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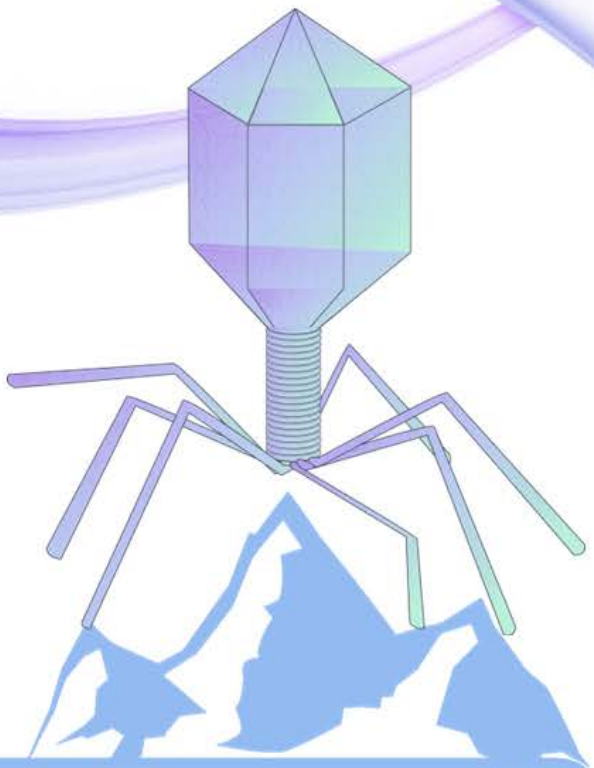

II SLOVENIAN PHAGE MEETING

23.01.2025

BOOK OF ABSTRACTS

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Second Slovenian Bacteriophage Meeting

23th of January 2025, Ljubljana, Slovenia

Book of abstracts

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Welcome from the organizers

There are approximately 10^{31} phages on Earth, making them the most abundant biological entities on the planet. It is estimated that these phages may kill up to 50% of the total bacterial biomass every day. Phages are key modulators of microbiomes. They are extremely abundant and important, yet they remain among the least understood entities on Earth.

In recent years, this has been slowly changing—phage research has flourished, including here in Slovenia. This progress is reflected in the diverse and comprehensive program of this meeting. In a country of only 2 million people, we have assembled a program featuring 18 speakers across four thematic sessions: 1) Phage Ecology and Genomics; 2) Phage-Host Interactions; 3) Phage Applications; 4) Pro- and Antiviral Approaches. Over 150 participants have registered for the meeting, representing both academia and industry, including national institutes of Slovenia, healthcare institutions, and several foreign universities.

This is already our second meeting on phages. The first symposium took place exactly 2 years ago (23th of January 2023). The idea for this event was born during an informal gathering over beer at Lotus Bar, where researchers from two faculties and colleagues from two companies discussed the need for such a platform. The first meeting was a small afternoon symposium, where we curated a program by inviting colleagues working on phages. This time, the event has expanded into a full-day meeting, allowing for open abstract submissions. We are grateful to our sponsors for supporting this event. We also deeply appreciate our support team for their efforts in organizing and promoting this meeting. Most importantly, we thank all of you for attending. We hope this gathering will become a traditional phage-focused event in Slovenia and the surrounding regions of Europe.

Senior research associate, Anna Dragoš, PhD

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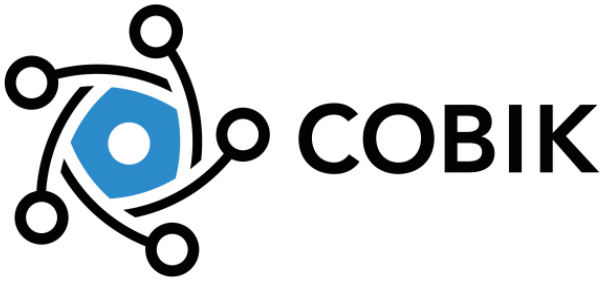

