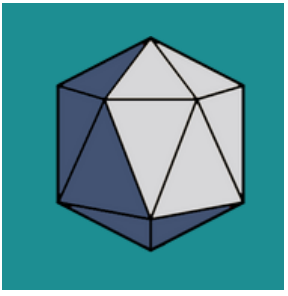
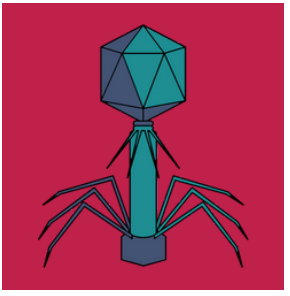
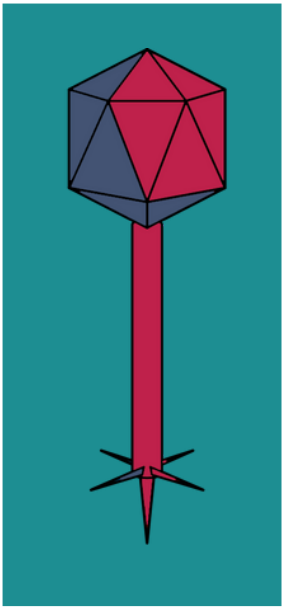
PHAGE CANADA
2022

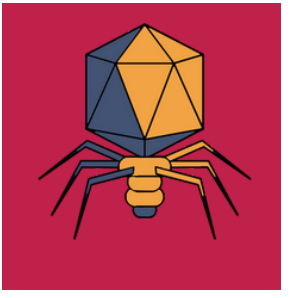






Symposiums Virtuels 2022
Virtual Symposia

August 17, 2022, 12:00 - 16:15 EDT
August 18, 2022, 11:30 - 15:30 EDT
August 19, 2022, 12:00 - 14:30 EDT



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English

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PHAGE CANADA 2022
Welcome

Message from Dr. Alexander Hynes

Welcome to the second - I think I can now safely say "of many" - Phage Canada Symposia. As in 2020, I can take no credit for the programme. The unique format, themed days, and broad outreach are the result of an amazing, engaged body of trainee volunteers working as the organizing committee under my vague insistence we engage the diversity of phage researchers in Canada.

An underlying theme of Viruses of Microbes this year was the emergence of many new societies/networks for phage researchers across the world. Some extend beyond phages to other viruses of microbes, others centre on phage therapy, some span multiple countries, some focus on academic research. These organizations have estimated memberships ranging from 30 to at most 150-200 people. As I write this, we have 253 registered attendees, with affiliations spanning 35 Canadian Institutions.

I know that this is possible because of a conscious choice to make this affordable and accessible. To reach out to companies, to government regulators and researchers, to academics. And this year, in particular, to undergraduates - who submitted a third of the abstracts we received. But, above all, this is possible because you - all 253 of you, have made time for and recognize the value of an organization like Phage Canada.

I hope you will remain engaged after the meeting as I reach out for assistance shaping and defining the organization. For me, this is the year Phage Canada coalesces into something that will, I hope, outlast me, and serve as a home for every phage aphicionado [sorry] in the country.

Alexander Hynes

Message de Dr. Alexander Hynes

Bienvenue au deuxième – et je pense que je peux maintenant dire « de plusieurs » - symposium de Phage Canada. Comme en 2020, je ne peux pas m'attribuer aucun crédit pour le programme. Le format unique, les journées thématiques et la vaste diffusion sont le résultat d'un groupe incroyable et engagés d'étudiants bénévoles en tant que comité organisateur sous ma vague insistance pour que nous mobilisons la diversité des chercheurs sur les phages au Canada.

Un thème sous-jacent de Viruses of Microbes cette année était l'émergence de nombreuses nouvelles sociétés/réseaux pour les chercheurs sur les phages à travers le monde. Certains s'étendent au-delà des phages à d'autres virus de microbes, d'autres se concernent sur la phagothérapie, certains couvrent plusieurs pays, certains se concentrent sur la recherche universitaire. Ces organisations ont des adhésions estimées de 30 à au plus 150-200 personnes. À ce moment, nous avons 253 participants inscrits, affiliés à 35 institutions canadiennes.

Je sais que cela est possible d'un choix conscient de rendre cela abordable et accessible. Pour atteindre les entreprises, les régulateurs gouvernementaux et les chercheurs, les universitaires. Et cette année, en particulier, aux étudiants de premier cycle - qui ont soumis un tiers des résumés que nous avons reçus. Mais, surtout, cela est possible parce que vous - tous les 253 d'entre vous, avez consacré du temps et reconnu la valeur d'une organisation comme Phage Canada.

