ABSTRACT BOOK

2nd BSVOM SYMPOSIUM

Friday
September 8
2023

BSVOM

Belgian Society for Viruses of Microbes

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CONTENT TABLE

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The Belgian Society for Viruses of Microbes was founded in 2022, on the initiative of Belgian phage researchers at different national universities and university colleges, hospitals, and research centers. The mission statement of BSVoM includes the promotion of research on viruses of microbes, including the fundamental study of these viruses as well as the development of applications.

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SYMPOSIUM ORGANIZING COMMITTEE

The symposium was organized by a group of enthusiast phage researchers. A big thanks to all their efforts, whether small or large, to make this second symposium a success. If you have a remark, comment, or evaluation, please pass them on to one of the organizers so we can improve the next edition!
BSVoM symposium 2023, here we come!

Prof. Dr. Damien Thiry
Prof. Dr. Yves Briers
Prof. Dr. Abel Garcia-Pino
Prof. Dr. Annika Gillis
Prof. Dr. Gipsi Lima-Mendez
Prof. Dr. Jelle Matthijnssens

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We also like to thank all the helping hands on the side, printing things, putting up poster boards, breaking down poster boards, running small errands, taking care of the presentations, mic switches…

BSVoM kindly thanks the University of Liège for the availability of the venue hall.

WEBSITE

The website was conceptualized and maintained by Jolien Venneman. Do not forget to add this website to your bookmarks, so all important information can be found in just one click. For your convenience, you can use the QR code below.

https://www.bsvom.be

SPONSORS

BSVoM is a non-profit organization. The organizing committee, a group of volunteers affiliated with the different universities in Belgium, try to keep the BSVoM conference available for only the small fee to enable broad participation of early-career researchers.
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PROGRAMME

2nd BSVoM symposium, Liège 2023

8h30: Registration & poster set-up

9h00: Welcome by **Prof. Damien Thiry** (ULiège)

9h10-10h40: Fundamental research in phage ecology and biology – Part I

Chair: Prof. Jelle Matthijnssens (KU Leuven)

9h10: Keynote lecture: **Prof. Bas Dutilh** (University of Jena, Germany) - Mapping the Microverse and modelling its drivers (30 min talk/5 min QA)

9h50: Ella Sieradzki (Ecole Centrale de Lyon) - Phages succession follows microbial resuscitation during seasonally dry soil rewetting (10 min talk/5 min QA)

10h05: Lore Van Espen (KU Leuven) - Exploration of >1000 human gut viromes and matched metagenomes discovers phages with unexpected integration potential and broad host range (10 min talk/5 min QA)

10h20: Invited talk: **Prof. Daniel Nelson** (University of Maryland, USA) - Structure of the Escherichia coli O157:H7 bacteriophage CBA120 tailspike complex (15 min talk/5 min QA)

10h40-11h10: Coffee Break

11h10-12h10: Fundamental research in ecology and phage biology – Part II

Chair: Prof. Gipsi Lima-Mendez (University of Namur)

11h10: Susanna Grigson (Flinders University) - Phynteny: Synteny-based annotation of viral genes (10 min talk/5 min QA)

11h25: Ekaterina Jalomo-Khayrova (Philipps-Universität Marburg) - Structural and biochemical dissection of the SPβ prophage master repressor MprR (YopR) (10 min talk/5 min QA)

11h40: Albinas Cepauskas (ULB) - Direct activation of an innate immune system in bacteria by a viral capsid protein (10 min talk/5 min QA)

11h55: Sponsor talk: Biolog - 15 min

12h10-12h45: Student pitches - 3 min each, no QA

- **Laura Bessems** (UZ Leuven) - Optimization of bacteriophage therapy for difficult-to-treat musculoskeletal infections: from bench to bedside and vice versa
- **Salomé Desmecht** (ULiège) - Isolation, in vitro characterization and efficacy assessment in *Galleria mellonella* larvae of four bacteriophages targeting *Aeromonas salmonicida*
- **Lene Bens** (KU Leuven) - Hidradenitis suppurativa: a challenging opportunity for phage therapy
- **Céline Antoine** (ULiège) - Genomic analysis and in vitro/in vivo characterization of phage resistant *E. coli* K1 isolates
- **Wouter Magnus** (VUB) - Transcription regulatory program of the ssv1 virus infecting the thermoacidophilic archaeon *Saccharolobus solfataricus*
- **Claudia Campobasso** (KU Leuven) - Antibiofilm activity of a *Staphylococcus aureus* phage: potential key role of its baseplate protein
- **Diana Bittremieux** (ULB and UNamur) - Discovery of new antiviral mechanisms in oceanic bacteria using adam
- **Fanny Laforêt** (ULiège) - Impact assessment of vb_kpnp_k1-ulip33 bacteriophage on the human gut microbiota using a dynamic in vitro model

12h45-13h00: Group picture

13h00-14h30: Lunch & poster session

14h30-16h55: Present and future applications of phages

Chair: Dr Pieter-Jan Ceyssens (Sciensano)

14h30: Keynote lecture 1 (online): **Prof. Lone Brøndsted** (University of Copenhagen, Denmark) - Advancing our knowledge of phage-host interactions for the benefit of science and society (30 min talk/5min Q&A)

15h10: Keynote lecture 2: **Prof. Patrick Soentjens** (Queen Astrid military hospital/Institute of Tropical Medicine, Belgium) – How to start a phage therapy hub for human applications in your own clinic: the 5 year experience of the Military Hospital Brussels? (30 min talk/5min Q&A)

15h45: Eveline-Marie Lammens (KU Leuven) - Mining genetic parts and tools from bacteriophage genomes for Synthetic Biology (10 min talk/5 min QA)

16h00: Roberto Vázquez (UGent) - Structural and functional diversity of cell wall binding domains in staphylococcal endolysins (10 min talk/5 min QA)

16h15: Michele Molendijk (Erasmus MC) - Bacteriophage efficacy against *Staphylococcus aureus* in burn wounds, using an ex vivo skin model (10 min talk/5 min QA)

16h30: Manon Nuytten (UC Louvain) - Towards rapid detection of pathogens using phage-based lateral flow assay (10 min talk/5 min QA)

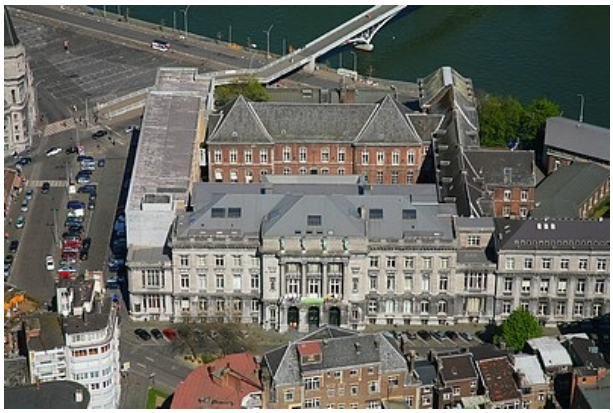
16h45: Sponsor talk: Vésale Bioscience - 15 min

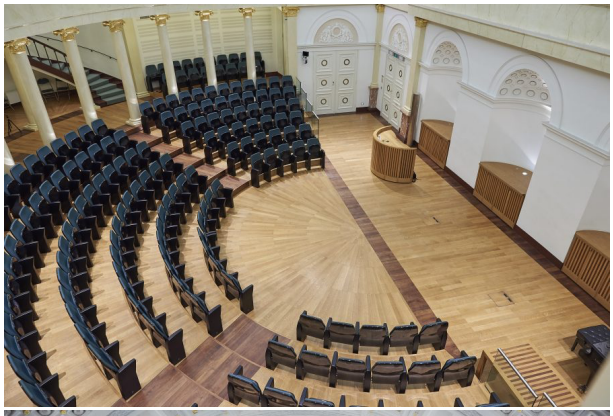
17h00: Prize winners' announcements, closing remarks & reception

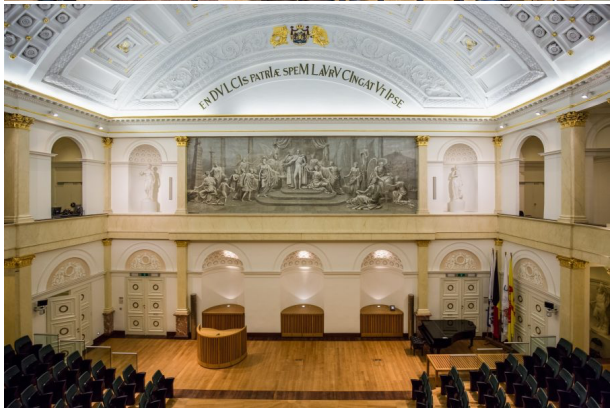
19h00: Symposium Dinner (optional) – Billie Jean *(Rue St Jean en Isle 20, 4000 Liège)*

LOCATION

University of Liège, campus du Centre-ville
main building, 'Academic Hall'
Place du 20-Août, 7-9, 4000 Liège







FUNDAMENTAL RESEARCH IN PHAGE ECOLOGY AND BIOLOGY LECTURES

Keynote lecture - Mapping the Microverse and modelling its drivers

Prof. Bas Dutilh, Viral Ecology and Omics Group, Friedrich Schiller Universität Jena, Germany

Biography

Bas E. Dutilh is professor of Viral Ecology and PI of the Viral Ecology and Omics Group (VEO) at Friedrich Schiller Universität Jena, affiliated with the Microverse Cluster, and of the Utrecht University Metagenomics Group (MGX), affiliated with Science4Life and the Utrecht Bioinformatics Centre. The Microverse is arguably the most complex system known to mankind. Dutilh and his team use high-throughput experiments and 'omics data of various flavors, combined with innovative computational analyses to understand how microbiomes come about.

