PHAGE: Therapy, Applications, and Research
Volume 2, Number 3, 2021

DOI: 10.1089/phage.2021.29019.eve

Conference Report

A Window into Evergreen 2021 from the Next Generation of Phage Researchers

Yuval Mulla, PhD,[1] Kyle Enriquez, MSc,[2] Jarin Taslem Mourosi, MSc,[3] Katharine Muscat, MSc,[4] and Michael Shamash, BSc[5]

Introduction

By Yuval Mulla

DURING THE FIRST WEEK of August 2021, the Evergreen State College held its 24th Biennial International Phage Meeting. The meaning of having a 24th biennial meeting truly hit home during the beautiful introductory speech of Betty Kutter—who has (co-)hosted every meeting since the very first in 1975 and seen the community vastly grow throughout this almost half a century. Owing to the ongoing pandemic, the 24th meeting was inevitably different from those before. With this Meeting Report, we would like to share the experiences of both in-person and online-only attendees—discussing both the science and the format of the meeting.

A key characteristic of Evergreen is an acute awareness of the need to welcome and encourage the scientific development of graduate students and postdoctoral researchers. This article has been written by myself and other students who are excited by the novel biology associated with phage and their potential to solve pressing problems associated with antimicrobial resistance. After this introduction, we present a series of ''stand out'' talks alongside a commentary of content and why they stood out to us.

Conference Format

The Evergreen phage community is well known for combining great science with a warm social embedding. In previous years, there were barbecues, wine-and-cheese tastings, nature hikes, and all attendees were hosted on campus. Owing to the pandemic, none of this was possible this year and travel restrictions reduced the number of in-person attendees from $\sim$300 down to 64. The organizers clearly had to ''reinvent the conference'' in these difficult times. The result was a hybrid online/in-person conference with a record number of participants (500+), and a digital infrastructure, currently containing >100 online talks that will continue to be a valuable resource and will hopefully set the standard for future conferences.

Although it is impossible to beat the socializing and networking capabilities of a fully in-person conference, the new hybrid format also had advantages. The online attendance enabled people from all over the world to enjoy the conference at a greatly reduced financial and environmental burden. The possibility to rewatch talks of interest allowed everyone not to worry about a ''once only'' performance. The organizers put significant effort in making sure that online attendees could interact with speakers afterward by posting questions. There were also dedicated cameras for both the speakers and the in-person audience asking questions, which helped online viewers feel part of the whole process. There were several platforms that helped to connect attendees from around the world, such as a dedicated slack channel, Zoom break-out rooms and poster sessions that allowed people to connect one-on-one.

The science covered in this conference was even more diverse than its format, and just the keynote speakers' topics ranged from phage therapy to phage-based sensors for agriculture and systems biology. The conference was genuinely multidisciplinary with contributions from synthetic biology, bioinformatics, biochemistry, molecular biology, and many more fields. Here we do not intend to give a ''complete'' summary of the conference but give some highlights based on the talks that stood out to myself and the contributing authors. We hope to present these in the spirit of Evergreen and that it will be a valuable and enjoyable summary to the readers of *PHAGE*. We start with an overview of the three keynote talks. For those wanting to dive more into the conference, it is possible to do so, the entirety of the program, including its drug development and genome annotation workshops, is still available online and it is possible to register even now, the conference is technically over already.

Summary of Keynote Talks

Presenters: Jean-Paul Pirnay, Martha Clokie and Sam Nugen
Recap author: Yuval Mulla

The first of the keynote talks was by Jean-Paul Pirnay, from Queen Astrid Military Hospital, Brussels Belgium, who sketched how phage therapy is currently being rolled out in Belgium in close collaboration with the Eliava institute in Georgia. He argued that the current regulatory framework is poorly suited for ''one-size-fits-all'' phage cocktails as