J'espère que vous resterez engagé après ce symposium alors que je demande de l'aide pour définir cette organisation. Pour moi, c'est l'année où Phage Canada devient quelque chose qui, je l'espère, me survivra et servira à tous les *aphicionados* de phages [désolé] du pays.

Alexander Hynes

PHAGE CANADA 2022
Policies/netiquette/recording

English

Each symposium will use the Webinar feature of Fourwaves. As an attendee, your video and microphone are disabled, but you can interact with the symposium through one of two features:

(1) The **Q&A tool** allows you to submit written questions for the presenters.

(2) The **chat** window allows you to interact with fellow audience members.

Attendees are not permitted to record talks, although you may take a picture/screenshot (with the intent to showcase the speaker, not their data).

The talks will be recorded and made available to the audience upon the presenter's permission.

Français

Chaque symposium utilisera la fonctionnalité Webinaire offerte sur la plateforme Fourwaves. Comme participant, votre micro et votre vidéo seront désactivés, mais vous pourrez interagir pendant le symposium grâce à deux fonctionnalités:

(1) **L'outil Q&A** vous permettra de soumettre des questions écrites aux présentateurs.

(2) **Le chat** vous permettra d'interagir avec les autres participants.

Il est interdit d'enregistrer les présentations. Vous pourrez cependant prendre des saisies d'écran dans l'intention de capturer le présentateur, et pas ses données.

Les présentations seront enregistrées et diffusées avec l'autorisation du présentateur.

Phage Phamily comes together!

Show us your phage art whether it's digital, wearable, edible, growable or anything you can (safely) dream up! Tag us on Twitter @phagecanada or use the #phagecanada to showcase your work! You could win a prize! Check out the amazing submissions from 2020 below.

La phamille des phages se réunit !

Montrez-nous votre art inspiré des phages ! Identifiez-nous sur Twitter @phagecanada et/ou utilisez le mot-clic #phagecanada pour présenter vos créations ! Vous pourriez gagner un prix! Découvrez les soumissions incroyables de 2020 ci-dessous.

Cayla Burk, University of Toronto

Mateus, University of Calgary

DAY 1

Undergraduate Research & Education

12:00 - 12:10 Welcome from the Organizing Committee

12:15 - 13:30 Undergraduate Phage Education Across Canada
1. Dr. Adam Rudner (University of Ottawa)
2. Dr. Helene Deveau (Université Laval)
3. Dr. Corinne Maurice (McGill University)

13:30 - 14:00 Undergraduate Phage Education - Panel Discussion

14:00 - 14:15 Break

14:15 - 14:27 Isolation and host range determination of lytic bacteriophage against *E. coli*, *Shigella* spp., and *B. cereus* for biocontrol applications (W.Dharmasiddhi, Agriculture and Agri-Foods Canada)

14:27 - 14:39 Prophages may be sensitizing *P. aeruginosa* strains to ciprofloxacin (M.S. Othman, McMaster University)

14:39 - 14:51 Characterizing the functions of Tequintavirus AKFV33's lateral tail fibers and receptor binding proteins during phage adsorption process to Shiga toxin-producing *Escherichia coli* O157 (K. Krura, University of Calgary)

14:51 - 15:03 Translational frameshifting in tail assembly chaperones in Actinobacteriophages (E. Galimova, University of Ottawa)

15:03 - 15:15 The Effects of viral infection of cyanobacteria on toxin production and release (V. Lee, University of Waterloo)

15:15 - 16:15 Poster Session

Isolation and host range determination of lytic bacteriophage against *E. coli*, *Shigella* spp., and *B. cereus* for biocontrol applications

Wiweka Dharmasiddhi, Hany Anany, Janet Lin, Shirlie Chan
Agriculture and Agri-Food Canada