Second Slovenian Bacteriophage Meeting Program

9:30-9:40	**Welcome by organizers**
9:40-10:30	**Keynote lecture by Rob Lavigne: Pseudomonas bacteriophages: tinkers, tailors, soldiers, spies** *Lab. of Gene Technology, Catholic University of Leuven*
Coffee break 10:30-11:00	
11:00-12:20	**Session 1:** Phage ecology and genomics *chaired by Tomaž Accetto*
11:00-11:20	**Aljoša Beber**: First insight into bacteriophages of *Xanthomonas campestris* pv. *campestris* in Slovenia *Plant protection Dep., Institute of Agriculture, Slovenia*
11:20-11:40	**Polonca Štefanič**: Prophage-like elements in *Bacillus subtilis* from global and local geographical scales *Dep. of Microbiology, Biotechnical Faculty, University of Ljubljana*
11:40-12:00	**Nejc Stopnišek**: Diversity, host-range, and temporal stability of gut prophages *Dep. for Microbiological Research, Centre for Medical Microbiology, National laboratory of health, environment and food, Maribor* *Dep. of Microbiology, Faculty of Medicine, University of Maribor*
12:00-12:20	**Virginie Grosboillot**: Using synphage to analyse and compare *Bacillus subtilis* prophage genomes *Dep. of Microbiology, Biotechnical Faculty, University of Ljubljana*
Lunch break 12:20-13:20	
13:20 – 14:25	**Session 2:** Phage-host interactions *chaired by Nina Vesel*
13:20-13:40	**Ana Lisac:** Induction of incomplete lytic cycle for *E. coli* – T4 pair by bacterial starvation *Dep. for Chemical Engineering and Chemical safety, Faculty of Chemistry and Chemical Technology, University of Ljubljana*
13:40-14:00	**Matej Butala**: The genetic switch of tectivirus GIL01 *Dep. of Biology, Biotechnical Faculty, University of Ljubljana*
14:00-14:20	**Jaka Jakin Lazar**: Investigating new role of phage-encoded bacteriocin, sublancin, in phage and host fitness *Dep. of Microbiology, Biotechnical Faculty, University of Ljubljana*
14:20-14:25 Flash talk	**Špela Blaznik**: Evaluating the role of *Staphylococcus capitis* physiology in bacteriophage K growth and efficiency *Dep. for Chemical Engineering and Chemical safety, Faculty of Chemistry and Chemical Technology, University of Ljubljana*
Coffee break 14:25-15:00	
15:00 – 16:30	**Session 3:** Phage applications *chaired by Aleš Podgornik*
15:00-15:20	**Katja Molan**: Antibacterial surfaces using bacteriophages: a proof-of-concept *Faculty of Health Sciences, University of Novo mesto* *Dep. of Microbiology, Biotechnical Faculty, University of Ljubljana* *Institute of Metals and Technology University of Ljubljana*
15:20-15:40	**Andrej Cör**: Melanoma immunotherapy using engineered bacteriophage vaccines *Valdoltra Orthopaedic Hospital*
15:40-16:00	**Tomaž Kasunič, Gašper Dokl**: Bacteriophage production for various application from R&D to the commercial scale *Jafral*
16:00-16:20	**Martina Durcik:** From phage isolation and *in vivo* testing to phage therapy for patients in special clinical need *Cobik*
16:20-16:25 Flash talk	**Ana Zupančič**: Autoantibody neutralisation in autoimmune disease using short peptides *Dep. of Pharmaceutical Biology, Faculty of Pharmacy, University of Ljubljana*
16:25-16:30 Flash talk	**Klemen Gnidovec**: Subunit antigen-decorated filamentous bacteriophages as a modular vaccine platform *Dep. of Pharmaceutical Biology, Faculty of Pharmacy, University of Ljubljana*
Coffee break 16:30-17:00	
17:00-17:45	**Session 4:** Pro and antiviral approaches *chaired by Hannah Bonham*
17:00-17:20	**Ažbe Žnidaršič**: Host cell impurity reduction in bacteriophage samples using CIMmultus® monolithic technology *Sartorius BIA Separations*
17:20-17:40	**Arijana Filipič**: Novel SuperPlasma technology for inactivation of viruses in water *Dep. for biotechnology and systems biology, National Institute of Biology Ljubljana*
17:40-17:45 Flash talk	**Saša Kaloper**: Colloidal formulations of polysaccharide/phenol-based antiviral coatings for pandemic control *Lab. for Characterization and Processing of Polymers, Faculty of Mechanical Engineering, University of Maribor*
Closing by organizers ***Snacks and drinks until 19:30***	

