Klebsiella phages tail fibers engineering as step towards host range modification

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

In this study we analysed the genes encoding the Klebsiella phage attachment apparatus with a special attention to tail fibers/tail spikes possessing depolymerase domains. Two structurally different anchoring systems have been found. Tail fibers/tail spikes can be linked to the virion by an N-terminal anchoring domain or by linking to a separate adaptor protein that acts as an intermediate. The second step was the application of VersaTile Shuffling – a new high-throughput method for the construction of chimeric proteins - to generate chimera composed of specific depolymerase domains and different anchor domains or adaptor proteins. All chimeric proteins maintain their enzymatic activity and specificity for the capsular serotype. It was demonstrated that the VersaTile Shuffling method enables the construction of chimeric tail fibers/tail spikes with specific anchors or adaptors that can be attached to different phage virions. In this way, we are able to generate modified phages equipped with depolymerases with a different specificity. Chimeric depolymerases allow more accurate phage engineering. In future, modification of host range of well characterised viruses could make phage therapy more controlled, predictable and consequently safer, providing excellent tool for fighting multi-drug resistant bacteria.

[ID: 95] Functional analysis of *Streptococcus thermophilus* bacteriophage-encoded baseplate complexes

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Streptococcus thermophilus is one of the most valuable members of the Lactic Acid Bacteria (LAB) in the global dairy industry where it is primarily utilised in the production of various cheeses and yogurts. Strains of *S. thermophilus* are susceptible to bacteriophage predation that often results in delayed fermentation, poor quality products and economic losses. Despite this established threat, few studies have focused on the phage interactome of *S. thermophilus*. The importance of defining the molecular moieties which underpin *S. thermophilus* phage-host interactions has been further highlighted by the recent emergence of novel *S. thermophilus* phage groups. In the current study, structural proteins that are assumed to form part of the adhesion device - namely the distal tail protein (DIT), receptor binding protein (RBP) and baseplate protein (Bpp) - of selected *S. thermophilus* bacteriophages isolated from a global biodiversity study, were subject to a detailed in silico analysis. Structural domains were identified via HHpred and CDD analyses whilst functional annotations were assigned through collective Blast(p) and Pfam searches. Our findings illustrate that the constitutive proteins of the adhesion devices harbour multiple and variable carbohydrate binding domains, which further upholds the assertion that phages of *S. thermophilus* recognise a saccharidic cell surface receptor on the bacterial cell surface.

[ID: 135] Addressing knowledge gaps in the taxonomy of phages of Lactic acid bacteria : phage-host interactions in the underrepresented genera *Oenococcus*, *Weissella* and non-dairy *Leuconostoc* spp

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Phages infecting lactic acid bacteria have been the focus of significant research attention over the past three decades. Phage infection of dairy starter cultures remains the main cause of fermentation failures in the dairy industry. Owing to their economical importance, dairy phages became the most thoroughly sequenced phage group in the databases. In contrast, our knowledge of phage diversity in non-dairy food fermentations is still in its infancy. This is true for the "Leuconostocaceae" family, including the members of the *Leuconostoc*, *Oenococcus*, and *Weissella* genera. *Oenococcus oeni* drives the second fermentation of wine, called malolactic fermentation (MLF), which reduces acidity, stabilizes wines and protects it from spoilage. *Weissella cibaria*, *W. confusa*, *Leuconostoc mesenteroides* and *L. citreum* have been recently associated with different French sourdough bread, and also with different vegetable fermentations.

In this study, we have collected lytic and temperate phages from food samples and from indigenous strains isolated from sourdough breads and other vegetable fermentations. DNAs have been extracted from 250 phages/prophages. The genetic diversity of phages belonging to different taxonomic groups was further explored. Insights into phage-host interactions provide essential data for the current or future selection, biomass production and inoculation of commercial starters to be used in fermentations and other biotechnological applications.

[ID: 156] Bam35 P2 is a single-stranded DNA binding protein that stimulates processive DNA synthesis

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Tectiviridae are tail-less, membrane-containing viruses that includes two genera, alphatectivirus infecting Gram-negative bacteria, like PRD1, and betatectivirus, preying Gram-positive bacteria, exemplified by Bam35. Tectivirus genome is a linear dsDNA with ITRs and a terminal protein (TP) covalently attached at the DNA 5'-ends of DNA as consequence of the protein-primed DNA replication initiation mechanism. We have recently reported that Bam35 full-length genome replication can be obtained in vitro with only two proteins, the DNA polymerase and the TP. This replication mechanism has been also characterized in other viruses, including PRD1 and other distant viruses, like Φ 29 or adenovirus. In all those models, a number of accessory DNA binding proteins have been shown to increase the efficiency of the genome replication in vitro and/or in vivo. To further characterize the Bam35 genome replication mechanism, we have analyzed the biochemical properties of Bam35 protein P2, which contains a predicted OB-fold structural domain, characteristic of single-stranded DNA binding proteins (SSBs). Electrophoretic mobility shift assays showed that P2 binds to single-stranded DNA (ssDNA) but binding to dsDNA and RNA was negligible. Stable P2-ssDNA complexes can be detected with ssDNA oligonucleotides of 33 nt, and the binding capacity improves as we increase the size of ssDNA fragments. Although P2 is a monomer in solution, cross-linking experiments showed that P2 can form large oligomers in the presence of ssDNA, with a minimal binding site of 3-5 nt for each monomer. Altogether, our results indicate that P2 binds with high efficiency to ssDNA cooperatively. Moreover, P2 does not improve early steps of TP-primed replication in vitro, but it stimulates processive DNA synthesis by Bam35 DNA polymerase, increasing DNA synthesis velocity as well as reaction yield. Finally, to further study the molecular basis of ssDNA binding of P2, several protein variants with modifications in conserved residues allowed us to disclose different requirements for the interaction with DNA and for protein multimerization.

[ID: 68] A reverse genetic system for the *Pseudomonas aeruginosa* leviphage, PP7

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PP7 is a leviphage with single-stranded RNA genome that can infect *Pseudomonas aeruginosa* PAO1. Unlike other RNA viruses, however, not much comprehensive reverse genetic approach has been performed for PP7 due to the lack of infectious cDNA clones. Here we have created the reverse genetic system for PP7 using two full-length PP7 cDNA clones (PP7-S and PP7-O) linked to the T7 promoter: PP7-S was chemically synthesized by using the sequence deposited in the database; PP7-O was generated by RT-PCR using the isolated PP7 RNA. PP7-O and PP7-S displayed 19 nucleotide differences. Infectious PP7 virions were produced not from PP7-S, but from PP7-O, whereas the comparable amount of RNA-containing viral particles was observed for PP7-S. Reverse genetic analyses of the 19 different nucleotides revealed that 2 nucleotide differences for PP7-S (G1125A and C1157G) were critical in the infectivity in PP7, both of which resides in the maturation protein (R349Q and S360C). Further point mutation and complementation analyses showed that the two nucleotide differences of PP7-S resulted in the dysfunction of the maturation protein. These results substantiate the roles of maturation protein in phage infectivity and, more importantly, provide a lesson that the viral RNA genome sequencing needs functional verification possibly by a reverse genetic system.

[ID: 223] Can a phage application reduce E. coli in a chicken shed?

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Escherichia coli is a major pathogen in poultry farming, causing significant economic losses. Standard therapies include the use of colistin and enrofloxacin, which already selected for bacterial strains resistant to antimicrobial therapy. Consequently, farmers have to use higher concentrations of antibiotics or switch to alternative antibiotics, with a risk to select for multidrug-resistant bacteria.

To interrupt this vicious circle a phage therapy approach was tested. Bacteriophages showing a broad range of activity against 48 ESBL-E. coli isolates from commercial poultry farms and slaughterhouses were identified and characterized for their ability to inhibit bacterial growth.

In an in vivo trial with 16 broiler chicken per group, in a controlled facility of the University of Veterinary Medicine Hannover, the application of a four-phage cocktail to freshly hatched chicken did not result in the eradication of E. coli from the chicken intestine. The obtained data suggested a delayed colonization of the intestine by E. coli in treated birds. Such a delayed establishment of the E. coli population might alleviate problems associated with the rapid growth of E. coli in freshly hatched chicken.

As a next step a seven-phage cocktail was tested in a commercial poultry farm with 33.000 chicken. The delayed colonization observed in the small scale trial could not be reproduced. Detailed analyses revealed that the E. coli population in this shed was not very sensitive to the applied cocktail (26% of 34 E. coli isolates were sensitive). A new cocktail, which covers 74% of 46 field isolates was composed and will be tested in a follow-up study. However, the low coverage observed in the commercial setting raises the question just how diverse the E. coli populations in chicken could be and indicates that stable specific cocktails might be necessary to cover the wide diversity.

[ID: 276] Diversity of bacteriophages against clinical strains of Klebsiella

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The widespread growth of antibiotic-resistant bacterial strains is becoming a serious problem in the World. Multi-drug resistant beta-lactamase producing bacteria - *Klebsiella pneumoniae* is an important causative agent of different diseases, including hospital and healthcare associated infections. These pathogens are resistant to many antibiotics, including third-generation cephalosporins. The type of lactamase enzymes and severity of infections are important considerations to select antimicrobial therapy. Bacteriophage therapy is considered as an alternative way to prevent and treat bacterial infections, including those caused by *Klebsiella* spp.

In this study we have collected 67 bacterial strains of *Klebsiella pneumoniae*, 17 *Klebsiella oxytoca* and 4 *Klebsiella terrigena* from the clinical samples isolated in Georgia and outside of the country. 53 Clinical strains of *Klebsiella pneumoniae* were received from different countries (Singapore, China, Switzerland and France). Ten phages specific to the *Klebsiella* strains were isolated from environment. Electron microscopic studies showed that the phages belong to Myoviridae, Siphoviridae and Podoviridae family. Phages were also different according to host specificity and DNA restriction. Three phages from Myoviridae family revealed wider spectrum of lytic activity than the others - from 41% up to 65% strains were sensible to these phages. Phages did not show species-specificity- as they lysed bacterial strains of *K. oxytoca* and *K. terrigena*, as well as *K. pneumoniae*. The next step is detailed characterization of specific phages.

[ID: 402] Metastable (pseudolysogenic) associations of virulent coliphages and their hosts

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Bacteriophages can be considered to be ultimate survivors in natural environments, successfully coping with long periods of survival when their host bacteria are in scarce supply. Temperate phages achieve this by integrating into bacterial genomes, but the mechanisms of virulent phages long term persistence are largely unknown.

We characterized the metastable associations that we termed pseudolysogenic associations (PA) of three coliphages: a siphovirus 9g (Kulikov et al. 2014) and N4-related podoviruses G7C (Kulikov et al. 2012) and St11Ph5 (Golomidova et al. 2018) with their cognate host strains of *E. coli*.

In all the cases we were able to obtain long lasting cultures producing the phage active against the parental bacterial strain by 20 passages and longer. In all the cases we observed in situ evolution that gave rise to bacterial strains with highly reduced sensitivity to the original phage and to the phage strains with reduced activity against the parental host but infecting more effectively the evolved hosts. The PFU/CFU ratio in PAs were higher in 6-10 passages and gradually decreased in most of the PAs in later passages that coincided with increase of bacterial clones resistant to all types of the phage present in PA. Eventually this increasing resistance should lead to complete cure of the bacterial population from phage highlighting metastable nature of PA.

However the subcloning of PAs unexpectedly revealed that many of the cells (from 5% in G7C-*E. coli* 4s to almost 100% in 9g-*E. coli* C600) present in them form colonies producing phage. This result indicates the existence of a mechanism of tight association of the individual cells with virulent coliphage that may ensure proliferation of PA.

The work supported by RSF grant #15-15-00134-P

[ID: 409] Meromictic lake Trekhtsvetnoe as a model of global phage-bacteria interactions

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The bacterial communities in free living ecosystems are generally very complex and diverse, with every component comprising only for a small fraction of total microbial population.

Our study was focused on the microbial community at the sharp biogeochemical barrier developed in the stratified water column of the meromictic lake separating from the White sea. We analysed in substantial detail the matter and energy flow in Trekhtsvetnoe Lake located in Kandalaksha bay near the White Sea Biological Station of MSU.

This layer is dominated by green sulfur bacteria that use energy of the light to oxidise H₂S and produce organic matter in anoxygenic photosynthesis process. The total microbial cell count in this layer reaches 2x10⁸ cells/ml. Metagenomic analysis revealed that 70-95% of this biomass is represented by *Chlorobium phaeovibrioides*. Interestingly genetic diversity of the dominant strain population in Trekhtsvetnoe Lake is extremely low and close to that of the clonal bacterial populations.

Among the metagenomic contigs we also detected four highly covered contigs apparently obelonging to a single myovirus genome. The search for CRISPR-Cas systems in *Chlorobium* contigs revealed two CRISPR arrays containing multiple spacers that targeted our phage contigs.

We were able to visualize an abundant myovirus by TEM examination of the water samples. Interestingly the viruse like particles (VLP) observed in biofilter layer water samples taken with the intervals as small as 2.5 cm were significantly different suggesting the ultra-fine stratification within this narrow horizon.

Despite high VLP concentration associated with high density homogenous host population the biofilter layer is very stable over many years. This suggests that some mechanisms limiting lytic phage growth should exist in this habitat. So the comparative biogeography of the viruses and bacteria between this closely located objects may reveal the mechanisms of the virus – bacteria coexistence in the communities of the biogeochemical barriers.

[ID: 148] In silico identification of prophages from *Streptococcus agalactiae* genome sequences

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Streptococcus agalactiae (Group B streptococci, GBS) is an opportunistic pathogen colonizing gastrointestinal and urogenital tract of healthy adults. These gram- positive β - haemolytic bacteria are leading cause of serious neonatal infections such as pneumonia, sepsis and meningitis. Newborn infections occur by pathogen transmission from asymptomatic mother. GBS also cause invasive diseases in adults, particularly in older and immunocompromised individuals. Several virulence factors are critical to disease development, many of them are encoded on mobile genetic elements. Among others, integrated prophages could be beneficial for bacterial survival during colonization and infection. In the present study we applied in silico method for identification and characterization of prophages from genomes of five *S. agalactiae* clinical isolates. Analysed strains belonged to three serotypes (III, V, VII) and were assigned into four different MLST sequence types (ST-1, ST-17, ST-19 and ST- 130). Genome sequences were generated by whole genome sequencing using Illumina platform. The sequences were assembled with program CLC Bio (Qiagen) and annotated with RAST. Prophage regions were identified in genomes by Phast software combined with manual analysis of annotated sequences. We found out that each strain contained 3 to 4 prophages with the size range 15 - 41 kbp. By BLAST comparison no significant homology (> 70%) with known phages was found in any prophage regions. According comparative analysis prophages were divided into nine groups (A-I). Six groups contained unique prophages while three groups possessed multiple phages present in different strains. Since no lytic bacteriophage infecting *S. agalactiae* is currently known, temperate phages are the only source of GBS phages. Induced prophages or prophage proteins with antimicrobial activity are thought as potential antimicrobial agents for treatment of GBS vaginal carriage.

[ID: 374] Lytic phage and sublethally injured Salmonella Typhimurium: A new challenge for phages in food safety applications?

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Intact host membrane receptors are crucial for phage infection. High hydrostatic pressure (HHP) is a non-thermal food processing technology that inactivates bacterial cells by damaging the bacterial membrane. However, depending on the properties of the food matrix and intensity of pressure applied, HHP can lead to the production of sublethally injured cells. Hence, the objective of this study is to investigate the use of bacteriophages as a biocontrol agent to control sublethally injured cells of Salmonella Typhimurium generated during HPP treatment. *S. Typhimurium* (109 CFU/mL) were subjected to HHP treatment at 400 MPa for 3 minutes to produce sublethally injured cells. Then a Siphovirus (Sal-11) or a Myovirus (Sal-16) phage suspension (109 PFU/mL) was added before incubation at 22°C for up to 5 days. Changes in phage titers and healthy and sublethally injured *S. Typhimurium* populations were enumerated at pre-determined time intervals. Two hours post-HHP treatment, approximately 90% of the sublethally injured cells recovered and total recovery was observed within 24 hours. Interestingly, Sal-11 and Sal-16 phages were unable to infect the recovered cells, while the phage-treated controls (no HPP treatment) were below the detection limit. Continued incubation showed that by day 2, Sal-16 phage titer increased by 1.5 log₁₀ with a 0.5 log₁₀ reduction in bacterial population that had recovered after HPP treatment. By day 5, more than 1 log₁₀ reduction in the bacterial count was observed with a 2 log₁₀ increase in the phage titre. The recovered cells were resistant to Sal-11 phage for up to 5 days. These results suggest that HPP-induced cellular injuries leads to a loss of phage susceptibility of *S. Typhimurium* and despite recovery of the sublethally injured cells, they remain resistant to phage infection. However, based on the nature of phage receptors, phage infection can be observed during a prolonged incubation. Further investigations are needed to understand the full recovery and phage susceptibility of sublethally injured cells.

[ID: 181] Evaluation of phage cocktail efficacy against *V. parahaemolyticus* infection during temperature and salinity variation

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Aquaculture industry has severe economic losses by bacterial infections. Antibiotic therapy was recently prohibited due to the proliferation of multidrug-resistant bacteria and antibiotics residues in the food products. Phage therapy is an alternative treatment for bacterial infectious diseases based on phage ability to recognize and reduce specific pathogen populations. However, has been previously observed that environmental parameters as temperature and salinity could interfere the effectiveness of phage therapy. In this study, we evaluated the effectiveness of phage therapy under different values of salinity (20, 30, and 40 ‰) and temperature (25, 30, and 35 °C) during a brine shrimp challenge test against *V. parahaemolyticus* (VP). Under our experimental conditions, temperature and salinity have not a significant effect in phage efficacy ($p > 0.05$). In all cases, phage therapy was successful to mitigate VP infection with at least 80 % survival, whereas controls reached only <55 % survival. In the controls, the highest mortality occurred with the highest temperature and lowest salinity (85 % of mortality). Phage cocktail reduced VP counts to 2-5 fold, whereas an increase in 4-fold in VP counts was recorded in non-treated samples. We did not record any effect in phage replication due to temperature and salinity, except in the lowest salinity at 35 °C with a one-fold reduction. We conclude that phage therapy efficacy could be stable during temperature or salinity changes. These results are significant for a future application of phages in aquacultures conditions where environmental parameters change between the production seasons..

[ID: 356] Mining the genomes of *Pseudomonas aeruginosa* in search for the link between genomics and phage infectivity

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Pseudomonas aeruginosa has a complex population structure where given pairs of strains can differ by hundreds of genes, including entire genetic systems (DISARM, CRISPR-Cas, RM, BREX, etc). Such discrepancies in accessory genome content can be expected to form the bedrock of differences in phage infectivity revealed by host-range assays, but we currently miss much of the link between the specific genome profile of a given strain, and which phages can infect it.

Although genetic systems linked to phage infectivity can be identified and functionally annotated, the annotation of prophage elements – routinely found in *P. aeruginosa* - proves to be tedious. However, prophage presence has been linked to transformative host phenotypes including modified virulence and antibiotics resistance, and they are crucial determinants of infectivity via superinfection exclusion.

Identifying prophages, tallying anti-phage genetic systems, and establishing infectivity patterns is a challenge appropriately met by combining lab techniques and computational approaches:

- 1) In silico, we leverage the abundant genomic data available for *P. aeruginosa* in search for (potentially domesticated) prophages, determinants of phage resistance, and establish core/accessory genome population structures, in the search for clustering patterns.
- 2) In vitro, we induce prophages from a collection of characterized clinical strains and identify them with nanopore-based, single-plaque sequencing. Separately, we cure prophages and establish new lysogens to verify changes in host-range.

Statistically linking genomic data to phage infectivity helps to reveal associations which can be used to build predictive models of infection phenotypes. Such algorithmic approaches would be useful in therapeutic settings when confronted with the task of selecting potent bacteriophages from a large library in order to clear an infection caused by a given strain.

[ID: 349] Comment to the taxonomy of Sk1virus. Suggestion of reconsidering the “936 phage group” as the 936species without a genus status

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DNA-DNA hybridization (DDH) has been used for prokaryote and phage classification at species level over decades. However, with an increasing amount of genomic information appeared, DDH may soon be an outdated taxonomic method. It may be replaced by the average nucleotide identity (ANIb). So far, there is a suggestion that classify the prokaryotes shared >95% ANIb as belonging to the same species. To evaluate if similar a suggestion is useful for phages, we collected 60 fully sequenced phages genomes (11 from GenBank and 49 newly sequenced) from the lactococcal Sk1virus genus (former known as the 936 phage group or the 936 phage species), and analyzed the effect of using >95% ANIb for species differentiation based on whole genome sequence and based on core genome sequence. The results showed that, if a simple >95 % ANIb was used, 35 new species of SK1virus would be generated. Most of core genes from SK1virus phages were located in the conserved late-expressed region and a small part were located in the early- and middle-expressed regions. Using a >95% ANI for the core genes for designation of new species also resulted in 35 new species. So both methods generated on average a new species almost for every second new sequence. The highest correlation was between where the phage was isolated. This will mean using the proposed subdivision ANIb will generate an artificial taxonomy below genus level, that will not be used. We therefore suggest that no species should be assigned to the SK1virus genus based on whole genome or whole core genome using >95 % ANIb, or preferably that the 936 group should be assigned to the 936 species and should not have genus status. As it is right now, the taxonomy for the SK1virus phages does not make sense bellow genus level.

[ID: 263] Characterization of bacteriophages with lytic activity against multidrug-resistant *Staphylococcus aureus*

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Staphylococcus aureus is associated with a number of diseases in humans, ranging from superficial to invasive infections such as pneumonia and sepsis. The increase in the prevalence of multidrug-resistant *S. aureus* (MDRSA) isolates creates a need for research into alternative methods of treatment. Much hope is placed in bacteriophages that have been shown to be an effective therapy for antibiotic-resistant infections.

The aim of this study was to characterize four phages isolated from sewage which show lytic activity against clinical isolates of multi-drug resistant *Staphylococcus aureus*. Morphology and biological properties, including plaque morphology, host range, adsorption rate, latent time, and phage burst size were studied for all four phages.

Three of the phages exhibited high rates of lytic potency on 60-70% of *S. aureus* clinical isolates and suppressed planktonic cells of selected MDRSA strains with multiplicities of infection (MOIs) ranging from 0,01 to 1 for 4 h without apparent regrowth of bacterial populations. One-step growth experiments indicated that one of tested phages has a short latent period, which takes 20 min, and large burst size (about 120 pfu×ml⁻¹ per infected cell) suggesting effective host infection and lytic activity.

[1] Chang Y., Shin H., Lee J.H., Park C.J., Paik S.Y., Ryu S.: Isolation and genome characterization of the virulent *Staphylococcus aureus* bacteriophage SA97. *Viruses*, 2015; 7: 5225–5242

[2] Wang Z., Zheng P., Ji W., Fu Q., Wang H., Yan Y., Sun J.: SLPW: A virulent bacteriophage targeting methicillin-resistant *staphylococcus aureus* in vitro and in vivo. *Front. Microbiol.*, 2016; 7: 1–10

This work was financially supported by Ministry of Science and Higher Education ST-02-0543/07/289 project of Medical University of Gdansk.

[ID: 280] Phage therapy in agriculture

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Modern agriculture is expected to face an increasing global food demand while concurrently living up to higher standards with regard to sustainability. Therefore, control of crop pathogens requires new, green and feasible alternatives to current methods. To strengthen the development of biological control in agriculture, we have compiled an extensive biobank of more than 180 phages targeting important plant pathogens: *Xanthomonas campestris*, *Erwinia amylovora*, *Pseudomonas syringae* and the soft rot enterobacteriaceae (*Dickeya* sp. & *Pectobacterium* sp.). Furthermore, to evaluate the efficacy of some of the isolated phages in an agricultural context, we set up an in vivo assay using soft rot enterobacteriaceae (SRE) as a model pathogen of potatoes.

Potatoes are susceptible to several bacterial diseases, with SRE playing a large role. There are currently no effective ways of combating SRE, therefore we sought to develop an approach, which could easily be incorporated into the potato production pipeline. To this end, the more than 80 of the phages in our biobank infecting SRE were thoroughly characterized using next-generation sequencing and relevant biological attributes including growth dynamics, host range and the presence of virulence genes. A subset of these phages was selected and included in a phage cocktail, which was applied in a proof-of-principle experiment, to treat soft rot in potatoes under in vivo conditions mimicking potato storage conditions.

We show that newly isolated phages show potential as biological control agents. Using an in vivo approach, we aim to examine the effectiveness of phage therapy under conditions relevant for agricultural use, while broadening our understanding of phage-bacteria interactions for the betterment of phage therapy in general.

[ID: 393] Construction of a panel of T4 phage variants with altered protein composition of capsid head and the role of individual head proteins in phage survival in acidic pH

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Objectives: Molecular engineering offers useful tools to generate bacteriophage mutants. In this study, a panel of T4 phage mutants lacking one or more head proteins was created and compared regarding sensitivity to highly acidic pH.

Methods: Site-directed mutagenesis of T4 phage and T4 Δ Hoc was applied to construct gp24 bypass mutants and Soc-deficient phages. Nonsense mutations were introduced in a PCR reaction with mutagenizing primers and resulting products were cloned into plasmid vectors used to transform *Escherichia coli* cells. Recombination cultures followed by selection of mutants from the overall phage progeny based on either: 1) PCR, or 2) microbiological plating were then performed. Mutations introduced to phage genomes were confirmed by direct sequencing, additionally bypass mutations in the gene 23 were identified in case of Δ 24 mutants. Protein composition of phage heads was verified using ELISA immunoassay with protein-specific sera. Phage sensitivity to highly acidic pH was tested in vitro. Phages (10e6 pfu/ml) were incubated in standard (pH 7.2) and acidified (pH 3) saline in 37°C for 30 minutes. Number of viable phage particles was determined using serial dilutions and spot method.

Results: In case of all gp24-deficient mutants, viable phage titers after exposition to pH 3 were reduced to zero, regardless of the absence or presence of the decorative proteins Hoc and Soc. This effect was not observed for single Δ Soc or Δ Hoc mutants, nor Δ Hoc Δ Soc double-mutant. Moreover, primary results of in vivo studies in mice indicated impaired survival of gp24-deficient phages in the stomach when compared to wild type T4 and T4 Δ Hoc.

Conclusions: Presented data strongly suggest that although Soc protein has been previously indicated as a key component facilitating T4 phage survival in extreme pH, when it comes to highly acidic pH its gp24 rather than Soc that is vitally important for T4 phage viability.

Acknowledgements: This work was supported by the National Science Centre in Poland grants: UMO-2017/25/N/NZ6/02372 and UMO-2012/05/E/NZ6/03314.

[ID: 347] Phage-encoded capsule depolymerases mediate innate host protection against *Klebsiella pneumoniae*

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The idea of using antivirulence compounds, including phage-encoded enzymes, therapeutically against bacterial infections, has recently gained traction in response to the emergence of multi-drug resistant pathogens. Because the polysaccharide capsule (CPS) is the most important virulence factor of *Klebsiella pneumoniae*, CPS depolymerase treatment in combination with the innate immunity seems to be an efficient mechanism to defend the host from *K. pneumoniae* infection. In this study, we identified and characterized two proteins encoded by the dual-host *Klebsiella* phage KP32 (Podoviridae, KP32virus), i.e. the tail spike and a hypothetical protein, that are able to recognize and digest the capsules of strains representing K3 and K21 serotypes. Based on serum bactericidal assay, we have found that following depolymerase-induced CPS degradation, the survival rate of bacteria was profoundly decreased compared to untreated cells in a largely serotype-dependent manner. Unlike the observed dependence on serotypes in terms of the ability to resist complement after depolymerase treatment, such dependence was not seen for the susceptibility to phagocytosis. Flow cytometry analysis revealed that UV-killed depolymerase-treated bacteria of both serotypes were taken up by monocytes more effectively than unmodified parent bacteria. Likewise, the number of bacterial cells killed by human monocyte-derived macrophages was significantly higher after removing of their capsule layer by these enzymes. These findings suggest that phage-derived enzymes can defeat the resistance of encapsulated bacteria to complement and phagocytic killing, likely through uncovering surface-exposed activators (porins and rough LPS) for complement components and ligands for phagocytic cell attachment. Thus, CPS depolymerases can be efficient antivirulence agents. Considering the mode of action and the efficacy of both depolymerases as adjunct therapeutic approach for the control of *K. pneumoniae* infections, biochemical and biophysical properties of both proteins were also determined.

[ID: 346] Demonstration of therapeutic potential of Samonella bacteriophages

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Phage therapy is one of the promising "new" methods for treatment of infectious diseases nowadays attracting increased attention worldwide. Salmonellosis is one of the leading diarrheal diseases. The severity of the disease depends on host defense, and on Salmonella serotype. Recently, a number of resistant serotypes appeared in the food chain, often leading to fatal consequences. Severe poisoning of 16 people by chicken burgers infected with S.Agona serotypes was registered in February 2018 in Georgia in one of the supermarkets.

The aim of the work to test susceptibility of the outbreak related strains against 13 single Salmonella specific bacteriophage clones and two batches of the commercial preparation Intesti-bacteriophage.

The strains were identified as Salmonella enterica subsp. enterica serovar Agona (O:4,O:12; H1:f,g,s; H2:1,2) according to Kauffman-White classification. All clinical S. Agona strains showed resistance to Ampicillin, two strains # 55 and # 46 showed Intermediate resistance to Nalidixic acid as well. Strain #64 showed a multidrug-resistance phenotype against Ampicillin, Tetracycline, and Nalidixic acid, and intermediate resistance to Ciprofloxacin and Azithromycin. The results of streak test demonstrated the presence of 7 phage susceptibility groups. Two pairs of isolates, namely # 45 and # 48 and # 55 and # 69 showed identical susceptibility profiles. The Isolates # 50 and # 64 appeared to be resistant to all phage clones and preparations. The phage patters of the isolates # 47, # 56 and # 57 showed individual patterns and significantly differed from other paired groups.

The broadest lytic spectrum and highest lytic activity were showed by phages: GE_vB_CT (Phage Preparation), followed by phages GE_vB_HIL (Siphoviridae), GE_vB_B3 (Myoviridae) and GE_vB_M5 (Siphoviridae) showing similar strength and GE_vB_INT (Commercial Phage Preparation), respectively.

This is a remarkable observation demonstrating high therapeutic potential of the commercial Intesti-bacteriophage that is ready for use in case of similar food outbreaks.