Traditional biocontrol methods such as chemical and heat treatment can have negative effects on the natural food microflora and nutritional value. With an increasing demand for minimally-processed and safe food products, lytic bacteriophages offer promising alternatives that address this need. Hence, the objective of this work was to isolate bacteriophages against four critically important foodborne pathogens; antimicrobial resistant (AMR) E. coli, shiga toxin-producing E. coli (STEC), Shigella spp. and B. cereus.
Primary sewage sludge and soil samples were collected and enriched using a cocktail of strains of each bacterial target, selected based on their unique toxin and AMR profile, or/and clinical prevalence. After three purification rounds using the double agar overlay method, phages with clear plaque morphology were selected. All phage isolates were subjected to a high-throughput turbidimetric assay to determine re-isolates and the host range patterns.
Thirty-nine E. coli, seventeen Shigella spp., and nine B. cereus phages were isolated. One E. coli phage, J12, was found to completely inhibit the growth of E. coli O157, the leading cause of STEC infections in Canada, for twenty-four hours. Shigella phage HS02 was shown to delay growth of three of the four dominant Shigella serovars in Canada by at least ten hours. Finally, B. cereus phage CR7 was able to delay growth of all B. cereus strains used in this study. The inhibitory effects of these lytic phages on a wide variety of pathogens demonstrates their potential as biocontrol agents. Further characterization through TEM imaging and genome sequencing will be conducted.

Prophages may be sensitizing *P. aeruginosa* strains to ciprofloxacin

Maryam S Othman, Alexander Hynes
McMaster University

The Comprehensive Antibiotic Resistance Database (CARD) can predict whether a given bacterial strain is resistant to antibiotics using sequence data. Intriguingly, CARD occasionally over-predicts resistance to certain drug classes like ciprofloxacin, a DNA-damaging antibiotic. For example, of 102 Pseudomonas aeruginosa clinical isolates tested for ciprofloxacin sensitivity, 71 were over-predicted to be resistant. We hypothesize that bacteriophages (phages) are behind this disparity. Ciprofloxacin can induce prophages, resulting in host cell lysis — which can explain the strain's increased sensitivity. To test this hypothesis, we sought to identify a clinical isolate over-predicted to be ciprofloxacin-resistant and predicted to contain the fewest phages possible. C0098 is one such strain as it satisfies the first criteria and has a 30-kb prophage region annotated as a tailocin. Through spot test assays, we learned that C0098 results in plaque formation across four different clinical isolate hosts, all of which have over-predicted resistance, and that induction with ciprofloxacin increases titre by 100-fold. Currently, we are working on curing this strain of its prophage using spontaneous phage loss assays and subjecting it to varying concentrations of ciprofloxacin. If, as we expect, removal of the prophage increases the strain's resistance to ciprofloxacin, then this could highlight a generalizable phenomenon that allows CARD to correct over-resistance predictions. In the future, this research can be extended to other bacterial strains and antibiotic classes to further improve CARD's predictive accuracy.

Characterizing the functions of Tequintavirus AKFV33's lateral tail fibers and receptor binding proteins during phage adsorption process to Shiga toxin-producing *Escherichia coli* O157

Kirti Krura, Annie Nghi Nguyen, Jieting Lin, Dongyan Niu
University of Calgary

Shiga toxin-producing Escherichia coli (STEC) is the cause of many of North America's foodborne infections for humans and feedlot animals. Resistance to antimicrobials is an arising problem, and phage therapy is a potential alternative. Lytic phages can also be used to kill specific bacterial pathogens without affecting commensal bacteria. AKFV33 is a Tequintavirus highly lytic and specific to common O157:H7 strains of Shiga toxin-producing Escherichia coli (STEC). For phage adsorption, AKFV33's lateral tail fibers (Ltfs) reversibly bind to the O antigen of the host lipopolysaccharide. The phage's receptor binding protein (RBP) then irreversibly binds to the host's outer membrane proteins. The detailed mechanism of how AKFV33 executes this has not been extensively studied. For example, does co-recognition between RBP and the Ltfs occur? This project aims to verify the functions of AKFV33 Ltfs (Ltfa, LtfB, and orf136) and RBP in phage adsorption. The target genes were expressed in two plasmids (pETDUET and pBAD) and transformed into DH5α E.coli cells. Through colony PCR, sequencing, and plasmid extraction, the positive transformants' plasmid DNA were isolated and expressed in BL21 E.coli. The target proteins will be purified, and their function will be evaluated by phage adsorption inhibition assay. This study is essential since this interaction can be used to kill STEC in food sources, infected people, and animals. This study can pave the way for future studies on the functions of Ltfs and RBP of other phages.