Keynote speaker

Professor Rob Lavigne

Rob Lavigne is a professor at KU Leuven, leading the Laboratory of Gene Technology. His research focuses on molecular microbiology, particularly in developing Artilysins™ and studying the Pseudomonas/phage interplay. He has pioneered work in phage diversity and engineered bacterial metabolism, earning an ERC consolidator grant. Lavigne has an international profile, serving as president of the International Society for Viruses of Microorganisms and chairing the ICTV committee on bacterial virus taxonomy. He co-founded P.H.A.G.E., a European non-profit focused on bacteriophage therapy, and has delivered over 50 keynote lectures worldwide. In his keynote lecture, entitled *"Pseudomonas Bacteriophages: Tinkers, Tailors, Soldiers, Spies,"* he highlights recent advances in the use of phage-encoded enzymes for precise bacterial gene editing, as well as Magistral phage therapy developments in Belgium, including the expansion of phage use to treat less conventional conditions such as Hidradenitis Suppurativa**.**

Session 1: Phage ecology and genomics

First insight into bacteriophages of *Xanthomonas campestris* pv. *campestris* in Slovenia

Aljoša Beber[1], Janja Lamovšek[1], Irena Mavrič Pleško[1]

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Black rot of brassicas, caused by *Xanthomonas campestris* pv. *campestris* (Xcc), is a globally significant disease that severely impacts the production of cabbage and other brassica crops. In recent years, black rot has become an increasingly challenging issue in cabbage production in Slovenia, highlighting the urgent need for effective management strategies. Bacteriophages are becoming promising biological control agents known for their targeted and species-specific action. However, in plant protection their use is still limited. Bacteriophages infecting Xcc were isolated from environmental samples in Slovenia for their potential use in the control of black rot of brassicas. Here we present their basic characteristics, mainly in physiology and stability. Determined properties and diversity of isolated bacteriophages are the basis for further research. Additionally, the determination of the host range provided valuable information on the diversity of Xcc strains acquired across Slovenia in recent years. These initial findings lay the foundation for development of phage-based biocontrol strategy that could be a reliable option for managing black rot in the future.

Prophage-like elements in *Bacillus subtilis* from global and local geographical scales

Polonca Štefanič[1], Eva Stare[1], Valentina Floccari[1], Jasna Kovač[2], Robert Hertel[3], Ulises Nunes da Rocha[4], Ákos T. Kovács[5.6], Ines Mandić-Mulec[1], Mikael Lenz Strube[6], Anna Dragoš[1]

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[4] Department of Environmental Engineering and Biotechnology, Helmholtz Center for Environmental Research, Germany

[5] Institute of Biology Leiden, Leiden University, Netherlands

[6] Department of Biotechnology and Biomedicine, Technical University of Denmark, Denmark

Although the majority of bacteria dedicate a substantial part of their genome to harbor prophage elements, our understanding of their ecological significance remains limited. We also still know little of the factors shaping the abundance and distribution of prophages within their host species, especially evident in non-pathogenic or beneficial bacteria. In this study, we investigate prophage elements within Bacillus subtilis, a well-studied model of beneficial bacteria representing the Firmicutes phylum known for its prevalence of prophages. By comparing bacterial genomes archived in the NCBI database and isolates from a narrowly defined geographical area, we unveil local effects on prophage abundance and distribution. Furthermore, we compare predicted prophage elements with currently known groups of Mobile Genetic Elements (MGE) discovered and studied in laboratory strains, for the first time revealing their abundance, distribution and potential role in shaping B. subtilis mobilome. Our investigation reveals intricate relationships, including both coupling and antagonism, among specific types of prophages. This suggests that superinfection exclusion and co-infection mechanisms play pivotal roles in determining prophage distribution within B. subtilis. Finally, through experimental studies, we demonstrate prophage activity. Although not explicitly linked to individual prophages, the magnitude of response to a phage-inducing agent can be predicted based on the number of prophage elements present. Our work provides profound ecological insights into the prophage elements of B. subtilis, laying the groundwork for future experimental studies to explore their roles in the ecology and evolution of this bacterial species.

Diversity, host-range, and temporal stability of gut prophages

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Phages are critical components of the gut microbiome, influencing bacterial composition and function as predators, parasites, and modulators of bacterial phenotypes. Prophages, integrated forms of these phages, are prevalent in many bacterial genomes and play a role in bacterial adaptation and evolution. However, the diversity and stability of prophages within gut commensals, particularly in the genera Bacteroides and Phocaeicola, remain underexplored.