[ID: 7] Electrospinning bacteriophages in nanofibers for wound dressing applications

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Multi-drug resistant (MDR) bacterial species such as methicillin-resistant *Staphylococcus aureus* (MRSA) pose a serious healthcare challenge in treating wound infections. In the recent years, phages have shown promise as alternative form of therapy due to their ability to infect and lyse bacterial cells. However, delivery of phages to the site of infection can be a challenge due to influence from external factors on phage viability. Also, delivery of the correct dosage form is an important aspect when tailoring therapy for specific bacterial infections. Therefore, in wound infections it is important to continuously release phage at the site of infection to allow diffusive interactions with the infected tissue as apposed one sprayed dose which can be prone to drying and washing away.

Here, we demonstrate the encapsulation of phage K (a model *staphylococcus aureus* phage) in nano and micro fibres using an emulsion electrospinning process where phage K was emulsified in an organic solution of Eudragit S100 (commercially available, pH responsive polymer). Defect-free, continuous fibres were fabricated, and their morphology was optimised to facilitate the encapsulation of phage K, serving as a proof-of-concept for novel wound dressing applications. SEM analysis revealed that fibre diameters and diameter distributions were altered by changes to the applied voltage and infusion rate. Increasing the concentration of either the organic phase or aqueous phase, which subsequently increased the viscosity and conductivity of the emulsions had the most influence on fibre diameters, ranging from 100-1000 μm . Phage release revealed that phage viability dropped from 109 PFU ml⁻¹ to 107 PFU g⁻¹ for ES100 fibres fabricated at the most ideal optimised conditions of 1 ml hr⁻¹ infusion rate, 15 kV applied voltage and 10% w/v ES100 concentration. Release kinetics revealed, phage was completely released from the fibres over a period of 4 hours.

[ID: 167] The activity of type I-E, I-F and III-A CRISPR-Cas systems during phage infection

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Bacteria can harbour multiple defence systems within their genome to counteract phage infection. Among them, CRISPR-Cas systems constitute the adaptive immune response of bacteria. During phage infection, bacteria acquire new spacers from the phage DNA into CRISPR arrays. In the expression phase, crRNAs are transcribed and, in conjoint action with the Cas protein complex, recognise and degrade the targeted DNA, preventing infection. Bacteria can have multiple CRISPR-Cas systems; such is the case of *Serratia* sp. ATCC 39006 that contains type I-E, I-F and III-A CRISPR-Cas systems. Little is known about how different systems are regulated and whether they present a coordinated or independent response upon phage infection. In this study, we aim to understand the importance of maintaining different functional CRISPR-Cas systems and their role in arresting phage propagation. To achieve this, three new *Serratia* ATCC sp. 39006 phages were isolated, sequenced and used to evaluate the activity of the different CRISPR-Cas systems. Interestingly, a variable range of infection resistance against the different phages was observed. This study will provide further insight into phage interaction with CRISPR-Cas systems and will help to understand the changes undergone in the cell during phage infection.

[ID: 49] How *Lactococcus lactis* bacteriophages recognize their host: a study on a newly isolated member of the rare species 1706

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Lactococcus lactis is a Gram-positive bacterium widely used as starter culture for the production of different fermented foods, especially a big variety of cheeses. Infection of these cultures by bacteriophages is one of the main causes of fermentation failure.

Ten different groups of *L. lactis* bacteriophages have been identified. Members of the groups C2, 936 and P335 are the most frequently isolated in dairy environments, and thus they have been extensively studied in the past two decades.

Phages belonging to rarer groups can also be found in dairy plants and contribute to fermentation failures. However, very little is known about their life cycle or host range. A better understanding of how these phages recognize their host and proliferate in dairy environments would widen our knowledge about rare lactococcal phages, and help design better starter culture rotation programs.

We isolated a new phage, named 26MP, belonging to the rare group 1706 (Garneau, Tremblay, Moineau 2008). As already shown for the reference phage of this group, it is able to infect the host strains of different lactococcal phage species. Therefore we aimed to get a better understanding on the type of receptor it recognizes.

We isolated four phage resistant mutants and analyzed their genomes and behavior towards the phage. In vivo assays and microscopy analysis confirmed that 26MP shows a considerably reduced adsorption to each of the mutants. In silico analysis of their genome revealed that they all harbor a different mutation on a specific gene of the cell wall polysaccharide (CWP) cluster. It is known that sugars composing the CWP, also called polysaccharide pellicle, are often recognized by many of the well characterized lactococcal phages. These findings strongly suggest that the rare phage 26MP also recognizes, at least in the first step of infection, a specific part of this sugar receptor.

[ID: 159] Viruses and Technology 1.0

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Increasing number of biological discoveries, many related to viruses, is being transformed into new technology, broadly defined as application of scientific knowledge for practical purposes. Most breakthroughs take place in settings outside the viewfield of biology undergraduates, thus the innovators of the future have limited opportunities to develop an invention-driven mindset. Timely acquisition of skills and confidence for primary literature analysis is a key asset in the toolset of budding scientists. I have designed a writing-intensive elective course *Viruses and Technology* aiming to equip students with skills for primary literature analysis and communication in the context of an independent research project. Enrolled students (n=17) self-associated in teams of 3-5 members, according to their interests to explore topics in the broad fields of Phage Therapy, Viral Nanotechnology, CRISPR/Cas 9 Gene Editing, Virus-driven Biocontrol, and Phage Display. By design, the course is built on the hypothesis that student interest in technology is likely to facilitate the learning process by softening the steep learning curve and frustration frequently associated with developing initial proficiency in primary literature reading. Students received instructions on key concepts in virology, were guided through reading primary articles with increasing level of complexity, and worked on a topic of their choice compiling a team introduction and an individual case study focusing on a single technological development. At the end of the semester, students assembled a project portfolio and reflected on their experience identifying perceived achievements and challenges. The majority of students reported that they were proud of their work, were most challenged by the expected level of detail, and were most frustrated by the process of integrating complex information from various sources. Technology-focused undergraduate instruction can serve as an effective first step in the process of guiding future biologists on the path of innovation.

[ID: 243] Phage resistance of sessile *Pseudomonas aeruginosa* after biofilm treatment

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Phage therapy is a promising alternative method for treatment of bacterial infections showing many advantages over traditional antibiotic therapy. Phage therapy exhibits bacterial strain specificity and lacks the negative effects on patient's microbiota. However, phage-resistant clones readily emerge upon treatment. Changes in phage-resistant mutants affect key bacterial properties associated with pathogenicity (i.e. biofilm formation, motility and other virulence factors). Interestingly, these changes may be associated with reduced growth and an overall decrease in bacterial fitness including higher susceptibility to immune system clearance.

The purpose of the study was to examine the extent of the modifications of *P. aeruginosa* after controlled phage infections of biofilm grown bacteria with preparations consisting of one, two or three phages. Three well-characterized were selected: phiKZ (fimbriae-dependent, giant Myoviridae); LUZ7 (LPS-dependent, Podoviridae) and KTN6 (LPS-dependent, Myoviridae). The phage typing in a two-layer assay was performed on isolated bacterial stains. Selected mutants were tested for motility, the amount of pyoverdine, pyocyanin, elastase and biofilm production, LPS patterns, sensitivity to antibiotics, and serum complement susceptibility. The virulence of phage-resistant mutants were also verified *in vivo* using the *Galleria mellonella* larvae infection model. As the control, sessile cells isolated from untreated biofilm were used.

Isolated phage-resistant mutants often exhibited cross-resistance to phages recognizing the same extracellular structures. They displayed LPS O-chain truncation and downregulation of type IV fimbriae expression (fimbriae-dependent phages). There was a correlation between increased serum susceptibility of strains and LPS truncation. These changes also affected colony morphology, and amount of biofilm production. Increased susceptibility to antibiotics and significant decrease in pathogenicity tested *in vivo* were particularly noticeable for phage-resistant strains obtained after cocktails application. These results indicate that mutants arising as a result of phage therapy display multiple defects with a global detrimental effect on virulence, presumably allowing their clearance by the host immune defenses.

[ID: 202] Assessment of phage SPP1 dependence on host molecular chaperonins

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SPP1 is a lytic bacteriophage infecting the bacterium *Bacillus subtilis*. SPP1 is a dsDNA virus that belongs to the Siphoviridae family and acts as a model system for viruses of Gram-positive bacteria.

During its infection cycle, SPP1 hijacks massively the host's resources, synthesizing 9 Mbp DNA and 150 000 polypeptide chains to produce 200 virions in 30 minutes, imposing a substantial challenge to the cell. Under normal conditions, molecular chaperones play an essential role in the folding of nascent chain polypeptides, refolding or degradation of misfolded and aggregated proteins, and other house-keeping and stress-related functions. GroEL is known to play a role in viral infection mediating the folding of capsid proteins during morphogenesis of several phages, namely λ (1,) T4 (2) and RB49 (3).

In this work we used a *B. subtilis* isogenic groEL knock-down mutant strain to show the dependence of SPP1 on host GroEL. Fluorescence microscopy showed that phage DNA replication was not severely impacted, as supported by qPCR analysis, while capsid assembly is strongly impaired in the groEL knock-down mutant. This was confirmed by a qualitative analysis of phage structures found in lysates by electron microscopy. Our preliminary results suggest that several components acting at different steps of the SPP1 capsid assembly pathway require GroEL for stable folding. Their complete repertoire and analysis how the cell protein folding machinery impacts on SPP1 infection will be presented.

1. J. Mol. Biol. (1973) 76:1, 45 – 60

2. Nature (1994) 368, 654 – 656

3. J. Biol. Chem (2001) 276:, 8720 - 8726

[ID: 396] Engineered M13-bacteriophages to target amyloid- β peptide in oligomeric/fibrillar form for Alzheimer's Disease diagnosis

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Alzheimer's Disease (AD) is an age-dependent neurodegenerative disease with very high incidence worldwide and detrimental consequences. Manifests as a decline in cognitive functions and memory loss, characterized at molecular level by an increased deposition of amyloid-beta (AB) plaques. Nevertheless, it is AB in the still-soluble oligomeric/fibrillar form that impairs synaptic function and memory encoding [1]. Therefore, new tools that selectively target AB oligomers/fibrils in the brain hold great potential to halt AD at an early stage.

Specific amyloidogenic peptide motifs grafted into an antibody, were described to react with AB fibrils: AB30-39, and with fibrils and oligomers: AB33-42 [2]. However, the blood-brain-barrier, by sheltering the central nervous system from the systemic circulation, is a major bottleneck for peptide- and antibody-based applications. To overcome this limitation, bacteriophages and phage display can be applied [3,4].

We have successfully cloned peptides AB30-39 and AB33-42 into the M13 filamentous phage genome. The binding ability of AB-specific phages towards AB-oligomers was tested *ex vivo* and *in vitro* in mouse AD-tissue and AB42 peptide, respectively. Results showed that AB36-39 peptide selectively target AB-protofibrils, but not plaques, in brain slices from AD-model mice and not from wild-type (WT) littermate. In addition, control phage (carrying no AB-selective peptide), did not stain AD or WT tissue.

For future work, this system will be tested in AD-mouse models to assess the inhibition of the oligomeric AB-mediated synaptic loss and memory impairment.

The outcomes will allow the development of novel phage-based tools for the detection/treatment of early AD, with a direct impact on society and well-being, minimizing the economic burden due to late AD diagnosis and consequent medical treatments.

[1] Kessels H.W., et al. PNAS, 2013. 110: p.4033-8.

[2] Perchiacca, J.M., et al. PNAS, 2012. 109: p.84-9.

[3] Pasqualini, R. & Ruoslahti, E. Nature, 1996. 380: p.364-6.

[4] Dabrowska, K. et al. J Appl Microbiol, 2005. 96: p.7-13.

[ID: 158] Interaction of three-phage cocktail with antibiotics on *Proteus mirabilis* biofilm

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The formation of *Proteus mirabilis* biofilm on urological catheters contributes to the development of catheter associated urinary tract infections (CAUTI) and is the cause of antibiotic treatment failure. Nowadays, the use of bacteriophages alone or in combination with antibiotics as a biofilm eradication method is intensively studied. The aim of the project was to assess the possibility of using phage-cocktail and the combination of phages with antibiotics for the eradication of *P. mirabilis* biofilms. In the study phage cocktails consisting of two or three viruses: 39APmC32, 65APm2833 and 72APm5211, were used alone or in combination with norfloxacin (NOR), amikacin (AMK) or cefotaxime (CTX) against *P. mirabilis* biofilms. Bacteria viability in the biofilms was assessed by MTT assay. Biofilms of 16 uropathogenic *P. mirabilis* strains tested have shown different susceptibility to phage preparations. The decrease in bacterial metabolic activity in phage-treated biofilms ranged from 10% to 90%. Phage-cocktails destroyed biofilms comparably to their most potent phage components. For the further investigation three strains (C8, C15 and C31) were selected. On the basis of the EUCAST breakpoints, biofilms formed by these isolates were considered resistant to tested antibiotics. The biofilm of C31 strain was reduced by about 75%, while biofilms of C8 and C15 strains were not disrupted by three-phage cocktail. Synergistic effect of the phage cocktail and antibiotics against the tested biofilms was observed only when cefotaxime was used. The C31, C8 and C15 strains biofilms were destroyed to the extent of about 100%, 50% and 75-90%, respectively. No similar effects were observed for the combination with amikacin or norfloxacin. What is more, AMK and NOR suppressed the activity of the phage cocktail at certain concentrations. Our results suggest that the combination of the phage cocktail with cephalosporins has a high potential in eradication of the *P. mirabilis* biofilms.

[ID: 151] Sequencing high efficiency transducing bacteriophage A25: the story of lysogenic escape

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Bacteriophage A25 has a rich history within streptococcal research, yet remained un-sequenced. A25 is a high efficiency transduction capability. We were interested in exploring the genome to better understand A25's role in horizontal gene transfer amongst streptococci. Sequencing was performed at The University of Oklahoma Health Sciences Laboratory for Genomics and Bioinformatics and revealed an unexpected, ~6.5 kb region of near identity with toxigenic prophages from *S. pyogenes*, including a partial lysogeny module comprising an operator and cro-like antirepressor. An integrase gene was absent indicating lysogenic escape. The A25 genome is mosaic with homology to phages from other streptococcal species including *S. suis* (ΦSMP) and *S. pneumoniae* (ΦMM1) within the lysin gene and the DNA packaging module respectively. Phylogenetic analysis of the large subunit terminase revealed homology with pac-type packaging phages; confirmed by restriction enzyme digest. Pac-type mechanisms are noted for lower packaging stringency, likely explaining characteristic transduction capabilities. *S. pyogenes* strains of a variety of M types were incubated with serial dilutions of A25 lysate and plated to assess host susceptibility range. The majority of strains were susceptible to A25 infection, but M-type 2, 3, and 4 strains sharing the lysogeny module with A25 and both M49 strains were resistant to A25 infection. Lysogeny escape effected A25 host range. Strains containing high homology prophages were resistant via superinfection immunity. M49 strains that were resistant failed to contain high homology prophages, suggesting an alternative resistance mechanism. Thus, genome analysis showed that phage A25 is partially descended from a common type of *S. pyogenes* prophage although having acquired genetic modules from phages of other species.

[ID: 117] The dynamics of a “cheater” phage detected in experimentally evolved MS2 phage populations

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RNA viruses provide several advantages for studying evolution in action due to their large populations, short generation times, small genomes and high mutation rates. In this study, we used MS2, an RNA bacteriophage with a tremendous burst size and a tiny genome. We characterized the phage response to a higher than usual temperature, comparing the identity and fitness effects of mutations at 41°C, vs. the optimal growth temperature of 37°C while performing twenty serial passages at a MOI of one. Unexpectedly, following next generation sequencing of the serial passages, we observed the emergence of a deletion mutation at position 1764 ($\Delta 1764$). This deletion affected two overlapping reading frames of the phage, and hence is expected to result in a truncated lysis protein but more importantly in a completely non-functional replicase protein. During the experiment, $\Delta 1764$ reached a population frequency of around 50% which remained stable for several passages and was then followed by a dramatic decline down to a population frequency of almost zero. We hypothesized that $\Delta 1764$ is a “cheater” phage, able to replicate only in the presence of WT phage. Substantiating our hypothesis, we showed that $\Delta 1764$ cannot form plaques initiated by an individual particle. Moreover, we were able to use real-time PCR to quantify ten times more “cheaters” than WT phages at passage fifteen. A mathematical model of phage replication suggests that in order to recapitulate the experimental results, one must assume differential fitness of the cheater phage which depends both on the ratio of cheater to WT co-infection, and on the density of the host population. The mechanism for $\Delta 1764$ cheating is yet unknown, a factor that can contribute to cheating success is that the mutant lysis protein produced by the cheater alters the timing of host cell lysis during co-infection. Work to fully elucidate the cheating mechanism is currently underway.

[ID: 307] Comparative study of phages active against *Mycobacterium abscessus* complex

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Mycobacterium abscessus complex comprising three subspecies is a representative of clinically relevant species of rapidly growing mycobacteria (RGM). Lately, lung infections caused by *M. abscessus* has become increasing problem in cystic fibrosis patients. Clinical isolates of *M. abscessus* are characterized by intrinsic and acquired resistance to wide range of antimicrobials. Therefore phage therapy can be considered as an alternative treatment for infections caused by this species.

A new phage 8UZL active against clinical strains of *M. abscessus* was isolated from sewage water sample and studied together with two other phages _DSM 105283 and DSM 105284, received from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) collection. Activity of phages was studied against representatives of RGM, including *M. fortuitum* and *M. chelonae*. The studied phages demonstrated different host range activity. Phage 8UZL showed high activity against majority of clinical isolates.

Optimal growing conditions of three phages also proved to be different: 8UZL can be propagated on its host strain at 37 °C, while optimal temperature for two other phages is 28 °C and they show no activity at 37 °C. 8UZL demonstrated only adsorption ability at 28 °C. Based on electron microscopic study phage 8UZL was defined as a representative of Siphoviridae family with long non-contractile tail of 170 nm in length and icosahedral head of 60 nm in diameter.

Further studies include comparative characterization of phage genomes.

[ID: 272] Prophages in marine Vibrios: implications for the carriage of virulence and antimicrobial resistance genes

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Vibrios are some of the most abundant bacteria present in aquatic environments. Although the majority of Vibrios are non-pathogenic, the taxa contains both clinically relevant human pathogens such as *Vibrio cholerae* or *Vibrio vulnificus* and economically important aquaculture pathogens, for example *Vibrio parahaemolyticus* and *Vibrio harveyi*. Acquisition of virulence factors by some Vibrios is linked to lysogenic conversion by temperate phages. The best known example is cholera, caused by expression of the CTX Φ phage-encoded cholera toxin in lysogenic *Vibrio cholerae*. Similarly, *Vibrio harveyi* VHML phage and *Vibrio coralliilyticus* prophage sequences encode virulence factors that increase the pathogenicity of their respective hosts. Although the aforementioned lysogenic conversion-based increases in *Vibrio* pathogenicity are well documented, the wider implications of prophage latency in other *Vibrio* hosts, including the potential to increase antimicrobial resistance, are still relatively unknown. As such, we set out to determine the distribution of prophages including both virulence and antimicrobial resistance genes encoded by them within the *Vibrio* taxa.

We analysed over 5000 *Vibrio* genomes to search for chromosomally embedded prophages. In an all-v-all comparison, prophages were clustered into groups based on their nucleotide identity, which roughly equates to a bacteriophage species. Phylogenetic methods were then used to gauge where each group fits within current bacteriophage taxonomy. We were able to discover a plethora of putative prophages that likely represent new phage species. We then went on to analyse the presence of both putative virulence genes and antimicrobial resistance genes within prophages. Both virulence and antimicrobial resistance genes are widespread in *Vibrio* prophages and often associated with specific prophage clusters. Subsequently, we experimentally confirmed that prophage encoded antimicrobial resistance genes are functional and can confer resistance when expressed in *E. coli*. Our research shows, that *Vibrio* lysogens are common and lysogenic conversion might be prevalent within the taxa.

[ID: 376] The first retrospective analysis of long term results of the application of phage preparations in patients with chronic bacterial infections

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In February 2018 a group of patients with chronic bacterial bone, joint, soft tissue, or periprosthetic infections receiving experimental phage therapy (PT) at the Phage Therapy Unit of the Hirsfeld Institute of Immunology and Experimental Therapy PAS in Wrocław were interviewed by phone. Information was collected from 33 subjects (70% *S. aureus* monoinfection) who completed PT between 15 months to 7 years ago. Patients used phage preparations topically and also by oral route in two cases. Ten patients were treated for free as a part of "Phages from POIG" program. Cumulative treatment time was from 3 to 168 days (median: 51 days). Good response to treatment assessed by a physician according to the scale described previously by Międzybrodzki et al. (*Adv Virus Res.* 2012; 83: 73-121) was observed in 12 of patients immediately after completing PT (data available for 31 patients), while eradication or healing in was observed in 4 cases. During next twelve months 8 of 26 responders still reported good results of PT (eradication or healing in 8 cases). Interestingly, 21 patients assessed the PT result as a good (17 reported eradication or healing) at the time of interview. Although the majority (79%) of responders applied another treatment after PT, 12 patients linked their current good clinical state to PT. None of 33 interviewed persons reported adverse reactions that could be directly related to the application of phage preparations. Twenty two patients confirmed that they were satisfied with PT (13 of them were highly satisfied).

These results confirm the safety of PT. Maintenance of a good response to PT for prolonged time after its completion suggests that the beneficial results of PT may be long lasting. Moreover, we cannot exclude that PT may support patient's clinical improvement or cure for the long term.

[ID: 414] Interspecies interactions of Salmonella Enteritidis and Escherichia coli dual-species biofilms and their control by bacteriophages

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Salmonella Enteritidis and Escherichia coli are foodborne pathogens forming challenging biofilms that contribute to their virulence, antimicrobial resistance and survival on food contact surfaces (1,2). Interspecies interactions occur in dual-species biofilms promoting different outcomes to each species (3,4). Here we describe bacteriophage control of biofilms focusing also in the interactions between two strong or weak biofilm producers of each species. Mono and dual-species 24 h-old biofilms, in all possible combinations, were formed in vitro and infected with S. Enteritidis and E. coli bacteriophages PVP-SE2 and vB_EcoM_CEB1. Treatment outcome was determined by viable cells counts and visualized by fluorescence microscopy. Biofilms presented higher pathogen loads in mono-species biofilms and weaker biofilm producers increased in number in the presence of strong biofilm producers. Fluorescence microscopy visualization showed different 3D biofilm from microcolonies and uniform layers to more complex and heterogeneously organized arrangements, and differences in carbohydrates in the biofilm matrix assessed by Fourier-transform infrared spectroscopy. Bacteriophage cocktail was efficient until 8 h resulting in greater reductions of S. Enteritidis cells highly due to bacteriophage PVP-SE2 growth characteristics. Furthermore, differences in bacteriophage effectiveness can also be a result of the biofilm 3D structure and matrix strength and compositions.

1. Giaouris, E. et al. *Front. Microbiol.* 6, 1–26 (2015).
2. CDC. Pathogens causing US foodborne illnesses, hospitalizations, and deaths, 2000–2008. (2012).
3. Esteves, C. L. C. et al. *Society* 73, 5198–5203 (2005).
4. Wang et al. *J. Food Prot.* 77, 805–813 (2014).

[ID: 137] Characterization of phages escaping restriction by LlaDCHI restriction modification system

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Lactococcus lactis is an industrial bacterium known for manufacture of fermented dairy products. Virulent bacteriophage, commonly found in the industrial environment, can cause significant economic loss due to end-product quality issues and failed manufacturing processes. Research into *L. lactis* phage-host interactions has revealed an extensive repertoire of phage defenses including restriction modification. The LlaDCHI R/M system, previously described on pSRQ700 (1), confers resistance to lactococcal phages including those of the C2Virus, Sk1Virus, and P335 groups which are the most commonly found in the dairy environment. The LlaDCHI R/M, composed of 3 orfs (endonuclease IlaDCHIC and methylases IlaDCHIB and IlaDCHIA), recognizes the site 5'-GATC-3'. Stronger resistance was reported against P335 and Sk1Viruses versus C2Viruses as their genomes contain more GATC sites. A survey of sequenced lactococcal genomes in the DuPont collection identified eleven strains containing the LlaDCHI R/M. The majority of phages in the DuPont collection which are able to infect these strains are C2Viruses, likely due to the lower incidence of GATC sites. Three of these C2viruses were sequenced and found to contain no GATC sites at all. Additionally, the DuPont collection contains multiple Sk1Viruses against LlaDCHI R/M-containing strains, five of which were sequenced. Sequence analysis indicates that, like the previously described C2Viruses, phages D2947 and D5604 contain no GATC sites, and D753 contains only two GATC sites. Phages D2123 and M6167 contain multiple GATC sites, 7 and 4, respectively; however, they encode a methylase that has previously been shown to methylate GATC sites (2). This data demonstrates phage evolution in response to lactococcal defense mechanisms which should be taken into consideration when exploiting these natural mechanisms in directed strain construction for improved phage resistance in starter cultures.

References

1. Moineau S, et al. *Appl. Environ. Microbiol.* 1995, 61:2193–2202.
2. Murphy J, et al. *BMC Genomics.* 2014, 15:831.

[ID: 271] Tail spike protein of Dickeya bacteriophage PP35 interacts with bacterial O-antigens to launch the infection, and, thus may propagate on alternative non-pathogenic environmental hosts with similar structure of O-polysaccharide

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The limitations in antibiotic use in the agriculture promote an application of alternative strategies to combat bacterial infections in plants. One of major threats is *Dickeya solani*, a recently emerged virulent agent causing black leg and soft rot of potato. An application of bacteriophages to control *Dickeya* infections is considered as promising approach. One of possible candidate phages PP35 was characterized in details. This contractile-tailed phage belongs to the newly proposed genus *Limestonevirus*. Similar viruses were isolated in all European locations where infection outbreaks caused by *D. solani* have been detected.

The host range of PP35 was shown to be governed by the function of the tail spike protein, PP35 gp156, and the sequences of corresponding genes are highly conserved among *Limestonevirus* genomes. High-resolution electrospray ionization mass spectrometry shows that the recombinant tail spike protein degrades the O-polysaccharide of *D. solani* into octameric fragments. The polysaccharide structure, $\rightarrow 2$ - β -D-6-deoxy-D-altrose-(1 \rightarrow , is unique among soft-rot *Pectobacteriaceae*. However the same structure was identified in non-virulent environmental bacteria also susceptible to phage PP35. This alternative host, *Lelliottia* spp. strain F154, was isolated and characterized. Some genes encoding O-polysaccharide biosynthesis shared with *D. solani* were identified. Non-pathogenic bacteria may play an important role in the ecology of environmental microbiome maintaining the threshold population of the phage aimed against potential pathogenic stranger bacteria. Also, such bacteria may serve as non-pathogenic host for industrial production of therapeutic phages.

[ID: 291] Isolation of 5 new hyperthermophilic archaeal viruses, all of different viral families

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Only limited information is known about the viruses infecting the archaea, the third domain of life. Despite the small number of isolates, which is hardly just over a hundred, viruses of the archaea are known to have astonishingly diverse virion morphologies and genomic sequence diversity. These features are clearly different from those observed in the bacterial virosphere. In contrast to the fact that several thousands of bacterial viruses are currently classified into just 10 viral taxonomical families, limited number of archaeal viruses are already classified into 18 families. And these numbers are still counting, even within the past 5 years, indicating that we only have a tip of the iceberg information about the true diversity of microbial viruses, especially those from extreme environment.

We have recently isolated 5 new viruses infecting hyperthermophilic archaea, *Pyrobaculum*, from various hot springs in Japan. These viruses range from filamentous, globular, spindles etc. Both detailed and preliminary analysis of these viruses indicate that all five of these belong to different families, and that four of them are potentially worth establishing novel viral families. One of those, *Pyrobaculum* linear virus 1 (PLV1), tentatively proposing “*Pyrolipoviridae*” family, will be described in detail. This linear filamentous virus has a linear dsDNA genome of 16.4 kb (35ORFs). By biochemical analysis, its major capsid protein gene has been identified. In addition, the capsid proteins are shown to be glycosylated, which is often the case of archaeal viruses, but further characterization indicate that it may be done through a very rare mechanism.

Lastly, one of the newest isolate seem to have a rare genotype. Preliminary analysis indicate that it may be a ssDNA virus of hyperthermophilic archaea. The updated analysis of all these virus will be presented.

[ID: 11] Gut microbiome and stable core virome after human fecal transfer

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We will discuss the follow-up of a recently described 4.5-year time course of the enteric bacterial microbiota and virome of a patient cured from recurrent *Clostridium difficile* infection (rCDI) by fecal microbiota transplantation (FMT). We analyzed bacterial and viral compositions of recipient and donor using 16S rRNA gene and metagenomic sequencing. The virome contained dsDNA viruses, mainly Caudovirales phages. Unexpectedly, sequences related to giant algae-infecting *Chlorella* viruses were also identified. Our findings indicated that intestinal viruses can be implicated in the establishment of gut microbiota, as phages and their host bacteria were frequently co-detected (1,2). Moreover, we found the patient's phage population to exhibit highly donor-similar characteristics, which remained stable for up to 7 months. This was unexpected since enteric viromes are normally highly variable, assumed to influence the bacterial host community and change with environmental conditions. In contrast to the virome, the bacterial microbiota varied indeed for more than seven months with ongoing dysbiosis before it reached donor similarity 4.5 years post-FMT (3,4). Our findings that are based on sequence information and protein domain analysis seem to suggest that stable phage properties correlate with successful FMT better than the changing bacterial communities. We speculate that we here preferentially detected a stable core virome, which dominated over a variable flexible virome that may have been too heterogeneous for experimental detection, or was underrepresented in the databases. The virome is possibly the determining factor in the composition of the gut microbiome and stool transfer.

It will be interesting to analyze whether the enteric virome allows for predicting the clinical outcome of FMT for rCDI and other diseases such as inflammatory bowel disease or obesity.

References:

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

[ID: 124] Genomic analysis of *Acinetobacter baumannii* prophages reveals remarkable diversity and suggests profound impact on bacterial virulence and fitness

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The recent nomination by the World Health Organization of *Acinetobacter baumannii* as the number one priority pathogen for the development of new antibiotics is a direct consequence of its fast evolution of pathogenicity and multidrug resistance. While the development of new antibiotics is critical, understanding the mechanisms behind the crescent bacterial pathogenicity is equally relevant. Often, resistance and other virulence elements are contained on highly mobile pieces of DNA that can easily spread to other bacteria. Prophages are one of the mediators of this form of gene transfer, and have been frequently found in bacterial genomes, often offering advantageous features to the host. Here we question the contribution of prophages for the evolution of *A. baumannii* pathogenicity.