The focus is on predictability: by building computational models of the various processes driving microbial functioning and dynamics, they try to understand microbiomes in their context. For his work, Dutilh has received awards including NWO Veni/Vidi, ERC Consolidator, and Alexander von Humboldt Professur.

Phages succession follows microbial resuscitation during seasonally dry soil rewetting

Sieradzki, E., Nicolas A.M. (1), Pett-Ridge J. (1,3,4), Banfield J.F. (1,5), Taga M.E. (6), Firestone M.K. (1), Blazewicz S.J. (7)

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Phages are highly active during ecosystem perturbations in aquatic systems, but these dynamics in soil ecosystems are unknown. Previous studies have shown that soil rewetting propels a succession of microorganisms with specific lineages growing and dying at different times. This varied taxonomic mortality could be driven by host specific viral lysis.

We investigated lineage-specific phage-host dynamics in grassland soil following soil rewetting, when resident microbes are both resuscitated and lysed after a prolonged dry period. To characterize actively infecting phages and host succession, we used a replicated time-series including a combination of viromes and H218O stable isotope probing targeted metagenomics.

We found that dry soil held a diverse but low biomass reservoir of virions, of which only a subset thrived following wet-up. Viral richness decreased by 50% within 24 h post wet-up, while viral biomass increased four-fold within one week. Counter to recent hypotheses suggesting temperate phages predominate in soil, our evidence indicates that wet-up is dominated by phages in lytic cycles.

Using isotope incorporation into viral and microbial DNA we characterized phage-host temporal dynamics and found taxon-specific trends in viral-host dynamics wherein phages may follow their microbial hosts or, perhaps, control host populations.

Constraining the rate of viral cell lysis revealed phages cause up to 46% of bacterial death one week following wet-up, resembling rates in marine systems that yield 20% of the dissolved organic carbon pool. Thus, phages contribute to turnover of soil microbial biomass and the widely reported CO2 efflux after wet-up of seasonally dry soils.

Key words: wetup; virome; SIP; mortality; succession

Exploration of >1000 human gut viromes and matched metagenomes discovers phages with unexpected integration potential and broad host range

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The virome is a crucial component of the complex microbial gut ecosystem, potentially influencing human physiology through the interplay between phages and bacteria. We explored the phage population in more than 1,000 fecal samples in terms of taxonomic classification, lifestyle and bacterial host using high-quality MAGs from the same samples.

Most phages were predicted to be virulent (69%), although prophages were also identified for Crassphages, Inoviruses and Microviruses, which are generally assumed not to be temperate. About 5% of the phages could be linked to multiple bacterial hosts belonging to different phyla despite stringent criteria to establish phage-host pairs. This broad host range of a limited number of phages has been sporadically suggested before, however it is not widely accepted. Within phage genera, the predicted host and lifestyle of phages is relatively consistent. Yet, some virulent-dominated genera unexpectedly contained prophages. Conversely, non-temperate phages in temperate-dominated genera are more common, however these phage genomes were less complete than their temperate genus members, potentially indicating that lysogeny-associated genes are encoded on the missing part of their genome. Finally hundreds of potentially novel phage genera were discovered, including temperate phages infecting Erysipelotrichaceae and virulent phages infecting Bifidobacteriaceae.

Optimizing the taxonomical classification, lifestyle and host prediction method for phages has resulted in more insights into the features of the previously undescribed phages in the human gut virome. This will allow us to better interpret virome associations and understand the potential role of phages in human health and disease.

Funding: MicrobLiver: the Novo Nordisk Foundation (Challenge Grant NNF15OC0016692) - GALAXY: EU's Horizon 2020 research & innovation programme (668031) - LVE: Fonds Wetenschappelijk Onderzoek (1S25720N)

Key words: human gut virome; phages; phage host prediction; phage lifestyle

Invited talk - Structure of the *Escherichia coli* O157:H7 bacteriophage CBA120 tailspike complex

Prof. Daniel Nelson

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Abstract

Phage vB_EcoM_CBA120, discovered by Betty Kutter and colleagues, belongs to the recently defined Kuttervirus genus of contractile-tailed phages, within the Akermannviridae family. The genomes of Kuttervirus phages encode up to four tailspike proteins (TSP1-4) responsible for breaking down bacterial host lipopolysaccharide substrates. Initially identified as an *Escherichia coli* O157:H7-specific phage, CBA120 has now been found to infect *E. coli* O77, O78, and *Salmonella enterica* serovar Minnesota via its different TSPs. Specifically, TSP2, TSP3, and TSP4 cleave the O157, O77, and O78 lipopolysaccharide O-antigens, respectively. These TSPs are multidomain trimeric proteins that contain a large, central β-helix domain that comprises the enzyme machinery for glycosidic bond cleavage of their specific substrates. Furthermore, TSP2 and TSP4 possess additional N-terminal domains that facilitate attachment to other TSPs and, in the case of TSP4, attachment to the phage baseplate. In this study, we employed mutagenesis and analytical ultracentrifugation techniques to investigate the interactions of CBA120 TSPs that form a branched complex. The insights obtained from our research present a paradigm for understanding the assembly of TSPs from other members of the Kuttervirus genus.

Phynteny: Synteny-based annotation of viral genes

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Bacteriophages (phages), viruses that infect bacteria, are Earth's most abundant and diverse biological entities. Phages play essential roles in driving fundamental biological processes, functioning as microbial predators and facilitating genetic exchange. In addition, phages may be used as phage therapy agents, to treat antibiotic-resistant infections. Accurate and efficient annotation of phage genes is crucial for understanding these viral processes. While routine methods employed to annotate phages rely on sequence homology, phages are vastly under-represented in genomic databases. As a result, only 35% of phage genes can be assigned a known biological function.

It has been shown that viral genomes are arranged according to the order in which proteins must be produced to support viral replication. This 'gene-order', also referred to as synteny, is highly predictive and conserved across viral genomes. Here, we present Phynteny, a novel bioinformatics tool that harnesses gene synteny, to annotate unknown phage genes. Phynteny employs a long short-term memory model, a type of neural network capable of capturing order dependencies in sequence prediction, trained on a dataset containing approximately one million viral sequences. We demonstrate that Phynteny generates high-quality annotations, consistent with the predictions made using the protein structural prediction software, AlphaFold, and annotates 64% of phage genes in our testing dataset.

By leveraging the power of gene order, Phynteny enables researchers to uncover the functional potential of uncharacterized bacteriophage proteins. Phynteny is freely accessible as a user-friendly command-line tool and will significantly contribute to the field of phage research.

Key words: protein prediction; bacteriophage bioinformatics; machine learning; sequence annotation; proteomics

Structural and biochemical dissection of the SPβ prophage master repressor MprR (YopR)

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Prophages can either reside in the genome of their bacterial host or enter the lytic cycle resulting in lysis of the cell. Hence, they must have evolved sophisticated strategies to control their lifestyle. The genome of Bacillus subtilis contains a prophage called SPβ whose lysogenic state strongly depends on the master repressor MrpR (YopR) [1,2,3]. The systematic analysis of a historic B. subtilis strain harboring the heat-sensitive SPβ c2 mutant, allowed us to analyze MrpR as a key component of the lysis-lysogeny decision system. We demonstrate that the heat-sensitive SPβ c2 phenotype is due to a single nucleotide exchange in the mrpR gene, rendering the encoded MrpRG136E protein temperature sensitive. The structural characterization of MrpR revealed that the protein is a DNA-binding protein resembling the overall fold of tyrosine recombinases. Yet, further biochemical, and functional analyses indicate that MrpR has lost the recombinase activity and evolved to function as a repressor protein. Finally, we found that the MrpR master regulator binds to several SPBRE (SPβ repeated element) intergenic regions within the genome of the SPβ prophage repressing downstream gene expression and thereby hindering activation of the lytic lifecycle, thus maintaining its lysogeny.

[1] Brady A., et al., 2021. Curr. Biol. 31, 5037-5045

[2] Kohm K., et al., 2022. Environ. Microbiol. 24, 2098-2118

[3] Bremer E., et al., 2023. Microb. Biotechnol. 00, 1-29

Key words: prophage; regulation; repressor, MrpR, recombinase

Direct activation of an innate immune system in bacteria by a viral capsid protein

Cepauskas A. (1), Tamman H. (1), Tong Z. (2), Coppieters K. (3), Tatsuaki K. (4), Talavera A. (1), Marteens C. (3), Hauryliuk V. (2), Garcia-Pino A. (1), T. Laub M. (2)

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Bacteria have evolved sophisticated and diverse mechanisms to protect themselves against a perpetual onslaught of bacteriophages. While indiscriminate defense, such as estriction-modification systems exist, many bacterial immune systems that respond to phage infection require a phage-specific trigger to be activated, which is not dissimilar to how eukaryotic immune systems sense foreign invaders through pathogen-associated molecular patterns (PAMPs).

To this day, identities of these triggers and mechanistic bases for sensing remain largely elusive. To rectify this, here we present a fused toxin-antitoxin (TA) system called CapRelSJ46 that provides specific protection to E. coli against diverse set of phages. Via genetic, biochemical, and structural analysis, we show that the toxic N-terminal region of CapRelSJ46 pyrophosphorylates tRNAs, blocking translation in bacterial cells and this activity is auto-inhibited by its C-terminal region which serves as an antitoxin element. Interestingly the C-terminal region has a double function as it also acts as a sensor for a specific phage infection, upon which a newly synthesized major capsid protein binds directly to the C-terminal domain of CapRelSJ46, disrupting auto-inhibited state and freeing the N-terminal region to exert its toxicity, suppressing the spread of specific phage via abortive infection mechanism.