[1]Faculty of Mathematics and Natural Sciences, Institute for Biological Physics, University of Cologne, Cologne, Germany.
[2]Department of Pathology, Microbiology & Immunology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA.
[3]Department of Biology, The Catholic University of America, Washington, District of Columbia, USA.
[4]School of Life and Environmental Sciences, Faculty of Science, University of Sydney, Sydney, Australia.
[5]Department of Microbiology and Immunology, McGill University, Montreal, Canada.

obtaining approval is expensive, yet not lucrative as the product is not patentable. Furthermore, clinical trials of phage cocktails have shown disappointing efficacy, likely because even carefully assembled phage cocktails lack the broad-spectrum activity of antibiotics. He, therefore, argued for a more personalized approach where a phage or phage cocktail is specifically assembled and possibly even trained for each patient separately. Since 2015, Belgium has implemented a regulatory framework in which personalized preparations are subjected to fewer marketing authorization and production constraints. This has allowed the Queen Astrid military hospital in Belgium to set up pipeline that has helped treat >70 patients since 2017. Bacterial eradication was observed in more than a third of the patients. However, efficacy remains unclear due to a lack of control groups and statistical power. Therefore, it will be important to set up randomized control trials, for which funding is currently being requested.

The second keynote talk was by Martha Clokie from the University of Leicester, United Kingdom (and our editor in chief), who proposed a new and enticing framework to group phages. Phages are incredibly diverse, and we currently struggle in classifying that diversity in an ecologically meaningful way. In botany, diverse plants are commonly classified along three different dimensions—namely their robustness to stress, their success in nutrient-rich environments, and their ability to cope with temporary disturbances. She proposes a similar classification system wherein phages can be grouped according to the number of early, middle, and late-expression genes. She hypothesized that early expression is key for phages growing in nutrient-rich environments as it allows them to rapidly take over the host, whereas stressful environments likely require more regulatory machinery typically seen in the intermediate category. Fluctuating environments require rapid viral assembly in case of good conditions—and such genes are typically expressed late. Strikingly, different phages that have been analyzed to date according to their transcriptional takeover do seem to be well separated in this three-dimensional space. For future study, it will be interesting to see whether this framework can be used to predict which types of phages are found in specific environments and how this information relates to phage types that work most optimally in disease settings. Furthermore, it will be interesting to test whether this framework can help to streamline and optimize phage cocktail compositions.

The third and last keynote talk was by Sam Nugen, whose study is motivated by the importance of monitoring water for agricultural and drinking use. In both cases, detection of pathogens at very low concentrations is required, yet the costs of detection should be minimal. Current detection methods are based on plating and phenotypic characterization—making quality control slow and relatively expensive. His laboratory has developed phage-based methods by genetically modifying pathogen-targeting phages with luminescent, colorimetric, or electrochemical reporter enzymes. They improved the sensitivity of these assays by binding these phages to magnetic beads, allowing for pathogen-specific enrichment assays. Compared with state-of-the-art commercially available immunomagnetic separation, the phage-enrichment approach showed greater efficiency in harsh environments such as extreme pH or salinity. Recently, this whole detection assay was integrated into a single chip as part of a UNICEF challenge. For future study, it will be interesting to engineer the host range of phage-based detection.

Filamentous Bacteriophages Delay Wound Healing

Presenter: Paul Bollyky
Recap author: Kyle Enriquez

Among an excellent set of talks discussing and challenging the ways we think about, study, and manipulate phage at the Evergreen conference, the talk I found particularly salient at the meeting was given by Prof. Paul Bollyky on work surrounding filamentous phages and their impacts on chronic wound re-epithelialization. The concept was an interesting and unique application of phages. Presenting work completed in his group by a host of trainees as well as collaborators, the talk focused on the *Inovirus* Pf phage, which facilitates robust biofilm formation in *Pseudomonas* strains. For me, a biochemist who has spent most of his scientific career studying biofilms, this presentation was a reminder of the breadth and relevance of phage to clinical practice and basic scientific discovery across the spectrum of disease.