About 954 strains were analyzed for the presence of prophages. A total of 4860 prophages were found, with 71% of the strains encoding intact prophages. Curiously, a few prophages were also found in plasmids, suggesting a higher potential for dissemination. A subset of intact prophages were analyzed in more detail. These were classified by comparing specific structural proteins to those of previously classified phages, and found to belong to the Caudovirales order, with family prevalence similar to that found in nature. Prophage sequences were aligned for the construction of genomic and proteomic distance matrices and phylogenetic trees. These clearly indicated a high diversity among prophages, which may contribute to the diversity of *A. baumannii*, since some strains differ only on the integrated prophages. Remarkably, *A. baumannii* prophages encode for numerous putative virulence factors that may be implicated in the bacterium's capacity to colonize host niches, evade the host immune system, subsist in unfavorable environments, and tolerate antibiotics.

Overall our results point towards a significant contribution of prophages for the dissemination and evolution of pathogenicity in *A. baumannii*, and highlight their clinical relevance.

[ID: 410] Temperate bacteriophages of the Roseobacter group

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Bacteriophages impact at least a third of the bacterial genomes in marine environment. However, to date little is known about the temperate phages infecting marine Rhodobacteraceae (the Roseobacter Group), which are major heterotrophic players in polar to temperate marine waters, especially in coastal regions. Our work on bioinformatic analysis of temperate phages in the Rhodobacteraceae family indicates that their genomes abound in prophages. We used two different bioinformatics tools (Phaster and VirSorter) to predict prophages in 346 genomes from 80 genera. The combined predictions gave 1810 putative prophages, which were grouped in three classes: class I, intact prophages, class II, questionable prophages, and class III, incomplete prophages. Class I and II are more likely to have inducible prophages. Class III is more likely to have prophage remnants, but also prophages which are hard to predict, due to the lack of known homologue genes in other phage genomes. Out of the 346 genomes, only 17 don't have predicted prophages. We have identified three major types of prophages: i) lambda similar, ii) Mu similar prophages and iii) plasmid prophages. Further work is focused on improving the prophage detection, phylogenetic classification of the prophages, auxiliary metabolic gene detection and elucidation of prophage life styles.

[ID: 407] genePROBER – a webtool for designing geneFISH and phageFISH probes

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Gene based methods, as for example phageFISH (1) and direct-geneFISH (2), allow intracellular detection of viruses at single cell level, during infection of their bacterial or archaeal hosts. These methods hold much promise for the study of phage-host interactions and infection life-cycles of both lytic and temperate phages. Because the two methods use a mixture of polynucleotides as probes, their application has been hampered by the lack of tools to automatize probe design. Here I present genePROBER, a web-based, graphical user interface tool developed for the design of polynucleotide probe mixtures. In several steps, it assists the user through the design process: i) detection of similarities in between the target and non-targets, ii) polynucleotide generation, iii) selection of polynucleotides based on probe %GC, probe and primer specificity and primer efficiency, and iv) probe mix generation. GenePROBER is accessible online at <http://kronos.icbm.uni-oldenburg.de/shiny/web-probe-designer/> and its main applications are the designing of probes to target genes in pure cultures or enrichments, as for example for detection of viruses in bacterial and archaeal cultures.

1. Barrero-Canosa, J., Moraru, C., Zeugner, L., Fuchs, B., Amann, R. (2017). Direct-gene-FISH: a simplified protocol for the simultaneous detection and quantification of genes and rRNA in microorganisms. *Environ Microbiol* 19: 70-82.

2. Allers, E., Moraru, C., Duhaime, M.B., Beneze, E., Solonenko, N., Canosa, J.B., Amann, R., and Sullivan, M.B. (2013). Single-cell and population level viral infection dynamics revealed by phageFISH, a method to visualize intracellular and free viruses. *Environ Microbiol* 15: 2306-2318.

[ID: 300] Characterization of Salmonella phage and prophage diversity on cattle, swine, poultry, wild birds in Chile

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Salmonella phages play a role in Salmonella ecology and diversity; in addition, phages are promising tools to control Salmonella in animal production. This study characterized the diversity of Salmonella and Salmonella phage through Chile, with the aims of mapping Salmonella diversity along with their phage and prophage contents and developing a collection of phages to be used in food safety. A total of 3,614 samples were analyzed, from wild animals (wetland birds and reptiles), animals in backyards (flocks, cows, and camelids), and animals confined in production systems (dairy cattle, swine, equines). We isolated 113 isolates of Salmonella and 385 isolates of phages. On 74 Salmonella isolates we sequenced the genomes and characterized the prophage and CRISPR contents. On the isolated phages we characterized the host range on a panel of 23 different Salmonella serovars and we sequenced the genomes of 26 phages. Prophage content ranged from zero prophage identified in *S. Anatum* and *Corvallis* to up to nine in *S. Santiago*. Diversity on prophage content also varied, being *S. Typhimurium* the serovar with the most variable content and *S. Enteritidis* with the least variable. Similarly, CRISPR spacer were found to be mostly identical for *S. Enteritidis* (2 pro<https://www.conference-service.com/w18-96/image/35> isolates) and very diverse for *S. Typhimurium* (5 pro<https://www.conference-service.com/w18-96/image/10> isolates). On phages we identified three cluster: i) phages from wetland birds that showed very wide host ranges, ii) phages from backyards animals that showed wide host ranges, and iii) phages from confined food animals that showed narrow host ranges. Phage genomes identified known phage genus (*Felixo1virus*, *Spo1virus*, *Sp31virus*) widely distributed on different animals and locations within Chile. This study represents the first comprehensive characterization of Salmonella and phages in Chile, our study generated a collection of phages to be used for control of this important foodborne pathogen.

[ID: 41] Characterisation of DNA interference by a minimal Type I-F CRISPR-Cas system

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CRISPR-Cas systems represent adaptive immune systems in Archaea and Bacteria. They use ribonucleoprotein complexes containing CRISPR RNAs (crRNAs) to target and degrade viruses in a process termed interference. In Type I CRISPR-Cas systems, the ribonucleoprotein complexes (termed Cascade) are able to discriminate between self and non-self by identifying a Protospacer Adjacent Motif (PAM) near the target. In Type I-E systems, this process is carried out by the large subunit of Cascade and PAM recognition results in the formation of a locked Cascade-DNA complex and subsequent target DNA degradation by Cas3 [1].

Here, we analyze a minimal Type I-Fv CRISPR-Cas system, identified in *Shewanella putrefaciens* CN-32. This system lacks a large subunit and encodes two previously uncharacterized Cas proteins, found to be homologues of Cas5f and Cas7f. We expressed the heterologous complex in *Escherichia coli* BL21-AI and showed Cascade-mediated interference against bacteriophages and plasmids in a sequence-, PAM- and Cas3-dependent manner.

The crystal structure of the Type I-F variant Cascade was solved, revealing that a unique Cas5fv domain is responsible for stringent GG PAM identification [2]. Additionally, we utilized Single-Particle Tracking Photoactivated Localization Microscopy and Biolayer Interferometry to determine the targeting specificities of Cascade.

We propose that this minimized Cascade is an evolutionary response to the appearance of viral anti-CRISPR (Acr) proteins found to block interference by I-F and I-E Cascades. In agreement with this hypothesis, known Acr proteins did not impair the interference activity of this variant complex.

1. Hayes RP, Xiao Y, Ding F, van Erp PB, Rajashankar K, Bailey S, et al. Structural basis for promiscuous PAM recognition in type I-E Cascade from *E. coli*. *Nature*. 2016;530(7591):499-503.
2. Pausch P, Müller-Esparza H, Gleditsch D, Altegoer F, Randau L, Bange G. Structural Variation of Type I-F CRISPR RNA Guided DNA Surveillance. *Molecular cell*. 2017; 67 (4):622-635 e4.

[ID: 99] Uncovering mycoviruses: The use of NGS for three Mitovirus spp. identification in the aggressive phytopathogen *Fusarium circinatum*.

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Fusarium circinatum is the causal agent of pine pitch canker disease, one of the most devastating pathologies for pine forest and nurseries around the world. Despite the high economic and ecologic importance of this disease there is not an effective method for biocontrol. Mycoviruses (virus infecting fungi) have been proved as a successful tool for controlling forest diseases as in the case of chestnut blight (causal agent: *Cryphonectria parasitica*) when mycoviral infection promote hypovirulence to the host. We use next generation sequencing (i.e. high throughput sequencing of small RNA) following by de novo assembling in order to identify the infection of three Mitovirus spp. (Narnaviridae; FcMV1, FcMV2-1 and FcMV2-2) in ten isolates of *F. circinatum* from different regions of Spain. As a result, we recovered the three mycoviruses (genome coverage by contigs >50% in all cases), being viral prevalence ensured by RT-PCR with eleven specific primer pairs. In addition, several recognition sites of dicer protein along viral genome were identified. In conclusion, these results reveal the mechanism of anti-viral response of *F. circinatum* against three different viral strains hosted in fungal mitochondria.

Keywords: Dicer protein, Narnaviridae, NGS, RNA silencing, vsRNA.

[ID: 316] The Bacteriophage Bank of Korea

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The bacteriophage Bank of Korea (<http://www.phagebank.or.kr>) was established in 2010 and serves as the center of isolation, characterization, stocking, and distribution of bacteriophages. Bacteriophages are viruses infecting bacteria. Since first discovery in early 20th century, they served as antibacterial agents until the emergence of antibiotics. Beside their use as alternatives to antibiotics, phages were main object for understanding molecular biological aspects of life. In addition, phages are used as food additives, feed additives, means for displaying proteins and peptides, and targets for elucidating novel mechanisms such as CRISPR system. The Bacteriophage Bank has collected more than 2000 different phages from various environmental sources. They are characterized for host range, virion protein composition, mass spectral analysis of virion proteins, genomic DNA sequences, and morphological analysis using transmission electron microscope. Host bacteria include *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Acinetobacter baumannii*, *Enterococcus faecalis*, *Enterococcus faecium*, *Cronobacter sakazaki*, *Serratia marsescens*, *Campylobacter jejunii*, *Pseudomonas syringe*, and more entities are being added in the list.

[ID: 317] Targeted delivery of phages induced tumor regression in mice models

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Oncolytic animal viruses are well documented and proven to be effective against cancer. Their anticancer effect was generally based on two ways; one is their ability to infect and lyse host cells while the other is enhancement of host immune responses. Phages are viruses and can be engineered to display peptides targeting specific cancer cells. We wanted to see whether phages can act as oncolytic viruses. In this experiment, bacteriophage T7 was engineered to display target specific peptides against mouse tumor cell lines. Two different cell lines used were CT-26 (colorectal cancer) and B16-F10 (melanoma). Two peptides targeting each cell lines were TCP1 and pep42, respectively. Mice were grafted intraperitoneally with each cell line, and tumor mass was allowed to grow for 6 days. Then phage T7 displaying targeting peptides were injected into tumor mass and mice were further observed until day 17. Mice grafted with CT-26 showed 85% reduction in tumor mass when treated with phages. Mice grafted with B16-F10 showed 76% reduction in tumor mass when treated with phages. Cytokines IL1-alpha and TNF-alpha increased significantly in mice treated with phages. Macrophage infiltration into tumor mass was observed from immunohistochemistry. Thus phage treatment could be another option as oncolytic viruses for cancer.

[ID: 125] Comparative study on the efficiency of three methods for *Campylobacter* phage detection

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Campylobacteriosis is the most commonly reported food-borne illness worldwide and the consumption of poultry is the most widespread route for human infection. Moreover, the use of antimicrobial agents promotes the spread of multidrug resistant *Campylobacter* strains. The need to overcome this problem highlights the requirement of further mitigating measures, such as the use of bacteriophages as pre- and post-harvest biocontrol strategies in poultry.

Campylophages have been isolated wherever their hosts are present with variable success rates from 0 to 100%, which could be due to differences in the isolation method or in the kind of samples. This study aimed to compare the efficiency of three methods on campylophage recovery from food and environmental samples.

For this purpose, several chicken skin and feces samples were inoculated with phage AZT501 at 10⁴ PFU/g and processed by a stabilization method and two enrichment methods in BHI and Bolton broth, respectively. After, chloroform was added and lysates centrifugated to remove remaining cells. The presence of phages and their titer were then assessed by the spot test.

Concerning the recovery of campylophages from chicken feces, no differences were found among tested methods, observing recovery efficiencies from 63 to 80 %. Similar efficiencies of 65 and 69 % were obtained in skin samples treated by the first two methods. Nevertheless a significantly higher efficiency of 160 % was achieved in samples enriched with Bolton which could be consequence of a great phage replication. Therefore, Bolton broth enrichment could be considered the most efficient methodology to recover campylophages from chicken skin. However, phage recovery in feces was not improved by using this method, which could be probably due to a matrix effect. Further research is being carried out to study the effect of the matrix, the phage titer and the kind of phage on the recovery efficiency.

[ID: 265] Attempt to analyze the similarity of the sequences of endolysins genes of new phages lytic against Bacillus anthracis

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Occurrence of *Bacillus anthracis* spores in soil, though rare nowadays, is still a problem in some European or Asian countries. This pathogen poses a threat also as a means of bioterrorism activities. Phages and their endolysins may be found helpful in fighting human and animal anthrax infections but also as decontamination agents.

A couple of new bacteriophages lytic against *Bacillus anthracis* were identified. The phages came from the Institute own collection and from new environmental collection sites. Twenty two different strains from *B. cereus* group were used in a host test experiment. Only *B. anthracis* 34F2 vaccine strain and three pathogenic *B. a.* strains were sensitive to tested phages. DNA of the phages was extracted and sequenced.

Genes encoding for endolysins were identified in each of these phages. According to BLAST these phages show DNA sequence similarities (query cover) of 72-77% to the best known, flag Gamma phage.

Although these phages differ, their endolysins share high level of similarity in a nucleotide and aminoacid sequence. The lysins show different secondary protein structure so their activity may not be identical. All endolysin proteins encoded by these viruses share the same length of 351 aminoacids, compared to 301 aa in a PlyG Gamma phage lysin. They all are N-acetylmuramoyl-L-alanine amidases.

Further experiments will include cloning of the lysins' encoding genes and obtaining purified proteins. Lytic activity of these proteins will be tested against *B. cereus* group members and *B. anthracis* endospores and their potential therapeutic and decontamination properties will be assessed.

[ID: 29] Optimization of virus purification by CsCl density gradient using general centrifuge

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[Introduction] Virus purification by cesium chloride (CsCl) density gradient ultracentrifugation, which generally requires an expensive ultracentrifuge, is an essential technique in virology. Some general centrifuges, such as large capacity centrifuge, can achieve high gravity but are not as expensive as ultracentrifuge. In this study, we optimized the virus purification by CsCl density gradient using general centrifuge.

[Materials and methods] Phages S13' and ϕ EF24C, and fowl adenovirus (FAV) strain JM1/1 were used. The viruses were purified by CsCl density gradient using ultracentrifuge and general centrifuge. The concentrations of the purified viruses were measured. And observation of virus particles by transmission electron microscopy, SDS-PAGE of virion proteins, and/or restriction of genomic DNA were done.

[Results and discussion] Phages S13' and ϕ EF24C were purified by CsCl density gradient ultracentrifugation ($100,000 \times g$, 1 h, 4 °C) and centrifugation using a large capacity centrifuge under higher gravity conditions ($40,000 \times g$, 2 h or 4 h, 4 °C) and under lower gravity conditions ($30,000 \times g$, 2 h or 4 h, 4 °C). The virus concentration was not significantly different among centrifugation under higher gravity condition ($40,000 \times g$, 2 h), under lower gravity condition ($30,000 \times g$, 4 h), and ultracentrifugation ($100,000 \times g$, 1 h). In addition, the centrifugation using a large capacity centrifuge showed sufficient purification ability, which was verified by observation of virus particle and virion proteins. Moreover, testing the feasibility of this method, FAV was also purified by general centrifugation ($40,000 \times g$, 2 h). From the purified viruses, high quality virion proteins and genomic DNA were sufficiently obtained. Considering these, we believe that the virus purification method by CsCl density gradient centrifugation ($40,000 \times g$, 2 h, 4 °C) or ($30,000 \times g$, 4 h 4 °C) will encourage research in virology.

[ID: 404] What if antibiotics shouldn't be used? Bacteriophage vB_Eco4M-7 as an alternative in fight against Escherichia coli (EHEC).

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Enterohemorrhagic *Escherichia coli* (EHEC) is a group of pathogens responsible for outbreaks of bloody diarrhea and hemolytic uremic syndrome (HUS). The best known member of EHEC group is *E. coli* O157:H7 and major source of these bacteria is cattle. Humans can get infected with EHEC mostly through consumption i.a. raw or undercooked ground meat products, raw milk, and faecal contamination of vegetables. These pathogens are also capable to form biofilm on both biotic and abiotic surfaces. The major virulence factors of EHEC are Shiga toxins, encoded by genes located on genomes of Shiga toxin-converting prophages (Stx phages). Effective production and release of toxins occurs only after induction of these prophages and start of a lytic life cycle. Many antibiotics used to treat bacterial infections stimulate induction of Stx prophages, causing escalation of the disease symptoms. Also, the use of medications that slow down intestinal peristalsis is not recommended. At present, only symptomatic treatment is used and it is important to search for alternative methods.

A new hope against EHEC may be bacteriophages, which are currently used i.a. in phage therapy. In earlier biodiversity studies 83 bacteriophages were isolated from urban sewage [1]. One of phages i.e. vB_Eco4M-7 have an ability to lysis only *E. coli* O157:H7 strain (ATCC 700728) and clinical strain *E. coli* O157:H7 with ST2-8624 prophage. Our study shows that bacteriophage vB_Eco4M-7 is characterized by short life cycle and high multiplication rate (about 1000 phage particle). This phage also have ability to reduce biofilm formed by *E. coli* O157:H7(ST2-8624). The above facts improve potential of bacteriophage vB_Eco4M-7 in the fight against Enterohemorrhagic *Escherichia coli* (EHEC).

[1] Jurczak-Kurek A, Gąsior T, Nejman-Faleńczyk B, et al., (2016). *Scientific Reports.*, 6:34338.

[ID: 285] Phages: nature's alternative to antimicrobial resistance crisis

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Antimicrobial resistance is already a global health crisis. Without effective and dynamic alternatives, drug-resistance pandemic is imminent. Among all alternatives, phage therapy has been used since before the advent of antibiotics yet largely ignored by western science. Here, we aim to isolate lytic phages from environment against drug-resistant clinical pathogens, study lytic efficiency in varying temperatures, pHs, and analyze its genomes.

Twenty river water samples were screened against 75 MDRO's representing ten different genera using double-layer-agar assay. Phages were purified, amplified and assayed for multiple-host-range. Ability of phages to lyse bacteria in their lag, log and stationary phase was analyzed using broth-assay, spectrophotometry and confirmed by culture. Lytic efficiency at different temperatures and pHs were analyzed using spectrophotometry. Phages were identified using electron microscopy and phage-genomes were analyzed for presence of potent 'lysin' enzyme and absence of lysogenic genes, virulent factors and/or resistant genes.

Eighty-two phages were isolated that could effectively lyse drug-resistant MDROs. Among them, 78 showed multiple-host-range within own genus and also did not induce any BIM up to 5th generation of host's life cycle. Phages were equally efficient in killing lag, log and stationary phase host bacteria, were most efficient near human body temperature and blood pH. TEM confirmed them as Caudovirales. No any 'bacterial genes' were identified within phage genome, which ruled out the concern for transfer of virulent genes. Specific 'lysin' enzyme was identified in all three phages that were conserved and thus could serve as broad range 'antibiotics'. Natural phages can be effective life-saving alternative when all available antibiotics fail as it can effectively kill pathogens not only in the idle environmental conditions but also at human blood pH and temperature as well. Absence of any virulent genes further makes phages excellent candidate in therapeutics. As phages play role in limiting pathogens in environment, study of their role in balancing human microbiome may yield a significant discovery.

[ID: 190] Mycovirus-induced Ochratoxin A (OTA) overproduction and mycovirus induced osmotic stress resilience in *Aspergillus ochraceus*

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Mycotoxins are metabolites produced by fungi and often found as contaminant in different crops and foods. These molecules can have toxic and carcinogenic effect on humans and represent a major problem in agricultural productions. Ochratoxin A (OTA) is the second most important mycotoxin produced by a range of species, both Aspergilli and Penicilli, and normed by the European Commission. Many different scientific groups tried to correlate the production of mycotoxins, both qualitative and quantitative, with the presence of several different mycoviruses. We here report for the first time a direct effect of *Aspergillus ochraceus* virus (AoV) which specifically induces overproduction of ochratoxin A (OTA) in *Aspergillus ochraceus* isolate MUT2036. The specific interaction between MUT2036 and AoV is further demonstrated by the observation that the isogenic MUT2036 isolate, transfected with *Penicillium aurantiogriseum* Totivirus 1 (PaTV1), did not show any statistically supported difference in OTA production. In addition to this specific effect on ochratoxin production, both isolates transfected with AoV and PaTV1 display osmotic stress resilience, suggesting a more generic effect of mycoviral infections similar to what we already described in *Chryphonectria parasitica* transfected with a partiti-like mycovirus, and possibly involving a generic antiviral response. Taken together this data could demonstrate a specific interaction between the mycovirus and the natural fungal host opening new insights for mycovirus-fungus-environment interaction studies.

[ID: 168] Susceptibility to lytic bacteriophages and comparison of methicillin resistant staphylococci from samples collected in European and African countries, Japan and Canada.

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Antimicrobial treatment against staphylococci has led to the selection of Methicillin Resistant Staphylococci (MRS) which represent a potential hazard for public health via the inter-Staphylococcus transferability of the mobile “Staphylococcal Chromosome Cassette” (SCC) carrying the *mec* genes encoding the resistance. The aims of this study were to characterize MRS, including *Staphylococcus aureus* (SA), strains originating from different countries and to test their susceptibility to lytic bacteriophages.

A total of 1068 staphylococci were isolated between 2005 and 2014 from different sources in Belgium, Italy, Switzerland, Senegal, Niger, Japan and Canada. After growing on “Chrom-MRSA ID[®]” agar and testing for hybridization targeting *mecA* and *mecC* genes, positive isolates were tested by disc diffusion assay for ceftiofur resistance and then submitted to several PCRs targeting the *mecA*, *mecC* genes and the SCC-*mec* types. The lytic activity of 3 bacteriophages (Romulus, Remus and DSMZ_105264) was then assessed on the MRS(A) strains.

A total of 138 isolates were positive to “Chrom MRSA ID[®]” agar and/or hybridization. Of these, 94 isolates were resistant to ceftiofur. Moreover, 94 isolates were PCR *mecA* positive yet none tested positive for the *mecC* gene. The SCC-*mec* typing showed 63 positive isolates subdivided into 8, 3, 37, 12 and 3 isolates belonging to the types II, III, IV, V and VII, respectively. The other 31 PCR *mecA* positive isolates were “not-typeable”. Lysis was observed for 28 MRS(A) isolates, encompassing 23 isolates with the phage Romulus (22 *S. aureus* and 1 non-*aureus*), 24 with Remus (23 *S. aureus* and 1 non-*aureus*) and 1 *S. aureus* with DSMZ105264. These results show no direct correlation between methicillin resistance and the phage lytic activity. Almost all isolates susceptible to lytic bacteriophages in this study were *S. aureus*. The 31 “not-typeable” isolates will be submitted to whole genome sequencing to identify, among others, their SCC-*mec* cassettes.

[ID: 162] Genomic variations and temporal dynamics among fifty-five novel phages infecting the Baltic Sea Gammaproteobacteria *Rheinheimera baltica*

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Aquatic virology has been improved by culture-independent methods, yet there is still a critical need for isolating novel phages to identify the large proportion of “unknowns” that dominate metagenomes and for providing new model-systems to study phage-host interactions. Here, we isolated 55 phages infecting *Rheinheimera baltica* (Gammaproteobacteria) from Baltic Sea seawater concentrates obtained in August and September 2015. Whole genome sequencing (Illumina) showed that the phages had quite similar genome sizes and number of genes (76 – 84 kb; 129 – 145 genes). The phages had a large number of core genes (n=91 with > 60% average amino acid identity) and 32 phages shared all genes (n=136 >60% AAI), while the remaining 23 genomes contained between 109-135 of these. Gene comparison (NCBI nr; DIAMOND) showed that while 56% of the genes had no similarity to other sequences, 19% showed similarity to other Myoviruses. This corresponds to results from transmission electron microscopy, where the phages showed a Myoviridae-like morphology. The phages possessed regular viral functions involved in DNA replication and structural genes, but also several auxiliary metabolic genes (AMGs), such as genes similar to ribonucleotide reductase that occurred in all the isolates. Other AMGs, such as PhoH and mazG genes, occurred in 53 of the isolates. While 53 isolates had a highly similar (98-100% AAI) gene of thymidylate synthase, the last two contained another gene with the same function. Besides genomic variation, recruitment of metagenomic reads (25 Baltic Sea metagenomes from 2012-2015) to the phage genomes showed temporal variations with increases of *Rheinheimera* phages in August and September, synchronising with host abundances (16S rDNA sequencing). Overall, this dataset of previously unknown phages provides a unique opportunity for investigation of the genetic diversity within a phage population and in situ phage-host dynamics.

[ID: 333] Investigation of the adsorption complex of the giant phage *vb_KleM-RaK2* infecting *Klebsiella*

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Bacteriophages represent the most numerous population on the planet. Approximately 95% of known phages are tailed viruses that comprise three families: Myoviridae (with long and contractile tails), Podoviridae (with short tails), and Siphoviridae (with long noncontractile tails). Although bacteriophages have served as a model object in many of the key studies of molecular biology, the specific mechanisms of phage infection have been extensively studied only in the case of a limited number of bacterial viruses (e.g. *E. coli* phages T4, T7, or Lambda). Notably, in the case of giant phages with atypical structure, the molecular mechanisms of phage-host interaction have never been comprehensively studied thus far.

Here, we present the results of the investigation of the adsorption complex of *Klebsiella*-infecting giant bacteriophage RaK2. The virion of RaK2 is formed from at least 54 structural proteins, 28 of which are hypothetical proteins of unknown function. Phage RaK2 is an atypical myovirus with six spiked tail fibers that appear to be formed from at least 10 podovirus-like proteins. We obtained the polyclonal antibodies against all 10 putative tail fiber/tail spike proteins of RaK2 by immunization of rabbits with the recombinant proteins (gp526, gp529, gp531-533) or the C-terminal parts of tail fiber/tail spike proteins (gp098, gp527, gp528, gp530, and gp534) of RaK2. Based on ELISA and Western blotting, all polyclonal antibodies showed no significant cross reactivity, and – during phage propagation within a cell – the biosynthesis of all proteins tested began at 30 min post infection. Then, the polyclonal antibodies were used in immunogold labelling to map the position of the proteins on the virion. The test revealed that the antibodies specifically bind to the tail fibers of RaK2. In addition, the results of spot tests revealed that two out of five RaK2 tail fiber/spike proteins (gp529 and gp531) possessed K1 capsule depolymerase activity.

[ID: 210] Phage mediated toxins expression regulation under stringent control. The use of isothiocyanates

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Foodborne diseases have a great economic impact on global health and care systems. We observe a growing concern regarding the increasing frequency of antibiotic resistance in foodborne bacterial pathogens, also exacerbating their virulence. The main virulence factors of enteropathogenic *E. coli* O157:H7 and *V. cholerae* N16961 strains are encoded on bacteriophage genome integrated in the host chromosome. In this work we aimed to investigate how the induction of bacterial stress response, the stringent response, can regulate toxins synthesis in these dangerous human pathogens. In our recent works we showed that well known phytochemicals – isothiocyanates, have potent antimicrobial activity which involve stringent response activation through amino-acid starvation pathway. We decide to analyze how isothiocyanates can impair expression of virulence factors.

We adapted 32P nucleotide labelling method and thin layer chromatography separation on PEI cellulose plates to assess (p)ppGpp cellular level. Analysis of expression of virulence genes were assessed by RT-qPCR and western blotting.

We observed a strong negative regulation of toxin genes *stx1* and *stx2* in *E. coli*. *V. cholerae* toxin genes expression were also diminished as well as other virulence factors associated with toxin-coregulated pilus (TCP) and hemagglutinin *hapA*. Impairment of toxins synthesis was confirmed by western blot analysis in both cases. We can conclude that induction of stringent response using a bioactive compounds may be a promising strategy to decrease bacterial virulence.

[ID: 427] Isolation of vibriophages from the environmental waters in Kenya and their application as biocontrol agents against *Vibrio cholerae*

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Kenyans continue to experience epidemics of cholera more frequently than previously. In 2014, cholera outbreaks were reported in Kenya, and out of 47 counties, cholera spread to 30 of them, with 15,000 reported cases and about 250 deaths. In May, 2015, a total of 3301 cases had been reported and 65 deaths occurred, with case fatality rates of 2%. In March, 2016, 216 deaths took place and 13,000 were admitted to hospitals following a cholera outbreak that affected many in the country. Since the December, 2014, outbreak, cholera has been recurring in Kenya. In May 2017 three people died of cholera after eating food contaminated with *Vibrio cholerae* in a wedding ceremony, with seven admitted to the hospital. Cholera outbreaks have also been reported in refugee and internally displaced people camps.

The goal of this research is to isolate bacteriophages lytic to *Vibrio cholerae* from the environmental waters of Kenya and apply them as biocontrol agents against the pathogen.

A total of 122 environmental water samples used for domestic purposes were collected from two regions: Lake Victoria and Coast between March 2015, and October, 2016. Water samples were collected from ponds, lakes, rivers, wells and boreholes. *Vibrio cholerae* and other bacteria were isolated from these environmental waters, analyzed using PCR, and with products sequenced for further identification.

The environmental waters of Kenya are contaminated with the pathogenic *Vibrio cholerae* O1 El Tor. More work is ongoing to isolate and characterize more vibriophages

[ID: 100] Isolation and characterization of a novel *Staphylococcus aureus* bacteriophages

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Staphylococcus aureus is a bacterial pathogen of great significance in human and veterinary medicine. Emergence of multi-drug resistant *S. aureus* is a threat to the global health as some strains of the bacteria are highly resistant even to the current most potent antibiotics [1]. However, the development of new antibiotics has recently declined to a standstill as most pharmaceutical companies have ceased antibiotic production [2]. Use of bacteriophages for bioremediation and therapy is considered as a promising alternative. In this study we describe two novel lytic *S. aureus* phages with broad host range isolated from sewage and wastewater samples of the city of Tirana, Albania. The phages, named as Stab22 and Stab23, were isolated using a sausage-fermenting *S. xylosum* strain. Stab22 and Stab23 are myoviridae phages with genome sizes of 155,962 and 154,449 base pairs. In terms of physico-chemical properties the phages are stable at 39 °C, within pH range of 5.4 – 9.4, tolerate 25 – 50 µJ/cm² UV radiation and at 50% chloroform. The phages can infect several human MRSA and MSSA isolates originating from major hospitals in Helsinki. These results suggest that both phages could be potential candidates to be included in phage cocktails for phage therapy and biocontrol agent.