Collectively, our results reveal the molecular mechanism by which a bacteria use a pseudo TA system to directly sense an essential component of phages, suggesting a PAMP-like sensing model for TA-mediated phage immunity in bacteria. This provides a new fascinating insights into a new front of the eternal co-evolutionary war being waged between phages and bacteria.

Funding: FRIA PhD Fellowship 40009450

Key words: phage defense; toxin-antitoxin; abortive infection

PRESENT AND FUTURE APPLICATIONS LECTURES

Keynote lecture (online) - Advancing our knowledge of phage-host interactions for the benefit of science and society

Prof. Lone Brøndsted, Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

Abstract

Escherichia coli is a versatile and genetically diverse species inhabiting the gut of animals and humans as commensal or pathogenic strains. While the average *E. coli* genome carry ~4700 genes with ~2000 genes belonging to the core genome, the pangenome consists of more than 18.000 genes. This huge genetic diversity also includes antibiotic resistance genes carried on plasmids, transposons, phage defense mechanisms as well as prophages. Given this enormous genetic diversity, *E. coli* phages are highly diverse and are currently found in more than 11 phage families and 111 genera.

In this talk, I will present a new collection of phages infecting extended spectrum β-lactamase producing *E. coli* that are commensal in livestock but poses a risk for humans by transferring antibiotic resistance to pathogenic strains. I will present the different strategies employed by these phages to adjust to diverse host receptors yet retaining the essential specificity and discuss the prospects of using phages for decolonization of animals. Moreover, I will reveal other examples of phage-host interactions in *E. coli* and discuss how this knowledge contribute to our understanding of phage-host interactions in complex environments as well as can be used for the benefit of society.

Biography

Lone Brøndsted is Professor in Phage biology and Biocontrol for food safety at the University of Copenhagen, Denmark and has a MSc in Chemical Engineering and Public Governance as well as a Ph.D. in Molecular Microbiology. Her current research focuses on in-depth analysis of phage biology, phage-host interactions and exploiting the antimicrobial potential of phages and phage proteins for novel approaches to combat pathogenic bacteria. She works closely with relevant industries for implementing such novel approaches and phage biocontrol targeting foodborne pathogens as well as human and animal pathogens.

Keynote lecture - How to start a phage therapy hub for human applications in your own clinic: the 5-year experience of the Military Hospital Brussels?

Prof. Patrick Soentjens, Center for Infectious Diseases, Queen Astrid Military Hospital, Brussels, Belgium and Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

Abstract

Prof Dr Patrick Soentjens and team built a clinical coordination platform and a treatment register database for human applications of Phage Therapy to ease patient processes for human applications of phage therapy together with Sciensano, academic hospitals in Belgium and overseas. More than 100 patients were treated under their coordination with phages produced by the QAMH. Starting a Clinical Hub Coordination Center for Human Applications seems easier than thought. During this presentation he would like to guide us through some of the sub-aspects to successfully start a center in Belgium.

Biography

Prof. Dr. Patrick Soentjens is an infectious diseases specialist with clinical expertise in Belgium in the field of travel medicine, HIV, sexual transmitted diseases, tropical diseases and severe multiresistant infections. He is active in outbreak management and in the preparedness of outbreak teams. He diagnosed the first Covid-19 case in Belgium on February 2, 2020 at the Military Hospital in Brussels, was responsible in the start-up of Covid-19 wards at the University Hospital in Antwerp, and was in charge of the medical teams to manage the hundreds of monkeypox cases at ITM in 2022.

As Head of the Travel Clinic ITM since 2016, the core team to perform reference tasks in travel medicine was greatly expanded. A new website and app www.wanda.be were launched under his supervision in November 2019. As Chief Physician at the Polyclinic ITM, he put accents on the daily operation with attention to more output, people management, improved processes and further specialization in ITM's niche activities. He was appointed Associate Professor of the new Chair of Travel Medicine at ITM in April 2021.

Since January of 2016, he is Chair of the Belgian Study Group of Travel Medicine who gives advice on travel medicine guidelines in Belgium. In addition, he's a consultant for the National Immunization Technical Advisory Group and he is an advisor in other boards like the Federal Agency for Medicines and Health Products (FAHPM), the European Medicine Agency (EMA), the Scientific Committee of the Conference of the International Society of Travel Medicine (CISTM) and Journal of Travel Medicine.

For more than ten years, his team has carried out investigator-driven clinical trials in soldiers at the Queen Astrid Military Hospital in Brussels on alternative and shorter intradermal vaccine schedules with microdoses of existing rabies vaccines. He conducted two large pivotal studies (between 2011-2017) which greatly contributed to a revision of the WHO recommendations on rabies vaccination in 2018. He is constructing a research portfolio investigating novel ways to vaccinate travellers and soldiers with new and shorter regimens of new or existing vaccines. An entirely new clinical trial center with dedicated team of seven team members to conduct vaccine trials was started at ITM in 2021.

In addition, he's supervising a phage therapy coordination team at the Queen Astrid Military Hospital in Brussels. They built a clinical coordination platform and a phage therapy treatment register database to ease patient processes for human applications of phage therapy together with academic hospitals in Belgium and overseas. More than 100 patients were treated under their coordination with phages produced by the QAMH. In 2021 he served in the inauguration committee of the Non-traditional Antibacterial Therapies ESGNTA of ESCMID.

Mining genetic parts and tools from bacteriophage genomes for Synthetic Biology

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In recent years, several Pseudomonas species, with Pseudomonas putida as their archetype, have gained notable attention in the field of biotechnology and synthetic biology (SynBio) as highly versatile and robust production hosts. To fully unlock the potential of P. putida and related species for SynBio, a well-characterized and tailored set of genetic parts is indispensable for the creation of reliable and performant synthetic genetic circuitry. One potential source of genetic parts are bacterial viruses (bacteriophages), as they have co-evolved with their bacterial host and are master cell manipulators that have inspired many SynBio tools and parts in the past.

We mined the genomes of several T7-like Pseudomonas phages for novel genetic parts adapted for Pseudomonas, as these phages encode their own orthogonal transcriptional machinery for the expression of their genome within this host. Guided by full-length RNA sequencing, a set of highly potent phage terminators were accurately identified and validated in vivo in Escherichia coli, P. putida and P. aeruginosa. In addition, we introduced and optimized four T7-like RNAP polymerases, their corresponding phage promoters and lysozymes in P. putida and P. aeruginosa, resulting in a tailored version of the multifaceted T7 expression system for these species. Apart from their proven functionality, the remarkable orthogonality between the identified phage RNAPs and their promoters was showcased. Together, these tools and parts highlight that bacteriophages, as evolutionary-adapted, natural predators of the targeted bacteria, provide a valuable source of orthogonal microbial SynBio parts.

Key words: SynBio; Pseudomonas; RNAP; promoter; terminator

Structural and functional diversity of cell wall binding domains in staphylococcal endolysins

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Phage lysins are increasingly investigated as a novel antibacterial against multidrug-resistant bacteria. They have a broader spectrum compared to phages, usually at the species or genus level, but till today their specificity determinants are poorly understood. While a lot of interest has been paid to their modular structures, which typically contain enzymatically active domains and cell wall binding domains (CBDs), current literature tends to explore them in a case-by-case manner, which often neglects the bigger picture and fails to deliver insights to be integrated in engineering pipelines. This work tackles such deficit by systematically analyzing a set of almost 200 staphylococcal endolysins and characterizing their CBDs with state-of-the-art techniques both in silico (AlphaFold structural predictions) and in the wet lab (creating tens of reporter-CBD fusions to test their binding specificity against a collection of staphylococci). As a result, we have uncovered an evolutionary convergence of staphylococcal lysins towards a trimodular structure bearing CBDs with the ubiquitous SH3 fold in three different variants (two of them yet undescribed). Our binding measurements show a remarkable variability of binding profiles, which contrasts with the evolutionary convergence of the CBDs. This result suggests that this kind of domains display an impressive functional plasticity, since small structural adjustments apparently have radical functional consequences. Moreover, we have identified three key amino acid residues in a pair of related CBDs which may explain their differential binding, pointing out to a path to success in rationalizing the biotechnological research on endolysins.

Key words: endolysins; protein engineering; cell wall binding; evolution

Bacteriophage efficacy against *Staphylococcus aureus* in burn wounds, using an ex vivo skin model

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Yearly 11 million people require medical treatment for burn wounds globally. Mortality rates are high and mostly due to bacterial infections. Staphylococcus aureus (S. aureus) is a major causative agent of burn wound infections. Antibiotic resistance and biofilm formation complicate treatment of these infections with antibiotics. An alternative for antibiotics is the use of bacteriophages, viruses that infect and kill bacteria.

To gain more insight into the efficacy of bacteriophage therapy for burn wound infections, an ex vivo model was set-up using surplus human skin obtained after elective surgery. A burn wound was applied to the skin, which was inoculated with a methicillin-resistant S. aureus (MRSA) strain. After one hour, the skin was treated with either bacteriophages (phage ISP or RPCSa2) at different multiplicity of infection (MOI), antibiotics (Fusidic acid), or phosphate buffered saline (PBS). Colony forming units (CFU) were determined after 24 hours.

A single treatment with bacteriophages or antibiotics did not significantly decrease CFU after 24 hours. However, when the skin was treated every three hours (three times in total) with bacteriophages, a significant decrease was observed at the highest MOI of phage ISP. Finally, when the skin was pretreated with bacteriophages, one hour prior to S. aureus exposure, a significant drop in CFU was observed for both bacteriophages at two out of three MOI tested.