Translational frameshifting in tail assembly chaperones in Actinobacteriophages

Enzhe Galimova, Adam Rudner, Laura Giles, Elizabeth Williams
University of Ottawa

Translational frameshifting is a mechanism that allows cells to produce two versions of a specific protein, a N-terminal short form, and a longer form created by the ribosome slipping either forward or backwards during the mRNA translation process, causing a frameshift that extends the protein sequence. Frameshifting in bacteriophage tail assembly chaperones (TAC) has been previously studied, however, there is no experimental evidence of translational frameshifting of the TAC proteins in EA cluster bacteriophages. In this study, we use a polyclonal antibody that recognizes the long form of the TAC to demonstrate that Winzigespinne (an EA1 phage) and Quartz (an EA10 phage) produce the frameshifted TAC in vivo during an infection of their MIcrobacterium foliorum host. To determine the precise slippery sequence we performed mass spectrometry on frameshifted protein purified from E. coli and have identified a novel consensus slippery sequence, GGGXGA, which leads to a +1 frameshift. Our work will lead to changes in the annotation of many EA cluster bacteriophages. We have also explored TAC frameshifting in the phages Kharcho and Ottawa, which form a novel cluster of phages with limited homology to other phages that infect Arthrobacter globiformis. The Kharcho and Ottawa TAC contains the widely used slippery sequence, GGGAAAA, but does not frameshift when expressed in E. coli. Translational frameshifting also occurs in several animal viruses, including HIV and Influenza, and this frameshifting also regulates the ratios of viral proteins. Our work is broadly applicable to understanding and exploiting the fundamental mechanisms that trigger ribosomal slippage.

The effects of viral Infection of cyanobacteria on toxin production and release

Victoria Lee, Jozef Nissimov
University of Waterloo

The dynamics between cyanophages and harmful algal blooms (HABs) formed by cyanobacteria in freshwater are unclear. Specifically, the effects of viral infection on cyanobacterial toxin production and release needs to be investigated, as some in situ studies have previously suggested that virus infection may worsen the effects of HABs. We hypothesized that virus-induced cyanobacterial cell lysis will result in a larger amount of microcystin toxins being released from cells. Using a commercially available ELISA kit for the detection and quantification of microcystin, we showed that infection of Microcystis aeruginosa (M. aeruginosa) strain NIES 298 by the cyanophage Ma-LMM01, results in as high as 40-fold higher levels of microcystin 2 days post infection (dpi), compared to the non-infected treatments. We also showed that microcystin levels remained high 7 dpi, despite the infected cultures losing pigmentation and appearing translucent. Considering a calculated daily microcystin decrease of 53 ppb, this means that it would take up to 14 dpi for the levels of microcystin to reach control levels. This has enormous implications to natural systems where often bodies of water may be considered safe after a detectible decrease in cyanobacterial biomass and/or decrease in chlorophyll concentrations. We also show through sonication experiments (mimicking viral lysis), that M. aeruginosa strains that do not normally produce detectable levels of microcystin, have the potential to pose significant health risks to an environment if they were to be lysed and the internal reservoir of toxins was to be released.

Undergraduate Poster Session

In vitro evaluation of lytic bacteriophages against Shiga toxin producing *Escherichia coli* O157:H7 on human intestinal cells
Akeel Faizal | *University of Calgary*

Towards bacteriophage therapy: Identification and characterization of bacteriophages isolated from sewage water samples effective against two clinical strains of multidrug-resistant *Escherichia coli*
Aman Galymov | *Thompson Rivers University*

Optimisation of electrotransformation efficiency of *E. coli* EDL933 in preparation for transposon-directed insertion site sequencing (TraDIS)
Andrej Momiroski | *University of Calgary*

Phage lysates as bacterial chemo-attractants: investigating the relative contributions of spent media to lysate-induced chemoattraction
Ashlyn Chou | *McMaster University*

Isolating broad-host range bacteriophages for *Pseudomonas aeruginosa* strains
Gabrielle Jean-Pierre | *McMaster University*

Isolation and characterization of five bacteriophages Infecting *Streptomyces avermitilis*
Katia Koziel Ly | *University of Ottawa*

Cooperation between prophages in *Escherichia coli*
Lauren Stadel | *University of Alberta*

Isolation and characterization of Actinobacteriophage defense escape mutants (DEMs)
Leen Madani | *University of Ottawa*

Identifying novel I-E anti-CRISPRs using guilt-by-association and proximity to known anti-CRISPRs
Rotimi Williams | *University of Toronto*

Antiviral activities of *Microcystis aeruginosa* against viruses of microalgae and cyanobacteria
Sally Zheng | *University of Waterloo*

Using tangential flow filtration to investigate phageome in the food production chain
Sarayna Bala | *Agriculture or Agri-Food Canada*

Characterizing a novel prophage-encoded phage defense gene
Matthew McCarthy | *University of Toronto*