References

1. Kumar, M. Multidrug-Resistant *Staphylococcus aureus*, India, 2013–2015 - Volume 22, Number 9—September 2016 - Emerging Infectious Disease journal - CDC. doi:10.3201/eid2209.160044
2. Fernandes, P. & Martens, E. Antibiotics in late clinical development. *Biochem. Pharmacol.* 133, 152–163 (2017).

[ID: 102] Therapeutic potential of lytic bacteriophage against hematogenous *Staphylococcus aureus* meningitis

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Staphylococcus aureus is a major nosocomial infection leading to meningitis with high mortality rate [1]. The infection is further aggravated by multi-drug resistant *S.aureus* (MDRSA) [2]. Phage therapy could be applied against such infections to save life.

To evaluate the efficacy of phage therapy against hematogenous *S. aureus* meningitis using BALB/c mice.

Phages and MDRSA were isolated from sewage and waste water collected from Nairobi sewage treatment plant. Multi-drug resistant nature of the bacterium was established through antibiogram tests. Thirty BALB/c mice were randomly assigned into three groups; the MDRSA infection group (n=20), the phage-infection group (n=5) and non-infection group (n=5). Infected mice were either treated with a single dose of clindamycin (8mg/kg/bwt) or 108 PFU/ml of *S.aureus* phage or a combination (clindamycin and *S.aureus* phage) at 72 hours post-infection (p.i.). Thereafter, the animals' physical health and bacteremia levels were monitored for one week.

Administration of phage rescued 100% of the MDRSA infected mice. Brain tissues from the mice in phage therapy group had normal morphology unlike those from other treatment groups. This is a proof of concept that phage therapy is applicable against *S.aureus* meningitis as phages can cross the blood brain barrier.

References

1. Huang, W.-C., Lee, C.-H. & Liu, J.-W. Clinical characteristics and risk factors for mortality in patients with meningitis caused by *Staphylococcus aureus* and vancomycin minimal inhibitory concentrations against these isolates. *J. Microbiol. Immunol. Infect.* Wei Mian Yu Gan Ran Za Zhi 43, 470–477 (2010).
2. de Oliveira, A. P. R., Pappalardo, M. C., Dantas, D., Lins, D. & Vidal, J. E. Brain Abscess due to *Staphylococcus aureus* Of Cryptogenic Source In an Hiv-1 infected patient in use of Antiretroviral Therapy. *Rev. Inst. Med. Trop. São Paulo* 58, (2016).

[ID: 225] Anti-virulent properties of a phage-encoded depolymerase against *A. baumannii* K2 capsular type

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Capsular structures are seen as a universal virulence trait among gram-negative bacilli but still poorly studied in *Acinetobacter baumannii*. Recent reports suggest that capsule structures are involved in evading or overwhelming microbial defenses and macromolecular antibiotics. The fact that at least 106 capsular types exist in *A. baumannii* may reflect the sophisticated and diverse protective mechanisms developed by this pathogen. In this study, we isolated a 93,641-bp phage infecting *A. baumannii* K2 capsular type and cloned its tail-associated depolymerase (B3gp42). The enzyme showed to digest extracted exopolysaccharides in a wide range of pH values (5 to 9), ionic strengths (0 to 500 mM) and temperatures (20 to 70°C). Additionally, the enzyme is stable for at least 2 years. To assess the anti-virulence properties, the B3gp42 was tested against K2 strain using i) a human alveolar epithelial model, ii) a *Galleria mellonella* caterpillar model and finally iii) human blood (serum and neutrophil killing). In the human lung epithelium, B3gp42 demonstrated to be non-toxic and able to reduce the K45 strain virulence in a time-dependent manner. Complementary studies performed *in vivo* showed that B3gp42 was able to rescue larvae infected with either K2 strain pretreated with B3gp42 for 2 hours or with B3gp42 administered 30 min after bacterial inoculation. Additionally, we show that the B3gp42 could make the K2 strain fully susceptible to human serum and neutrophil killing, reducing the pathogen below detection limit (<10 CFU/mL). Overall, we show for the first time that the capsule is an important virulence factor of *A. baumannii* and that capsule removal via B3gp42 activity helps the host immune system to combat the bacterial infection. We conclude that capsular depolymerases represent a high therapeutic potential against *A. baumannii* drug-resistant infections.

[ID: 222] High-throughput phage isolation - from sample to sequence

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Enteropathogenic bacteria are a recurring concern in animal production. Many enteropathogenic strains can cause life-threatening infections in both humans and animals and several are also resistant to antibiotics. One example is post-weaning diarrhoea (PWD) in piglets caused by enterotoxins produced by Salmonella and E. coli. PWD is a key concern in the pig industry and numerous different approaches to cure or prevent this infection has been attempted, but so far, no strategy has been completely efficient.

Phage therapy is a promising alternative to the use of antibiotics but requires a collection of multiple highly virulent phages to a wide range of pathogenic hosts. Wastewater and other environments with high levels of enteropathogenic bacteria are the perfect places to search for such phages. A novel, high-throughput screening method based on incubation in microtiter well-plates allows for a faster and more efficient screening of a high number of samples requiring only small sample volumes.

Here we present screening of 188 individual wastewater samples applying three different host bacteria (E. coli, S. enterica and E. faecalis) which resulted in high numbers of identified phages. This novel screening method is highly effective and can become a valuable tool in the process of constructing efficient phage cocktails suitable for treating bacterial infections.

[ID: 172] *Pseudomonas aeruginosa* pseudolysogeny of phage-resistant mutants after PA5oct jumbo phage biofilm treatment

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The *Pseudomonas aeruginosa* PA5oct giant phage (formally vB_PaeM_PA5oct) was isolated and characterized previously (Drulis-Kawa et al., Arch Virol 2014).

The main purpose of this study was to evaluate the impact of PA5oct phage on biofilm host population (*P. aeruginosa* PAO1) and the emergence of phage-resistance. PA5oct phage-resistant isolates obtained as a result of controlled phage infection were tested in terms of virulence factors such as biofilm formation, type IV pili expression, LPS profile, pyocyanin, pyoverdinin and elastase production, serum resistance, TLR/NOD stimulation of THP1 Xblue (NF- κ B/AP-1- Reporter Monocytes) and *Galleria mellonella* larvae pathogenicity. In addition, the isolates were checked by PCR and pulsed electrophoresis (PFGE) for the presence of pseudolysogeny event.

A detailed biology analysis were done for randomly taken PA5oct phage-resistant mutants of PAO1 strain. In general, all tested isolates (compared to planktonic and sessile population of PAO1) exhibited no significant changes in the production of pyocyanin, pyoverdinin and elastase. Noticeable variability was observed in biofilm production and swarming motility. Investigation of LPS structure showed significant truncation of O-chain in 7/10 selected isolates. As a result, these mutants were also cross-resistant to other LPS-dependent phages (KTN6 and KT28). Interestingly, despite the LPS modification, the serum sensitivity of tested isolates has not changed.

In contrast to the controls, 9/10 selected of PA5oct phage-resistant mutants were able to activate the pro-inflammatory Nf- κ B transcription factor in THP1 Xblue macrophages. These isolates also showed reduced virulence tested in vivo on *G. mellonella* larvae model. The PCR and PFGE analysis revealed the presence of PA5oct phage (pseudolysogeny) in those mutants.

The PA5oct phage demonstrate high pseudolysogenization rate, resulting in permanent host physiology changes leading to overall virulence reduction. PAO1 mutants carrying the PA5oct episome are less pathogenic to *G. mellonella* larvae and stimulate the pro-inflammatory response, becoming an easy target for the immune system.

[ID: 218] Bacteriophages ordered in alternating electric field and immobilized by surface chemical modification as sensing element for bacteria detection

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Since the discovery of the bacteria and threat that they cause, detection of bacteria is important aspect of science and target of many studies. Bacterial infections concerns many fields, such as hospital care (90 000 deaths during hospitalization every year) or food industry (76 million illnesses annually). Thus, there is a constant need of development in this area.

Faster and more sensitive environmental monitoring should be developed to face the worldwide problem of bacterial infections. To remedy this issue, we demonstrate a bacteria-sensing element that utilizes dense and ordered layers of bacteriophages specific to the given bacteria strain. We combine (1) the chemical modification of a surface to increase the surface coverage of bacteriophages (2) with an alternating electric field to greatly increase the number of properly oriented bacteriophages at the surface. Usually, in sensing elements, a random orientation of bacteriophages results in steric hindrance, which results in no more than a few percent of all receptors being available. An increased number of properly ordered phages results in the optimal performance of phage receptors, manifesting in up to a 64-fold increase in sensitivity and a limit of detection as low as 100 CFU mL⁻¹. Our sensing elements can be applied for selective, sensitive, and fast (15 min) bacterial detection. A well-studied pair T4 bacteriophage - bacteria *Escherichia coli*, was used as a model; however, the method could be adapted to prepare bacteriophage-based sensors for detection of a variety of bacterial strains.

[ID: 108] Genomic characterization of lytic *Yersinia pestis* bacteriophages fEV-1 and fD1

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Lytic bacteriophages fD1 and fEV-1 infecting *Yersinia pestis*, the causative agent on plague, were isolated from the sewage treatment plant of Turku, Finland, using *Y. pestis* strains D27 and EV-76, respectively, as hosts. As last cases of plague in Finland were reported in 1711, we anticipate that their present time natural host is not *Y. pestis*. Here we report the complete genome sequences and morphological characterization of the phages. Transmission electron microscopy revealed that both phages have the morphology of myoviruses. Phage fD1 is a T4-like myovirus with a 167-kb dsDNA genome (accession number HE956711) and phage fEV-1 is a Dwarf myovirus with a 38-kb dsDNA genome (accession number LT992259). Annotation of fEV-1 genomic sequence revealed that most of the potential genes had no homologs in the databases. We also determined their host ranges in several species of Enterobacteriaceae and one-step growth curves. We additionally identified virion-associated phage and host proteins via LC-MS/MS. Further attempts to identify their host receptors could improve their potential use in phage therapy, including treating infections caused by *Y. pestis*.

[ID: 235] Characterization of phages and antibiotics synergistic (PAS) effect in E. coli

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The emergence of antibiotic-resistant bacteria is one of the most serious threats for healthcare and public safety worldwide. The therapeutic use of bacteriophages could be one of the effective alternatives to antibiotic therapy. Recent data obtained by several groups have re-actualized the synergistic effect of phage and antibiotics when administrated in combination [1, 2].

In the present study we measured the combined action of different bacteriophages and various classes of antibiotics on E. coli growth and mutagenesis. Our results show that the production of T5 phage particles was stimulated in the presence of low concentrations of antibiotics and particularly by beta-lactams such as ampicillin, but also by chloramphenicol and spectinomycin. Bacterial resistance acquisition and mutagenesis frequencies were reduced in the presence of phages combined with sub-lethal concentrations of antibiotics compared to sub-lethal concentrations of antibiotics only. These results suggest that the combined use of phages and antibiotics for treatment of bacterial infections could be more efficient and less prone to resistance appearance than antibiotic therapy or phage therapy by themselves. Our studies pave the way to phage therapy used in combination with antibiotic therapy to treat infectious diseases and make particular sense for misused or post-administration antibiotics.

1. Comeau AM, Tétart F, Trojet SN, Prère M-F, Krisch HM (2007) Phage-Antibiotic Synergy (PAS): β -Lactam and Quinolone Antibiotics Stimulate Virulent Phage Growth. PLoS ONE 2(8): e799. doi:10.1371/journal.pone.0000799.

2. Fatima Kamal and Dennis Jonathan J. Burkholderia cepacia Complex Phage-Antibiotic Synergy (PAS): Antibiotics Stimulate Lytic Phage Activity. Appl. Environ. Microbiol. February 2015 vol. 81 no. 31132-1138.

[ID: 66] Characterization of shiga toxin encoding bacteriophage and transfer of shiga toxin genes to nonpathogenic E. coli

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Food-borne pathogen having shiga toxin (stx) has caused diarrhea and hemolytic uremic syndrome. Stx genes from bacteriophages might be suggested to be transferred to non-pathogenic host bacteria. E. coli host infection bacteriophage were isolated 19 phages from river and waste water. Isolated nine phages of the 19 phages were encoded stx1 or stx2 genes or both. Analysis of basic physiological characteristics of bacteriophage with both stx1 and stx2 and stability analysis in various environments revealed that ϕ NOECP 49 belonged to the Myoviridae family. One-step growth analysis showed a latent period of about 15 min and a burst size of 41.8 PFU/infected cells. The time required for one cycle was about 25 minutes. When exposed to high temperature, it showed stability at 60 ° C, but showed a low stability at 70°C. As a result of exposure to pH 2, pH 3, pH 5, pH 7, and pH 10 for 1 hour, it showed stability at various pH, and showed high stability even at pH 3, strongly acidic. As a result of exposure to 30%, 50%, and 70% ethanol for 1 hour, it showed low stability at 50% and 70% ethanol. 0 to 500 ppm of sodium hypochlorite for 30 minutes showed overall stability. Stx genes encoded in five bacteriophages were transferred to non-pathogenic host bacteria, which were non-O157 E. coli and did not be encoded with stx gene. Transferred non-O157 E. coli by ϕ NOECP 49 phage was encoded with stx1 and stx2 genes. When the E. coli was exposed in osmotic conditions, shiga toxin were expressed. The E. coli was incubated at salt conditions of 1%, 3%, 5%, 7% and 9% during 18hr. Stx1 expressed highest at 5% salt conditions. Therefore, bacteriophage in environment might be transferred to E. coli and be related to the food-borne diseases.

[ID: 4] Exploring properties of Russian and Georgian therapeutic cocktails

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Greater understanding of how bacteriophage infection progresses in environmental conditions and the potential impacts of these infections on the broader microbial community will be important in achieving full regulatory approval worldwide. Furthermore, it will aid in the development of more effective therapies. One area of potential concern is the recently described “super spreader” phenotype reported for at least two Felix 01-like E.coli phages. These lytic phages purportedly facilitate the spread of antibiotic resistance plasmids, possibly through their inability to break down the circular plasmid DNA and its subsequent uptake by unrelated, naturally competent bacteria (Keen et. al. 2016). The role of temperate phages in the horizontal transfer of bacterial genes through transduction is well studied, and leads to their avoidance. However, the possible transmission of antibiotic resistance through transformation by plasmids released by phage lysis is an important new consideration in the context of phage therapy. In order to better characterize the existing therapeutic phage preparations, we are isolating and characterizing a number of E.coli phages from Georgian and Russian Intestiphage and Pyophage cocktails, including testing for the possibility of the “super spreader” phenotype.

[ID: 269] Transcription analysis of *L. monocytogenes* 10403s prophage during lysogeny, lytic and active lysogeny cycles, reveals a unique regulation of prophage genes during bacterial infection of mammalian cells

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The human bacterial pathogen *Listeria monocytogenes* harbors a prophage within its genome, which is known to reproduce by both lytic and lysogenic cycles. We have previously shown that this prophage adapts an unusual behavior when *L. monocytogenes* infects mammalian cells. During macrophage cells infection the prophage, which is inserted within the *comK* gene, excises its genome leaving an intact *comK* gene that is necessary to facilitate bacterial phagosomal escape. Even though, phage excision occurs, it does not lead to generation of progeny virions and bacterial lysis, suggesting that the prophage cooperates with its host to promote successful mammalian cells infection. We termed this novel phage behavior active lysogeny, as the prophage is highly active transcriptionally and genomically during this novel lysogenic mode.

The objective of this work was to decipher the regulatory mechanisms of active lysogeny. We performed a transcriptional analysis of the three life cycles of the phage: lytic, lysogenic and active lysogenic; using genome-wide RNA-seq and Nano-string analyses. A unique transcriptional profile was discovered for each state.

Here we reveal for the first time a unique transcriptional behavior of the prophage during active lysogeny demonstrating specific downregulation of the late lytic genes during mammalian infection. These findings strengthening the premise that *L. monocytogenes*' prophage evolved to cooperate with its host during intracellular infection.

[ID: 79] The bacteriophage T4 MotB protein, a bactericidal DNA binding protein, improves phage fitness

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Viruses have evolved multiple means for overcoming defense mechanisms in their host to allow for optimal viral proliferation. In bacteria, histone-like proteins, such as H-NS and StpA, are DNA binding proteins that form higher-order nucleoprotein complexes that typically repress transcription by targeting AT-rich DNA sequences. As phage genomes often display a high AT content, H-NS can protect bacteria from the expression of phage-encoded genes by preferentially binding these sequences.

Bacteriophage T4 (65.5% AT) is a lytic virus that infects *E. coli*, (45% AT) resulting in cell lysis after ~20 min. We found that despite being nonessential, *motB* is conserved among T4-type phages and that a *motB* amber mutant (T4*motB*am) produces a two-fold lower burst size compared to T4 wild-type infections. Transcriptome analysis of a T4*motB*am infection revealed impaired expression of several late genes at 5 minutes post-infection. Primer extensions indicated that differences are no longer present 10 minutes post-infection, suggesting that delayed expression of select late genes is not sufficient to limit burst size.

We discovered that MotB production is extremely toxic when expressed in *E. coli* resulting in the decondensation of host DNA, cell lengthening, significant reduction in actively dividing cells compared to a vector control, and cell lysis. MotB binds tightly and nonspecifically to both host and T4 DNA and co-purifies with bacterial H-NS and StpA. Footprinting assays revealed the MotB, like H-NS, spreads along large regions of DNA.

Our results indicate that the T4 *motB* gene encodes a bactericidal DNA binding protein that improves the fitness of T4 infections. We hypothesize that the interaction of MotB with DNA may be part of a mechanism used by T4 to disrupt H-NS dependent DNA condensation, leading to a more productive infection.

[ID: 90] The power law of CRISPR-Cas systems

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Systems of adaptive immunity in bacteria and archaea are composed of the fingerprints of viruses and other mobile genetic elements. Spacers inserted into CRISPR arrays in microbial genomes match DNA of potential invaders, and are collected for future use. We demonstrate that global distribution of numbers of spacers between CRISPR arrays, across multiple biomes worldwide, exhibits scale invariant, power law behavior. The same pattern is found within single ecosystems of diverse nature.

Heavy-tail behavior of power law type distributions indicates rather high probability of microbes resistant to tremendous number of phages. These all-resistant super hosts can survive virtually any well adapted virus' infection and re-establish bacterial population. Due to the fitness cost of possessing and operating long CRISPR arrays [1], microbes that are losing spacers more rapidly, replicate faster and reproduce resource base for the next, yet to come, infection in a given ecosystem. Thus, the Red Queen dynamics in host-pathogen coevolution is limited to the bottom (fewer spacers) less resistant part of microbial populations. The same logic applies to macrosystems (biomes, countries, continents, oceans, and the Globe), due to the scale-invariance of power law distributions. In general, equilibrium microbial population would be observed for relatively long periods, interspersed with rare unpredictable mass extinction events. Newly established populations may organize into exactly the same ecosystem, or they may form new interspecies relationships, which may create or extinguish some ecological niches, depending on various biotic and abiotic factors. This behavior is similar to self organized criticality models [2] and suggests no contradiction between the Red Queen and the Court Jester [3] hypotheses.

References

1. Westra, E. R., S nderhauf, D., Landsberger, M. & Buckling, A. Nature reviews Immunology (2017)
2. De Los Rios, P. & Zhang, Y.-C. Physical Review Letters (1999)
3. Benton, M. J. Science (2009)

[ID: 368] Emergence of phage resistance in *Listeria monocytogenes* treated with individual and combined phages

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Bacteriophages are approved for use on food in a number of countries to control *Listeria monocytogenes*, a problematic foodborne pathogen with a high mortality rate. Here, we explore the dynamics of phage-resistance emerging in populations of *L. monocytogenes* following treatment with bacteriophages.

We infected *L. monocytogenes* 10403S (n = 9) at a concentration of $\sim 8 \times 10^7$ CFU/mL with phage LP-048 (binds rhamnose), LP-125 (binds N-acetylglucosamine and rhamnose), or both phages (MOI: 1.3 ± 0.2 for all conditions). Optical densities of *L. monocytogenes* cultures were monitored for 60 hours to observe cell lysis and subsequent regrowth of bacteria.

Average bacterial survivor counts for cultures infected with LP-048, LP-125, or both phages were 357 CFU/mL (39 CFU/mL standard error [SE]), 87 CFU/mL (31 CFU/mL SE) and 156 CFU/mL (64 CFU/mL SE), respectively. Phage-resistance assays performed 6-7 hours post-infection resulted in LP-048-infected cultures that were $\sim 96\%$ resistant to both phages and $\sim 4\%$ resistant to only LP-048; LP-125-infected cultures were $\sim 38\%$ resistant to both phages and $\sim 60\%$ resistant to only LP-125; cultures infected with both phages were $\sim 100\%$ resistant to both phages. Emergence of phage-resistant *L. monocytogenes* was observed by spectrophotometry and plate assay under all conditions between 24 and 36 hours post-infection, where LP-048-infected cultures showed visible regrowth between 24-26 hours, and cultures infected with either LP-125 or both phages showed visible regrowth between 25-36 hours. Early regrowth (≤ 26 hours) was consistently observed in all LP-048-infected cultures and LP-048-infected cultures yielded the greatest number of bacterial survivors post-infection. Phages isolated from late post-infection, particularly LP-125 isolates, demonstrated the ability to overcome phage-resistant strains of *L. monocytogenes*.

Further research is needed to model the emergence of phage-resistant populations of *L. monocytogenes* in food processing facilities post-phage treatment to better predict and improve the efficacy and long-term use of phage biocontrol in food safety applications.

[ID: 36] Temperate and virulent coliphages isolated from 1 year-old children gut microbiota have contrasted infectivity in this ecosystem

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A link between infant gut microbiota composition and asthma predisposition starts to emerge. Since bacteriophages constitute an important component of the gut microbiome, we investigated if they could influence early life microbiota colonization and maturation, and whether they play a role in the risk of asthmatic disease development, in the frame of the 'Earlyvir' european-canadian project, based on the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) cohort.

Because host prediction from phage sequences still represents a challenge, culturomics represents a biologically relevant alternative to determine which species of the microbiota are phage targets. *Escherichia coli* is one of the first bacterial species to colonize the infant's gut, and still represents 6% of total OTUs at 1 year. We extracted viromes from feces of 700 1 year-old children and spotted them on 2 indicator strains. In 24% of 339 virome samples tested, coliphages could be cultivated.

This collection consisted in 45 temperate and 30 virulent coliphages, and was further studied for its host range, over 90 *E. coli* strains also isolated from of 1 year-old infant feces and representative of the cohort. Strain response was markedly different when challenged with the two phage groups: 82% of strains were resistant to all temperate phages, while only 7% of them were resistant to all virulent phages. The host range also differed, with 30% of the virulent phages killing at least 30% of the strains, whereas none of the temperate phages could kill 30% of the strains. We conclude that the virulent fraction has a broad host range and contains most of the lytic activity. In the light of virome sequence analyses, our results suggest that not all phages will be equal in terms of impact on the microbiota.

[ID: 339] Xenogeneic silencing of cryptic prophages in *Corynebacterium glutamicum*

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Horizontal gene transfer is a pivotal driver of bacterial evolution and allows bacteria to rapidly acquire novel beneficial traits. However, the introduction of foreign DNA into the host regulatory networks needs to be tightly regulated as the high and uncontrolled expression, especially of viral genes, may cause high metabolic costs and/or will lead to cell death. To enable a decent integration of newly acquired DNA, cells harness the activity of a special group of nucleoid-associated proteins (NAPs) acting as so-called xenogeneic silencers (XS) [1].

In our studies, we investigate cryptic prophages encoded in the *Corynebacterium glutamicum* strain ATCC 13032. The largest phage element, CGP3 (187 kb), was shown to undergo spontaneous activation in a small fraction of cells even under standard cultivation conditions [2]. In recent studies, we – for the first time – reported on a prophage-encoded XS protein named CgpS. This protein shares intriguing similarities with the mycobacterial XS Lsr2. By genome-wide binding analyses, we confirmed the preferred binding of CgpS to foreign, AT-rich regions, especially to CGP3, but also to other horizontally-acquired regions. Furthermore, using a truncated variant of CgpS prevented the correct oligomerization of CgpS, thereby causing prophage induction and severe growth defects. Bioinformatical analysis revealed orthologous proteins in almost all Actinomycetes, but remarkably, also in several phage and prophage genomes [3]. Our results emphasize CgpS as a key factor for the control of CGP3 activity and highlight the importance of small NAPs for the control of foreign DNA in bacterial host strains.

References:

- [1] Singh K, JN. Milstein & WW Navarre. (2016). *Annu Rev Microbiol*. doi: 10.1146/annurev-micro-102215-095301
- [2] Helfrich, S., E. Pfeifer, C. Kramer, C.C. Sachs, W. Wiechert, D. Kohlheyer, K. Noh & J. Frunzke. (2015). *Mol Microbiol*. doi: 10.1111/mmi.13147
- [3] Pfeifer, E, M. Hünnefeld, O. Popa, T. Polen, D. Kohlheyer, M. Baumgart & J. Frunzke. (2016). *Nucleic Acids Res*. doi: 10.1093/nar/gkw692

[ID: 127] Enological environment as a source of a novel Tectivirus

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The Gluconobacter phage GC1 is a novel member of the Tectiviridae family isolated from a juice sample collected during dry white wine making. The bacteriophage infects *Gluconobacter cerinus*, an acetic acid bacterium which represents a spoilage microorganism during wine making, mainly because it is able to produce ethyl alcohol and transform it into acetic acid. Transmission electron microscopy revealed tail-less icosahedral particles with a diameter of ~78 nm. The linear double-stranded DNA genome of GC1 (16,523 base pairs) contains terminal inverted repeats and carries 36 open reading frames, only a handful of which could be functionally annotated. These encode for the key proteins involved in DNA replication (protein-primed family B DNA polymerase) as well as in virion structure and assembly (major capsid protein, genome packaging ATPase and several minor capsid proteins). GC1 is the first tectivirus infecting an alphaproteobacterial host and is thus far the only temperate tectivirus of gram-negative bacteria. Based on distinctive sequence and life-style features, we propose that GC1 represents a new genus within the Tectiviridae, which we tentatively named "Gammatectivirus". Furthermore, GC1 helps to bridge the gap in the sequence space between alphatectiviruses and betatectiviruses.

[ID: 119] Isolation and characterization of a Salmonella virulent phage Jersey-like virus from Thailand

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Salmonella enterica subsp. enterica is a major cause of a worldwide food-borne disease called Salmonellosis. Although many Salmonella phages have been isolated and studied in Western countries, very few completed characterizations have been reported from Southeast Asia. In this study, a total of 285 samples, including chicken dropping and sewage from Thailand, were subjected to Salmonella phage isolation. Of 285 samples, the positive Salmonella phage isolation is 68% (194/285 samples). The isolated phages contained both of the temperate and virulent phages. Among the isolated virulent phages, phiSE-W109 showed a broader host range of Salmonella infection. All of the 121 isolates of Salmonella could be lysed by the phiSE-W109 and these isolates included 48 isolates of drug-resistance Salmonella. In contrast, none of other Gram-negative (n=26) and Gram-positive (n=17) tested bacteria were lysed. Transmission electron microscopy indicated that phiSE-W109 belongs to the Siphoviridae family. Phage receptor identification indicated that LPS is the main receptor for the phiSE-W109 infection. Genome sequence analysis indicated that the phiSE-W109 has a genome size of 42,147-bp and share 94.0% homology with Salmonella phage SE2 (a member of the Jersey-like virus genus). The completed genome sequence of this phage does not contain any genes involved in lysogeny or bacterial virulence genes, indicating that it is a strictly virulent phage. In conclusion, we report herein the first completed genome sequences of a Jersey-like virus isolated from Thailand. Broad host range and strongly lytic properties of isolated Salmonella phage SE-W109 indicated its high potential to be developed as biocontrol agent for Salmonella infection.

[ID: 377] Bacteriophage interaction with *P. aeruginosa* and *A. baumannii* biofilms and colonizing human airway epithelium

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Antibiotic resistance constitutes one of the major worldwide public health problems. According to the World Health Organization, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are currently considered as top priority pathogens urgently requiring the development of effective therapies. These bacterial species are a common cause of hospital-acquired pneumonia, which is considered the leading cause of mortality among nosocomial infections. Besides displaying resistance to a wide range of antibiotics, both bacterial species have an ability to form biofilms on different surfaces, including biomedical devices and human epithelium. The complete eradication of biofilms is currently an almost impossible task and unless new and effective antibacterial strategies quickly emerge, the implications on public health will be devastating.

In this work, the interaction of phages with biofilms of different ages (24h, 72h, 7d) was analysed, as well as their efficacy against bacteria colonizing human bronchial epithelium. Two phages were used in this study: a newly isolated and characterized *P. aeruginosa* phage (vB_PaeP_PE3) and the previously characterized *A. baumannii* phage vB_AbaP_B5. After 3 and 6h of phage treatment, the number of viable cells and total biomass present in *P. aeruginosa* and *A. baumannii* biofilms was significantly reduced in most cases, depending on biofilm age and culture medium used for biofilm formation. After 24h of biofilm infection, as expected, phage insensitive mutants (BIMs) emerged causing an increase of biofilm cells. Among the isolated bacterial colonies at this time point, the percentage of BIMs found was approximately 77% for *P. aeruginosa* and 100% for *A. baumannii*. Nonetheless, phage treatment of NuLi-1 airway epithelial cells colonized with each species resulted in a significantly reduced cell death.

Overall, bacteriophages vB_PaeP_PE3 and vB_AbaP_B5 demonstrated to be a valuable approach for the treatment of biofilms formed in both abiotic and biotic surfaces.