This study highlighted the role of Pf phage as a bona fide ''mutualist.'' It showed a myriad of evidence suggesting that Pf has several important roles in preventing bacterial clearance and delaying wound re-epithelialization. In this case, the use of animal models helped to establish these findings. Furthermore, the data suggest that the virulence effects of Pf phage can affect wound healing without its bacterial host. In addition, the physician-scientists on the study were able to quantify both the relatively high prevalence and the significant morbidity/mortality effects of Pf phage. They then determined that even alone, Pf phage applied in these translational systems inhibits keratinocyte adhesion, and therefore migration, by binding to collagen fibers in the milieu. This, in turn, results in decreased re-epithelialization of chronic wounds affected by Pf phage and its *Pseudomonas* host. By establishing the clinical relevance of their model, they are able to walk the fine line across the wide expanse of translational work, and truly was an excellent model for how these research programs can be developed to address other pressing issues in the study of phage.

I found it compelling through my virtual time at Evergreen and the time I have spent looking into the literature that there seems to be a yet-to-be elucidated disconnect between the basic and consistent clinical application of phage. This is seen throughout the field through emerging mechanisms of phage resistance and numerous molecular mechanisms warranting future study. Although this is encouraging to young scientists like myself, who aim to contribute to the field translationally, it presents a unique challenge to the phage community to establish translational models and develop effective clinical tools from the wealth of data collected in the laboratory.

Phages on the Mend: DNA Repair As a Novel Anti-CRISPR and Immune Evasion Mechanism

Presenter: Shweta Karambelkar
Recap author: Jarin Taslem Mourosi

Bacteriophages (phages) are the most abundant entity of the Earth and are the natural enemies of bacteria. Bacteria have developed a powerful CRISPR defense against phage

predation. There is an evolutionary arm race between bacteria and phages, where phages have also developed different anti-CRISPR systems to counterattack CRISPR and various restriction-modification systems. Phages use different anti-CRISPR mechanisms, and have evolved several anti-CRISPR proteins (Acr) to counteract the CRISPR action, and a jumbo phage ΦKZ produces a physical barrier by creating a nucleus-like structure around its replication machinery to protect its genetic material from its host (*Pseudomonas aeruginosa*) nuclease attack.

In the Evergreen 2021 meeting, Dr. Karambelkar's talk particularly appealed to me as they presented a novel anti-CRISPR mechanism that they have elucidated, which targets various CRISPR-Cas and restriction-modification systems. When they tested different CRISPR-Cas and restriction-modification systems of different phages, they observed a unique and broad resistance pattern to different CRISPR-Cas (Cas9, cas12a, Cas13, Cas3 I-F) and RM systems (type-I, II) in phage ΦKMV that is not similar to any other anti-CRISPR mechanisms (nucleus-like compartment or anti-CRISPR protein or RM system), even some non-native *Pseudomonas* CRISPR-Cas systems showed resistance to the phage ΦKMV. When ΦKMV phage was plated on solid agar assay, it had resistance to CRISPR defenses; however, in liquid culture at low multiplicity of infection, a low level of sensitivity to CRISPR was observed.

The team targeted different locus of ΦKMV genome using Cas12a, a pattern of a single-nucleotide deletion (with a high frequency) and two-nucleotide deletion or a single-nucleotide substitution (with a low frequency) was observed in protospacer Cas12a target site.

Interestingly, the mutations do not significantly affect phages similar to other rare mutations in the protospacer region or another deletion event in T4 bacteriophage that is generated during phage-encoded recombinase (UvsX) assistant repair system. There is also an association between the protospacer site and mutation; when the protospacer site was changed, mutation shifted its position to protospacer that indicates that this indel mutation has been created as an error during the repair mechanism. Even the single mutation is susceptible to further CRISPR-12a attack until a different kind of mutation accumulates in the protospacer region. Among the CRISPR systems, only Cas3-IC shows sensitivity on ΦKMV, and its repair system works efficiently. As other CRISPR systems are resistant to ΦKMV phage, there are different bacterial factors (NHEJ pathway, transposon mutagenesis) and phage factors (e.g. early gene of ΦKMV) that are thought to be involved in other repair mechanisms. In summary, the complexity and efficiency of different CRISPR systems and repair mechanisms might play a role in different immune escape strategies, and this talk built on our knowledge of this fascinating complexity of different anti-CRISPR mechanisms.