This study aims to screen and characterize prophages in these genera, providing insights into their diversity, host range, and temporal dynamics in the human gut. Using a combination of three bioinformatic tools we conducted a comprehensive analysis of prophages in Bacteroides and Phocaeicola. Cenote-Taker 3 identified the most diverse set of prophages, with significant overlaps observed between the tools. After clustering high-quality prophages, we identified 22 unique viral operational taxonomic units. Notably, comparisons between prophages identified in isolated bacterial genomes, metaviromes, and large public gut virome databases revealed a broader host range than initially observed in single isolates. The identified prophages were not only prevalent but also exhibited broad host ranges and temporal stability. The presence of antibiotic resistance and toxin genes suggests that these prophages may significantly influence bacterial community structure and function in the gut, with potential implications for human health. These findings highlight the importance of using diverse detection tools to accurately assess prophage diversity and dynamics.

Using synphage to analyse and compare *Bacillus subtilis* prophage genomes

Virginie Grosboillot[1], Anna Dragoš[1]

[1] Department of Microbiology, Biotechnical Faculty, University of Ljubljana, 1000 Ljubljana, Slovenia

Prophages represent one of the key sources of genetic and likely phenotypic diversity within Bacillus subtilis. Prophage genomes are characteristically mosaic and the majority of their gene functions unknown. Knowledge on gene conservation or uniqueness within prophage would help to predict core structural genes required for horizontal transmission or unique accessory genes which can modulate specific host phenotypes.

Visualisation and comparison of genome maps can be very effective to identify conserved and unique regions, however none of the tools available on the market allows visualising gene conservation within multiple sequences at a glance. To gain better insight into core and accessory genes in prophage genomes, we developed a bioinformatic pipeline, synphage, to generate synteny graphics within group of related prophages. As proof of principle, we used SPbetaviruses, phages with relatively large and mosaic genomes, residing in over 30% of natural B. subtilis strains. The comparison between nucleotide and amino-acid based synteny diagrams, reveals that predicted protein sequences are better conserved among SPbetaviruses than the nucleotide sequences. We identified that 5% of SPbeta coding regions are conserved at the nucleotide level against 25% at the protein level. Among the 11 well-conserved genes, only 4 have a predicted function. The conserved proteins mainly encompass hypothetical and proteins of unknown function, putative transcriptional regulators and enzymes. Synphage can be used for the selection of subgroups of prophages based on their genomic diversity, namely accessory genes. Together with additional analyses such as transcriptomics and microscopy, these insights could reveal potential gene functions.

Session 2: Phage-host interactions

Induction of incomplete lytic cycle for *E. coli* – T4 pair by bacterial starvation

Ana Lisac[1], Aleš Podgornik[1]

[1] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

High quantities of bacteriophages are currently used in food industry and agriculture. However, the increasing antibiotic resistance of bacteria has recently sparked interest in the use of bacteriophages to treat bacterial infections in humans, suggesting that even larger quantities will be needed in the future. The high demand combined with a wide range of applications also requires efficient bacteriophage production processes that operate at low production costs. In addition, bacteriophages are being intensively investigated as an alternative to antibiotics, including for the treatment of chronic bacterial infections, where bacteria exhibit low metabolic activity. It is therefore important to understand the effects of the physiological state of the bacteria on the bacteriophage parameters. While mathematical correlations have been proposed for burst size, adsorption constant and latent period beyond certain specific bacterial growth rate, less is known about what happens under conditions of extreme substrate limitation. Here we will present results from the E. coli – T4 pair showing that, depending on the physiological conditions of the bacteria, an incomplete lytic cycle without cell lysis such as the scavenger effect and hibernation can be induced in addition to the conventional cell lytic cycle. We believe that this is a general response of lytic phages that significantly affects the efficacy of phage therapy in chronic bacterial infections.