[ID: 80] Crystal structure and functional analysis of the Ts2631 endolysin of *Thermus scotoductus* phage vB_Tsc2631 with the unique N-terminal moiety

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In order to escape from their hosts after replication, bacteriophages often use endolysins which can degrade the bacterial peptidoglycan. While mesophilic phages have been extensively studied in the past, their thermophilic counterparts are not well characterized. We analysed the sequence, structure and function of the Ts2631 endolysin from the thermophilic phage vB_Tsc2631 which is a zinc-dependent type 2 amidase (EC 3.5.1.28). Adaptation of this protein to the thermophilic lifestyle is mediated by an unusually high number of proline and tryptophan residues, plus an intramolecular disulphide bridge. The endolysin contains a positively charged N-terminal extension of 20 residues which mediates dimerization in the crystal structure via 3D domain swap. The active site of Ts2631 consists of three essential residues His30, His131 and Cys139 involved in Zn²⁺ coordination and catalysis. Active site residues are dispensable for peptidoglycan binding but not lysis. To elucidate residues important for peptidoglycan binding, we tested single aa substitution variants for their activity and identified six to be essential. Substitution of Cys80, resulting in lack of the disulphide bridge, causes inactivation of Ts2631 similar to substitutions of H31, T32 (being a part of conserved His/His/Thr triad), Y60, K70, and N85 residues. Out of the 24 residues tested, Y60 and K70 turned out to be necessary for peptidoglycan binding. Moreover, we showed that the wild type Ts2631 endolysin has bactericidal effect against *Thermus thermophilus* HB8 and deletion of 20 amino acids from the N-terminus (delta2-22 derivative) abolished this activity. The N-terminus is also essential for peptidoglycan binding. Because Ts2631 endolysin features intrinsic antibacterial activity and unusual thermal stability, it is perfectly suited as scaffold for the development of an antimicrobial protein against human or food pathogens.

[ID: 430] Characterization of novel podoviruses infecting different *Acinetobacter baumannii* capsular types.

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Acinetobacter baumannii is one of the most clinically important pathogens associated with hospital-acquired infections worldwide. This microorganism is capable to produce a vast variety of capsular polysaccharides differing from each other by composition. Being important virulence factor, CPS is a powerful element of bacterial defense against environmental conditions and biological systems. By now, more than 130 types of chromosomal capsule loci have been identified in *A. baumannii* strains and their number constantly increases.

In this study, we present seven novel viruses, specifically infecting and lysing *A. baumannii* strains of K2, K32, K37, K44, K48, K87, and K89 capsular types or K-types. The phages were identified as members of the family Podoviridae by transmission electron microscopy. The overall genomic architecture and homology of genes analyses showed that the viruses are the representatives of Friunavirus genus of the Autographivirinae subfamily. The linear double-stranded DNA genomes of these phages share high nucleotide sequence identity with the most variable regions falling in genes encoding structural depolymerases or tail spikes which determine host specificity.

Deletion mutants lacking N-terminal domains of the tail spikes were cloned, expressed and purified by immobilized metal ion affinity chromatography with subsequent ion-exchange chromatography and gel-filtration. Purified depolymerases are highly specific and form opaque haloes on the bacterial lawns of *A. baumannii* strains belonging to corresponding K-types. Bacterial CPS digestion products were analyzed by nuclear magnetic resonance spectroscopy, and mechanisms of enzymes action have been established.

This work was supported by the Russian Science Foundation, Grant # 18-15-00403

[ID: 50] Evolutionary history of bacteriophages in the genus *Paraburkholderia* and novel inducible prophage from *P. terrae* strain BS437

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The genus *Paraburkholderia* encompasses mostly environmental isolates with diverse predicted lifestyles. Genome analyses have shown that bacteriophages form a considerable portion of some *Paraburkholderia* genomes. Here, we analyzed the evolutionary history of prophages across genus *Paraburkholderia*. Specifically, we investigated to what extent the presence and distribution of prophages affect the diversity/diversification of *Paraburkholderia* spp., and coevolution of these partners. Moreover, particular attention was given to the presence of CRISPR-Cas arrays as a reflection of past interactions with phages. Analysis of 39 genomes of *Paraburkholderia* spp., including those of 11 new strains, and three *Burkholderia* species, revealed the presence of at least one prophage per genome. The highest number was found in *Paraburkholderia* sp. MF2-27, in which nine prophages were found, amounting to up to 4% of the genome. Among all prophages, a key moron gene, DNA adenine methylase, was found, that might be advantageous for host fitness. Cophylogenetic analyses showed complex evolutionary scenarios between the different *Paraburkholderia* hosts and their prophages, including short-term cospeciation, duplication, host switching and phage losses. Analysis of the CRISPR-Cas systems showed a diverse record of past, potentially recent, phage infections. Reflecting their interaction over evolutionary time. To isolate phage from mycosphere inhabitants, we screened mycosphere and bulk soils for phages able to produce plaques, however found these to be below detection. Based on bioinformatics analysis, one contiguous sequence predicted to encode a complete phage was found in the genome of *P. terrae* BS437. Thus, mitomycin C was used to produced high-titered phage suspensions. The prophage (denoted ϕ 437) progeny was validated by TEM and phage major capsid gene molecular detection. We obtained the full sequence of ϕ 437, which, remarkably, had undergone genome reshuffling. One predicted moron gene was found, and it is currently analyzed to understand the extent of its ecological significance for the host.

[ID: 432] Host recognition by podoviruses G7C and Alt

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The O22 E. coli strain 4s is a host to a number of bacteriophages that compete and, possibly, help each other to survive in the complex and dense microbiological environment of the lower intestine of a horse. N4-like phages G7C and Alt are close relatives with essentially identical genomes, except for a single gene the product of which is responsible for host recognition and forms a tailspike on the phage particle. We used a combination of X-ray crystallography and cryo-electron microscopy to determine the structure of G7C tail in atomic details. It carries twelve copies of two different tailspikes that form a branched structure. The long tailspike has a remarkable multidomain structure and bear an attachment platform for the other spike. This protein is identical in both phages. The short spikes of G7C and Alt have a set of nearly identical N-terminal domains that interact with the long one and strikingly different C-terminal receptor-binding domains. The short spike of Alt is a lyase. It degrades the E. coli 4s O-antigen into small fragments. In contrast, the short spike of G7C keeps the backbone of the same O-antigen polymer intact but removes one O-acetyl group per its repeating unit. G7C and Alt utilize non-homologous domains and different types of enzymatic activities to recognize and bind to the same cellular receptor molecule. Functional analysis of host recognition by G7C, Alt, and their viable tailspike mutants revealed that O-antigen binding and modification are critically important for infection. Moreover, the kinetics of O-antigen processing by the tailspikes appears to control the transition from primary host recognition to irreversible adsorption and subsequent DNA release. Our findings demonstrate that phages with identical particle structure and tail architecture can utilize strikingly different biochemical mechanisms to efficiently recognize, bind, and infect host cells.

[ID: 387] Inhibitory effect of bacterial viruses on early and late gene expression of adenoviral mRNA in vitro

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During our studies on the interactions between eukaryotic and bacterial viruses we have observed a significant reduction in human type 5 adenovirus (HAdV-5) titer in the A549 cell line when coinocubated with *E. coli* T4 bacteriophage or staphylococcal A5W/80 bacteriophage. The aim of the current study is to assess the influence of T4 and A5W/80 phages on adenoviral mRNA expression in in vitro cell culture.

A549 cells infected with low (100 TCID₅₀/mL) and high (1000 TCID₅₀/mL) titers of HAdV-5 were incubated with purified phage preparations. Effective phage doses were calculated on the basis of previous results from experiments investigating phage influence on HAdV-5 titer. Time dependent assays with initial (before HAdV-5 infection) or continuous phage incubation (after HAdV-5 infection) were performed and mRNA expression of early (DNA binding protein) and late (hexon) genes of HAdV-5 were measured.

Expression of early HAdV-5 gene was affected only in cells with high HAdV-5 titer continuously incubated with phage T4. Preincubation, as well as continuous cell incubation with both phages significantly decreased expression of late adenoviral genes when cells were infected with a low HAdV-5 titer. When a high HAdV-5 titer was applied we observed the same effect for phage T4 whereas phage A5W/80 could only inhibit late gene expression during continuous incubation but not after preincubation with the cells.

Results of this study suggest that the inhibitory effect of phages on HAdV-5 infection may be partially explained by HAdV-5's influence on the expression of early and late adenoviral genes at mRNA level.

Acknowledgments: This work was supported by funds from the National Science Centre, Poland for the project grant based on decision No. DEC-2013/11/B/NZ1/02107.

[ID: 247] Characterization of bacteriophage T5 pre-early genes: elucidating the minimal set of genes for host takeover

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Bacteriophage T5 uses a two-step strategy to deliver its DNA into its host *Escherichia coli*. First, 8% of the genome enters the cell and the injection stops. During the pause, phage pre-early genes are expressed, inactivating host defense systems and triggering the massive degradation of the host genome; then the genome transfer resumes, allowing phage replication cycle. This unique mechanism of DNA delivery makes T5 is an attractive model to study the role of pre-early genes in the host takeover process.

Among the sixteen T5 genes introduced in the bacteria in the first step, only A1 (encoding a DNase) and A2 (a DNA-binding protein) have been studied. The role of most other genes is still unknown. To assess their function we follow two strategies: i) reverse genetics to study the effects of T5 gene inactivation on infection and ii) testing the impact of phage gene ectopic expression on host growth kinetics.

For the reverse genetic approach, we optimized the CRISPR/Cas9 system¹ to improve the screening of T5 mutants engineered by homologous recombination. Hence, conversely to what had been proposed previously², we observed that some pre-early genes conserved in all T5 viruses are dispensable (O2, O5). In the second approach, ectopic expression of viral genes O5 and A1 was toxic to *E. coli* while that of genes A2, dmp, O2, O7 did not affect bacterial growth. Further work will aim to understand the nature of this toxicity.

Thus, our work aims to describe the minimal set of proteins a phage must deploy for host takeover, opening opportunities for the conception of biotechnological tools and developing new antimicrobial strategies.

References

1. Tao et al. Engineering of Bacteriophage T4 Genome Using CRISPR-Cas9. *ACS Synth. Biol.* (2017).
2. Davison. Pre-early functions of bacteriophage T5 and its relatives. *Bacteriophage* (2015).

[ID: 431] Staphylococcus aureus mutants resistant to bacteriophage K

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Staphylococcus aureus is an opportunistic pathogen colonizing roughly thirty percent of the population. The number of staphylococcal infections continues to increase in the hospital as well as the community; however, treatment has become difficult because of the emergence of staphylococcal strains resistant to multiple, and often last resort, antibiotics. With the urgent need for new treatments against multidrug-resistant bacteria such as *S. aureus*, bacteriophages have received renewed interest. While there are advantages and disadvantages of phage therapy, one notable disadvantage is the ability of the bacteria to become phage resistant. Because this can be a major barrier to phage therapy, it is important to understand phage-host dynamics and resistance mechanisms. We have isolated eighteen spontaneous *S. aureus* mutants resistant to bacteriophage K, a strictly lytic broad host range staphylococcal phage. Whole genome sequencing identified single-nucleotide polymorphisms (SNPs) in each of the resistant strains. Mutations that conferred resistance to phage K were found within the wall teichoic acid biosynthesis operon, RNA polymerase beta-prime subunit, ligase, and tRNA(Gln). These mutations provide insight into phage receptors, replicative pathways, and other cellular components that are crucial for phage growth. We also screened a *S. aureus* transposon library for phage K-resistant mutants. Many transposon mutants were resistant to phage K. Of interest are the mutants in which the transposon disrupted genes in the polyamine operon transport system, potABCD. This has led to several testable hypotheses that we will pursue regarding interactions between this transport system, polyamines, and phage K growth. Our findings identified bacterial mutants that are resistant to a strictly lytic broad host range staphylococcal phage. Understanding phage resistance mechanisms is crucial to successfully using phage as a therapeutic. By characterizing resistance mechanisms this could provide insight on ways to genetically modify phage or use characterized phage cocktails to overcome the resistance barrier.

[ID: 309] Are phages of wide host range a better alternative for their use for biocontrol?

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Phages have been lately used during as biocontrol agents in different foods. The main limitation of their use for biocontrol is the appearance of bacterial resistance against phages. Phages of wide host range could delay the appearance of phage-resistant mutants, since they select lower frequency of resistant mutants or because they can easily overcome bacterial resistance.

The purpose of this study was to compare the frequency of resistant mutants of *Salmonella* Infantis (SI) and their phenotypic changes in response to two phages of the Felixo1virus genera, one of wide host range (vb_SI_SF20-2) and one of narrow host range (vb_SI_QUI-1). Both were previously sequenced and characterized by host range and TEM. Two independent assays were conducted at 37 ° C with stirring. Assay A (AA) contains SI (OD600 = 1) and phage vb_SI-SF20-2 and assay B (AB) SI (OD600 = 1) and phage vb_SI-QUI-1. MOI 0.0001 and 3 controls (TSB, TSB-phage, SI-TSB) were used. Phage-specific primers were designed to control assays. Two samples of 100 uL were taken every hour, for 12 hours. One sample was plated on an overlay of TSA and the other sample was 10-fold diluted, and dilutions 10-5 and 10-10 were plated. Both assay were independently replicated. Efficiency of plating (EOP) was calculated using *Salmonella* after 12 hour-incubation and wild type phages.

In both assays, a similar frequency of resistant mutants was observed on dilutions 5 and 10 since the first hour. The EOP showed a reduction of 20-50% in the resistant mutant in both assays. The morphology of phages in AA assay was changed and the plaque size increased. This is a pioneering study that shows the existence of phenotypic changes in phages during *Salmonella* challenge, which could indicate that phages of wide host range are more efficient when used for biocontrol.

[ID: 233] Infection dynamics of lytic phage phiPLA-RODI during biofilm formation of *Staphylococcus aureus*

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The human opportunistic pathogen *Staphylococcus aureus* forms biofilms on a wide range of biotic and abiotic surfaces, which protect the microbe from routine disinfection procedures and antibiotic treatment. Therefore, it is necessary to develop novel strategies to prevent and eradicate biofilms from inert surfaces and human tissues. One interesting option is the use of bacteriophages as antibiofilm agents. However, we still do not fully understand the interactions between phages and their bacterial hosts during biofilm formation.

In a previous study we showed that low-level predation of *S. aureus* by the virulent phage phiPLA-RODI led to significant transcriptional changes in the bacterial population, as well as the development of highly stable DNA-rich biofilms. This work aims to study further the dynamics of the interaction between the phage and bacteria throughout the establishment and development of staphylococcal biofilms. On the one hand, we monitored expression changes in selected genes at different time points (3, 5, 7 and 24 h) during biofilm formation. This experiment revealed that most gene changes were associated with biofilm maturation. We also assessed the evolution of phage titer in the planktonic phase and the biofilm in relation to the bacterial cell number when the initial MOI was 10-5. Our results showed that phage numbers increase steadily until 7 h of biofilm development and then decrease between the 7 and 24 h time points. This suggests phage inactivation by extracellular enzymes such as proteases, produced by *S. aureus* during stationary phase. Also interestingly, phage propagation in the biofilm never reached levels akin to those observed in the planktonic phase. This phenomenon would facilitate phage: host coexistence in attached multicellular communities.

Overall, these results contribute to our understanding of the differences in bacteria: phage dynamics between planktonic and biofilm lifestyles. Moreover, this information can be useful for the development of antimicrobial phage-based products.

[ID: 34] The bacterial host determines the life-cycle of Sa3-bacteriophages

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Staphylococcus aureus possesses a set of virulence factors necessary for infection of the human host some of which are encoded on bacteriophages. Sa3-phages integrate specifically into the *hlyB*-gene thereby leading to a loss of β -hemolysin production. However, these *hlyB*-converting phages provide additional virulence genes coding for human specific immune modulatory factors. About 90% of all *S. aureus* strains of human origin carry these *hlyB*-converting phages, whereas animal strains are usually devoid of them.

We aimed to analyze the interference of the bacterial host background with the biology of these Sa3-phages, especially phage Φ 13. Phage-cured *S. aureus* strains 8325-4, USA300-c, Newman-c (clonal complex 8) and MW2-c (clonal complex 1) were lysogenized with Sa3-bacteriophage Φ 13Kana carrying a kanamycin resistance cassette. Phage transfer and phage induction was quantified by co-culture experiments and qPCR. Transfer and phage induction was significantly higher in the lysogenic strains of MW2c and Newman-c compared to 8325-4 and USA300c. Phage integration into the *hlyB*-gene was validated by Multiplex-PCR and pulsed-field gel electrophoresis (PFGE). PFGE analysis followed by Southern hybridization revealed multiple phage copies integrating into the *hlyB*-gene during first step of lysogenization. Changes on the transcriptional level of different genes were investigated by northern blot hybridization. A gene with unknown function, *orfC*, is located in the antisense direction between genes coding for integrase and *cl*-repressor. Expression of *orfC* was significantly increased after mitomycin C treatment. The expression of *orfC* also correlated with the strain specific phage inducibility. In summary, the bacterial background has a severe impact on phage mobilization. Strain specific regulation of *orfC* may be crucial for phage-host interaction.

[ID: 35] Isolation and characterization of bacteriophages from *Bacillus anthracis*

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Bacillus (*B.*) *anthracis*, the causing agent of anthrax mainly infects herbivores but is also a serious threat for humans due to its potential as a biological weapon. Investigations of environmental and animal *B. anthracis* isolates indicate that at least 20% are stable infected with one or more bacteriophages (1). The aim of this work was the isolation and characterization of bacteriophages from *Bacillus anthracis* isolated from animals which died from anthrax and environmental strains as well which were collected in South Africa (SA) and Namibia (AF). For phenotypic analysis phages were isolated from virulent strains and stably introduced in an avirulent recipient to form lysogens. Phage morphology was determined via electron microscopy. Experiments were carried out for investigation of any influence of the phage onto the biology of *B. anthracis*. So far 27 bacteriophage lysates were investigated. All phages belong to the order of Caudovirales, representing either Myoviruses or Siphoviruses. 3/4 of the phages tested enabled biofilm formation in the experimental lysogens and 7 phages seem to have an influence on sporulation capacity. Phenotypic changes in colony morphology were observed which were attributable to stable phage infection. Sequence analysis give first evidence for monophyletic evolution of these bacteriophages.

In summary, our results indicate that (i) bacteriophages are present in *B. anthracis* isolated directly from the blood of dead animals, (ii) such phages may have an impact on the live cycle and survival strategies of its host by active lysogeny (2).

(1) Raymond Schuch, Vincent A. Fischetti (2009). The secret life of the Anthrax agent *Bacillus anthracis*: Bacteriophage-mediated ecological adaptations. PLoS ONE, 4: e6532

(2) Tal Argov et al., (2017). Temperate bacteriophages as regulators of host behavior. Current Opinion in Microbiology, 38: 81–87

[ID: 187] Engineered peptidoglycan hydrolases as antimicrobials for treatment of staphylococcal infections

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The increasing emergence of antibiotic-resistant bacteria is one of the most urgent problems of our time. Methicillin-resistant *Staphylococcus aureus* (MRSA) causes a broad variety of diseases, posing a threat to human and animal health. In addition, *S. aureus* can invade and persist within mammalian cells, thereby evading the host immune response and antibiotic therapy. For these reasons, novel antimicrobial agents effective against these pathogens are needed. Peptidoglycan hydrolases (PGHs), including phage endolysins, hold promise as antimicrobials due to their strong bactericidal activity, high specificity, and low chance of resistance development. Moreover, these modular enzymes can be engineered to create protein chimeras with desired properties. Despite these advantages, systemic administration of PGHs is currently hampered by several factors, including reduced activity at the site of infection, lack of cell-penetrating properties, and insufficient serum circulation half-life.

Our lab has generated a large collection of engineered staphylococcal PGHs, and we have developed a method to rapidly screen this library for enzymes with high staphylolytic activity under relevant conditions, such as in human serum and intracellular environments. In an effort to render our PGHs active against intracellular staphylococci, we fused them to cell-penetrating peptides (CPPs) to enable transduction into eukaryotic cells. In co-culture experiments with *S. aureus*-infected eukaryotic cell lines, PGH-CPP fusion proteins reduced intracellular *S. aureus* concentrations by up to 5 logs compared to PGHs without CPPs. Furthermore, we demonstrated that the circulation half-life of PGHs in mice can be extended by fusion to an albumin binding domain (ABD). Upon intravenous injection, the ABD mediates high-affinity binding to serum albumin, thereby reducing renal filtration and lysosomal degradation of the complex. Finally, we showed anti-biofilm activity of PGHs in dynamic models and in synergy with an exopolysaccharide depolymerase. Overall, our results demonstrate the high potential of PGHs as therapeutics for treatment of staphylococcal infections.

[ID: 146] Novel enzybiotics against intracellular and drug-resistant *Staphylococcus aureus*

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Staphylococcus aureus is a major concern in current health care due to the widespread resistance against antibiotics, such as methicillin (MRSA) and vancomycin (VRSA). Intracellular persistence has been demonstrated to be responsible for recurrent infections and protection of *S. aureus* from antibiotic therapy as well as immune response. Phage-derived peptidoglycan hydrolases (PGHs) are active against drug-resistant *S. aureus* and hold promise as novel antimicrobial agents. Among other advantages, their modularity, high specificity and low chance of resistance formation make PGHs interesting candidates for drug development. However, there is only limited knowledge regarding their capability to target and kill intracellular *S. aureus*.

We developed multiple cellular infection models to mimic intracellular persistence of different *S. aureus* strains in different tissue types. In order to target these intracellular bacteria, we fused PGHs to various cell-penetrating peptides (CPPs). The PGH-CPP fusions showed enhanced efficacy, reducing intracellular *S. aureus* by up to 5 log units compared to PGHs without CPPs. Time-resolved confocal laser-scanning microscopy was used to visualize intracellular killing and supports our findings. In addition, we demonstrated increased uptake of Europium-labeled PGH-CPP fusion constructs into eukaryotic cells by time-resolved fluorescence measurement.

To further enhance intracellular killing efficacy, we screened a collection of 320 parental and engineered PGH constructs for enzymes with high staphylolytic activity in buffers mimicking intracellular conditions. The 35 most promising constructs were comparatively characterized in three in vitro activity assays, yielding a final selection of six enzymes. These highly active PGHs in combination with our pool of CPPs are expected to further drive the development of potent protein therapeutics for treatment of infections caused by intracellular and drug-resistant *S. aureus*.

[ID: 212] Identification of a novel mechanism for genetic regulation of *Bacteroides cellulosilyticus* WH2 prophage induction

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The human gut microbiome is composed of a variety of bacteria many of which perform essential metabolic functions. One such bacterium is *Bacteroides cellulosilyticus* WH2 a commensal symbiont of the human gut necessary for the breakdown of otherwise indigestible polysaccharides of the human diet. Its presence in the gut contributes to a mature composition of the microbiome in healthy individuals. The genome of WH2 harbors two prophages, one of which is inducible both in vitro and in vivo. This study seeks to unveil a new mechanism of prophage induction in WH2. To identify candidate genes involved in this mechanism, a *Bacteroides cellulosilyticus* WH2 INSeq library was treated with a known prophage inducer and mutagenic antibiotic, Carbadox. This method allows for the genetic screening of over 90,000 isogenic mutants, each of which harbor a transposon insertion in one gene of the genome. Our results showed two isogenic mutants enriched after Carbadox treatments, revealing candidate genes for an alternative mechanism of prophage regulation. A gene located within the WH2 genome codes for Guanosine-3'2C5'-bis(diphosphate) 3'-pyrophosphohydrolase ((p)ppGpp), a sensor for phage lambda induction whose expression levels may affect the switch between lysis and lysogeny. Another candidate gene, LexA, is located within the prophage genome and represses the Coliphage 186 lysis antirepressor (Tum). The presence of LexA-binding boxes in WH2 has yet to be determined. This study demonstrates that when (p)ppGpp or LexA are disrupted by a Tn, the prophage is non-inducible, suggesting new mechanisms for lytic repression in a lamboid prophage of the human gut. These results propose novel induction mechanisms for lamboid prophages, that contribute to the knowledge on human gut microbiome composition dynamics.

[ID: 18] CRISPR-mediated immunity and phage-encoded anti-CRISPR mimicry in *Pseudomonas aeruginosa*

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Bacteria and archaea have developed sophisticated adaptive immune systems called CRISPR-Cas systems. Like all immune systems, CRISPR-Cas systems must facilitate destruction of foreign material (like viruses) while guarding against toxic self-targeting. In particular, Type I CRISPR systems must regulate the endonuclease activity of the CRISPR-specific nuclease-helicase Cas3, to ensure only verified DNA targets are degraded. Here we present the ~ 3.4 Å structure of the Type I-F crRNA-guided surveillance complex (Csy complex) from *Pseudomonas aeruginosa* bound to a dsDNA target. Structural and biochemical analyses reveal large conformational changes in the Csy complex that are driven by target binding, and culminate in a recruitment signal for Cas3. The result is an elegant mechanism that ensures only bona fide DNA targets are degraded. However, a *P. aeruginosa* bacteriophage subverts CRISPR immunity by encoding an anti-CRISPR protein that is a molecular mimic of a key subunit in the Csy complex. We exploit the anti-CRISPR mimicry to discover the putative docking site for Cas3 on the target-bound Csy complex. Our results highlight the ability of phage-encoded anti-CRISPRs to serve as evolutionary arrows, pointing toward critical processes in CRISPR-mediated defense.

[ID: 408] The effect of growth temperature of *Aeromonas salmonicida* on sensitivity to phages with a potential use in phage therapy

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Aeromonas salmonicida is a major fish pathogen and the causative agent of furunculosis that causes significant economic losses in the aquaculture industry worldwide. Possible alternative to antibiotic treatment of this disease could be phage therapy. For its proper functioning it is essential to know the properties of the bacterium. *Aeromonas* behaves differently at various temperatures. It has been found that cultivation of these bacteria at a higher than optimal temperature causes loss of some virulence factors. Therefore, we have decided to test if the cultivation temperature has also effect on the sensitivity of *A. salmonicida* to phages, which is crucial for the use of phage preparations *in vivo*.

Nine *A. salmonicida* phages were isolated from waste water plants in various cities in the Czech Republic. Some of them were studied in more detail, sequenced and their morphology determined by electron microscopy etc. Host spectrum of these phages was determined on bacterial strains originating from Czech fish farms and two of them had broad spectrum. Sensitivity of the bacterial strains to phages was also tested in a range of temperatures and in some cases the sensitivity was decreasing with lower temperatures. This finding could have a significant impact on the practical use of phages against *A. salmonicida* strains because the temperature for *in vivo* application in the fish farms stands in the lower range.

We kindly acknowledge the financial support from the Ministry of Industry and Trade of the Czech Republic #CZ.01.1.02/0.0/0.0/15_019/0004353 & #CZ.01.1.02/0.0/0.0/16_084/0009987.

[ID: 123] Characterization of the long elusive nuclease of bacteriophage T5

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Phage T5 infects *Escherichia coli* and injects its genome in an original two-step mechanism: in the First Step Transfer (FST), only 8% of the phage DNA enters the host cell. The transfer pauses then for several minutes before DNA entry resumes to completion (Second Step Transfer, SST). The FST is accompanied by a very rapid and massive destruction of bacterial DNA (50 % decrease in labeled DNA within 4 min of infection). The identity of the phage T5 DNase has remained elusive for sixty years, as none of the phage proteins encoded on the FST-DNA resemble known nucleases. However, A1, a gene carried by FST-DNA, appears to control host DNA degradation as well as the SST. In this study we investigated the role of the 62-kDa protein A1 in DNA degradation *in vitro* and in the bacterial cell.

In the C-terminal half of A1, we identified several motifs that are conserved in a large family of metallophosphatases including the DNA repair and recombination nucleases Mre11/SbcD/gp46. Purified A1 exhibited manganese-dependent DNase activity on linear or plasmid DNA *in vitro*, suggesting that A1 is a Mn-dependent nuclease. Using fluorescence microscopy of *E. coli* cells, we observed a rapid decrease in bacterial DNA staining with DAPI upon ectopic expression of A1. Moreover, we frequently saw the formation of a focus of fluorescence, suggesting a dramatic reorganization of the bacterial nucleoid. Mutations in putative catalytic amino-acid residues abolished nuclease activity *in vitro* as well as *in vivo*. Taken together, our results indicate that A1 is the long elusive early-encoded DNase of phage T5. Interestingly, T5 phage DNA is not modified and is sensitive to A1 *in vitro*. How the DNase activity of A1 is regulated to control the SST without digesting the T5 genome remains to be elucidated.

[ID: 258] Genome-wide CRISPR-dCas9 screens in E. coli identify essential genes and phage host factors

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High-throughput genetic screens are powerful methods to identify genes linked to a given phenotype. The catalytic null mutant of the Cas9 RNA-guided nuclease (dCas9) can be conveniently used to silence genes of interest in a method also known as CRISPRi. Here, we report a genome-wide CRISPR-dCas9 screen using a pool of ~ 92,000 sgRNAs which target random positions in the chromosome of E. coli. We first investigate the utility of our screen for the prediction of essential genes and various unusual features in the genome of E. coli. We then apply the screen to discover E. coli genes required by phages λ , T4 and 186 to kill their host. In particular, we show that colanic acid capsule is a barrier to all three phages. Finally, cloning the library on a plasmid that can be packaged by λ enables to identify genes required for the formation of functional λ capsids. This study highlights the usefulness and convenience of pooled genome-wide CRISPR-dCas9 screens in bacteria in order to identify genes linked to a given phenotype.

[ID: 420] Phages in a fibrin glue: new possibilities against bacterial infections in surgery

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Our first results of incorporation of bacteriophages into a fibrin glue were promising in terms of the stability of the fibrin scaffold, the survival of phages inside the fibrin glue and the kinetics of their release. In the present study we develop this concept of prolonged local control of bacterial infections in surgery. In vitro tests showed antibacterial efficacy of the phage-incorporated fibrin glue layered on solid surfaces. Structure of the fibrin glue scaffolds with and without bacteriophages was investigated with a scanning electron microscopy. Fibrin glue has potential to be a convenient substance for the local delivery of bacteriophages.

[ID: 193] Isolation of bacteriophages specific to a fish pathogenic bacteria from genus *Pseudomonas*

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Bacteria are probably the most significant pathogens of cultured fish, causing the high levels of morbidity and mortality. The rapid development of the aquaculture industry has led to a highly motivated understanding of the pathogenesis of bacteria in farmed fish and ways of treatment. But information about bacterial flora and impact of bacteria on fish organisms remains relatively poor compared with our understanding of bacterial diseases in humans and other animals (Woo and Bruno, 2011). In addition, aquaculture systems contain high numbers of diverse bacteria, which exist in combination with the current and past use of antibiotics, probiotics, prebiotics, and other treatment. Extensive antibiotic resistant strains are now being detected and the spread of these strains could greatly reduce medical treatment options (Watts et al. 2017).

There are lots of different drugs and solutions, which have been widely investigated as potential antimicrobial agents. Bacteriophages are able to penetrate through bacteria cell membranes specifically and have strong antimicrobial and lytic properties. Thus, the purpose of this work was to isolate the bacteriophages from pathogenic bacteria of genus *Pseudomonas* in cultured rainbow trout *Oncorhynchus mykiss*, common carp *Cyprinus carpio* and bighead carp *Hypophthalmichthys nobilis*.