DAY 2
Graduate & Post-graduate Research

11:30 - 12:00 **KEYNOTE** Dr. Andrew Kropinski History of Canadian Phage Research

12:00 - 12:15 Break

12:15 - 12:30 A novel phage defense system inhibits phage assembly without friendly fire (P. Pateli, University of Toronto)

12:30 - 12:45 Investigating mutation patterns that arise from spontaneous resistance to type IV pilus-targeting bacteriophages (V. Tran, McMaster University)

12:45 - 13:00 Commensal *Clostridium* species host inoviruses that are secreted in response to bile acids and increased osmolality (J. Burckhardt, University of British Columbia)

13:00 - 13:15 Whole genome characterization and tail proteins function verification of Shiga toxin producing *Escherichia coli* phages (A. N. Nguyen, University of Calgary)

13:15 - 14:30 Poster Session

14:30 - 14:45 Optimization of a phage cocktail against *Salmonella enterica* from Kenyan chicken farms and their BIMs (K.M.D. Gunathilake, Université Laval)

14:45 - 15:00 Synergistic interactions among Bcc phages reveal a novel therapeutic role for lysogenization-capable (LC) phages (P. Lauman, University of Alberta)

15:00 - 15:15 Control of *Salmonella* in pigs at the primary production level using spray dried pH-responsive encapsulated bacteriophage cocktail (G. Islam, University of Guelph)

15:15 - 15:30 Understanding phage-phage interactions through population dynamics for the design of a more efficacious phage-based biopesticide (S. Gayder, Agriculture and Agri-Food Canada)

Dr. Andrew Kropinski, Adjunct Professor
University of Guelph

In 1972, Dr. Kropinski started on an academic track in the Department of Microbiology and Immunology (currently Department of Biomedical and Molecular Sciences) at Queen's University (Kingston, Ontario) - a career which lasted 32 years. During this time, his work examined lipopolysaccharide biosynthesis in *Pseudomonas aeruginosa*, lysogenic conversion, development of shuttle vector systems, and cytochemistry of the sheath of the saprophytic spirochete, *Spirochaeta aurantia*. Sabbatical leave in 2004 resulted in a career change to a Government of Canada research scientist with the Public Health Agency of Canada's Laboratory for Foodborne Zoonoses (Guelph, Ontario) where his research centred on the development of a molecular typing system for *Salmonella* leading to the genome sequencing of several hundred *Salmonella* strains. In 2014, Dr. Kropinski "retired" for the second time, and took up two adjunct positions at the University of Guelph. As a consulting virologist, he now works on analyzing the genomes of bacteria and phage for students, their supervisors, and NCBI.

In his spare time, he is bringing order to the burgeoning chaos of bacteriophage classification through his role in the Bacterial Viruses Subcommittee of the International Committee on Taxonomy of Viruses. He tells us that the only constants in his life are a love for problem-solving, bacteriophages, and his partner in the adventure of life, Peggy Pritchard, the author/editor of "Success Strategies from Women in STEM: A Portable Mentor."

A novel phage defense system inhibits phage assembly without friendly fire

Pramalkumar Patel, Karen Maxwell
University of Toronto

Bacteria and their viruses are locked in a perpetual arms race that has given rise to an impressive defensive and offensive arsenal. Temperate phages integrate their genome into the host chromosome as a latent prophage and exhibit a symbiotic relationship with their host by expressing genes that increase bacterial fitness. Since phage predation is a major threat to bacterial survival, prophages have evolved proteins that protect host cells from further phage infections. We showed that phages infecting Pseudomonas aeruginosa frequently express genes that protect their host from phage attack. Since prophages are common, understanding these defense systems and how they protect their host will provide insight into the phage-host evolutionary arms race. Here, I characterize a novel anti-phage system encoded by the JBD26 phage, Gp31, that inhibits a step-in phage assembly. To our knowledge, this is the first known anti-phage system mediated via inhibition of phage assembly.
Since Gp31 provides resistance to phages closely related to JBD26, a question arises: how does JBD26 escape Gp31 activity? Strikingly, we noticed that gp31 is always found in combination with another gene, gp30, suggesting a functional link. We found that Gp30 is both necessary and sufficient to evade Gp31. We propose that when JBD26 itself wishes to enter the lytic cycle, Gp30 is expressed along with the virion assembly proteins, binds to Gp31, and shuts down its inhibitory activity. Our work underscores that prophages encode an anti-phage defense to inhibit phage infection but also encode a counter-defense to prevent autoimmunity.