The genetic switch of tectivirus GIL01

Ana Pavlin[1], Gregor Bajc[1], Nadine Fornelos[2], **Matej Butala**[1]

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Betatectiviruses are prophages with linear extrachromosomal genomes, such as GIL01, which infects *Bacillus thuringiensis* serovar *israelensis*. We show that in contrast to typical temperate phages, GIL01 relies on the host SOS response repressor LexA, which in complex with the phage's gp7 protein supports the lysogenic cycle. Gp7 enhances the DNA-binding ability of LexA and simultaneously inhibits its autocleavage, thereby modulating the host SOS response and suppressing the induction of the lytic cycle of GIL01 and cohabiting prophage pBtic235 after DNA damage. In addition, the gp1 protein encoded by GIL01 represses transcription from the strong promoter *P2* and thus influences prophage maintenance. GIL01-encoded gp6 protein, which is homologous to LexA, activates late gene transcription and drives the phage into the lytic cycle. These mechanisms illustrate the sophisticated interplay of GIL01 with host and viral factors to regulate its life cycle and SOS response, and highlight the complexity of prophage-host dynamics.

Investigating new role of phage-encoded bacteriocin, sublancin, in phage and host fitness

Jaka Jakin Lazar[1], Anna Dragoš[1]

[1] Department of Microbiology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Bacteriocins encoded by prophages play critical roles in shaping microbial interactions, yet their influence on phage biology and phage-host coevolution remains underexplored. In this study, we investigate sublancin, a bacteriocin encoded by the *Bacillus subtilis* prophage SPβ, to uncover its potential new roles in bacterial and phage ecology. We use experimental and analytical approaches, to examine how sublancin impacts both vertical and horizontal phage transmission, specifically by modulating lysogenic stability, lytic induction, and interactions with closely and more distantly related bacteria.

Using a mushroom isolate *Bacillus subtilis* MB8_B7 which is naturally lysogenic for SPβ, we created a series of mutant strains lacking key sublancin genes within the prophage region. We next exposed these strains to DNA-damaging agent mitomycin C, using a concentration that was optimized for maximal phage titer. Thus far, we have demonstrated that the naturally lysogenized strain MB8_B7 shows a significant drop in titer following phage induction with mitomycin C when the entire sublancin-encoding biosynthetic gene cluster is deleted. Study of single mutants within sublancin cluster have demonstrated that the genes *bdbA* and *sunT* do not alter phage titer significantly after induction with mitomycin C. We also observed that strains lacking gene *sunI*, which encodes for sublancin immunity protein SunI, displayed a significantly lower susceptibility to detergent Triton X-100, while showing a significantly higher susceptibility to lysozyme. This suggests that SunI destabilizes cell wall and/or stabilizes the membrane.

We will further compare the specificity range of sublancin and host range of SPβ phages, revealing potential interactions and specificity patterns that shape microbial community dynamics. For that purpose, we have already developed a method for quantifying the zone of inhibition on LB agar, which occurs when a strain producing sublancin interacts with strain lacking sublancin immunity.

This work will provide new insights into the interplay between bacteriocins and prophages, with implications for microbial community structure and evolution.

Evaluating the role of *Staphylococcus capitis* physiology in bacteriophage K growth and efficiency

*** Flash talk**

Špela Blaznik[1], Ana Lisac[1], Aleš Podgornik[1]

[1] Faculty of chemistry and chemical technology, University of Ljubljana, Ljubljana, Slovenia

Accurate determination of phage parameters is crucial for optimising bacteriophage production and evaluating the efficacy of phage therapy. In this study, we investigated clinical isolate Staphylococcus capitis as a bacterial host and lytic bacteriophage K to assess key growth parameters, including: the adsorption constant (ka) [mL/(CFU·h)], which reflects the rate of phage adsorption on bacterial cells, latent period (LP) [h], representing the duration between infection and lysis, and burst size (BS), indicating the number of viruses released per host cell.

Our results indicate that these parameters are strongly influenced by the physiological state of the bacterial host. The maximum specific growth rate of S. capitis in defined Luria-Bertani medium was determined to be 0.59 h−1. Notably, phage K adsorption to *S.capitis* occurs significantly faster than the well-studied phage T4 and E. coli. However, the multiplication of phage K in S. capitis leads to lower burst sizes. This study provides valuable insights into the dynamics of phage-host interactions and supports the optimisation of bacteriophage production and therapeutic applications.