The strains of *Ps. fluorescens*, *Ps. putida*, *Ps. intestinalis* and *Ps. cyprinisepticum* were isolated from carp and bighead carp, *Ps. fluorescens* and *Ps. chlororaphis* were identified in rainbow trout. These pseudomonads were tested for phages activity and among 15 isolated strains only 2 were sensitive to lytic activity of isolated phages. *Ps. fluorescens* and *Ps. cyprinisepticum* strains showed sensitivity to tested phages. Other strains of bacteria were not sensitive to isolated phages. The detailed study of biological properties of isolated phages and development of approaches for its using in aquaculture for effective prevention and treatment of bacterial fish diseases are needed and will be provided in our further investigation.

[ID: 288] Three-dimensional structure of novel ssRNA bacteriophage virus-like particles

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Bacteriophages of the Leviviridae family are small viruses with single-stranded RNA (ssRNA) genomes. The family had for long remained rather small with the known representatives infecting *E.coli* and related Enterobacteria, and a few isolates with some other Proteobacteria as their hosts. However, recent metagenomic studies (PMID: 27010970, 27880757) have revealed many novel ssRNA phage sequences, demonstrating that the true ubiquity and diversity of ssRNA phages in nature has been greatly underestimated and underexplored.

The metagenomic data have revealed more than 150 novel ssRNA phage coat protein (CP) sequences, which often have no similarity to the known phages or to each other, and additionally have a surprising variation in length (105 to 208 residues). ssRNA phage CPs alone can often assemble into virus-like particles (VLPs), morphologically almost identical to phage virions, which enables to study phage structure even in absence of the actual virus. We have obtained more than 100 of the novel CP sequences using gene synthesis and expressed them in *E.coli*. In the majority of cases, the CPs were able to assemble into VLPs, and we have determined the 3D structures of eight of them using x-ray crystallography. While all CPs have the same conserved core fold, the structures have revealed extensive shortening and expansion of protein loops, as well as addition or deletion of entire secondary structure elements in some cases. The different CP lengths do not result in significantly different particle sizes, but there is wide variation in the size of the pores that the ssRNA phage capsids have. In one case, the VLP appears to have a prolate icosahedral shape which has not been observed for ssRNA phages before. Given the very high sequence variability of the ssRNA phages, the structural data will be important to assist future classification efforts within the Leviviridae family.

[ID: 331] Isolation and characterization of new *Flavobacterium columnare* strains and its bacteriophages from fish farms

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Fish are an important source of protein and aquaculture has been the fastest growing food production sector over the past 20 years. However, the intensification of fish production in farming systems expose fish to bacterial infections. *Flavobacterium columnare* is a Gram-negative bacterium that causes epidemical columnaris disease at fish farms during the warm water period. This pathogen causes significant financial losses in the industry if the disease is not treated with antibiotics as soon as the first symptoms appear. In order to develop a bacteriophage-based control of *F. columnare*, we isolated 117 *F. columnare* strains and 64 *F. columnare* phages from 10 different fish farms in Finland and Sweden. Genetic characterization of the bacterial isolates was made using 16S RFLP and ribosomal intergenic spacer analysis, and the virulence of 25 isolates was tested using a rainbow trout challenge model. All *F. columnare* isolates could be assigned into four previously identified genetic groups: 73 to group C, 22 to group E, 14 to group A and eight to group G. Of these, bacterial isolates belonging to group C and E were the most virulent. Phage host range analysis against a collection of 229 bacterial isolates showed that the collection of phages together infected bacterial isolates from all the different genetic groups. Most phages infect bacteria in a genotype-specific manner, and only few isolates had wider host ranges. Phages infecting the most virulent bacterial groups were isolated. Together, the diversity, lytic capacity and combined host range of our phage collection demonstrates its potential to be used in phage therapy.

[ID: 178] Interactions of peptidoglycan hydrolases with staphylococcal cell walls

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We have gained new insights into structural bases of peptidoglycan recognition and mechanism of catalytic domain of M23 metallopeptidases and SH3b cell wall binding domain by combining biochemical and structural approach. Both these domains can be found in phage proteins but are also present in autolysins (e.g. LytM) and bacteriocins (e.g. lysostaphin).

M23 metallopeptidases comprise a group of enzymes that predominantly cleave cross bridges in peptidoglycans (PG), especially those present in various staphylococcal species.

SH3b domains recognize bacterial peptidoglycans (predominantly staphylococcal) and act as anchors for various catalytically active domains (eg. M23 in lysostaphin or CHAP peptidases and amidases in phages).

We have crystallized and analyzed structures of M23 catalytic domain and SH3b domains with peptidoglycan fragments. LytM catalytic domain was crystallized in complex with transition state analogue and lysostaphin SH3b domain with pentaglycine. These analysis supported by biochemical and biophysical tests allow us to propose and discuss the possible mechanism of hydrolysis for M23 peptidases and define some of the structural features determining substrate specificity for both domains.

This information is important not only for our deeper understanding of basic biological processes but might also have a great impact on application of these enzymes in combating staphylococcal infections. Our research might provide information necessary for engineering enzyme specificity in order to overcome existing resistance mechanism. We have attempted structure-based engineering of both domains to expand substrate specificity and generate mutants which would recognize and efficiently bind resistant bacteria.

1. Odintsov S et al (2004) JMB 335:775
2. Sabala I et al (2014) FEBS J 281(18):4112-22
3. Grabowska M et al (2015) Scientific Reports, 5:14833

[ID: 428] Transcriptomic analysis of T4-like phage NCTC 12673 infection of *Campylobacter jejuni* 11168

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Campylobacter jejuni is a frequent foodborne pathogen of humans. As *C. jejuni* infections tend to arise from handling and consuming poultry, bacteriophage treatments have been proposed to reduce *C. jejuni* load on farms and prevent human infections. We used RNA-seq to profile phage-host interactions between phage NCTC12673, a lytic T4-like myovirus with a 135-kB dsDNA genome, and *C. jejuni* NCTC11168 during infection.

We found that NCTC12673 phage transcripts made up roughly 1/3 of total transcripts post-infection, and did not observe the rapid degradation of host transcripts shortly after infection seen during T4 infection. We observed significant differential expression of *C. jejuni* genes during phage infection with up to 13.5% of all host genes upregulated and 4.8% downregulated upon infection. In contrast, we observed no change in phage gene expression during infection.

We sought to identify host pathways differentially expressed upon phage infection. Interestingly, we found that the three most highly upregulated genes are uncharacterized and within the same operon (cj0423-cj0425). This operon includes genes with homology to an antitoxin and to a superinfection exclusion gene. This operon is not universally conserved in *C. jejuni* species, and is absent in the propagating strain for NCTC12673. Other significantly upregulated genes include those involved in oxidative stress defense and multidrug efflux pumps. To confirm these findings, we tested phage infection efficiency on targeted *C. jejuni* NCTC11168 mutants and found that mutagenesis of catalase (*kata*) reduced phage infectivity, and further work to test the effect of mutagenizing other host genes upregulated during infection is ongoing.

Together our results shed light on the phage-host dynamics of an important foodborne pathogen during lytic infection by a T4-like phage

[ID: 70] Digging in the dirt for E. coli phage diversity – still something new to discover

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Escherichia coli is among the most common pathogens in poultry and the causative agent of colibacillosis. This frequent disease creates significant economic losses and is commonly treated by antibiotics, which in turn promotes the selection of multidrug resistant (MDR) bacteria. A high incidence of MDR bacteria is problematic not only for animal health but also because of the potential for zoonotic transfer to humans through contaminated food. Therefore, there is a high demand to develop new strategies as alternatives to antibiotic therapies. In accordance with the One-Health concept this project aims to isolate and characterize phages to fight *E. coli* infections in broilers.

Apart from generating a suitable phage mixture for a field trial with broilers, one further aim of this study is to get further insight into the diversity of *E. coli* phages followed by enhanced work on taxonomic issues in that field.

In this study, phages were isolated from different habitats, e.g. sewage, surface water, manure, poultry respectively horse dung, and hospital wastewater. Morphological characterization revealed a diversity of morphotypes (77% Myo-, 17% Siph-, and 6% Podoviridae), genome sequencing resulted in genomes sizes from 44 up to 370 kb. Annotation and comparison with databases showed similarities in particular to T4- and T5-like phages, but also to less known groups.

Though a lot of phages against *E. coli* are already described in literature and databases, we still isolated phages that showed no or only few similarities to other phages at the genomic level.

Overall taxonomic analysis of the genomes of the phages isolated in our study compared to known phages resulted in the proposal of at least three new genera.

[ID: 252] The efficacy of phage therapy using murine models of vancomycin-resistant Enterococci colonization

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The CDC estimates that 23,000 deaths/year in the United States are caused by antibiotic-resistant bacteria, such as Vancomycin-resistant enterococci (VRE). The inability to treat these infections with common antibiotics necessitates the development of alternative interventions. Bacteriophages (phages) represent one potential alternative therapeutic to combat these infections. Here, we sought to develop and characterize an effective phage therapy against VRE gut colonization using a murine model. Several phages with activity against VRE strains were isolated from sewage. We administered various antibiotics in mice to disrupt the normal gut microbiota at different levels and allow VRE to colonize. Using virulent phages, we designed a cocktail with high activity against VRE. VRE-colonized mice were treated with either the phage cocktail, a single phage, ampicillin (a standard antibiotic intervention), or ampicillin plus the phage cocktail. Depending on the VRE colonization model used, phage therapy success varied. In a model using vancomycin pretreatment to achieve VRE colonization, phage therapy lead to sustained VRE decolonization. This contrasts with ampicillin treatment, which resulted in an initial rapid decrease in VRE colonization that rebounded to high levels after ampicillin treatment ceased. In a model using vancomycin, polymyxin B, and clindamycin to achieve more stable VRE colonization, phage therapy had moderate success, reducing VRE in some mice but not others. Finally, in a germ-free mouse model lacking a gut microbiota, phage therapy did not alter VRE colonization levels. Our data suggests that a phage cocktail could be successfully utilized to decolonize VRE in mice. However, based on the results across three VRE colonization models, we hypothesize that members of the gut microbiota assist in phage-mediated VRE decolonization. These investigations will have a significant impact on the largely understudied field of phage therapy and will contribute to the development of new strategies for treating antibiotic-resistant bacterial infections.

[ID: 256] “Wild” Pseudomonas aeruginosa phages isolated from mouse wounds

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The efficacy of a 5-phage cocktail was tested against experimental *Pseudomonas aeruginosa* infection in a mouse wound infection model. Phages from the cocktail administered intraperitoneally spread into the wounds infected with *P. aeruginosa* but no significant difference was observed in bacterial burden in the treatment and control groups. Surprisingly, live phages were isolated not only from the phage-treated animals but also from the control group administered with vehicle solution, in comparable numbers, though the phages from vehicle-treated mice were different (“wild” or “endemic” phages). Two such wild phages from mouse wounds were isolated and characterized, a highly lytic phage belonging to the PB1 group and an undescribed temperate phage. These wild phages might play a role in natural control of *P. aeruginosa* wound infection.

[ID: 3] To evaluate the potential of bacteriophages to cure multiple drug resistance Salmonella species isolated from poultry

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Multiple drug resistant Salmonella serovars circulating in poultry with little or no incidence of disease are major cause of enteric infections in humans[1] . Poultry acts as main reserve of salmonella species where it causes little or no disease. Antibiotics are used as growth promoters in poultry industry that leads to emergence of multiple drug resistant salmonella varieties [2]. Due to infection with these MDR strains the choice of drug for the cure of human Salmonellosis and typhoid fever is limited [3]. Since Phage therapy is effectively employed in several European countries for treatment of multiple drug resistant pathogens; we aimed to produce effective lytic phage cocktails for treatment of such MDR salmonella strains. Six bacteriophage isolates were obtained from 55 human stool (n=2) and poultry samples (n=4) tested. These bacteriophages were characterized for pH, temperature and detergent tolerance. They were also subjected to Electron Microscopy. They grew well on MDR salmonella strains of choice. Bacterial growth reduction assays and titration of the phages revealed their good lyses potential. The phages were tested as phage cocktails in different combination for best salmonella control in vitro.

References;

1. Pui, C., et al., Salmonella: A foodborne pathogen. International Food Research Journal, 2011. 18(2).
2. Shahzad, A., et al., Prevalence of salmonella species in hen eggs and egg storing-trays collected from poultry farms and marketing outlets of Faisalabad, Pakistan. Pak J Agric Sci, 2012. 49: p. 565-568.
3. Imran, Z.P., et al., Molecular and epidemiological analysis of multidrug-resistant Salmonella paratyphi A obtained from different regions of Pakistan. Pak J Bot, 2010. 42: p. 2941-2947.

[ID: 173] Probing the ‘dark matter’ of the human gut phageome: culture assisted metagenomics enables rapid discovery and host identification for novel bacteriophages

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Recent years have been marked by a growing interest in the indigenous viral populations in the human gut. It has been shown that gut viral populations, predominantly composed of bacteriophages, markedly outnumber bacterial and archaeal gut symbionts. The phageome also displays high levels of inter-individual specificity and temporal stability. Numerous observations suggest an important role for the human gut phageome in host physiology and gastrointestinal diseases. Despite all that, the overwhelming majority of gut bacteriophages, often referred to as ‘the viral dark matter’, remain uncharacterised in terms of their hosts and persistence strategies.

Here, we describe a method that enables rapid discovery and host identification for novel bacteriophages in the gut via a combination of serial bacterial enrichment cultures and shotgun metagenomic sequencing of the viral fraction of faecal samples.

Using this approach tens of novel and known bacteriophages were detected, including a number infecting difficult-to-culture extremely fastidious gut anaerobic bacteria. One particularly interesting example was the isolation of a novel member of the expansive and highly predominant, yet poorly characterised family of crAss-like bacteriophages.

[ID: 305] Molecular characterization of *Pantoea* sp. infecting siphovirus vB_PagS_Vid5

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A novel low-temperature *Pantoea* phage vB_PagS_Vid5 (Vid5) has been isolated in Lithuania from thicket shadbush. Based on TEM results, phage Vid5 belongs to the family Siphoviridae and has a slightly prolonged head (about 75 nm in length and 54 nm in width) as well as a non-contractile flexible tail (about 189 nm in length). The host range determination test revealed that out of 20 bacterial strains tested, only *Pantoea agglomerans* isolate MMG is sensitive to Vid5. Plating tests revealed that phage can form plaques in the temperature range of 4 to 32°C. The 61.437 bp genome of Vid5 has a G+C content of 48.8% and contains 99 probable protein encoding genes as well as 1 gene for tRNA^{Ser}. Comparative sequence analysis revealed that 46 out of 99 Vid5 ORFs encode unique proteins that have no reliable identity to database entries. Based on homology to biologically defined proteins, 33 ORFs of Vid5 have been given a putative functional annotation, including genes coding for structural proteins as well as those associated with DNA metabolism, morphogenesis and phage–host interactions. In addition, a ~6.5 kb gene cluster encoding enzymes involved in queuosine biosynthesis has been identified. Phylogenetic analysis, based on the alignment of the essential structural and functional genes, revealed that phage Vid5 is the most closely related to *Escherichia* or *Salmonella*-infecting phages, most of which contain the aforementioned queuosine biosynthesis gene cluster. No close phylogenetic relationship between Vid5 and phages infecting *Pantoea* sp. has been observed. Thus, the data presented here will expand our knowledge of the genetic diversity and evolution of *Pantoea* phages, especially *Pantoea*-infecting siphoviruses, and their phylogenetic relatedness to other bacteriophages. This research was funded by a grant (No. SIT-7/2015) from the Research Council of Lithuania.

[ID: 306] Pantoea spp. infecting bacteriophages: morphologically divergent representatives within the order Caudovirales

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Erwinia and their close relatives – Pantoea are among the most predominant bacteria in phyllospheres. These genera comprise phylogenetically closely related species of bacteria and most of Erwinia phages described to date have been found to infect certain strains of Pantoea, as well. Moreover, despite the fact that siphoviruses are the most frequent phages in nature, only several Erwinia and Pantoea-infecting siphophages have been characterized to date.

In this study, five Pantoea bacteriophages have been isolated in Lithuania from the thicket shadbush berries using Pantoea agglomerans strains as the hosts for phage propagation. TEM analysis revealed different virion morphotypes and confirmed that phages belong to all three families within the order Caudovirales. With a head of about 119 nm in diameter and a long, contractile tail of about 290 nm in length, myophage vB_PagM_KTK4 (KTK4) fits A1 morphotype and is characterized as the largest phage of this study. Myovirus vB_PagM_LIET2 (LIET2) fits A3 morphotype and has a head of about 115x45 nm and a tail of about 88 nm in length. Siphoviruses vB_PagS_MED16 (MED16) and vB_PagS_AAM21 (AAM21) fit B1 morphotype and have a head of about 64 and 89 nm in diameter and a tail of about 145 and 193 nm in length, respectively. Podovirus vB_PagP_SOF1 (SOF1) shows C1 morphotype and is characterized by a head of about 59 nm in diameter and a tail of about 12 nm in length. Plating tests revealed that phages KTK4, AAM21, SOF1, LIET2 and MED16 can form plaques in the temperature ranges of 4 to 31, 34, 35, 36 and 37°C, respectively.

Results of this study not only extend our knowledge about the diversity of Pantoea viruses but also suggest that aforementioned broad-temperature range phages could be potentially used as phage-based bioagents to control plant, animal and human pathogens.

[ID: 394] The effect of the pR' region of Shiga toxin production and its inhibition by the Locus of Heat Resistance

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Shiga toxin genes (stx) are carried by lamboid bacteriophages with the stx genes located in the late transcription region, upstream of the lysis cassette and downstream of the antiterminator Q. Transcription of the stx genes are under the control of the late promoter pR', and thus the sequence diversity of the regulatory region between Q and stx affects Stx toxin production.

We defined the region between Q and stx as the pR' region and compared the sequence of this region and the stx subtypes of nineteen shiga-toxin producing *Escherichia coli* (STEC). Phylogenetic analysis suggested that the pR' region is not linked to stx subtype. Further, we selected representative pR' regions, established and validated a transcriptional fusion of the pR' region to the DsRed reporter gene using mitomycin C induction. The effect of the pR' region on Shiga toxin expression was measured with flow cytometry. Induction levels change when different pR' regions are placed in the same host strain under control of this strain's prophage-derived regulatory proteins. Moreover, the same pR' region regulates expression differently in different STEC strains. Not every toxin can be induced in its native host, indicating that the pathogenicity of an STEC is determined merely by the number of prophages it harbors.

To determine whether the proteins that confer stress resistance in the host affects stx expression, we examined the effect of the Locus of Heat Resistance (LHR) on stx expression. The LHR confers resistance to H₂O₂, an agent that induces oxidative stress and stx-prophages. Cloning of the LHR in strains carrying stx-prophages increased survival in presence of H₂O₂ by 2.49 log, decreased the proportion of cells with oxidized membrane lipids by 17.4%, and reduced the proportion of cells expressing stx-prophages by 20%.

In conclusion, the pR' region and host susceptibility to oxidative stress influence Stx production.

[ID: 89] Determination of non-sequencable bacteriophage genome sequence via RNA-seq of phage-infected bacteria

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YerA41 is a myoviridae phage that infects *Yersinia ruckeri*. Several attempts to determine its gDNA-sequence using traditional and next generation sequencing technologies failed, indicating that the phage genome carries a novel modification rendering it unsuitable as a template for any DNA-polymerase used for sequencing or PCR. The use of restriction enzymes also failed to digest the phage gDNA. To overcome this YerA41 DNA modification and to determine the genome sequence we isolated total RNA from phage-infected *Y. ruckeri* cells at different time points post-infection (0 – 90 min). After removal of rRNA, the remaining mRNA was reverse transcribed and the cDNA sequenced using the Illumina MiSeq platform. The host-genome specific reads were subtracted and de novo assembly performed on the phage genes that were being expressed over this time period. This resulted in 12 contigs with a total length of 145 kb. Annotation of the sequences revealed 192 potential genes most of which found no homologs in the databases. A few genes encoding nucleotide metabolism associated enzymes, including phage-encoded DNA polymerases, and structural proteins were identified. Proteome studies identified altogether 42 phage particle-associated proteins. Additionally, transcriptomics revealed a clear temporal expression profile of the phage genes.

[ID: 72] Development of novel chimeric endolysin with enhanced lytic activity against *Staphylococcus aureus*

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Increasing threat of antibiotics resistance in *Staphylococcus aureus* has created an urgent need for development of new antimicrobial agents. Endolysins (peptidoglycan hydrolase) are believed as one of the alternatives to antibiotics due to their narrow host-specific activity spectrum. However, the discovery of potent endolysin is still challenging because it requires labor-intensive work and has difficulties in getting soluble form with high lytic activity. Modular structure of endolysin can provide an opportunity to develop novel endolysins with improved properties by rearrangement of functional domains. In this study, a random domain swapping of *Staphylococcus aureus* endolysins was used to obtain engineered endolysins with higher solubility and lytic activity. The novel chimeric endolysin exhibited more rapid and efficient bacterial cell lysis than its parental endolysins against staphylococci including *S. aureus*, *S. heamolyticus*, *S. epidermidis*, and *S. warneri*. The minimum inhibitory concentration (MIC) of the chimeric endolysin against methicillin-resistant *S. aureus* (MRSA) was at least 2.25-fold lower than those of parental endolysins. These findings suggest that the novel chimeric endolysin has the potential to be an alternative therapeutic agent for the treatment of infections caused by MRSA.

[ID: 237] The McrB recognition subunit of the methyl-specific McrBC endonuclease cleaving modified DNA is associated with phage resistance in *Campylobacter jejuni*

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Campylobacter jejuni and their phages co-exist in chicken gut, providing an excellent environment for phage resistance development. Here we aim to elucidate novel phage resistance mechanisms in *C. jejuni* by investigating strains of chicken origin belonging to the most prevalent clonal complexes ST-21 and ST-45. To determine phage susceptibility, we screened 31 strains originating from broilers for plaque formation using our collection of 43 *C. jejuni* phages. Interestingly, sensitive ST-21 strains were only infected by CPS-dependent (capsular polysaccharide) phages, whereas sensitive ST-45 strains were exclusively infected by flagellotropic phages. Comparative genomics revealed that ST-21 strains contained the same genes in the CPS loci also encoding the phase variable O-methyl phosphoramidate (MeOPN) receptor of many CPS phages. In contrast, the CPS loci of the ST-45 strains were highly variable, yet none encoded genes necessary for MeOPN synthesis, explaining their resistance to the CPS-dependent phages. To elucidate novel mechanisms of phage resistance, we chose two ST-21 strains with highly similar genome sequences, but one being sensitive to all CPS dependent phages, whereas the other (CAMSA2038) was resistant. Interestingly, sequence divergence among the two strains was only observed in seven genes, three of which encode putative restriction modification systems including a methyl-specific McrBC endonuclease. While the C-terminal of the putative McrB subunit was conserved in the two strains, no sequence similarity was found in the N-terminal responsible for binding to the methylated recognition site. Remarkably, a *mcrB* deletion mutant in CAMSA2038 was sensitive to 18 out of 43 phages, three of these phages being flagellotropic phages that otherwise did not infect any ST-21 strains. Thus, our results indicate that McrB is associated with the highly phage resistant nature of CAMSA2038 and that internal phage resistance mechanisms are involved in the co-existence of *C. jejuni* and its phages in the chicken host.

[ID: 224] Characteristics of *Klebsiella pneumoniae* population treated with phage preparations

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Phage therapy is a promising alternative method for treatment of bacterial infections showing many advantages over traditional antibiotic therapy. Indeed phage therapy exhibits bacterial strain specificity and lacks the negative effects on patient's microbiota. However, phage-resistant clones emerge relatively quickly. This is often associated with the modification of surface structures recognized by phages. Changes in phage-resistant mutants affect key bacterial properties associated with pathogenicity (i.e. biofilm formation, surface adhesion, motility and other virulence factors). Interestingly, these changes may be associated with reduced growth and an overall decrease in bacterial fitness including higher susceptibility to immune system clearance.

In this study, we examined *Klebsiella pneumoniae* clones arising after controlled infections of planktonic and sessile bacteria by a single-phage preparation and cocktails composed of three phages. For experiments we selected *Klebsiella* phages from Caudovirales: KP15, KP27 (Myoviridae), KP34, KP32 (Podoviridae) and KP36 (Siphoviridae).

Clinical *Klebsiella* strains and their mutants were tested for cross-resistance to a set of phages, susceptibility to serum complement and antibiotic resistance. We also assessed the LPS patterns and the bacterial ability to form biofilms.

We observed that phage resistant clones have altered sensitivity to particular phages. The mutants became cross-resistant to other phages recognizing the same type of receptor. Interestingly, the modification of the capsular receptor targeted by podo- and siphoviruses led to sensitivity to Myoviridae phages and vice versa. Additionally, some of mutants changed its sensitivity to antibiotics. We also observed the truncation of LPS O-chain which was correlated with increased susceptibility to complement killing.

[ID: 188] Eradication of biofilm-forming clinical strains of *P. aeruginosa* by the novel bacteriophage cocktail

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Pseudomonas aeruginosa being a critical priority pathogen on account of emergence of carbapenem-resistant strains, is a global concern human health wise, especially in the hospital environments. Special difficulties with eradication of this bacteria comes with its ability to adhere to surfaces and form virulent biofilms. Developing of new antibiotics and antimicrobial agents is time consuming and complex, with most of the current drugs being modifications of existing classes of antibiotics, portending short-term solutions. Thus, new alternative strategies of treatment and prevention are necessary. The use of bacteriophages is an interesting possibility, considering their specificity in term of bacterial hosts, safety in context of natural flora, mutation capability, countering phage-resistant bacteria and easiness of isolation.

In this study 19 wild isolates and two reference strains of *P. aeruginosa* were used as bacterial hosts for 6 bacteriophages, which were formulated into cocktail for elimination of planktonic and biofilm cultures of bacteria. To estimate biofilm formation by used strains and the influence of phage cocktail on formed biofilm, metabolic test and crystal violet staining were applied, to determine reduction of the biofilm biomass. For phage treatment group, more than 90% of biofilm biomass was eliminated. In order to assess the *in vivo* efficacy of phage therapy against *P. aeruginosa* clinical strains, *Galleria mellonella* was used as an infection model.

Formulated cocktail has noticeable potential in the field of therapeutic purposes against biofilm-forming *P. aeruginosa* infections.

[ID: 310] Identification and characterization of lysogenized phages in methanotrophic bacteria

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Methanotrophs are a unique subset of bacteria with imminent industrial potential. Their capacity to metabolize methane from impure sources such as industrial waste streams, and convert it to valuable products such as bio-fuels and biomaterials provides a basis for new bioproduction platforms. However, key aspects of their fundamental biology, including the prevalence and induction of lysogenized phages, are still being elucidated, limiting their utilization in the large-scale industrial applications. Because prophage regions can be a hindrance in industrial processes, with unintentional induction of the phage resulting in the loss of an entire fermentation batch, more information on phages of methanotrophs is crucial to the development of new processes.

Overall, phage infection has been poorly characterized in methanotrophic strains. Thus the present study uses genomic data of several methanotrophs to identify potential prophages sequences and investigate induction factors. For instance, a prophage sequence was found in *Methylobacter marinus* A45 and was successfully induced using Mitomycin C. In other host strains, such as *Methylococcus capsulatus* str. Bath, *Methylosinus trichosporium* OB3b, and *Methylocystis* sp. ATCC 49242, as many as 6 potential prophage regions have been identified.

Induction of lytic phase of these prophages was attempted through chemical and UV induction. Successfully induced phages were isolated for characterization of DNA and infection parameters.

As prophage regions can often contribute unique genetic traits to a bacterium, this characterization can help us better understand the biology of methanotrophs. More importantly, the information on the mechanisms [...].

[ID: 284] BAFASAL® - a new antibiotic-free solution against Salmonella in poultry

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Most of foodborne bacterial diseases in humans are related to the consumption of animal products, and animal husbandry is therefore extremely important for the protection of human health. In treatment of such infections antibiotics are commonly applied. But in face of growing antibiotic resistance of bacterial strains other solutions shall be considered in order to protect animals against bacterial pathogens and minimize the potential side-effects for humans. Bacteriophages might be an important alternative to antibiotics. Although this novel approach seems to be very promising, the use of bacteriophages to prevent illness in food animals on the farm is relatively rarely studied. Here, we developed bacteriophage-based preparation BAFASAL®, a feed additive, which selectively eliminates human pathogenic Salmonella strains in poultry farms. BAFASAL® is a mixture of carefully selected and patented phages that inhibit the growth of Salmonella serovars important for poultry breeders.

The aim of this study was to investigate the efficacy of BAFASAL® in Salmonella eradication and to investigate its tolerance in broiler chickens.

Our results revealed statistically significant lower number of Salmonella in Salmonella exposed and BAFASAL® treated group of chickens compared to non-treated Salmonella exposed group. The reduction of Salmonella in caeca collected at the end of the trial (day 35) was more than 94%. What is more, BAFASAL® did not induce changes in zootechnical parameters of birds even at the tolerance concentration (100x dose). Also, no signs of toxicity were stated in a toxicity test.

This data suggest that the BAFASAL® feed additive is very efficient in prevention and reduction of Salmonella occurrence in gastrointestinal tract of chickens and may be applied against Salmonella infections in poultry.

[ID: 277] Phigaro - a novel command line tool for phage and prophage identification in metagenomic assemblies

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One of the steps in the study of bacteriophages is prophage prediction in bacterial genomes and metagenomic contigs. One of the problems in identification of phages and prophages is their striking diversity, that makes it difficult to predict novel prophages via sequence homology search.

At the moment, the existing tools for annotating prophages in bacterial genomes are either web-based or outdated, and there is no up-to-date command-line tool capable of predicting prophage-like structures from unannotated nucleotide sequences, including metagenomic contigs.

We designed a new command-line tool called Phigaro for predicting phage-like elements that is available at (<https://github.com/lpenguin/phigaro>). As Phigaro runs in the command line, it is potentially more scalable than web-based tools such as PHASTER [1] and easier to integrate in computational pipelines.

Phigaro takes fasta-formatted genomic data as input, annotates phage-like genes and then uses a triangular window algorithm that determines regions with high density of phage-like genes and prophage boundaries based on phage genes annotation. Phage-like genes are annotated using phage-specific pVOG [2] Hidden Markov Models (HMMs). We estimated the best parameters such as window length, E-value threshold and score thresholds using a “golden standard” of 54 bacterial genomes with known prophage coordinates. With these parameters, Phigaro has a Positive Predictive Value (PPV) of 0.8. It’s performance is comparable to performance shown by PHASTER [1] both in accuracy and time.