These findings suggest that bacteriophage therapy could be effective against burn wound infections using a multiple treatment strategy. It would be most effective however, if used in a prophylactic manner.

Key words: S. aureus; burn wound infections; bacteriophage therapy; human skin model

Towards rapid detection of pathogens using phage-based lateral flow assay

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Recent epidemics have proven that diseases can spread rapidly in our globalized world. New innovative solutions for the rapid and specific detection of bacterial pathogens in food and the environment have become of primary importance, especially in a context of alarming growth of antimicrobial resistance. The Bacillus cereus group includes several species known for their pathogenic potential and their rapid detection in the agro-food sector is essential to avoid food poisoning.

Lateral Flow Assays (LFA) are one of the most used point-of-care sensors (e.g. Covid self-test) in various disciplines ranging from clinical diagnostics to environmental, food safety and veterinary analyses thanks to their user friendly and convenient format. While still being the gold standard bioreceptors for LFA, antibodies show limitations in terms of variability in specificity and stability, and high costs, which calls for urgent alternatives. Phage proteins involved in the adsorption and lysis have evolved specific binding modules that can be used as bioreceptors for LFA purposes. However, there is a lack of understanding of the interactions occurring at the interface level which reduces the ease of design of phage-based LFA. The emergence of accurate bioinformatics tools including AlphaFold2 has strongly facilitated the microstructural characterization and engineering of phage proteins which will contribute to the better performance of phage-based biosensors.

In our work, in silico analysis of phage affinity proteins targeting Bacillus cereus used in an LFA format highlighted the regions involved both in the binding to LFA interfaces and bacterial cells which opens the way to the optimization of the protein performances used in these tests.

Key words: Bacillus cereus s.l.; Lateral Flow Assays; bioreceptors; phage protein

FUNDAMENTAL RESEARCH IN PHAGE BIOLOGY POSTERS

Genomic analysis and *in vitro/in vivo* characterization of phage resistant *E. coli* K1 isolates

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E. coli K1 is involved in several types of human infections, including meningitis, urinary tract infections and bloodstream infections. The understanding of the bacterial resistance mechanisms to phages has implications for the development of phage-based therapies. The objective of this study was to investigate the resistance of E. coli K1 isolates to ULINTec4, a K1-dependent bacteriophage. Resistant bacterial colonies were isolated from an avian pathogenic E. coli strain APEC 45 and the human strain C5, both previously exposed to ULINTec4. After confirming their resistance, genomic analysis was carried out and several parameters were evaluated, such as growth capacity, phage adsorption, phenotypic impact at capsular level and virulence in the in vivo Galleria mellonella model. One of the resistant isolates exhibited a significantly slower growth rate suggesting the presence of a resistance mechanism altering its fitness. Comparative genomic analysis revealed insertion sequences at various capsular gene sites. Adsorption of the ULINTec4 phage was reduced on all resistant isolates. In addition, antigenic tests targeting the K1 capsule showed a very low positive reaction compared to the control. Nevertheless, microscopic images revealed the presence of capsules and a clustered organization of resistant strains. In the Galleria model, larvae infected with phage-resistant strains showed better survival rates than larvae infected with phage-sensitive strains. In conclusion, a phage resistance mechanism was detected at the genomic level, have an impact on capsular expression and was able to decrease the virulence of E. coli K1 in-vivo.

Funding: The project was financially supported by Wallonia in the framework of the call for projects organised by BioWin competitiveness cluster (Project Inteliphages).

Key words: phage resistance; E. coli K1; ULINTec4; genomic analysis; Galleria mellonella

Poster number: 1

Discovery of mechanisms of interactions between bacteria and bacteriophages in the human gut microbiome

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Bacteriophages (phages) are the most abundant viruses in the human virome and establish complex interactions with their bacterium and human hosts and with each other, thereby shaping microbiota composition. Phage-bacteria interactions may be beneficial or commensal through lysogeny, which confer beneficial traits to the bacterial hosts, and predatory through lytic infections.

The dynamics and outcome of phage-bacteria interactions depend on complex molecular interaction networks between genetic defense systems in bacteria that protect them from phage infection and counter-defense systems evolved in phages that allow them to bypass those defenses. Exploiting the clustering of defense systems in prokaryotic genomes have led to the discovery of many new systems in the neighborhood of known systems in complete genomes. However, it is currently unknown the relevance of these defenses in the gut microbiome.

This work is focused on discovering the molecular crosstalk mediating the interactions between bacteria and bacteriophages in the human gut microbiome to gain insights into their coevolution and ecology. For this, we use ADAM (Automatic Detection of Antiviral Mechanisms), a bioinformatic tool developed to predict novel defense mechanisms, which uses a set of criteria derived from known defense systems. ADAM has provided a first list of potential defense modules, which will be prioritised for experimental confirmation.

Key words: bioinformatics; gut microbiome; defense systems

Poster number: 2

Enhancing phage therapy safety: reliable and sensitive phage genome annotation with rTOOLS2 high-throughput pipeline

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Phage therapy is an exciting and promising approach to fight bacterial infections. However, ensuring the safety and efficiency of phages for therapeutic use requires a thorough understanding of their genomic properties [1]. Traditional bioinformatics tools designed for bacterial genomes are not well-suited for phage genomes due to their unique structure, leading to poor gene calling and function annotation [2].

rTOOLS2 is a multi-hypothesis, phage-focused annotation pipeline: its advanced algorithm uses the output produced by widely-used annotation tools to find more gene functions, with high evidence thresholds to avoid false positives.

In this study, 135 phage genomes published in Genbank were annotated using Pharokka and rTOOLS2's high-thoughput version, and the results were compar

Pharokka was able to improve the average published annotation, as the average number of genes functionally annotated grew from 29.5% to 35.9%. rTOOLS2's high-throughput version was able to significantly increase the rate of annotated genes, reaching 54.6%.

rTOOLS2's high-throughput version can rapidly provide a strong genome characterization. The use of curated databases ensure that meaningful annotations are provided, and results can be published with low risk of public database poisoning. Moreover, rTOOLS2 produces more information, as it nearly doubled the number of annotated genes in the published genomes.

[1] Culot A., et al., 2019. Aquaculture 513, 734423

[2] McNair K., et al., 2019. Bioinformatics 35, 4537-4542

Key words: bioinformatics; genome; annotation

Poster number: 3

Discovery of new antiviral mechanisms in oceanic bacteria using ADAM

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Bacteria and phage engage in an arms race, where bacteria evolve strategies that prevent or abort phage infections, collectively known as defense systems. Genes encoding defense systems cluster in bacterial genomes, forming defense islands. These defense islands are often within prophage and other mobile genetic elements regions. The most used approach to discover new defense mechanisms is to locate known systems in the genomes and to extract the neighboring genes, hypothesizing they are part of a defense island. This strategy provides a list of candidates to be validated experimentally. While there are software to detect known defense systems, at present there are no bioinformatics tools to predict de novo defense systems.

We have developed ADAM, an automatic tool to discover new anti-phage mechanisms. ADAM analyses metagenomics and genomic data to predict novel defense systems. ADAM algorithm includes prophage prediction, protein function prediction and gene-context analyses. We applied ADAM to the Tara Oceans dataset and selected 5 systems for experimental validation. Heterolog expression in E.coli of two these systems showed anti-phage activity when challenged against coliphages, both in solid and liquid media. Challenges of E.coli expressing the systems at varying multiplicity of infection indicates the systems act via abortive infection.

Key words: defense systems; phage-bacteria interaction; bioinformatics

Poster number: 4

Bacteriophages against *Pseudomonas aeruginosa* are inhibited by complement in human serum

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Bacteriophage (phage) therapy constitutes a promising alternative to antibiotics. However, therapeutic phages are often chosen based only on specificity to bacteria, without evaluating the role of factors such as the patient's immune system. In particular, the complement system, a cascade of proteases that recognizes and targets invading organisms, may interact with phages and limit their efficacy.

Objective: To study the influence of the complement system on different phages targeting Pseudomonas aeruginosa.

Methodology: We used the membrane-impermeant dye Sytox green, which stains damaged bacteria, to assess the killing of P. aeruginosa by myophages and podophages in presence of human serum. Phage binding was studied through flow cytometry and confocal microscopy using phages labeled with a fluorophore by means of click chemistry.

Results: Serum inhibited myophages in a concentration dependent manner, while podophages were unaffected by the presence of serum. Myophage activity could be rescued by inactivating the complement system through heat treatment or use of a specific inhibitor of the early stages of the complement cascade. Likewise, antibodies isolated from serum did not affect phage activity. A binding assay with fluorescently labeled myophage PB1 coupled with surface-bound complement staining showed that active complement interferes with phage infection at the stage of receptor recognition and binding.

Conclusions: Our results show that the complement system impairs the activity of myophages against P. aeruginosa. The inhibitory effect of serum on these phages, often found in therapeutic cocktails, highlights the importance of using physiologically relevant conditions when choosing phages.

Funding: Netherlands Center for One Health

Key words: phage therapy; innate immunity; serum; complement system; P. aeruginosa

Poster number: 5

Understanding the RBP modules function of KP32 as a podophage model

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Klebsiella pneumoniae phage KP32 possesses two receptor-binding proteins (RBPs), specifically recognizing capsular serotypes K3 and K21/KL163 using RBP1 and RBP2, respectively. Both RBPs have a modular build-up.