Session 3: Phage applications

Antibacterial surfaces using bacteriophages: a proof-of-concept

Katja Molan[1], Tjaša Danevčič[2], Marko Volk[2], David Stopar[2], Barbara Šetina Batič[3]

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The failure of medical implants due to biofilm problems is a major health problem. Bacteria are the main cause of implant-related diseases. In dentistry, peri-implantitis is a serious inflammatory disease that affects the tissue around dental implants and often leads to bone loss and implant failure. Conventional methods of treating peri-implantitis, including mechanical debridement and antibiotics, often prove inadequate due to the resilience of bacterial biofilms and the growing problem of antibiotic resistance. Bacteriophages, viruses that specifically infect and kill bacteria, offer a novel and targeted approach to the prevention of peri-implantitis. By immobilizing bacteriophages on implant surfaces, antibacterial coatings can be developed that actively combat the formation of biofilms. To test this approach, we used T4 phages and their host *Escherichia coli* (*E. coli*). Confocal laser scanning microscopy (CLSM) was used to determine the presence and extent of *E. coli* biofilms on treated and untreated titanium surfaces. Scanning electron microscopy (SEM) was used to confirm the presence of T4 bacteriophages on the titanium surface and to observe the biofilm structures, if present. Our proof-of-concept experiments have shown that phages (T4) randomly immobilized on the implant material (titanium) can prevent the formation of *E. coli* biofilms. This innovative methodology is promising for the prevention of peri-implantitis.

Melanoma immunotherapy using engineered bacteriophage vaccines

Andrej Cör[1,2], Nuša Brišar[2], Simona Kranjc Brezar[3], Katja Šuster[1]

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Melanoma represents only 3% of all skin cancer but the vast majority of deaths are from melanoma. Treatment is often ineffective due to aggressive tumor growth and resistance to therapy. The introduction of nanotechnology and nanoparticles, including bacteriophages opens new opportunities for the development of anti-tumor vaccines. The aim of our study was to prepare a bacteriophage-based vaccine and test its anti-tumor efficacy in vivo in a murine model of malignant melanoma.

Using phage display technology, M13 filamentous bacteriophages were genetically engineered to express the tumor peptides MAGE-A1, gp100 or MAET-1/MELAN-A on phages capsids surface. The therapeutic potential of bacteriophage vaccination alone or in combination with gene electrotransfer (GET) of plasmid encoding IL-12 was tested in vivo using C57BL mice bearing B16F10malignant melanoma.

We demonstrated that the growth of the tumors was inhibited and the life time of mouse was prolonged in the group treated with genetically engineered phages, compared to untreated and wild-phage treated groups. In the group treated with GET-IL12 in combination with genetically engineered phages 30% of mice had a complete response. Experiments showed no adverse effects in treated mice.

Results demonstrate that vaccination comprising genetically engineered bacteriophages and GET of plasmid encoding IL12 exhibit considerable potential and warrant further investigation. As understanding of phage-based anti-cancer vaccines grows, there is hope that they may become routine tools for treating and preventing melanoma, as well as other cancer types, with potential applications in future human clinical trials.

Bacteriophage production for various application from R&D to the commercial scale

Tomaž Kasunič[1], **Gašper Dokl**[1]

[1] Jafral, Ljubljana, Slovenia

It is of critical importance that bacteriophage production is efficient, high-quality and economically-feasible in order to bring bacteriophage-based drug products to the patient.

In the talk we will present experience and requirements needed to develop bacteriophage-based products; from the process of selection of suitable bacteriophage, development of manufacturing processes, development and validation of analytical methods, process scale up and preparing regulatory dossiers, all while adhering to good manufacturing practice (GMP) principles; a cumulation of activities which allow to bring phage-based products to clinical trials/market.

From phage isolation and in vivo testing to phage therapy for patients in special clinical need

Vida Štilec[1], **Martina Durcik[1]**, Monika Marušić[1], Klara Gregorič[1], Nika Janež[1], Lucija Rebula[2], John Dunn[3], Alasdair MacInnes[4], Daniela I. Munteanu[5], Simon Horvat[6], Josh Jones[7], Matjaž Peterka[1]

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Phage therapy is considered an experimental treatment in Europe and is mainly available through named patient use or compassionate use programs. Typically, it is prescribed by the responsible clinician, with informed patient consent, when licensed alternatives fail [1,2].