The research was made possible by the financial support of RFBR (grant No. 16-54-21012).

References:

1. Arndt, David, et al. "PHASTER: a better, faster version of the PHAST phage search tool." *Nucleic acids research* 44.W1 (2016): W16-W21.
2. Graziotin, Ana Laura, Eugene V. Koonin, and David M. Kristensen. "Prokaryotic virus orthologous groups (pVOGs): a resource for comparative genomics and protein family annotation." *Nucleic acids research* (2016): gkw975.

[ID: 301] Creating custom genomic databases for metagenomic analyses

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A big problem facing biologists is the challenge to quickly download a large amount of genomic data at once in order to analyze metagenomic data sets. My attempt to gather all phage genome data from the NCBI showed that the methods to quickly get all of the data you need at a click of a button are not readily discernible. The lack of an obviously quick and easy way to grab all genomic data for a particular group of organisms given precise taxonomic data creates a major hurdle to doing metagenomic analysis, and means that far more time is spent downloading and assembling the data needed in order to do the research as opposed to doing the research itself. The task is complicated enough that it can only be done by somebody with advanced computer and research skills. On top of this, the documentation is still limited and can be significantly improved, which we have attempted to do here. We wrote an introduction to Entrez, which is a straight forward way to gather genomes from Genbank given precise parameters. We hope that this tutorial will help speed up metagenomic research without all of the data cleaning other methods often require. (Data cleaning is the process of organizing data in a fashion so it can be effectively used by a computer program. It is a major problem in data analysis which slows down research.)

[ID: 244] MALDI-TOF mass spectrometry allows identification of Kayvirus genus phages in phage preparations

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Staphylococcus aureus is a major causative agent of infections associated with hospital environments, where antibiotic-resistant strains have emerged as a threat. Phage therapy could offer a safe and effective alternative to antibiotics. Phage preparations should comply with quality and safety requirements; therefore it is important to develop efficient production control technologies. This study was conducted to develop and evaluate a rapid and reliable method for identification and distinguishing of staphylococcal phages based on detecting their specific proteins using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) profiling. This technique is among the suggested methods for meeting the regulations of pharmaceutical authorities. Five different phage purification techniques were tested in combination with two MALDI-TOF MS matrices. Phages, either purified by CsCl density gradient centrifugation or as resuspended phage pellets, yielded mass spectra with the highest information value if ferulic acid was used as the MALDI matrix. Phage tail proteins yielded the strongest signals whereas the culture conditions had no effect on mass spectral quality. Thirty-seven phages from Myoviridae, Siphoviridae or Podoviridae families were analysed. 23 siphophages belonging to the International Typing Set for human strains of *S. aureus* were included as well as phages belonging to Kayvirus genus in preparations produced by Microgen, Bohemia Pharmaceuticals and MB Pharma. The data obtained demonstrate that MALDI-TOF MS can be used to effectively distinguish between *Staphylococcus*-specific bacteriophages.

Acknowledgements:

This work was supported by the Ministry of Health of the Czech Republic (grant number NT16-29916A). CIISB research infrastructure project LM2015043 funded by MEYS CR is gratefully acknowledged for the financial support of the MALDI-TOF MS measurements at the Proteomics Core Facility.

[ID: 76] Bacteriophage predation selects for attenuated virulence in *Listeria monocytogenes*

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The typically foodborne intracellular pathogen *Listeria monocytogenes* is distinguished by its ability to invade and replicate within mammalian cells. Remarkably, though there are 15 defined serotypes within the *Listeria* genus, strains belonging to serotype 4b cause a majority of listeriosis cases and most outbreaks. The antigenic basis of serotype in *Listeria* is attributed to the monomeric wall teichoic acids on the cell wall of the bacterium, which can be differentially glycosylated or acetylated. In *Listeria*, bacteriophages adsorb to these decorations with a high specificity, which defines phages' host range. Here, we use an engineered lytic version of the siphovirus A500, which possesses a 4b-specific host range, to target a serotype 4b model strain. Upon predation of the bacterium, surviving resistant *Listeria* strains lose the galactose decoration from their wall teichoic acid and lipoteichoic acid via inactivating mutations in genes required for teichoic acid galactosylation, and in doing so convert themselves to the avirulent 4d serotype. Upon further scrutiny, we found that loss of galactosylation led to a notable inability of the *L. monocytogenes* strain to invade human cells *in vitro*, and a significant virulence attenuation *in vivo*. We attribute this loss of invasiveness to the classical *L. monocytogenes* virulence factor internalin B, which we observe dissociates from the bacterial cell wall upon forfeiture of galactosylation, due to a loss of direct adherence with the teichoic acids. We therefore theorize a stimulating evolutionary model, which depicts 4b strains maintaining their virulence directly through the serotype antigens via internalin B, at the cost of being susceptible to bacteriophage predation, thus defining bacteriophage A500 as an antivirulence agent.

[ID: 82] Characterization of a dominant phages group; Salmonella Chi-like bacteriophages isolated from poultry farms in Thailand

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Salmonellosis in humans is generally occurs through the consumption of Salmonella- contaminated food of animal origin such as poultry and porcine. Little is known about poultry-associated Salmonella bacteriophage (phage) in Thailand. In this study, 421 chicken droppings were randomly collected from the poultry farms in Thailand using Salmonella enterica serovar Typhimurium as a host strain to screen the phages. Of 108 isolated of phages, phiSTm-101, phiSTm-118 and phiSTm-374 showed different plaque morphologies and host ranges, so were selected for whole genome sequencing. Genome sequence analysis indicated that the isolated Salmonella phages have genome sizes around 59 Kb and shared 91.9%-92.9% nucleotide homology to phiSPN19, a member of Chi-like virus genus. PCR amplification of the other 105 phages with Chi-like virus capsid protein E primers revealed that 54/108 (50.0%) of phages gave positive reaction. This suggests that the Chi-like virus is predominated among all Salmonella phages in our collection. Data from various Salmonella mutants infection indicated that phiSTm-101, phiSTm-118 and phiSTm-374 infected Salmonella via bacterial flagella and LPS. Interestingly, phiSTm-374 can use Ton-B dependent receptors as an additional receptor to infect Salmonella, which is correlated with its broader host range of Salmonella infections than the other two phages. Electron micrographs of these three phages indicated that they are members of Siphoviridae family. A phylogenomic tree reconstructed from a shared core set of 28 orthologs (from sequences of 14 Chi-like phages), illustrated that phiSTm-101 is more closely related to the phiSTm-118 whereas the phiSTm-374 appeared to be distantly related to the phages STm-101 and STm-118. Taken together, these data indicated the heterogeneity among Salmonella Chi-like phages and that they are predominant, at least, in poultry farms in Thailand.

[ID: 199] Improving virome assembly through hybrid sequencing

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The gut microbiome is widely accepted to have a significant impact on human health yet, despite years of research on this ecosystem, the forces driving microbial population structure remain poorly understood. We propose that the virome is one of the main agents responsible for shaping the gut microbiome. Characterization of this “gut virome” is suited to a metagenomic approach, due to difficulties in culture based methods such as unidentified or uncultivable viral hosts. However, viral metagenomes present a number of difficulties which hamper assembly algorithms and cause fragmented or inaccurate assemblies of individual viral genomes.

Technologies such as single molecule real-time (SMRT) sequencing, generate long, low-coverage reads with relatively high error rates. Hybrid sequencing is an approach which uses these long reads in conjunction with accurate short reads to generate high quality assemblies and addresses the individual failings of each sequencing technology. Here we demonstrate as a proof of concept, improved assembly of putative viral genomes from a human gut virome using a hybrid sequencing approach. To our knowledge, this is the first application of hybrid sequencing using virome data.

[ID: 74] Diversity of bacteriophages that infect *Aggregatibacter actinomycetemcomitans*.

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Aggregatibacter actinomycetemcomitans (A.a.) is generally considered to be a commensal oral bacterium. Yet the carriage of highly toxic A.a. JP2 clone by healthy Moroccan adolescents poses a high risk of periodontitis (1). A.a. has been also isolated from non-oral infections like endocarditis, soft tissue abscesses, and osteomyelitis. Only a tiny fraction of A.a. phages has been explored so far (2).

Our aims are to estimate the diversity of A.a. phages, elucidate their role in oral ecology and pathologies, and evaluate their biotechnological potential.

We identified 830 phage-like elements in 274 genomes of *Aggregatibacter* and related *Haemophilus* species. Thirteen putatively intact A.a. prophages could be classified into three main types. We developed a PCR for detection of these prophage types. Nine sets of primers, three for each type, were designed based on conserved regions of their genomes. PCR-based screening was performed on 157 A.a. strains and 32 strains of related species. Our A.a. strain collection encompasses isolates representing 6 serotypes, 7 countries of origin, health and 3 main clinical conditions (i.e., adult periodontitis, localized juvenile periodontitis, and abscesses). Overall, prevalences in A.a. strains of 29 %, 7 %, and 5 % were observed for types I – III, respectively. Lambdoid type I prophage was highly prevalent in serotype a strains, whereas transposable types II and III prophages were commonly found in genomes of serotype e strains. Genetic and functional characterisation of selected phage isolates has been performed.

(1) Haubek D, Ennibi OK, Poulsen K, Vaeth M, Poulsen S, Kilian M. (2008) Risk of aggressive periodontitis in adolescent carriers of the JP2 clone of *Aggregatibacter* (*Actinobacillus*) *actinomycetemcomitans* in Morocco: a prospective longitudinal cohort study. *Lancet*. 19;371(9608):237-42.

(2) Szafrąński SP, Winkel A, Stiesch M. (2017) The use of bacteriophages to biocontrol oral biofilms. *J Biotechnol*. 20;250:29-44.

[ID: 268] Characterization of lytic Lactococcus 936-type phages from dairy environments by physiological, molecular and whole-genome sequencing approaches

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Irrelevant of multiple attempts, bacteriophage infections significantly contribute to production disturbances observed in milk factories worldwide (1). This in turn, translates into serious economical consequences. *Lactococcus lactis* strains are commonly used as starters for manufacture of cheeses, sour cream, kefir, etc. (2). Studies on *Lactococcus* phage populations isolated from failed fermentations conducted in various geographical locations indicate the predominant presence of 936-type species in dairy settings (2-5). This is due, at least in part, to their high heat and desiccation resistance (6, 7). Understanding the biology of industrially-emerging bacteriophages is a fundamental factor in controlling their prevalence in this environment. To gain more knowledge on *Lactococcus* 936-type phages persisting in dairy environments in Poland, we characterized eight distinct phages from different geographical regions. For this purpose, we applied various approaches, including plaque assays, electronic microscopy, host range and one-step growth studies, as well as PCR-based techniques. Additionally, by high-throughput sequencing, we unraveled their genomic composition. Phage isolates were determined to exhibit a narrow host range, fast growth rate and fairly high burst sizes. Comparative analyses of phage genomes revealed their close relatedness, suggesting low frequency of evolution. Thus, we postulate that the Polish 936-type isolates examined in this work have recently diverged from one ancestral phage, irrespectively of geographical region and time of isolation.

1. Szczepankowska AK. et al. (2013) *InTech*. 2: 23-71.
2. Moineau, S. et al. (1996) *J. Dairy Sci.* 79: 2104–2111.
3. Josephsen J. et al. (1994) *Int. Dairy J.* 4: 123-140.
4. Bissonnette F. et al. (2000) *J. Dairy Sci.* 83: 620–627.
5. Brüssow H. (2001) *Annu. Rev. Microbiol.* 55: 283–303.
6. Atamer Z. et al. (2009) *Int. Dairy J.* 19: 228–235.
7. Verreault D. et al. (2011) *Appl. Environ. Microbiol.* 77: 491–497.

[ID: 411] P5 protein of Pseudomonas bacteriophage ϕ 6 is a lytic transglycosylase.

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P5 originates from Pseudomonas phage phi 6 and displays lytic activity against Gram-negative bacteria. It has been classified as a peptidoglycan protease of “Unknown Catalytic Type”, which may potentially function through a novel mechanism of catalysis. Different results are, however, conflicting and P5 was also suggested to function as lytic transglycosylase. The aim of this study was to define the catalytic mechanism of P5 through biochemical, mutagenesis and structural characterization.

Our crystal structure of P5 as well as the other published results (Desseau et al., PLoS Genet., 8:e1003102) suggest that P5 functions as a transglycosylase rather than a protease. Accordingly, substrate specificity analysis using synthetic substrates and bacterial sacculus allowed us to document typical lytic transglycosylase products. Mutagenesis of key residues further documents such mechanism of action. Additionally, the crystal structures documented flexible N and C terminal extensions which assume different positions in different structures and have unknown function. Site directed truncations allowed us to dissect the role these extensions. While the N-terminal part does not seem to have any influence on catalytic activity and its function remains unknown the C-terminal helix is involved in the regulation of P5 activity.

[ID: 354] Gut microbiome composition in mice after 100 days of oral treatment with staphylococcal bacteriophage A3R

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Objectives: Nowadays, rapidly developing techniques of Next-Generation Sequencing allow for fast and accurate examination of different microbiomes. Because of that technological progress more and more people associated with scientific society are getting involved in experiments investigating the impact of various factors on microbiomes habitats. Among those factors are bacteriophages. Their potential application in phage therapy evokes questions on phage effects on bacterial communities. These effects may determine safety of therapeutic phage applications, due to the high impact of microbiomes on human and animal health.

Methods: Staphylococcal phage – A3R was selected because of their lytic properties. Phages were given to male C57Bl/6 mice (N=6) in drinking water/PBS solution in mixed by 1:1 ratio. The control group of animals received only water/PBS mixture without phages. Fecal samples were collected after 100 days of the treatment. That material was used for microbial DNA isolation with QIAamp Fast DNA Stool Mini preceded by 0,22 mm zirconia beads physical homogenization. DNA samples were processed to 16S rDNA targeted sequencing in the Ion PGM system.

Results: Sequencing revealed that microbiome of control mice consisted of 70% Bacteroidetes, 25% Firmicutes, 3% Proteobacteria and 1% Actinobacteria. Microbiome of A3R-treated mice was covered in 30 % by Bacteroidetes, 54% by Firmicutes, 13% by Proteobacteria and 2 % of Actinobacteria. There were no significant differences in alpha diversity in experimental groups. In each sample, in all representative phylums the composition of bacterial classes remained unchanged. An altered Firmicutes/Bacteroidetes ratio was observed in comparison to control group, but it is unclear if it has and impact on animals' physiological condition.

This work was supported by the Wrocław Centre of Biotechnology, programme The Leading National Research Centre (KNOW) for years 2014–2018

[ID: 75] To bind or not to bind? New insights on phages and phage receptors of the dairy bacterium *Streptococcus thermophilus*

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Streptococcus thermophilus is among the most common starter bacteria used in cheese and yoghurt production worldwide. Bacteriophage infections represent a major concern during dairy fermentations, because they lead to acidification failures, resulting in a lower quality of final products. This work focuses on characterizing *S. thermophilus* phages and identifying cell wall receptors involved in phage adsorption to the host.

Firstly, genome sequencing and comparison was performed for a number of *S. thermophilus* phages from the Chr. Hansen A/S collection. Based on their overall genetic similarities, the studied phages could be divided into four clusters. One of the groups comprised representatives of a new type of *S. thermophilus* phages. Following phage characterization, interactions of selected phages with their hosts were visualized using conventional and super-resolution fluorescence microscopy. The assays revealed two adsorption patterns, suggesting that cell wall components which serve as receptors for phages were differently distributed around the bacterial cell surface.

Subsequently, six industrial *S. thermophilus* strains were used to develop a range of spontaneous phage-resistant mutants. Analysis of their genome sequences unveiled SNPs in genes putatively involved in the biosynthesis of the cell surface components. Cell walls of selected industrial strains and their mutants were further characterized using biochemical approaches. The relations between different cell wall associated compounds and phage binding were verified with adsorption assays.

In summary, we characterized four types of *S. thermophilus* phages and proposed cell wall structures involved in phage-host interactions. These data are expected to assist developing better strategies to control phage attacks in dairy plants.

[ID: 324] Evaluation of lytic activity of bacteriophages in lipid-based formulations

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Application of bacteriophages as an alternative therapeutic agents becomes of growing interest as the infections caused by antibiotic-resistant bacteria poses a critical problem and burdens health system. Renewed interest in phage therapy is accompanied by research in development of chemical carriers facilitating their application. Among these, natural based nanocarriers might be a promising delivery system, due to their biocompatibility and small size (1).

The objective of this study was to evaluate in vitro the lytic efficacy of E. coli-specific bacteriophages by formulating three different lipid-based carriers. Model bacteriophage T4 and BF9, which is highly active against ESBL/AmpC producing E.coli(2), have been successfully immobilized by emulsion, glycerosomes and conventional liposome systems. Prepared formulations were described by physicochemical parameters and biological activity. The lytic activity of bacteriophage BF9 after release from all preparations have been retained and reached the value at the level of initial phage population. Although, viability of phage T4 has been affected after release from classical liposomes and glycerosomes (about 3.26 log₁₀ pfu/ml and 2.0 log₁₀ pfu/ml reduction, respectively), it may still represent a therapeutic dose. This results indicate that developed formulations might be a promising delivery system of bacteriophages for efficient treatment of bacterial infections for different applications, including topical agents or systemic delivery.

1. Malik DJ, Sokolov IJ, Vinner GK, Mancuso F, Cinquerrui S, Vladislavjevic GT, Clokie MRJ, Garton NJ, Stapley AGF, Kirpichnikova A. 2017. Formulation, stabilisation and encapsulation of bacteriophage for phage therapy. *Adv Colloid Interface Sci* 1–34.

2. Skaradzińska A, Śliwka P, Kuźmińska-Bajor M, Skaradziński G, Rzaśa A, Friese A, Roschanski N, Murugaiyan J, Roesler UH. 2017. The efficacy of isolated bacteriophages from pig farms against ESBL/AmpC-producing *Escherichia coli* from pig and Turkey farms. *Front Microbiol* 8.

[ID: 109] Diversity and evolution of arbitrium systems in phage genomes

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Upon the infection of a host cell, a temperate phage is able to choose between two developmental pathways, the lytic pathway and the lysogenic one. In a previous study, we showed that a genetic system called arbitrium utilizes peptide-based communication to regulate the lysis-lysogeny decision in phages belonging to the SpBeta group, infecting *Bacillus subtilis*. This system was shown to rely on 3 genes: *aimP*, which produces a secreted peptide; *aimR*, an intracellular peptide receptor; and *aimX*, a ncRNA negative regulator of lysogeny. Here, using extensive homology searches, we found that arbitrium and arbitrium-like systems are widely spread among phages infecting soil bacteria. We show that the original arbitrium system, described in our previous study, is only a subset of a much wider array of peptide-based communication systems. These new systems vary in their peptide codes and can be detected in numerous phage types. We further experimentally verified that distant versions of arbitrium are used to regulate lysogeny decisions among genetically distant phages. Finally, we show how the *aimX* ncRNA executes the downstream effect of the lysogeny decision via an antisense-based mechanism.

[ID: 390] The use of Raman spectroscopy and chemometrics to detect and discriminate low-titer phages in raw milk

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Phages are among the leading causes of problems in the dairy industry. The phage presence in the raw milk affects the starter cultures, causing fermentation to slow down and sometimes even stop completely. This situation can lead to serious financial losses in dairy businesses. Therefore, it is crucial to be able to detect phage presence in the raw milk just before the process. Since conventional detection methods require a long analysis time, can be affected by naturally occurring inhibitory substances in the environment and may not be sufficiently specific, the need for a faster and reliable analysis is increasing day by day. For this purpose, a method has been developed to detect and discriminate phages in raw milk using the combination of Raman spectroscopy with chemometric methods. For this purpose, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* phages, which are among the most problematic phages in the dairy industry, were chosen. Phages were added separately into raw milk, and Raman spectra of the samples were collected after some pretreatments. Then the obtained spectra were analyzed by one of the chemometric analysis methods, namely principal component analysis (PCA) to discriminate these phages in raw milk. After that, different concentrations of *S. thermophilus* phages in raw milk were prepared and Raman spectra were collected. These spectra were analyzed by using partial least squares (PLS) method to detect phages in low titer. As a result, it has been demonstrated that *S. thermophilus* and *L. bulgaricus* phages could be discriminated even when they have low titers (10^2 - 10^3 pfu/ml) not enough to fail fermentation. It has also been found that low concentrations of *S. thermophilus* phages (10^2 pfu/ml) could be detected through Raman spectroscopy with a short analysis time (60 min) and high regression square (0.98).

[ID: 227] Isolation, characterisation and delivery of giant bacteriophages infecting the pig enteric pathogen *Salmonella enterica*

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The World Health Organisation has reported 1 in 10 people worldwide suffer from food poisoning each year and 11.7% of infections are caused by consumption of pig meat contaminated with *Salmonella* spp. This is likely due to over a third of pigs being infected with *Salmonella* when slaughtered. However, treating infections by antibiotics in pigs is becoming less effective due to a surge in infections caused by multi-drug resistant (MDR) *Salmonella* strains on farms. Natural bacteriophages can provide a safe and effective alternative in controlling *Salmonella* in agriculture and food and have already been granted the status 'Generally Regarded as Safe' (GRaS) by the FDA. The aim of our research was to isolate a panel of bacteriophages that could be developed therapeutically to prevent *Salmonella* infection in pigs and data will be presented on progress made.

We liaised with farmers to collect pig faecal samples, from which 21 myoviruses were isolated. All phages could infect 74 MDR strains from the most prevalent *Salmonella* serotypes associated with pigs, with significantly similar efficiency of plating. Genetic analysis revealed all phages had what is referred to as 'giant' genomes at approximately 240 kbp. According to predicted protein similarity they cluster together with two other *Salmonella* phages previously isolated in Korea. No phages contained predicted integrases or virulence genes. From isolated phages two phage cocktail could reduce *Salmonella* by 4-logs in 2 hours when they were applied at an MOI of 100 both in vitro and in the in vivo *Galleria mellonella* infection model. Furthermore the phages can be converted from liquid to powder via spray drying with less than 1 log₁₀ reductions in titres. We are currently collaborating with farmers and end users to determine the optimum intervention point at which phages could provide maximum benefit in reducing *Salmonella* in pigs.

[ID: 328] Implementation of the *Galleria mellonella* larvae model to evaluate phage therapy against *Klebsiella pneumoniae*

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Klebsiella pneumoniae is an encapsulated Gram-negative bacillus belonging to the Enterobacteriaceae family. *K. pneumoniae* strains display a large number of capsular serotypes. It is an opportunistic pathogen causing severe therapeutic challenges due to the continuous emergence of multidrug resistant strains. The aim of this work is to develop an infection model of *Galleria mellonella* larvae with *K. pneumoniae* strains of interest to evaluate phage therapy strategies.

Three bacteriophages, isolated from wastewater plants of Paris against two *K. pneumoniae* strains of epidemiological interest, were selected for further analysis. Two against strain ST258 (phages Kunk-ULIP47 and Kunk-ULIP54) and one against ST23 (phage K1-ULIP33). The host range of these three bacteriophages displays a high specificity for the *K. pneumoniae* capsular type. Their genomes were sequenced using an Illumina platform. In a first in vivo experiment, 110 larvae were used to assess the optimal inoculation dose of *K. pneumoniae* to use for phage therapy experiments. A dose of 104 CFU (in 10 µl) resulted in a 70-90% killing of larvae in 4 days. In the second and the third experiment, 480 larvae were inoculated to assess both prophylactic and curative treatment of the selected bacteriophages.

The whole genome sequencing shows that Kunk-ULIP47 and Kunk-ULIP54 and K1-ULIP33 belong to the Podoviridae family and Autographivirinae subfamily. More than 80% of survival is observed in the larvae treated with the bacteriophages at a multiplicity of infection of 10 compared to the non-treated *K. pneumoniae* infected control in which over 90% of larvae died. The group of larvae inoculated with bacteriophages only showed comparable survival rate as the PBS control group. Both prophylactic and curative groups showed similar survival rates.

These results show that *G. mellonella* could be used as a preliminary model to test phage therapy against *Klebsiella pneumoniae* infection.

[ID: 86] Identification of phage BFK20 adsorption receptors on bacterial host *Brevibacterium flavum* CCM 251

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Bacteriophages are viruses that infect procaryotic organisms with high specificity and efficiency. Phage ability to infect certain host cell depends on capability of adsorption onto specific receptors on bacterial cell wall. Phage adsorption is a crucial step in infection of bacteria that triggers injection of phage DNA into the host. Interaction between phage's receptor binding protein and host's adsorption receptor is very specific. The adsorption receptors may have various character from peptides to polysaccharides depending on the composition of the bacterial cell wall. BFK20 is a lytic phage of its host *Brevibacterium flavum* CCM 251, industrial producer of L-lysine. We used EZ-Tn5TM Tnp TransposomeTM Kit to create a library of transposon mutants of *B. flavum* for identification of BFK20 adsorption receptors. We optimized method of electrocompetent cells preparation to achieve high transformation efficiency (105 CFU/ml). The result of several transposition with EZ-Tn5TM Tnp was 2×10^4 colonies and we analysed 693 transposon mutants of *B. flavum* in detail. First we determined their sensitivity (resistance) against lysis of BFK20. Measurements were taken in a microtiter plate reader and assays were repeated in triplicate. Transposon mutants (TM) that resisted to BFK20 lysis were subsequently analysed in 50 ml media in cultivation flasks. We localized insertion site of transposon into chromosomal DNA of *B. flavum* TM by single primer PCR and analyzing the sequence of PCR products. The resulting sequences were used as a query to search the Blast non-redundant database using BLASTX. We identified the highest similarity with genes coding MFS transporter, aldehyd dehydrogenase, putative ATPase and septum site determining protein. We suppose that these proteins could have a role in a biosynthetic pathway of specific receptors involved in adsorption of BFK20 virion.

Acknowledgement: This work was supported by the VEGA Grant 2/0139/18 from Slovak Academy of Sciences

[ID: 364] Morphological and biological characteristics of new isolated bacteriophage vB-EcoS-95 and use of this phage to control the formation of biofilms

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Bacterial viruses, or bacteriophages, are estimated to be the most widely distributed and diverse entities in the biosphere. Bacteriophages are viruses that infect and lyse bacterial cells and they have played and continue to play a significant role in bacterial genetics and molecular biology. In recent years research on bacteriophages has become more popular, among others the use of phages to combat environmental biofilms.

In this part of the research we would like to present a virulent Siphoviridae phage, named vB-EcoS-95 that was isolated from sample of urban sewage. The aim of the study was determined on the morphological and biological characteristics of vB-EcoS-95. This phage was found to infect *Escherichia coli* bacteria giving clear plaques. Genome sequence analysis of vB-EcoS-95 revealed 74% similarity to genomic sequence of *Shigella* phage pSf-1. Compared to pSf-1, phage vB-EcoS-95 does not infect *Shigella* strains and has an efficient bacteriolytic activity against *E. coli* strains. One-step growth analysis revealed that this phage has a very short latent period (5 min) and a large burst size (115 PFU/cell) which points to its good infectivity of host cells and strong lytic activity. The bacteriolytic effect of vB-EcoS-95 was tested also on biofilm-producing strains. Based on the preliminary research we can notice that this bacteriophage can destroy the bacterial biofilm, which has been confirmed by several different experiments. In conclusion, the obtained results show that vB-EcoS-95 is a novel *E. coli* phage that may be used to control the formation of biofilms.

Reference list

- [1] Jurczak-Kurek et al.,(2016). " Biodiversity of bacteriophages: morphological and biological properties of a large group of phages isolated from urban sewage".
- [2] Jin Woo Juna, et al., (2013). "Characterization and complete genome sequence of the *Shigella* bacteriophage pSf-1".

[ID: 379] Biohackers identify four lytic phages as decontamination agents for *Enterobacter hormaechei* subsp. *Steigerwaltii*

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Biohackers from the Do It Yourself Biology (DIYbio) community identified phage therapy as the best solution to the antimicrobial resistance crisis, as it follows an alternative rationality to existing therapeutic regimes. Since 2014 biohackers develop automation technology to facilitate phage therapy. A recent case of a contaminated reagent kit was used as a test run to merge phage therapy protocols with our latest automation technology.

In March 2017, the authorities for health and food safety (LGL) of Bavaria, Germany, issued a public warning about a pathogen contamination in a kit supplied by The Odin, a company selling biotechnology reagents over the web. The educational kit was contaminated with *Enterobacter* species and *Klebsiella pneumoniae* and subsequently banned from import to Germany. After the public warning, biohackers of Europe took a proactive attitude and gathered three samples independently from LGL and performed WGS on an MiSeq Illumina platform (ENA database ID PRJEB23486). The analysis returned *Enterobacter* species and after alignment 83% of the reads, contigs were mapped to the genome of *Enterobacter hormaechei* subsp. *Steigerwaltii* which is in accordance with the LGL report. Subsequently, de novo phage isolation was conducted at Eliava IBMV. Four *Enterobacter hormaechei* subsp. *Steigerwaltii* specific lytic bacteriophages were isolated from river water in Tbilis, Georgia and of the black sea coast. Phages were plaque purified and high-titer phage stocks were prepared according confluent lysis plate method. TEM and sequencing of the phage genomes are currently under way.

All protocols were analysed and are now being adapted to automate procedures using open source digital microfluidics, incubation and filtration systems. To enable widespread application of phages as therapeutic or decontamination agents further effort is needed in automating laboratory procedures to avoid time consuming manual practise. The goal of the DIYbio community is to automate all phage protocols to establish open source phage libraries.

[ID: 57] Efficacy of coliphages to prevent chicken embryo mortality

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Avian colibacillosis, induced by specific *Escherichia coli* strains, is the major bacterial disease in poultry and is mainly treated by antibiotics. Increasing resistance to different antibiotic classes and restrictions on the use of antibiotics by the European Union has prompted a search for alternatives to antibiotic chemotherapy, such as phage therapy. To date, efforts to protect chickens from APEC infections have had limited success.

We have therefore isolated and characterized a new set of 21 coliphages as potential biocontrol agents. Among those phages, five are highly related to phAPEC8, and three to phage phi92. When mixing *in vitro* two phAPEC8-related phages, we observed the appearance of a recombinant phage, which exhibited a broader host range and a higher lytic activity compared to parental phages. We tested in a chicken embryo lethality assay the therapeutic potential of the recombinant phage either alone or in combination with a phi92-related phage. We observed that the combination of both phages allowed 100% of chicken embryos to survive an infection by an avian pathogenic strain, in contrast to the control, which gave a survival rate of 20%. Our results strengthen the effective potential of phages to control avian colibacillosis.