The function of RBPs modules was analyzed at protein and phage levels by preparing C-terminally truncated proteins, chimeric protein fusions as well as engineered phages lacking the modules of interest. Chimeric RBPs, truncated RBPs, and engineered phages were produced using the VersaTile method and Gibson assembly. The activity and specificity of RBPs were analyzed using a spot test; binding ability was tested with a GFP-based binding assay; engineered phages infectivity was checked with EOP and propagation ability; and protein trimerization was established with MALS.

Deletion of the lectin domain (LEC), located at the ultimate C-terminus, does not disturb the enzymatic activity of RBP2. Deletion of both C-terminal modules (carbohydrate-binding module (CBM) and LEC) leads to enzymatic activity loss. Engineered phage particles lacking LEC or CBM_LEC lose the ability to infect K21/KL163 host. There was no serotype specificity switch/extension due to C-terminus deletion nor when the CBM and LEC domains were used in chimeric fusions with other RBPs. In addition, the CBM and LEC were not able to bind to encapsulated bacteria surface, in contrast to the full-length protein.

The central enzymatic domain and at least the CBM are required for RBP enzymatic activity. CBM and LEC domains are necessary for proper protein trimerization but are not responsible for the specificity of RBP2 nor protein binding to the capsule.

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Key words: receptor-binding proteins; carbohydrate-binding modules; phage engineering; chimeric proteins

Poster number: 6

Isolation and characterization of spontaneously induced *Pseudomonas* temperate phages

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The autoplaque phenomenon is defined as the spontaneous appearance of plaques that can be observed in the bacterial lawn without exposure to any external phage. Different causes have been proposed for this phenomenon, including programmed cell death, exposure to lethal factors or prophage induction. However, mechanisms underlying this phenomenon remain unknown.

In this work, we have observed spontaneous induction in 63 Pseudomonas aeruginosa strains from a collection of 313 strains from different sources. Autoplaque isolation and test of 39 of these strains in a range of diverse Pseudomonas indicator strains have shown the presence of phages as the probable origin of these autoplaques. Isolation, amplification and genome sequencing of these phages resulted in the isolation of 18 unique Pseudomonas temperate phages, principally belonging or related to the Casadabanvirus, Hollowayvirus and Beetrevirus genera, as well as a novel group of phages. On the other hand, other known and common Pseudomonas temperate phages such as Detreviruses (similar genomic structure as lambda) were not found in autoplaques. This points to a possible link between the autoplaque phenomenon and the type of prophages involved in it. We performed a genomic characterization of both hosts and isolated phages and transcriptomic characterization of the lytic cycle of representative phages to shed some light on this phenomenon and expand the biological knowledge on the still understudied temperate phages of Pseudomonas.

Funding: This work is part of a project that has received funding from the European Research Council under the European Union's ERC consolidator grant number [819800].

Key words: Pseudomonas; temperate; autoplaque; induction; prophage

Poster number: 7

Viral acetyltransferases: a novel, yet widespread host-hijacking strategy in *Pseudomonas* phages

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In continuously changing environments, cells need to adapt rapidly to remain competitive and survive. One particularly fast method to modulate metabolism is through protein post-translational modifications, such as lysine acetylation. Recently, we discovered that phages manipulate bacterial acetylation levels by encoding their own acetyltransferases. Given the reversible nature of lysine acetylation and the short time frame of phage infection cycles, these acetyltransferases present a compelling new host-hijacking strategy. However, the viral acetyltransferase space remains vastly underexplored, and insights into these enzymes and their targets are lacking.

Here, we present the first catalog of phage-encoded acetyltransferases, and show their impact by a case study on the prime targets of one of the identified acetyltransferases. We mined 197 Pseudomonas phage genomes and identified 130 putative acetyltransferases. AlphaFold2 predicted a conserved acetyltransferase fold in 98 of these enzymes. Remarkably, the majority of Phikmvvirus phages consistently encodes the same pair of acetyltransferases: a known one (Rac) and a novel one. Acetylomics analysis revealed that the latter facilitates acetylation of nine proteins (logFC > 2) in P. aeruginosa, including two key enzymes of the cysteine and methionine biosynthetic pathway: MetE and MetK. Through analysis with AlphaFold2 and molecular dynamics, we discovered that one of the acetylations found in MetE stabilizes its active site and its interactions with single substrates. In conclusion, this study shows that acetyltransferases are commonly encoded by Pseudomonas phages and have the potential to influence enzyme activity in key metabolic pathways in exciting new ways.

Funding: This research was supported by the KU Leuven project C1 'ACES' [C16/20/001].

Key words: lysine acetylation; AlphaFold2; protein post-translational modifications; Pseudomonas phages

Poster number: 8

Transcription regulatory program of the SSV1 virus infecting the thermoacidophilic archaeon *Saccharolobus solfataricus*

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Fuselloviridae infecting thermoacidophilic Sulfolobales are undoubtedly the best-studied archaeoviruses, with the SSV1-Saccharolobus solfataricus interaction as a model. Around one-third of fusellovirus core genes encode putative DNA-binding proteins (DBPs), which, although little is known about their functions and mechanisms of action, suggests that DNA-binding processes such as transcription regulation are a critical aspect of the infection cycle.

In this work, we aim to identify and characterize SSV1-encoded DBPs to elucidate transcription regulatory mechanisms in the virus-host interaction.

Host and virus transcriptome dynamics post-infection and post-induction are being studied by performing high-resolution, long-read ONT-cappable RNA-sequencing. This end-to-end sequencing of primary transcripts will provide a full transcriptional blueprint of SSV1, including transcription start and stop sites, operon structures and UTRs. This transcriptomic study will be complemented with genome-wide binding profiles of all SSV1 putative DBPs by performing ChIP-seq experiments using engineered virus variants in which each DBP is epitope-tagged. On top of this, molecular mechanisms of individual SSV1-encoded DBPs are being characterized through protein-DNA binding assays and in vitro transcription.

This work is expected to greatly enhance our understanding of the SSV virus-host interaction by applying state-of-the-art techniques to study SSV1. By bridging critical knowledge gaps, we might contribute to the development of virus-based biological parts for gene expression engineering in Sulfolobales, an archaeal order with great biotechnological potential.

Funding: Research funded by Research Foundation Flanders (FWO), grant 1172723N

Key words: Archaea; SSV1; transcription; regulation; DNA binding

Poster number: 9

Analysis of Group A *Streptococcus* lytic phage interactions with human serum

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Group A Streptococcus (GAS) is a strictly human pathogen which causes mild to severe infections. They are currently treated exclusively with antibiotics, as a vaccine is not yet available. New treatment approaches, like phage therapy, require the characterization of phage-pathogen-host interactions. GAS is known to bind human serum/plasma proteins to escape immune attack. We found that the M25 strain is protected from infection by the virulent phage A25 when incubated in human serum. Infection and whole cell binding assays suggest a role of high molecular weight (HMW) proteins. When the serum was fractionated, the >100kDa fraction was able to protect from phage infection compared to the >100kDa fraction. Purified Immunoglobulins (150kDa) provided only a limited protection against infection. On the other hand, we tried to identify their binding partner(s) on the bacterial surface. We generated three single and one triple knock-out mutants of the three major surface proteins, M and M-like proteins (Δemm, Δenn, Δmrp) in the M25 strain. In the presence of serum, the Δenn and Δmrp mutants were protected from infection similarly to the wild-type control, whereas the Δemm and Δ3M populations were partially lysed. This lead us to believe that a part of the protection is mediated by the M-protein. We are currently purifying this protein to then determine the interacting proteins in the serum by co-purification and mass spectrometry. Moreover, we will perform whole cell binding assays to determine which serum proteins bind the bacterial surface using human serum on the different mutants. With these results, we hope to understand the masking strategy developed by GAS using human host proteins to prevent phage infection.

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Key words: Streptococcus pyogenes; lytic phage; human serum; anti-phage defense

Poster number: 10

FUNDAMENTAL RESEARCH IN PHAGE ECOLOGY POSTERS

Optimization of Virome Enrichment Method for Human Respiratory Samples

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The disease etiology of chronic inflammatory lung disorders, such as COPD (Chronic Obstructive Pulmonary Disease) is complex. The factors that determine whether a patient will develop a particular illness and whether they might benefit from a specific prophylactic or therapeutic intervention are only partially understood. Increasing evidence suggests that microbial dynamics play an important role in the host's health, immune system, and in disease pathogenesis. Several studies have already investigated the lung microbiome of COPD patients and compared it to healthy subjects. However, most of these studies focused on characterizing the resident bacteria, ignoring the role of viruses. Thus, the diversity and physiological roles of eukaryotic viruses and bacteriophages in COPD patients remain vastly underrepresented in the literature. To address this gap, my Ph.D. project aims at optimizing a wet-lab pipeline to enrich for viruses and bacteriophages from human sputum samples. As the homogenization of sputum is a key step of the viral enrichment protocol, we designed a study to find the best liquefaction method (i.e., ideally a method that completely disrupts the viscous/sticky matrix of sputum while conserving the microbial composition). First, we tested how different liquefying agents would affect the integrity of viral particles (with and without subsequent enrichment). A mock community of 7 viruses with different morphological and genetic properties was spiked in sputum samples and quantified by RT-qPCR after homogenization with different liquefaction methods. However, it was observed that the addition of an artificial mock virome did not accurately represent the complex virome/sputum structure. Therefore, we subsequently sequenced the same samples using Illumina technology, followed by bioinformatics analyses (including SPAdes, CheckV, and geNomad). These data provided new insights into the effects of different liquefying methods on the recovery of the naturally occurring virome in sputum. The most promising liquefying agent will be used to explore the respiratory virome in longitudinal samples of moderate to severe COPD patients.