Here, we describe the process from isolation and characterization of phage COP-80B to animal testing, production, and clinical application. *Staphylococcus epidermidis* phage COP-80B was isolated from wastewater at COBIK and was extensively characterized *in vitro* for its antibacterial and antibiofilm activity, and absence of unwanted genetic elements [3]. Furthermore, it demonstrated efficacy in a mouse periprosthetic joint infection (PJI) model. COP-80B was propagated in an animal component-free medium on an appropriate production strain. Phage lysate was clarified via filtration, purified with two-step chromatography using CIMmultus OH and QA columns and concentrated with diafiltration.

When a patient with recalcitrant *S. epidermidis* PJI was identified, COP-80B lysed all five bacterial strains isolated from the infection site and was thus selected for treatment. To prepare the final formulation, phage stock was diluted in 0.9% sodium chloride injection solution, filled in 2-ml syringes, and the formulation was tested for endotoxin content and sterility. After importation approval was granted, the phages were sent to the UK where the patient received phage treatment, locally applied during surgery, alongside ongoing antibiotic therapy. The patient experienced no side effects and was infection-free six months later.

The increasing number of successful cases highlights the significant potential of phage therapy in advancing the treatment of complex infections.

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Autoantibody neutralization in autoimmune disease using short peptides

*** Flash talk**

Ana Zupančič, Zala Celan, Mojca Lunder

A common feature of most autoimmune diseases is the production of autoantibodies targeting self-antigens. Autoantibodies directed against extracellular targets can cause numerous detrimental effects; therefore, inhibiting their interaction with their targets holds the potential to mitigate disease effects.

Thrombotic thrombocytopenic purpura (TTP) is a severe and life-threatening autoimmune disorder in which the pathogenic role of autoantibodies is well established. By screening phage display peptide libraries (composed of bacteriophage libraries displaying linear or cyclic peptides) against antibodies directed toward a selected autoantigen, we identified short peptide sequences. These peptides conformationally bind to antibody paratopes, mimicking epitopes on the surface of the autoantigens. Using ELISA, we identified phage clones with strong binding to the antibodies and determined their amino acid sequences through Sanger sequencing. Additionally, next-generation sequencing (NGS) provided deeper insights into the repertoire and frequency of peptide sequences that bind autoantibodies. This approach enabled the identification of recurring amino acid motifs within the peptide repertoire, suggesting their critical role in antibody binding.

The next steps involve selecting peptides, synthesizing them, and evaluating their interactions with patient-derived autoantibodies. Using microarray technology, we will map the specific target regions on the autoantigen most frequently recognized by autoantibodies in patient samples. Based on these findings, we will select, synthesize, and evaluate additional peptide sets that mimic the linear epitopes of the target.

Subunit antigen-decorated filamentous bacteriophage as a modular vaccine platform

*** Flash talk**

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Vaccination is one of the most cost-effective public health interventions. A general robust vaccine platform capable of triggering a broad range of immune responses is considered the holy grail of vaccinology. We propose to exploit the high valency heterologous peptide display capacity and unique immunomodulatory properties of filamentous bacteriophage to design a modular vaccine scaffold with adjuvant properties.

We designed engineered a hybrid phage vector capable of simultaneously displaying foreign peptides on pIII and pVIII capsid proteins. Those on pVIII are exploited to tether recombinant subunit protein antigens, while peptides fused to pIII are used to target dendritic cells with the goal of further potentiating antibody and cellular immune responses. Specifically, we have displayed the SpyTag peptide fused to the pVIII and optimized display efficiency, monitoring display levels with ELISA. A model antigen (sfGFP) was expressed in E. coli fused to the SpyCatcher protein. The SpyTag/SpyCatcher split protein spontaneously assembles and forms an isopeptide bond [3]. We have verified that sfGFP is tethered to the phage capsid by immunoblotting and measurements of relative fluorescence intensity. Additionally, the decorated phages were visualized using total internal reflection fluorescence (TIRF) microscopy. Dendritic cells can be targeted via a number of endocytic receptors [4]. Using phage ELISAELISA, we have demonstrated that nanobodies against Clec9a, expressed as a fusion with SnoopCatcher and conjugated to bacteriophage surface via the pIII protein displaying SnoopTag [5], enable phage binding to recombinant Clec9a.