[ID: 286] Characterization of four novel broad-temperature range *Pantoea myoviruses*

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Plants are usually populated by a complex microbial communities composed of algae, fungi, bacteria and their viruses. The composition of these communities varies across the hosts and depends on interspecies interactions and many other factors of the plant environment. Although the cellular microbiome of plants has been the subject of many studies, the impact of viral component is still poorly explored.

In this study, six bacterial strains that formed yellow-pigmented colonies on the Luria Broth agar were isolated from redcurrant, jostaberry and shadbush bushes. Based on 16S rDNA sequences, all isolates belonged to the genus *Pantoea*. By using these newly isolated strains as the hosts, we isolated a number of bacteriophages, four of which (AAM22, AAM37, PSKM and SSEM1) had similar virion morphology. Transmission electron microscopy images showed that all phages were members of the family Myoviridae, and had isometric heads (diameters ranging from 63 to 67 nm), and contractile tails from about 90 to 118 nm in length. Efficiency of plating test revealed that phages AAM22, SSEM1, PSKM1 and AAM37 could form plaques in the temperature ranges of 4–30, 4–34, 4–35, and 4–40 °C, respectively. The restriction analysis of phage genomic DNAs revealed distinct restriction profiles indicating that, despite the similar size and virion morphology, phages AAM37, AAM22, PSKM and SSEM1 have different genomic composition.

The genus *Pantoea* is one of the predominant taxa in a variety of phyllospheres. It comprises both pathogenic and beneficial species that can survive as epiphytes or endophytes on their hosts, but sometimes can cause infections in humans. Therefore, broad-temperature range bacteriophages could be useful for the prevention and therapy in both plants and humans or for food preservation. This research was funded by a grant (No. SIT-7/2015) from the Research Council of Lithuania.

[ID: 21] Recovery of mycobacteriophages from archival stocks stored for approximately 50 years in Japan

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Mycobacteriophages, viruses that infect *Mycobacterium* spp., are presently known to be one of the most genetically-characterized phage groups. We have obtained the archival stocks stored for ca. 50 years in Japan due to the retirement of principle investigators. In this study, the mycobacteriophages were recovered from the archival stocks, and their phylogeny and taxonomy were analyzed using the genome sequences.

The phages were recovered from lyophilized or liquid archival stocks by double-layered agar method, using *M. smegmatis* mc2155 as host. The genomes were sequenced using Roche GS Jr. Proteomic tree of phage genomes was constructed using a software VipTree. The mycobacteriophage genome reference data were downloaded from PhagesDB.

The phages were recovered from 50 stocks at 56% (28/50 stocks). Among them, 72.2% of the phages (13/18 stocks), which had been stored for 47–56 years in lysophilization, were recovered. Moreover, morphological analyses of 12 representative recovered phages, including A6, BK1, C3, D29, GS4E, B1, PP, HC, Y2, Y10, D12 and PR, led to their classification as belonging to the family Siphoviridae. Furthermore, the phylogenetic tree of 12 phages was constructed with 1528 mycobacteriophages that have recently been isolated. These reference phage genomes have been categorized in clusters/subclusters. According to these data, A6, BK1, C3, D29, GS4E, B1 and PP were categorized into cluster A, which represents the genus L5virus. HC was categorized into subcluster I1, which represents the genus Brujitaivirus. Y2 and Y10 were categorized into cluster K, which represents the genus Tm4virus. D12 and PR were categorized into cluster W, to which a viral genus has not been assigned yet. Considering the importance of such archival mycobacteriophages, high number of mycobacteriophages and their genetic diversity, the fine viral taxonomy in mycobacteriophage will be hopefully proposed.

[ID: 81] Unravelling the function of the toxic phage protein Gp21 as inspiration for new antibiotics to combat *P. aeruginosa* infections

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Bacteriophages are notorious for their effective bacterial-killing strategies. The ongoing coevolution with their bacterial hosts has resulted in the emergence of highly efficient strategies to manipulate the host metabolism and promote their own propagation. In turn, these strategies yield inspiration for the development of new antibacterial strategies. However, due to the lack of sequence similarity to known genes, functional annotation of predicted phage genes is highly challenging and many host-manipulating strategies so far remain elusive.

In this research, we aim to elucidate the function of a single protein Gp21 of *Pseudomonas aeruginosa* phage LUZ19. This late-early protein is one of the most abundant proteins during the LUZ19 infection cycle and induces immediate growth arrest upon synthetic expression in *P. aeruginosa*. At the molecular level, Gp21 causes a drop in bacterial transcription and translation, but not in DNA replication¹. Furthermore, this protein has the ability to bind both DNA and RNA, but has a clear preference for ssDNA, prompting the hypothesis that Gp21 is a ssDNA-binding protein. Cell biology studies using fluorescence microscopy revealed that Gp21 does not associate with the *P. aeruginosa* nucleoid, but rather assembles into mobile foci in the bacterial cytoplasm. Finally, determinants of Gp21 toxicity were identified by studying six loss-of-toxicity mutant proteins *in vivo* and *in vitro*. Both mutants that lost the ability to form foci and that showed aberrant nucleic acid-binding, were identified, suggesting that both characteristics are related to the Gp21 toxicity. Further experiments will focus on the elucidation of the specific biological role for this nucleic acid-binding phage protein.

[ID: 257] Post-harvest application of bacteriophages on beef as a natural intervention against E. coli O157

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Escherichia coli is a major food pathogen that remains a great concern for the food industry, in particular for beef processors. Especially shiga toxin producing E. coli (STEC) can cause serious illness, which can lead to Hemolytic Uremic Syndrome (HUS) and in some cases to death. The specificity and natural origin of bacteriophages potentially form an additional safe and effective intervention against E. coli O157 on beef. Therefore, the objective of this study was to determine the efficacy and feasibility of applying bacteriophages as a natural intervention against E. coli O157 on refrigerated beef.

For this purpose, bacteriophages were isolated from sewage water, after which lysis activity of the isolated phages was assessed by spotting serial dilutions on 88 E. coli O157 strains. Based on the resulting lysis activity, two phages with a complementing and broad hostrange activity were selected to form a phage cocktail, lysing 90 % of all E. coli O157 strains tested. Subsequently, cold beef cuts (4 °C) of 9 cm² were contaminated with four different E. coli O157 strains at a rate of 1×10^5 cfu/cm², after which they were treated with the two phage cocktail at 3×10^7 or 3×10^8 PFU/cm². The cocktail of the two selected phages was able to reduce the bacterial load by 1.5 -1.9 log₁₀ when contaminated cold beef was treated with 3×10^8 PFU/cm², while 0.8 – 1.5 log₁₀ reductions were observed with 3×10^7 PFU/cm². A time trial experiment showed that the majority of the reduction was already achieved 2 hours after phage application, and that no further reduction was observed after 6 hours.

All in all, we show that bacteriophages provide a natural, safe, and effective intervention for the beef industry to fight E. coli O157.

[ID: 219] Crystal structures of bacteriophage receptor binding proteins

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Bacteriophages use receptor binding proteins to recognise their host cells. They have specialised proteins for initial, reversible, host cell wall recognition. Once a suitable host is found, the phage commits to infection by irreversible attachment via a secondary receptor interaction. The crystal structures of several of these receptor binding proteins have been solved by X-ray crystallography, and have been shown to be mainly beta-structured, but structurally highly diverse and containing several new protein folds. Structures of the receptor-binding proteins of the coliphages T4, T5 and T7, of the Salmonella phage epsilon15 and of the Staphylococcus phages S24-1 and K will be shown. We also discovered that a eukaryotic virus (adenovirus) has a capsid protein (LH3) that contains a bacteriophage tailspike fold.

Ongoing structural, mutational and binding analysis of virus receptor-binding proteins with receptors and receptor analogues will be discussed. Bacteriophage receptor recognising proteins may be used for bacterial detection, while modification by natural or experimental mutation of bacteriophage receptor binding domains may allow retargeting of phages to alternative host bacteria. Their shape and stability may also allow their use in nanotechnological applications.

[ID: 184] Eradication of ST131 E. coli and cc258 K. pneumoniae using bacteriophage

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ST131 E. coli with blaCTX-M-15 and cc258 K. pneumoniae carrying blaKPC are globally disseminated highly virulent clones dominating the epidemiology of life-threatening multidrug resistant infections [1], and common colonizers of the human gut, able to persist asymptotically for up to 12 months thus increasing the risk of transmission to vulnerable hosts [2]. Alternative or adjuvant therapies to antibiotics are urgently needed to both combat refractory infections and limit dissemination of these problematic opportunistic pathogens [3]. Bacteriophage therapy may provide a valid clinical alternative, as obligately lytic bacteriophages can be readily isolated and have the potential to be effective against multidrug resistant bacteria [4]. However, routine implementation of bacteriophage treatment in the clinic is hindered by poor understanding of therapeutic applicability, penetration, and resistance [5]. We are currently investigating a rational design approach to the development of bacteriophage therapeutic cocktails to be used as a gut decontamination strategy. We have fully characterized sets of target bacterial strains (60 ST131 E. coli; 20 cc258 K. pneumoniae), and tested the infectivity of >30 bacteriophages from our extensive library (AmpliPhi BioSciences). Phages specific to each target population (n=8 for E. coli; n=15 for K. pneumoniae) were selected for detailed characterization. Using a combined genomic and molecular microbiology approach, we found that the genetic diversity of each bacterial population was associated with specific phage susceptibility profiles reflecting the structural specificity of the bacterial outer cell envelope. With this work, we aim to establish a robust scientific rationale for the selection of phages for the development of optimal therapeutic cocktails against problematic pathogenic clonal species.

[1] Woodford et al. 2011 FEMS Microbiol Rev; [2] Kang et al. 2005 AAC; [3] WHO (2014) Antibiotic Resistance: Global report on surveillance 2014; [4] Lin et al. 2017 WJGPT; [5] Abedon (2018) in Host-Pathogen Interactions. Methods in Molecular Biology.

[ID: 6] Encapsulation of enteric bacteriophages in pH responsive polymeric microcapsules using scalable microfluidic membrane microarrays

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Bacteriophages may be encapsulated in protective micro- and nanocapsules to improve stability e.g. for treatment of gastrointestinal infections affording protection of phages from gastric acidity during oral delivery (Malik et al. 2017). Previous phage encapsulation studies have utilised different mechanical emulsification techniques involving homogenisation and simple extrusion methods. These methods do not afford precise control over the uniformity of the resulting microcapsules. We have recently shown for the first time that advanced microfluidic based methods result in production of highly uniform droplets with exquisite control of the size of microcapsules containing the encapsulated enteric phages (Vinner et al. 2017). Single microfluidic droplet generators however have inherent limitations in terms of scale-up. In the present work we demonstrate for the first time use of microfluidic membrane microarrays for scalable production of uniform microcapsules encapsulating Salmonella myophage Felix O1.

We present results showing control over the microcapsule size using membrane microarrays with different pore sizes. We demonstrate the effect of microparticle size on phage protection upon exposure to simulated gastric fluid for exposure up to 2h mimicking the human stomach environment. Phage microencapsulation and subsequent release kinetics in simulated intestinal fluid revealed that the microparticles prepared using a composite alginate-methacrylate copolymer formulation possessed pH responsive characteristics with phage release triggered in an intestinal pH range. Following storage under refrigerated conditions (at 4 deg C) we show stability of encapsulated phage in excess of 3 months. In vitro results show encapsulated phage arresting bacterial growth and compare these with free phage. The results provide proof-of-concept data supporting the suitability of our approach for gastrointestinal delivery of phages.

References

1. Malik, D.J. et al., 2017. Formulation , stabilisation and encapsulation of bacteriophage for phage therapy. Adv. in Colloid and Interface Science, 249:100-133.
2. Vinner, G.K. et al., 2017. Microencapsulation of Clostridium difficile specific bacteriophages using microfluidic glass capillary devices for colon delivery using pH triggered release. PLoS ONE, 12(10): e0186239.

[ID: 283] Unravelling biofilm inhibitory phage mechanism

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Biofilms embed bacterial communities, protected to bactericidal treatment, including antibiotics. Since up to 80% of bacterial infections are biofilm-related, new anti-biofilm treatments are essential. Bacteriophages can effectively kill bacteria in biofilms and probably have evolved mechanisms to inhibit biofilm formation. We present phage proteins from different lytic phages that specifically inhibit biofilm formation of *P. aeruginosa* during the phage infection process. The biofilm inhibition tests were conducted with clinical, synthetic and laboratory strains, with individual phage genes integrated into their genome for expression under the control of an inducible promoter. From a collection of over 200 phage ORFs, we identified seven candidate phage proteins with an anti-biofilm effect. For this analysis, we used the Calgary device system for all bacterial clones and measured biofilm formation after 24 h, 48 h and 72 h. The biofilm was reduced up to 40% compared to the non-induced controls. Subsequently, the four most effective inhibitory phage proteins were tested in *P. aeruginosa* PAO1 in a flow-cell device. Biofilm formation after 48 h and 96 h was measured using confocal laser scanning microscopy. The results were analyzed with IMARIS, comstat1 (MATLAB) and Eigenvector. All four proteins inhibited biofilm formation extensively in the 48 h test, with the biomass being reduced up to 85%. Two of them still had a substantial inhibitory effect after 96 h, one reduced biomass by 99%, other by 97%.

The identification of these biofilm-inhibitory proteins forms the first step in the development of anti-biofilm molecules, inspired by phage. To achieve this, the molecular mechanism for biofilm inhibition is currently being analyzed.

[ID: 54] Prophages as regulatory switches

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Active lysogeny is a form of phage-host interaction in which temperate phages are integrated into functional open reading frames. Controlled excision under specific conditions leads to the complete reconstitution of the gene and provides thereby a regulatory mechanism of bacterial gene expression. Examples of such regulatory switch systems include the competence gene essential for bacterial phagosomal escape by *Listeria monocytogenes* during infection, and a sporulation-related gene in *Bacillus subtilis* as well as *Peptoclostridium difficile*.

Here, we present the screen of the complete genomes of 211 *Salmonella enterica* and 203 *Escherichia coli* strains for the presence of interrupted genes (by intact or cryptic prophages as well as other integrative elements) and examined them for their potential to be regulatory switches.

[ID: 397] Novel ssDNA-binding proteins from thermophilic sources

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Single-stranded DNA binding proteins (SSBs) bind to single-stranded DNA in sequence-independent manner to prevent formation of secondary structures and protect them from nuclease degradation. These ubiquitous proteins are present in prokaryotes, eukaryotes and viruses and play a pivotal role major cellular processes: replication, recombination and repair of genetic material. In DNA replication, SSB proteins specifically stimulate homologous DNA polymerase, increase fidelity of DNA synthesis, assist the DNA polymerase to advance forward by destabilizing DNA helix, organize and stabilize replication origin.

Here, we present characterization of four SSB proteins of different origin. One of them were isolated from *Clostridium* sp. phage phiCP130 (SSB C1: 124 aa, Mr=13,905). Three others (SSB M2: 136 aa, Mr=15,009, SSB M3: 144 aa, Mr=16,106 and SSB M5: 138 aa, Mr=15,851) were isolated from metagenomes. They show high similarity to SSB proteins from *Caldanaerovirga acetigignis*, *Caldanaerobius fijensis* and *Fervidobacterium gondwanense*, respectively. The recombinant proteins were overproduced in *E. coli* Rosetta [pRARE], except for SSB M5, which was overproduced in *E. coli* BL21. Proteins were purified using a metal-affinity chromatography as His-tagged fusion proteins. Electrophoretic mobility shift assay was used to examine their DNA binding activity with fluorescein-labelled oligonucleotide (dT40) used as a substrate. Thermal stability analysis revealed that they are stable at elevated temperatures with exception of SSB protein C1 which loses its activity above 65°C. Other proteins are active in high temperatures, SSB M3 up to 85°C, while SSB M2 and SSB M5 are active in 98.7°C. The subunit structure of proteins was analyzed by gel filtration on Superdex 75 column (AKTA). This allowed us to conclude that analyzed proteins in solution exist in oligomeric form, which feature is characteristic to other SSB proteins. Purified SSB proteins were tested to improve specificity of PCR-based DNA amplification.

[ID: 149] Interactions between crenarchaeal viruses and mobile genetic elements

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Our understanding of viruses has taken a new turn with the advent of different technologies with relevance to biology, especially metagenomics. Over the past two decades, many new viruses have been identified in diverse habitats, providing important insights into the diversity and ecology of the virosphere. However, there is still much debate concerning the origins of viruses in the evolutionary history of life. Viruses are related to hosts from different cellular domains including Archaea, Bacteria and Eukarya and these entities are considered to be a major evolutionary force shaping cellular life. The first archaeal virus was described in the 1970s, before Archaea was recognized as the third domain of life (Schnabel et al., 1982). Archaeal virosphere consists of viruses that are currently classified into 17 virus families (Prangishvili et al., 2017). Archaeal viruses can be broadly divided into groups: archaea-specific viruses and cosmopolitan viruses that possess morphological and genetic features that are observed among bacterial and eukaryotic viruses. *Sulfolobus turreted icosahedral virus 1* (STIV) and STIV2 from the family *Turriviridae* are the only two crenarchaeal viruses with morphological similarity to viruses from other domains. The double-jelly-roll topology of the major capsid proteins (MCP) of STIV shows great similarity to virions found in bacterial viruses (*Tectiviridae* and *Corticoviridae*) and eukaryotic viruses (adenoviruses, polintonviruses and the nucleocytoplasmic large DNA viruses). In this study, we use various approaches to study evolutionary connections among STIV, these other viruses and other mobile genetic elements. This study is part of the larger ERC EVOMOBIL project.

References:

1. Schnabel, H., Zillig, W., Pfäffle, M., Schnabel, R., Michel, H., Delius, H., 1982. Halobacterium halobium phage ϕ H. *EMBO J.* 1:87–92
2. Prangishvili, D., Bamford, D.H., Forterre, P., Iranzo, J., Koonin, E.V., Krupovic, M., 2017. The enigmatic archaeal virosphere. *Nat. Rev. Microbiol.* 15 (12), 724–739

[ID: 14] *Clostridium botulinum* group III BoNT phage genetic diversity

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Clostridium botulinum group III is the main organism responsible for animal botulism, a deadly paralytic disease caused by botulinum neurotoxins (BoNTs). The neurotoxin locus is carried on a large bacteriophage of the Siphoviridae family. This phage has a pseudolysogeny relationship with its bacterial host and does not integrate into the chromosome. Despite large botulism outbreaks - reported every year - and substantial economic losses in affected farms, the BoNT phage is poorly understood. One of the reasons is its instability, especially during laboratory handling. The mechanisms involved in the loss of the phage are unknown. Deciphering the mechanisms behind the BoNT phage's stability are a prerequisite for understanding neurotoxin production by *C. botulinum* group III strains and could provide the key for the development of novel measures to control animal botulism.

Laboratory cultivation experiments have shown that the instability of the BoNT phage is strain dependent. This could be explained by a different genetic composition. The comparison of different BoNT phage sequences using EDGAR (<https://edgar.computational.bio.uni-giessen.de>) allowed to identify core and accessory genetic content. Most of the identified core genes (60%) encode proteins of unknown's functions. The rest of the core elements encode phage structure and function proteins, toxins, segregation-partitioning systems and transcriptional regulators. The accessory phage genome contains transposons, CRISPR-elements, coding regions for restriction endonucleases, DNA-modification methyltransferases, toxin-antitoxin systems and hypothetical proteins (representing 61% to 89% of the accessory content). The conserved genetic position of the core genes suggests that the different analysed BoNT phages share a common ancestor. The diversity of the acquired accessory genetic content clearly indicates that they have evolved in different environments. With 75% of the phage's genetic content made of accessory elements, some of the acquired or lost genetic elements may play a role in its instability. These specific elements remain to be identified.

[ID: 44] The diversity of virus in the plant pathogenic fungus *Sclerotinia sclerotiorum*

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Rapeseed (*Brassica napus*) stem rot caused by *Sclerotinia sclerotiorum* is the most important disease on rapeseed in China. Mycoviruses are viruses that infect fungi and replicate in fungal cells, and they are found in nature commonly. Hypovirulence-associated mycoviruses of fungal plant pathogens have attracted much attention because of their potential as biological control agents against plant fungal diseases. Hypovirulent strains of *S. sclerotiorum* are increasingly recognized to harbor great diverse mycoviruses. They possess diverse genomes of mostly ssRNA, dsRNA and rarely circular ssDNA. Those newly founding mycoviruses associated with hypovirulence contribute to exploit new potential virocontrol agents for rapeseed rot disease. However, the spread of RNA mycoviruses is limited among vegetatively incompatible individuals, and this limitation is regarded as one of the critical factors responsible for reducing the efficacy of hypovirulence-associated RNA mycoviruses in controlling fungal diseases. The hypovirulence-associated *Sclerotinia sclerotiorum* mycoreovirus 4 (SsMYRV4), was found to function as a potent inhibitor of G protein signaling pathway, ROS production and vegetative incompatibility-mediated PCD. Furthermore, SsMYRV4-infected strain could easily accept other viruses through hyphal contact and these viruses could be efficiently transmitted from SsMYRV4-infected strain to other vegetatively incompatible individuals. Thus, we concluded that SsMYRV4 is capable of suppressing host vegetatively incompatible reaction and facilitating heterologous viruses transmission among host individuals. These findings may enhance our understanding of virus ecology, and provide a potential strategy to utilize hypovirulence-associated RNA mycoviruses to control fungal diseases.

[ID: 213] Regulators of CRISPR-Cas adaptive immune systems in *Serratia*

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The CRISPR-Cas systems represent an adaptive and heritable prokaryotic immunity that confers the ability to withstand invasion by selfish genetic elements, such as bacteriophages, and promote host survival. Over the past decade the majority of CRISPR-Cas research has been dedicated to unravelling the molecular function and exploitation of these systems. To effectively counteract foreign invaders, bacteria are proposed to employ timely and coordinated management of these systems, but the mechanisms of which are yet to be thoroughly elucidated. The aim of the current study is to determine genetic regulators that control the activity of the CRISPR-Cas systems in *Serratia*. We have identified a number of genes that modulate the activity of bacterial adaptive immunity. These include an RNA-binding stress response protein and a DNA-binding LysR-type transcriptional regulator. The CRISPR-Cas regulatory function of these genes was investigated by analysing the expression of the three specific CRISPR-Cas systems through reporter gene-fusions. In addition, the changes in CRISPR-Cas expression resulted in altered immune activity, as assessed by adaptation and interference assays. In summary, we have identified new regulators that play a significant role in controlling the levels of different CRISPR-Cas adaptive immune systems in *Serratia*.

[ID: 238] The two major *Campylobacter* phage genera control lysis genes differently

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Campylobacter phages are divided in two major genera; Cp220virus and Cp8virus, showing very little intergenus homology. Here, we aimed to identify the lysis genes of both *Campylobacter* phage genera. To identify the putative endolysins and holins, we used a bioinformatic approach searching for conserved domains based on protein homology. F379 and F352 phages were analyzed from our collection, as representatives for Cp220virus and Cp8virus phages, respectively. Putative endolysins were identified in both phages, containing a transglycosylase domain in the C-terminus and a strongly hydrophobic transmembrane domain in the N-terminus. However, the putative holins of the two phages were very distinct containing different number of transmembrane domains. To verify the function of the putative endolysins and holins, constructs were made and expressed in *E. coli*, followed by lytic assays. We found that both endolysins were translocated to the periplasm using the bacterial Sec-pathway, followed by a cleavage in the N-terminus. Furthermore, the holin of phage F352 was shown to work as an activator of the endolysin leading to cell lysis after co-expression, whereas the expression of the endolysin encoded by phage F379 led to lysis on its own. Interestingly, we found that a gene (gp28) located upstream and overlapping with the endolysin in phage F379 slowed lysis of cells, after co-expression with holin, indicating that Gp28 works as a regulator of lysis timing. Although Gp28 does not possess any predicted domains, it is conserved in all Cp220virus phages, securing phages from premature lysis. We are currently investigating the exact interaction between Gp28 and endolysin of Cp220virus phages to elucidate the exact control mechanism. In summary, we identified the *Campylobacter* phage endolysins and holins and we showed that the two groups of *Campylobacter* phages control lysis by distinct mechanisms.

[ID: 303] New isolated temperate phage from canine staphylococcal infection

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Interest in phage therapy has grown rapidly over the past few years. That brings attention to the isolation of new phages for further bacterial targets. *Staphylococcus pseudintermedius* is an important canine commensal and opportunistic pathogen and causes pyoderma and necrotizing fasciitis. The presence of this pathogen has also been observed in human infections. Manifestation of *S. pseudintermedius* infection and spread of resistance to methicillin is strikingly similar to that of *Staphylococcus aureus*. Methicillin resistant *S. pseudintermedius* (MRSP) has emerged during the last years and is becoming a serious problem in veterinary medicine of household pets.

Newly isolated phage designated as vB_Sps_QT1 belongs to family Siphoviridae according to electron microscopy. Growth characteristics for this phage were determined. Sequenced genome is 46 kb long and has cos ends. Phages with cos ends often contribute to horizontal gene transfer. Twenty six percent (n = 11 / 43) of the tested strains are sensitive to this phage. Some of the *Staphylococcus capitis*, *pasteuri* and *simulans* strains are also sensitive.

After preliminary tests the phage vB_Sps_QT1 will be suitable as a diagnostic and even a therapeutic phage for canine dermal infections caused by *S. pseudintermedius*.

This work was supported by grants from Czech Science Foundation GA18-13064S, Masaryk University MUNI/A/0824/2017, and Ministry of Agriculture QJ1510216.

[ID: 297] Broad spectrum vibriophage endolysin with lytic activity against *Vibrio parahaemolyticus* strains related to acute hepatopancreatic necrosis disease (AHPND)

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During the last decade AHPND has collapsed the shrimp aquaculture industry in Asia and the Americas. Originally *Vibrio parahaemolyticus* (VPAHPND) strains were described as the cause of this disease. However, the latest reports about the presence of the same virulence genes related to AHPND in other *Vibrio* species are concerning, since apparently the problem is more serious and complex than estimated. Attributed mainly to gene transfer versatility, the problem is exacerbated by the high degree of bacterial resistance recorded in VPAHPND strains and the lack of new control strategies available to address this aquaculture health problem. In this context, bacteriophage lytic enzymes (endolysins) are a promising antibacterial alternative to reduce the impacts of AHPND to shrimp industry, because their previous recorded high effectivity. In this study we compare the susceptibility of 17 VPAHPND strains and other six *Vibrio* strains (*V. alginolyticus*, *V. harveyi* and *V. campbelli*) to VPMS1 phage infection and its recombinant endolysin (LysVPMS1). The results showed broader spectrum of recombinant LysVPMS1 endolysin which contrast with the specificity of the parental bacteriophage VPMS1. LysVPMS1 had lytic effect against all tested strains while VPMS1 phage only had lytic effect against reference strain *V. parahaemolyticus* ATCC-17802. To our knowledge, this is the first report of bacteriophage lytic enzyme evaluation against *V. parahaemolyticus* strains associated to AHPND, with expanded lytic effect on other *Vibrio* species, which is especially relevant in AHPND context due to horizontal gene transfer problem, so that could be considered as a potential antibacterial strategy in aquaculture. More research is needed to generate enough evidence about advantages, disadvantages and limitations that can be attributed to the use of endolysins as an approach for bacterial control in aquaculture.

[ID: 61] Complete genome sequences of *Erwinia horticola* temperate bacteriophages 49 and 59

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The enterobacterium *Erwinia horticola* is a phytopathogenic bacteria causing black bacteriosis of apple, pear and beech [1]. This work presents the complete genome sequences of *Erwinia horticola* phages 49 and 59.

The genome of bacteriophage 49 consists of a circularly permuted dsDNA molecule, 46,835 base pairs in length with a G+C content of 50.8%. *Erwinia* phage 59 also contains a circularly permuted dsDNA molecule of 47,116 bp with a G+C content of 50.4%. Annotation of their genomes led to the detection of 81 putative ORFs for both phages. In addition, phage 59, but not phage 49, encodes an arginine tRNA gene. Phages 49 and 59 share extensive nucleotide sequence identity over about half of their genomes encoding proteins involved with capsid assembly, DNA packaging, and lysis.

The genomes of the newly sequenced phages are not significantly similar to that of any previously reported viruses of Enterobacteriaceae family members. At the same time, *Erwinia* phages share the higher amino acid identity with bacterial sequences rather than with their viral homologs. Because of remarkable functional synteny together with highly mosaic genomes, phages 49 and 59 are suggested to belong to the 'lambdoid' super-family of viruses.

The phages 49 and 59 differ from the formerly sequenced *Erwinia* phages. To date, there are only two *Erwinia* infecting viruses (PhiEaH2, PhiEaH1) from the Siphoviridae family whose complete genome sequences were determined [2]. Sequencing of more Siphoviridae phage genomes contributes to better understanding of bacteriophage diversity, biology and evolution.

References

1. Belyukova K. et al. *Microbiol Z.* 1972;34(1):104-6.
2. Meczker K. et al. *FEMS Microbiol Lett.* 2014;350:25–27.

[ID: 73] Genomic characterization of two novel Enterobacteriaceae bacteriophages

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We would like to present two novel annotated genomes of yet unpublished and locally isolated Enterobacteriaceae Siphoviridae phages along with their morphological and microbiological characterization. BLASTn [1] has shown that they both share a very little similarity with other objects available in the GeneBank (highest query covers being 3% for phage "Gungnir" and 20% for phage "Jormungandr" respectively as of March 2018). Furthermore, in silico [2] analysis and RFLP experiments [3] have revealed that one of the phages has circularly permuted genome what is an uncommon case among Siphoviridae lineage and adds to its novelty among myriads of phage genomes (2201 complete bacteria virus genomes at NCBI database as of March 2018 [4]) available at the moment. As was foreseen, genome architecture is composed of both "core" and "accessory" [5] elements and many of the ORFs found encode hypothetical proteins. Some of them contain evolutionary conserved domains with yet unknown function (DUFs), which leads to possibilities of future structural and functional studies using these objects (unpublished data).

1. Altschul et al. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403-410.
2. Garneau et al. 2017. PhageTerm: a tool for fast and accurate determination of phage termini and packaging mechanism using next-generation sequencing data. *Sci Rep.* 2017 Aug 15;7(1):8292.
3. Karlene et al. 2012. Comparative analysis of two phenotypically-similar but genomically-distinct Burkholderia cenocepacia-specific bacteriophages *BMC Genomics*, 13:223
4. <https://www.ncbi.nlm.nih.gov/genomes/GenomesGroup.cgi?opt=virus&taxid=10239&host=bacteria>
5. Cazares et al. 2014. Core and accessory genome architecture in a group of Pseudomonas aeruginosa Mu-like phages. *BMC Genomics*, 15:1146

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