Key words: COPD; sputum; respiratory viruses; protocol optimization; NGS

Poster number: 11

Comparative Assessment Reveals Significant Output Differences Between VIPCAL and Linear Regression in Viral Reduction Assays

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This study conducts an in-depth examination of methodologies employed in viral reduction assays, with emphasis on the Viral Increase Percentage Calculation (VIPCAL) and linear regression methods. Extensively utilized to estimate parameters such as viral production rates, viral-mediated bacterial mortality, lysogeny, and the viral shunt, these methods demonstrate significant divergence in outputs, as revealed by our comprehensive simulations. VIPCAL, while not definitively accurate, displays comparative superiority in extracting lysogenic values over linear regression.

We introduce an enhancement to the VIPCAL method, named VIPCAL-SE, which incorporates standard error measures to identify data peaks and valleys more accurately. This refinement mitigates the risk of off-replicate artifacts and reduces potential inflation in outputs. The relevance of this enhancement is underscored in the context of current meta-analyses, which aggregate data from both methodologies despite their significant output discrepancies.

Our study also unveils pronounced differences in bacterial production between treatments, due to the inductant, antibiotic Mitomycin-C. These findings suggest a potential increase in lytic viral production due to heightened bacterial production. This underlines the need for including bacterial counts to validate the assumptions inherent in the assays, adding a bacterial production endpoint.

Given the evident methodological divergence, an urgent reassessment of current datasets is warranted. To address these challenges, we are finalizing an R package. This package, featuring all 12 methodologies with emphasis on VIPCAL-SE and bacterial production endpoint, aims to enhance consistency and accuracy in the field.

Key words: viral reduction assays; VIPCAL; viral production; lysogeny; marine viruses

Poster number: 12

PRESENT AND FUTURE APPLICATIONS POSTERS

Hidradenitis Suppurativa: A challenging opportunity for phage therapy

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Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease in which patients develop painful skin abscesses, caused by a variety of triggers which lead to a bacteriome imbalance and inflammatory responses. Currently, some patients manage HS with phage cocktails of the Eliava Institute, but it is unclear whether disease relief is due to antibacterial or immunomodulating effects.

We believe that the application of phages in these patients provides a unique opportunity to learn more about both the disorder and phage therapy in general as HS differs in three key points from other diseases currently treated with phage therapy: (1) HS is not a stricto sensu infectious disease, (2) HS is a multifactorial, immunological disorder, (3) patients currently applying phages are using them for a long period of time.

Bacterial culturing and 16S rRNA profiling was carried out on skin swabs of HS patients to determine the target, revealing that Staphylococcus and Corynebacterium are on average less abundant in HS patients compared to healthy individuals. In contrast, Escherichia-shigella is more abundant, from which surprisingly only a low number of strains could be cultivated. Three microbiome profiles of HS patients could be distinguished based on a high abundance of obligate anaerobes in profile A and B, and high abundance of Escherichia-Shigella and Streptococcus in profile B and C.

Phages infecting 100% and 70% of E. coli isolates could be identified. Moreover, some patients were infected with the pathogen S. aureus, of which all could be infected by phage Romulus. This shows that there are phages with application potential to treat HS patients.

Key words: phage therapy; hidradenitis suppurativa; microbiome study; immunomodulation

Poster number: 13

Optimization of bacteriophage therapy for difficult-to-treat musculoskeletal infections: from bench to bedside and vice versa

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Given the increasing threat of antimicrobial resistance, scientists are urgently seeking alternative antimicrobial strategies, such as phage therapy (PT).

However, despite promising results for the treatment of musculoskeletal infections (MSI), crucial knowledge gaps remain regarding the optimal treatment protocol.

To address these knowledge gaps, a prospective observational study (PHAGEFORCE) study was set up as well as a multidisciplinary approach to achieve and optimize standardized treatment guidelines.

At our center, PT is strictly controlled and monitored by a multidisciplinary phage taskforce. Each phage follows the same pathway to ensure standardization and data quality.

PT for MSI is applied locally via a drainage system. The advantage of such an administration protocol is that local phage titers and bacterial load can be monitored during treatment.

After treatment, patients are followed for at least a year.

Within the PHAGEFORCE framework, we established a testing platform to gain insight in the safety and efficacy of PT, biodistribution, phage kinetics and the molecular interaction between phages and bacteria.

The draining fluid is collected after each application to determine the phage titer and bacterial load during treatment.

All isolates taken during and after phage treatment are fully characterized by genome sequencing to detect mutations in the phages that may increase the efficacy of the phages in the patient environment and to test the susceptibility of the bacteria to the applied phages.

We hereby present the implementation of this standardized bench-to-bedside protocol for six patients with difficult-to-treat MSI.

Key words: bacteriophage therapy; bench-to-bedside; optimisation; treatment

Poster number: 14

Antibiofilm activity of a *Staphylococcus aureus* phage: potential key role of its baseplate protein

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The efficiency of phages in targeting and killing bacteria could be impaired when cells are biofilm-embedded. Our work aimed to drive the evolution of phage Romulus (Silviavirus genus) within S. aureus biofilm and to analyse the mutations occurred in the evolved phage clones.

A one-month directed evolution protocol was based on a one-hour phage incubation with a pre-formed biofilm on porous beads, followed by an eight-hour bead incubation in fresh BHI broth. At the end of each cycle, the medium containing phages was collected and used in the following round. After the 31st round (R31), ten plaques (p1 to p10) were isolated and sequenced. The antibacterial effect of the wild type (wt), the R31 and the single mutant phages was assessed against planktonic (multiplicity-of-infection MOI 0.1) and sessile cells (from 10^7 to 10^9 PFU/ml), after 24 hours.

No reduction of planktonic cells was observed with the wt phage compared to the untreated control. Conversely, the eradication was obtained with R31 cocktail, p2 and p5 phages. In comparison to the wt phage activity, a statistically significant biofilm reduction (at least 3log10) was achieved with evolved phages, when tested at 10^8-10^9 PFU/ml. Genome sequencing revealed that most of the mutations occurred in gp58, annotated as baseplate protein, which is predicted to have depolymerase activity (98.0% certainty, by PhageDPO).

These results suggest a key role of the baseplate protein (as putative depolymerase) in targeting bacterial cells, including those embedded in biofilm. Ongoing experiments are aimed at better understanding gp58 function.

Key words: Staphylococcus aureus; biofilm; phage evolution

Poster number: 15

Burnzymes as anti-inflammatory, third-generation lysins targeting burns infected with *Acinetobacter baumannii*

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Phage-derived lysins have been tested in phase III clinical trials as a promising alternative to the conventional antibiotics. However, tailoring these lysins to their final application might result in a higher efficacy. This study aimed to provide a proof-of-concept of such a third-generation lysin improving efficacy when treating burn wound infections. A delicate inflammatory balance, crucial for burn wound healing, is disturbed by infection, resulting in chronic infections and eventually, scarring. Therefore, the previously engineered lysin, 1D10, targeting Acinetobacter baumannii, was fused with several peptides having anti-inflammatory properties. A library of 117 variants was screened for antibacterial activity, and three unique and intact hits were identified and characterized. The lead variant, BZAb1, was shown to be non-cytotoxic in a preliminary screen in HeLa cells and shown to specifically targeting A. baumannii strains at least equally effective as its parental lysin, 1D10. Moreover, this lead variant was able to bind LPS in vitro, confirming its potential anti-inflammatory property. In sum, this study provided the first third-generation lysin targeting a Gram-negative pathogen. By including additional properties, in this case the property to bind LPS, hence potentially reducing inflammation at the site of infection, the final clinical outcome might be improved.

Funding: This work was supported by the Research Foundation – Flanders (FWO) under grant [G066919N].

Key words: lysin engineering; drug discovery; Acinetobacter baumannii; burn wound

Poster number: 16

Isolation, in vitro characterization and efficacy assessment in *Galleria mellonella* larvae of four bacteriophages targeting *Aeromonas salmonicida*

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The Gram-negative bacteria Aeromonas (A.) salmonicida is a primary fish pathogen that causes furunculosis in salmonids as well as septicemia in a variety of fish. In one hand because this disease is responsible for significant losses in salmonid production worldwide and in the other hand because of the frightening tendency of this bacteria to exhibit antimicrobial (multi)resistances, phage therapy could represent a leading alternative to treat this infection in aquaculture. The aims of this study were to create a collection of A. salmonicida strains, isolate phages targeting these strains, phenotypically and genomically characterize these newly isolated phages and finally assess their potential for phage therapy in the preliminary in vivo model of Galleria (G.) mellonella larvae. Four new phages active against A. salmonicida were isolated from water samples collected in fish farms and natural aquatic environments in southern Belgium. Genomic analysis showed that 3 of these phages, named vB_AsaM_ULASA2 (170,823bp), vB_AsaM_ULASA3 (164,381bp) and vB_AsaM_ULASA4 (171,205bp), belong to the Straboviridae family while vB_AsaM_ULASA1 (47,813bp) stay in the unclassified part of the Caudoviricetes class. All 4 presented a myovirus morphotype. Four-day efficacy experiments in the preliminary in vivo model of G. mellonella larvae showed that 3 of these 4 phages were responsible for a significant extension in the larval survival time at the 2 treatment doses tested (MOI 10 and 100). In light of these results, these phages targeting A. salmonicida could represent potential new candidates for the development of anti-furunculosis phage treatments in aquaculture.