Long Read Viromics of Cattle Slurry Reveals a Diverse Community of Viruses That May Alter the Metabolism and Virulence of Their Hosts

Presenter: Ryan Cook
Recap author: Katharine Muscat

It has been 2 weeks since the Evergreen conference and I am still digesting the incredible work being conducted by the phage community. One talk that really impacted me was from PhD student, Ryan Cook, who works with Dr Andrew Millard at the University of Leicester and Mike Jones, Dov Stekel, and Jon Hobman from Nottingham University. The project involved developing a hybrid assembly approach (long- and short-read sequencing) to capture the virome of a ''slurry'' tank on a U.K. dairy cattle farm. These tanks contain a mixture of (mostly) cow feces along with other effluents such as urine, rainwater, bird feces, and contaminated milk from cows with mastitis infections. In other words, a phage biologist's jackpot! Admittedly, I am biased with my history of phage hunting through horse feces, so it was not difficult to get me excited about cattle slurry. With that said, there were many interesting findings from the study that have important implications, not only for the field of phage research but also for the agricultural industry and the ''One Health'' movement. Since slurry is spread on the land to fertilize crops, there are direct links between evolution in such slurry tanks and food consumption.

In terms of their scientific findings, the slurry contained an abundant and diverse pool of novel phages that were mostly lytic. Although the composition of phage types remained relatively constant over repeated samplings, the presence of diversity-generating retroelements suggested a strong potential for evolution of individual phages. These elements can allow for hypervariable tail fibers and thereby are potentially of importance in phage therapy and phage-mediated decontamination and biocontrol. Of concern, however, was the finding of phage-encoded auxiliary metabolic genes that could result in antimicrobial resistance or increased virulence of bacteria in the environment. The most notable example of this was virulence-associated protein E that is a virulence factor for mastitis-causing Streptococcus and other veterinary pathogens. Given the widespread use of this slurry as fertilizer for crops, and the existence of human gut-associated crAssphages in the cattle slurry, this highlights a significant connection between animal health and human health.

Microbiome and Phage Taxonomy: An Unnecessary Hindrance or the Ultimate Unifier?

Presenter: Evelien Adriaenssens
Recap author: Michael Shamash

This was my first time attending Evergreen and despite joining only virtually due to ongoing public health restrictions, I was still able to enjoy the diverse array of fascinating talks and posters. The topics presented ranged from artificial intelligence to microbiomes to phage therapy and more. Despite being a hybrid in-person and virtual event, the incredible coordination that was done behind the scenes made it such that the experience was enjoyable and engaging for all. Beyond the interesting talks, I especially enjoyed the ''Phage Phun'' breakout room networking sessions and am glad to hear they will be continuing outside of the conference as well! I look forward to attending many more Evergreen meetings to come!

The first talk of Wednesday morning's session was from Dr. Evelien Adriaenssens, group leader at the Quadram Institute in the United Kingdom and chair of the Bacterial Viruses Subcommittee at the International Committee on

Taxonomy of Viruses (ICTV), who presented the changes that are upcoming for phage taxonomy. These changes to the way scientists carry out phage taxonomy, although drastic, are much needed in a world where unannotated phage nucleotide records are doubling at an almost yearly pace, mostly due to the rapid accumulation of metagenomic data. Gone are the days of classifying phage families based on morphology alone, instead a genome-based taxonomy with clear criteria for demarcation of all taxonomic ranks will allow for a standardized approach when classifying new viral sequences. Applying this gene-sharing taxonomy approach to viral sequences from three human gut metagenomes revealed the highly complex and individualized nature of the gut virome, which was not immediately apparent when comparing samples using the current Caudovirales families alone. These changes are pending an ICTV ratification vote and are expected to be adopted in early 2022.