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Session 4: Pro and antiviral approaches

Host cell impurity reduction in bacteriophage samples using CIMmultus® monolithic technology

Ažbe Žnidaršič[1]

[1] Sartorius BIA Separations

This lecture will introduce CIMmultus monolithic chromatography, a cutting-edge technology developed by the Slovenian company Sartorius BIA Separations. Monolithic chromatography improves the purification of bacteriophages by addressing unique challenges such as the removal of endotoxins and host cell impurities, which are critical for ensuring the safety and efficacy of phage-based therapies. Traditional methods often struggle with efficiency, have long purification times, and can damage the phages. Monolithic columns offer a better solution and advantages over commonly known methods such as tangential flow filtration, ultracentrifugation, traditional packed-bed, and membrane chromatography, or other currently used purification methods. These columns provide high recovery rates, rapid processing, and scalability, making them ideal for the purification of large biomolecules like bacteriophages.

The lecture will explain how these columns work, focusing on a two-step process that effectively purify phages from bacterial lysates. This process reduces impurities while keeping the phages active and effective. The technology is not only efficient but also cost-effective, as the columns can be reused and are available in different column sizes to suit for both lab and industrial scales.

Participants will learn about the practical benefits of using CIMmultus columns in both research and industrial applications. The session will also discuss how this technology can advance phage therapy, making it a promising tool for future medical treatments.

Novel SuperPlasma technology for inactivation of viruses in water

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Water scarcity is one of the greatest challenges we face today. A major contributing factor is the increasing presence of waterborne pollutants, including viruses. Human waterborne viruses infect millions of people annually, leading to significant health and financial burdens. Environmentally friendly technologies that can inactivate these viruses and efficiently clean water are, therefore, essential. To address this need, we developed and patented SuperPlasma technology. This technology combines supercavitation—a phenomenon where vapor bubble forms in water due to a pressure drop—with cold plasma, the fourth state of matter. Cold plasma contains many different particles, some with strong antimicrobial properties, such as reactive oxygen species. These act as powerful oxidants, capable of damaging organic materials from proteins to nucleic acids. Plasma is increasingly used for decontamination for this reason. Due to the challenges of working with human viruses, surrogate viruses like bacteriophages are often used. Bacteriophage MS2 is a common surrogate for human enteric viruses like norovirus because of their structural similarities and comparable inactivation efficiencies with different technologies. In our study, we applied SuperPlasma technology to treat bacteriophage MS2 in water samples with varying properties, including pH, temperature, virus concentration, and organic and inorganic compounds, to assess its effectiveness across different water types. Our results show that this combined technology can inactivate viruses quickly, regardless of sample properties, offering a versatile solution for water decontamination.

Colloidal formulations of polysaccharide/phenol-based antiviral coatings for pandemic control

*** Flash talk**

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The 2019 coronavirus pandemic underscored the urgency for antiviral coatings on textile personal protective equipment (PPE). PPE lacks inherent antiviral activity and can harbor pathogens, facilitating viral transmission. Concerns over PPE coatings, utilizing metal nanoparticles and synthetic compounds, have shifted focus to natural polysaccharides and phenols for their antiviral potential, biocompatibility and biodegradability [1].

This study presents physicochemical and antiviral properties of two newly developed aqueous dispersions (AD) of quaternary chitosan (HTCC) micro/nanoparticles, linked by sodium tripolyphosphate (STPP) through ionic gelation, wherein one further incorporates encapsulated tannic acid (TAN). Characterization techniques revealed that the two mixed ADs and ADs of individual components contain characteristic and active functional groups (i.e., quaternary amines, esters of carboxylic acids etc.), potentially responsible for antiviral activity, and 0.34-7.27 mmol of charge/gram of material. Mixed dispersions exhibited characteristic zeta potential (ZP) values within the pH 3-9, representative of every component (e.g., high ZPs at low pH (≥ +35 mV), gradually decreasing beyond pH~5). Due to the application's nature, the ZP and charge amount are critical at pH 7–7.5 (i.e., average physiological and exhaled air condensate's pH). Double-layer plaque assay with bacteriophage phi6, a SARS-CoV-2 surrogate, revealed that all ADs exhibit antiviral activity (i.e., ≥ 2 log 10 PFU/mL viral titer reduction) at tested concentrations after 24- and 18-hour incubation, excluding 0.5 % STPP (w/v), showing promise as potential antiviral coatings on materials typically used for textile PPE (e.g., polypropylene), such as masks and gowns, increasing their overall safety, comfort and sustainability while reducing viral transmission risks.

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