Key words: bacteriophages; phage therapy; Aeromonas salmonicida; furunculosis; Galleria mellonella

Poster number: 17

Stability and lytic activity assessment of bacteriophages targeting *Staphylococcus aureus* causing bovine mastitis in milk

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Bovine mastitis is a major cause of culling in dairy cattle and the antimicrobial treatment of the infection contributes to the emergence and the spread of antimicrobial resistances. Phage therapy could be a promising approach but the biological and physicochemical properties of milk can affect the phages properties. The objective of this study was to compare the stability and lytic activity of phages targeting Staphylococcus aureus in raw and pasteurized milk.

A total of 28 bacteriophages previously isolated against S. aureus were spotted on 44 S. aureus strains isolated from bovine mastitis to evaluate the phage host range. The phage stability was assessed by titration in milk prior and after 6h incubation at 37°C. The lytic activity was assessed by inoculating milk with S. aureus and phages at a MOI of 1000. Bacterial and phage titration at different timepoints allowed to compare the lytic activity of the phages and their replication.

A broad host spectrum was observed for 24/28 phages. Stability analysis showed that all phages were still active after 6h incubation in both raw and pasteurized milk with an average stability rate of 8%. Regarding the lytic activity, 16/21 phages were able to replicate in milk but no decrease in bacterial count was measured, which could be linked to resistant bacteria. Pasteurized milk allowed significatively better stability and replication of the phages.

In conclusion, heat-sensitive components in milk alter the phages properties. Further tests in milk fractions should be performed to assess their effect on phages. The resistance of bacteria against phages should be investigated and the use of phage cocktails should be evaluated to circumvent the phage resistant bacteria emergence.

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Poster number: 18

Assessing the environmental biosafety of phage-based biocontrol applications

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Over the past decades, phage biocontrol as a means of treating bacterial plant diseases has regained keen interest. Indeed, pioneering trials have shown this is a promising strategy to treat different diseases. However, just like other plant protection products, the biosafety of bacteriophages needs to be validated and reported before registration on the European market is possible. In this regard, the EU has provided data requirements for viral biocontrol in EU Regulations 283/2013 and 284/2013. However, the guidelines on how to determine important characteristics with regard to phage biosafety, remain scarce. Based on the current data requirements and literature, we developed a pipeline based on taxonomic analysis using PCR-based 16S rRNA gene amplicon sequencing. As an illustration of the power of this approach, we show that FoX2 and FoX4, capable of infecting and killing Xanthomonas campestris pv. campestris, appear not to affect non-target species and hence, are environmentally safe.

Funding: European Union's Horizon 2020 Research and Innovation Program (grant agreement N. 773567)

Key words: phage biocontrol; environmental safety; black rot; 16S rRNA gene amplicon sequencing

Poster number: 19

Towards an Alternative Approach for Personalized Phage Therapy: Instant and On-Site Production of SynPhages (Synthetic Phages)

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Since 2018, phage therapy, the process of using the viruses that infect bacteria to cure bacterial disease, has been made a reality in Belgium. At the Queen Astrid Military Hospital (QAMH) in Brussels, and all Belgian university hospitals, patients are now being treated with phage preparations. These preparations are exclusively produced at the QAMH. However, due to the limited host range of individual phages, personalized phage therapy necessitates the maintenance of extensive therapeutic phage banks, periodically updated with novel phages. Moreover, the exchange of patient-specific bacterial strains and matching phages between QAMH and other medical centers is required. The present study will provide a proof of concept for an alternative and innovative phage production system, where both genome assembly and phage rebooting occur without interference of any bacterial cell. This will allow for instant and on-site production of synthetic phages and will not require phage banks or the circulation of bacterial isolates and phages. It would represent a paradigm shift in the production of personalized (phage) medicines.

Key words: phage therapy; Escherichia coli; Klebsiella pneumoniae; genome assembly; cell-free system

Poster number: 20

Impact Assessment of vB_KpnP_K1-ULIP33 Bacteriophage on the Human Gut Microbiota Using a Dynamic In Vitro Model

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The use of bacteriophages, shortened as phages, is a promising alternative treatment to fight antimicrobial resistant bacteria. In this context, the effect of the phage vB_KpnP_K1-ULIP33 (ULIP33) on the intestinal microbiota was assessed in the dynamic in vitro model called the SHIME (Simulator of the Human Intestinal Microbial Ecosystem). The host of this phage is a hypervirulent Klebsiella pneumoniae ST23 and capsular type K1. The phage was inoculated for 7 days in the model and its persistence in the different colons was studied until its disappearance from the system. No significant effect of the phage addition was highlighted regarding the short-chain fatty acid productions, the diversity parameters (α and β) and the qPCR targeting different genera of interest in chronic or acute bowel inflammation. Further studies are now needed to assess the efficacy of this phage against its bacterial host within the human intestinal ecosystem, but the phage ULIP33 exerted no significant change on the global colonic microbiota.

Funding: The project was financially supported by Wallonia in the framework of the call for projects organised by BioWin competitiveness cluster (Project Inteliphages).

Key words: SHIME®; hypervirulent K1 Klebsiella pneumoniae; intestinal microbiota

Poster number: 21

INNOLYSINS: Novel antibacterials against *Salmonella*

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Salmonella is a major cause of foodborne bacterial disease worldwide. Increasing antibiotic resistance and ability to persist, call for novel treatment solutions. Endolysins, phage-encoded enzymes degrading peptidoglycan, are promising antibacterials due to the low probability of bacterial resistance. However, only few endolysins can overcome the outer membrane to access the peptidoglycan and kill Gram-negative bacteria. Our research group has previously engineered endolysins to exert antibacterial activity against both Escherichia and Campylobacter. To do so, we developed Innolysins composed of endolysins and phage receptor-binding proteins (RBPs) that enable the fused endolysins to overcome the outer membrane barrier and act as antibacterials. Here, we aim to develop novel Innolysins against Salmonella causing systemic infections in Africa. We have already identified, multiple phages in our collection able to infect these strains by targeting distinct receptors including outer membrane proteins and lipopolysaccharides. Exploiting the diversity of these RBPs, we are currently using the VersaTile platform to develop an array of Innolysins against Salmonella. Once we generate the Innolysins libraries, the Innolysin with the highest antibacterial activity will be selected based on a high-throughput screening assay. Here, we will study for the first time the use of Innolysins as antibacterials in vivo and fuse them with various cell penetrating peptides (CPPs) to target persister cells hidden inside immune cells. Overall, we are expanding the use of Innolysins as novel antibacterials against enteric pathogens and study the pharmacokinetics of Innolysins to further optimize their use as antibacterials in humans.

Funding: This project has been co-funded by the Tres Cantos Open Lab Foundation.

Key words: innolysins; endolysins; phage receptor-binding proteins; antibacterials

Poster number: 22

Isolation and characterization of five lytic bacteriophages against *Pseudomonas aeruginosa* causing canine otitis externa

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Multi-drug resistant (MDR) Pseudomonas aeruginosa is a common bacterium isolated in canine chronic suppurative otitis externa. The presence of this bacterium is often correlated with a lack of response to the medical treatment. Its virulence factors, including biofilm formation, make it a fearsome pathogen. The use of bacteriophages appears to be a promising alternative for treating MDR infections. The objectives of this study were to isolate and characterize lytic P. aeruginosa phages from wastewater. The enrichment method was used to isolate bacteriophages from Belgian wastewaters that were active against P. aeruginosa. Host range and efficiency of plating were performed on approximately fifty strains of Pseudomonas spp. originating from different species (dogs, cats, horses and reptile) and organs (ear canal, skin, tracheal and broncho-alveolar lavage, nose, fluid, eye). Efficiency of plating was assessed for phages showing total and partial lysis. A total of five lytic P. aeruginosa phages were isolated from Belgian wastewaters. These phages showed a wide spectrum of lysis on Pseudomonas spp. Finally, the genomes of these phages were sequenced before considering the utilization of a phage cocktail in MDR P. aeruginosa suppurative otitis externa.

Key words: Pseudomonas; otitis; dog; treatment

Poster number: 23

In Vitro Effect on Piglet Gut Microbiota and In Vivo Assessment of Newly Isolated Bacteriophages against F18 Enterotoxigenic *Escherichia coli* (ETEC)

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Pig production is impacted by the negative effects of enterotoxigenic Escherichia coli (ETEC), which might result in post-weaning diarrhea (PWD) in piglets. F4 and F18 fimbriae are utilized by ETEC strains to adhere to the small intestinal epithelial cells of the host. If antibiotic resistance becomes a problem with ETEC infections, phage therapy would be an interesting alternative. Four bacteriophages, termed vB_EcoS_ULIM2, vB_EcoM_ULIM3, vB_EcoM_ULIM8, and vB_EcoM_ULIM9 in the present study, have been isolated against an O8:F18 E. coli strain (A-I-2110) and selected based on their host range. These phages have been investigated in-vitro and shown lytic activity throughout a pH (4-10) and temperature (25-45 °C) range. These bacteriophages have been categorized as Caudoviricetes according to genomic investigation. No lysogeny-related gene have been identified. Galleria mellonella larvae in vivo model revealed the therapeutic potential of one chosen phage, vB_EcoS_ULIM2, with a statistically significant increase in survival compared to untreated larvae. A static model of the piglet intestinal microbial environment was infected with vB_EcoS_ULIM2 for 72 hours in order to evaluate the impact of this phage on the piglet gut microbiota. In a Galleria mellonella model, this study confirms the effective replication of the phage and demonstrates the safety of the phage-based therapy for the piglet microbiota.