The Effect of Bacteriophage Treatment on the Healthy Gut Microbiota

Presenter: Teagan Brown
Recap author: Michael Shamash

In the same session, Dr. Teagan Brown, a postdoctoral researcher in the laboratory of Dr. Adriaenssens at the Quadram Institute, presented her work studying the effects of commercially available phage cocktails on the healthy human gut microbiome. Feces from three healthy human donors was inoculated into anaerobic batch fermenters before the addition of Intesti Bacteriophage, a commercially available phage cocktail from the Eliava Institute, indicated for dysentery, Salmonellosis, and infective colitis. Although the bacterial and viral communities did not change much from their initial composition in two of the three donor-seeded fermenters, the third fermenter had a remarkable phage bloom that recovered within 24 h. This was especially interesting as one may not normally expect these cocktails to influence healthy microbiomes, pointing to additional unknown interindividual ecological factors that are at play.

Conclusion

By Yuval Mulla

In summary we have presented a flavor of the conference content and shown some of the diverse topics associated with phages. It is, therefore, great to have such a multidisciplinary audience at the Evergreen International Phage Meeting and we hope there will be many more editions. It goes without saying that we hope the in-person attendance will no longer be limited by pandemic-induced travel restrictions. However, the hybrid format has shown its merits and we believe that, for example, the recording of talks is a practice that should outlive this pandemic. We would like to thank all the organizers in setting up such a great conference. In her concluding words, Betty Kutter promised at least another 15 more years of Evergreen phage meetings, and we would love to see it happen!

How to Access the Evergreen Video and Abstract Library

Want to register for Evergreen and access $\sim$100 video recordings, including those already described, as well as the full abstract book? It is not too late to register at (https://evergreen.phage.directory).

Authors' Bios

Yuval Mulla is a postdoctoral fellow at the University of Cologne interested in bacteria–phage coevolution, phage-mediated horizontal gene transfer, high-throughput techniques, and antibiotic resistance evolution (https://phage.directory/people#yuval-mulla).

Kyle Enriquez is a Vanderbilt MSTP-in-training (infectious diseases), Academic Foci in Microbiology, Phage, and Microbe-Host Interaction, Stanford Class of 2020 (MSc, BS) (https://stage.phage.directory/people#kyle-t-enriquez).

Jarin Taslem Mourosi is a third year PhD scholar at Catholic University of America and currently working on T4 bacteriophage-based dengue vaccine (https://phage.directory/people#jarin-taslem-mourosi).

Katharine Muscat is a late-stage PhD candidate (University of Sydney) working with bacteriophages of *Rhodococcus equi*. Her project focuses mainly on characterization of these phages for potential future use in biocontrol on farms and exploring genetic features that could be contributing to *R. equi* susceptibility/resistance to phage infection (https://phage.directory/people#katharine-muscat).

Michael Shamash is a master's student in the Maurice Lab at McGill University. His research focuses on studying the dynamics of bacteriophage–bacteria interactions in infant gut microbiota in the contexts of health and malnutrition. To do so, he uses single-cell techniques combined with viral and bacterial metagenomics, as well as animal models of early life development. He also developed and maintains OnePetri, an open-source mobile application that accelerates plaque counting and common assays using computer vision and artificial intelligence (https://shamash.me; https://phage.directory/people#michael-shamash).

Address correspondence to:
Yuval Mulla, PhD
Faculty of Mathematics and Natural Sciences
Institute for Biological Physics
University of Cologne
Zülpicher Str. 47a
Cologne D-50674
Germany

Email: ymulla@uni-koeln.de