Key words: ETEC; Galleria mellonella; bacteriophages; microbiota; phage therapy

Poster number: 24

Breaking Down Walls: Towards a Functional-Based Metagenomic Discovery Platform for Phage-Derived Lysins

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Although broad-spectrum antibiotics have proven effective in treating acute bacterial infections, there is an increasing recognition of their detrimental impact on the host its microbiome. Consequently, a current paradigm shift focuses on eliminating disease-causing bacteria, while preserving and even restoring a healthy balanced microbiome during infection treatment. Lysins, peptidoglycan-degrading enzymes encoded by bacteriophages, exhibit the capacity of breaking down the peptidoglycan layer of the bacterial cell wall, which could lead to osmotic cell lysis. While the first lysins are currently tested in clinical trials, emphasizing their potential as a new class of antibiotics, several challenges remain to render lysins effectively for a wide range of bacterial infections. One such obstacle is the development of a platform enabling the rapid discovery of novel lysins targeting diverse bacteria.

This research project aims to overcome this hurdle by establishing a functional metagenomics platform for the discovery of novel lysins targeting Enterococcus. Unlike conventional sequence-based approaches, functional metagenomics enables direct screening of enzyme activities. By leveraging this approach, potent novel lysins targeting Enterococcus could be identified. However, the platform's flexibility will allow for the use of various starting materials and the targeting of diverse bacteria. Consequently, such a versatile platform could contribute to the establishment of lysins as a new class of narrow-spectrum antibiotics, with numerous potential applications. The successful implementation of such a platform, therefore, holds significant promise for the development of lysins as a new class of narrow-spectrum antibiotics.

Funding: FWO SB 1SC9424N

Key words: enterococci; phage lytic proteins; antibiotics; functional metagenomics

Poster number: 25

Unearthing the Global Soil Microbial-Associated Virome and (u)MAPping the Tectonics of its AlphaFOLD2-Aware Structural Diversity Upon a One Health Framework

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Viruses of soil-inhabiting microbes are major drivers of ecosystems but can also mediate diseases to economically important crops or animals and therefore represent a serious burden to global health. Surprisingly, the microbial-associated virus diversity remains speculative. Insofar, and despite the significance of soil microbial lifeforms, no research focusing explicitly on large scale analysis of soil-associated viromes has been carried out making it challenging to mitigate the emergence of soil-associated pathogens. Here, through extensive metatranscriptomic analysis of retrieved soil genomic data, including rhizosphere, decaying compost and leaf litter, we demonstrate that soil microbes, are a remarkable niche to viruses, constituting one of the most biodiverse hosting ecosystems. Using our novel Modulome approach as a proof-of-concept, we accurately investigate viral proteomic data associated with soil-inhabiting cellular organisms, with a focus on emerging taxonomic lineages. By combining our computational Alphafold2-predicted structural information with Ai-driven statistics based on manifold deep learning for dimension reduction (UMAP), over 250.000 viruses across 20 well-supported RdRp-based phylogenetic lineages were uncovered in diverse soil-inhabiting microbial cryptic hosts, ranging from protists to fungi. Taken together, most speciose taxa were prevalently hosted by Fungi and Chromista kingdoms, thereby enriching our understanding of the uncharted viral diversity and evolution in their soil ecological niche. Finally, our enumeration can enable stakeholders to accurately advocate for better soil health policy while facing global biodiversity crisis and climate change-linked epidemic risks within a more comprehensive One Health framework.

Funding:

Key words: MetaVIRIA; AlphaFOLD2; Structural Evolutionary Proteomics; Riboviria; Meta-transcriptomics; Machine Learning; One Health; RNA virus discovery

Poster number: 26

In vitro efficacy of bacteriophages in the treatment of chronic rhinosinusitis-related biofilms

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High-quality data on the treatment of cystic fibrosis-related chronic rhinosinusitis (CF-CRS), targeting underlying bacterial colonization and biofilm formation, are lacking. The ability of bacteriophages to degrade CF-CRS-related biofilms was investigated in vitro.

Material & methods: Sinonasal isolates of CF patients were obtained and biofilms were grown for 24 hours in pathology-specific SCFM2 medium. After treatment with S. aureus phage ISP and P. aeruginosa phages PNM and 14-1, in monotherapy and combined with antibiotics and antibiofilm agents, differences in colony forming units (CFU/mL) were evaluated.

Results: Fourteen isolates (10 S. aureus, 4 P. aeruginosa), were obtained. PNM (p=0.0019) and 14-1 (p=0.0095) were able to reduce mature biofilms, compared to control, whereas ISP did not (p=0.2049). No additional reduction could be observed after adding sodium bicarbonate, xylitol or rhDNAse. A significant reduction was observed after combining sub-MIC levels of levofloxacin with ISP (p=0.0167), sub-MIC levels of ceftazidime to 14-1 (p=0.015) and supra-MIC levels of ceftazidime to PNM (p=0.0224).

Conclusions: PNM and 14-1 phage are able to reduce mature P. aeruginosa biofilms, whereas no reduction in bacterial load was observed after treatment of S. aureus biofilms with ISP. Combination with antibiotics further reduces biofilms and should be further investigated in future experiments.

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Key words: biofilm; phage therapy; cystic fibrosis; chronic rhinosinusitis

Poster number: 27

A VersaTile-engineered endolysin improves early cloxacillin treatment in a preclinical mouse model for Streptococcus uberis mastitis: a proof-of-concept study

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Bovine mastitis, an infectious udder disease with substantial economic implications in dairy cows, is often caused by Streptococcus uberis. This major pathogen is known to persist in the bovine mammary gland and evade early host immune detection. Conventional antibiotics used in veterinary medicine such as the penicillin-derivative cloxacillin have limited success in eliminating S. uberis as a stand-alone therapy, resulting in chronic infections. To address this challenge, the potential of the previously in vitro characterized VersaTile engineered endolysin NC5 as a supplemental therapy to cloxacillin was investigated in a mouse model of bovine S. uberis mastitis. First, the progression of a S. uberis infection in the lactating mouse mammary gland was monitored, tracking the bacterial load as well as the influx of blood neutrophils as mastitis hallmarks. Second, healthy murine mammary glands were challenged with endolysin to evaluate acute toxicity. Thirdly, the therapeutic efficacy of endolysin combined with cloxacillin was compared to cloxacillin alone and a placebo control. This combination therapy effectively reduced bacterial load, mitigated neutrophil infiltration, and modulated pro-inflammatory mediators including the mouse IL-8 homologue MIP-2, which displayed a dose-dependent response. However, two type of responder mice were observed that were identified as slow versus fast responders. This proof-of-concept in vivo study complements our previous in vitro data and highlights the potential of engineered endolysins as an adjunct therapy to enhance the effectiveness of β-lactam penicillins in the intramammary treatment of bovine S. uberis mastitis.

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Key words: endolysin; Cloxacillin, bovine mastitis; Streptococcus uberis; mouse model

Poster number: 28

PCR for the typing of the most common filamentous bacteria in wastewater treatment plants for targeted bacteriophage use

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Bacteriophages are viruses that infect and replicate within bacteria. Their unique bactericidal properties have garnered interest in various applications from medical applications to food- or wastewater treatment.

Foaming and bulking in wastewater treatment plants are the most common yet complex sludge separation problems They occur mostly due to a large range of known filamentous bacteria. Traditionally foaming is controlled by chemical additives, such as surfactants or chemical disinfectants, such as chlorine or hydrogen peroxide. These compounds have an adverse environmental impact, are costly and may lead to operational fallout of the wastewater treatment plant due to the non – selective disinfection mechanism. In contrast to the current controlling mechanisms, bacteriophages offer a promising alternative as a natural, targeted, and eco-friendly solution resulting in the preservation of the overall microbial balance in the wastewater treatment plant.

In this study, a multiplex approach is presented in which firstly, filamentous bacteria are identified, secondly, correct bacteriophage for eradicating these bacteria are used and, thirdly, progress of phage propagation and foam/bulking reduction is monitored.

In conclusion, the utilization of bacteriophages for the prevention and control of foam formation and bulking in wastewater treatment plants with this multiplex approach presents a promising approach with ecological impact. Further research and implementation of phage-based foam control systems may lead to significant improvement in the field of wastewater treatment plants and contribute to a greener and more efficient future.

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Key words: wastewater treatment; filamentous bacteria; foam formation; phage propagation

Poster number: 29

Pyrogen testing of phage therapeutic products

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The use of phage therapeutic products has gained popularity in Belgium these last years, with treatments provided to more than 100 patients, against more than 10 different bacterial species. Therapeutic phage products are produced in a bacterial host strain, hence pyrogen contamination (e.g. cell wall debris) is a major safety concern as these pro-inflammatory agents can cause unwanted clinical reactions with potential detrimental outcome. The most common and potent pyrogens are endotoxins, components of the cell wall of gram-negative bacteria which are released upon bacterial lysis.

In our routine phage quality control (QC) workflow, endotoxins are quantified using the Limulus amoebocyte lysate (LAL) assay and the animal-friendly alternative, the recombinant factor C (rFC) test. However, other (non-endotoxin) pyrogenic substances including compounds of the gram-positive bacterial cell wall, flagellin, etc. are not detected by those tests. As phages are being produced in a growing variety of bacterial host strains, including gram-positive bacteria, an expansion of the QC workflow is pivotal to limit the risk of non-endotoxin contamination. These pyrogens can be detected by the monocyte activation test (MAT), which employs human blood cells to quantify pyrogenicity in vitro. In this work, we discuss and compare the applicability of the LAL, rFC and MAT tests for pyrogen testing of clinical grade phage products based on the analysis of various productions manufactured by the Queen Astrid Military Hospital in Brussels. With a few exceptions, the results on different phages demonstrate that in general pyrogenic contamination is low for most samples.

Key words: phage therapeutic products; quality Control; pyrogens; endotoxins

Poster number: 30