Investigating mutation patterns that arise from spontaneous resistance to type IV pilus-targeting bacteriophages

Veronica Tran, Lori Burrows
McMaster University

Antibiotic resistance has driven the pursuit of novel therapeutics such as bacteriophages (phages). While phages have promise as therapeutics, the issue of phage resistance is of concern as evolutionary pressures exerted by phages can drive spontaneous development of resistance in the host during infection, rendering the treatment ineffective. However, phage resistance can also be used to our advantage as mutations that arise due to resistance may result in changes in bacterial traits such as decreased virulence and increased antibiotic sensitivity. These traits are favoured because they reduce pathogenicity and may be selected for through the types of phages to which bacteria are exposed.
In Pseudomonas aeruginosa, type IV pili (T4P) act as phage receptors and virulence factors. We hypothesize that P. aeruginosa strains resistant to T4P-targeting bacteriophages will have mutations in genes involved in T4P regulation and assembly. Here, we show that P. aeruginosa mutants resistant to PO4 have lost T4P-dependent twitching motility, suggesting impaired pilus function. Using whole genome sequencing, followed by comparison of the parent strain to phage resistant isolates, we identified mutations in genes required for pilus assembly and function in many of the phage resistant strains. Since T4P are an important virulence factor, modifications to T4P, that arose and conferred phage protection, can have implications on the infectivity of P. aeruginosa following phage exposure. These findings provide fundamental information for the implementation of phage therapy. Understanding resistance mutation patterns will guide decisions in phage selection in phage therapy in clinical settings.

Commensal Clostridium species host inoviruses that are secreted in response to bile acids and increased osmolality

Juan Burckhardt, Derrick Chong, Katharine Ng, Carolina Tropini
University of Toronto

Inoviruses are unique bacteriophages with filamentous morphologies and distinct lysogenic life cycles. Instead of lysing their host cell to release progeny, inoviruses establish non-lethal chronic infections in their host, where they constantly assemble and secrete virions. Inoviruses have been broadly studied in gram-negative human pathogens. However, no inoviruses have been characterized in resident commensals from the gut. In this study, we utilized in silico, in vitro and in vivo methods to characterize gut microbiota inoviruses. By screening a representative genome library of gut commensal bacteria, we detected inovirus prophages in Clostridium spp. and, using imaging and molecular methods, we confirmed the secretion of inovirus particles in vitro. To assess how the gut abiotic environment, bacterial physiology and inovirus secretion may relate to one another, we deployed a tripartite in vitro assay that progressively evaluated the growth dynamics of the bacteria, biofilm formation and inovirus secretion in the presence of bile salts and changing osmotic environments. Counter to other inovirus-producing bacteria, inovirus production was not correlated with biofilm formation in Clostridium spp. Instead, the Clostridium strains showed heterogeneous responses to changing abiotic factors. With a mouse model of osmotic diarrhea, we confirmed inovirus secretion from Clostridium strains in vivo. Consistent to our in vitro observations, inovirus secretion was regulated by the osmotic environment of the gut. Gut commensals harbouring inoviruses reveals a new layer of complexity to the gut microbiome. These phages can fill unknown niches and affect our current perceptions of bacteria-host relationships, evolution, and immunity in the gut.

Whole genome characterization and tail proteins function verification of Shiga toxin producing *Escherichia coli* phages

Annie Nghi Nguyen, Kirti Krura, Jieting Lin, Matthew Walker, Kim Stanford, Tim A. McAllister, Alexei Savchenko, Jeroen De Buck, Yan D. Niu
University of Calgary

Shiga toxin-producing Escherichia coli (STEC) continually challenges the food safety system worldwide. Bacteriophages show great potential as the biocontrol agent of STEC in food supply chain. This study characterized six STEC lytic phages isolated from cattle, Alberta and elucidate the function of tail proteins in host range determination. Phages in the study had a relatively broad host range. They were capable of lysing more than 1 strains from the top 6 STEC serogroups commonly associated with human infection. Additionally, phage adsorption rate of 4 phages was fast ranging from 5.2x10^-10 to 2.2x10^-9 ml/min. Based on genomic data, they belonged to Tequintavurus genus, Siphoviridae family. They have a 110 kb dsDNA, encoding 155 – 166 genes and 22-24 tRNAs. Their genomes were highly identical (92.5% identity) to 0157 Tequintavirus AKFV33, but their tail fibers (TFP) and receptor binding proteins (RBP), which were known to be responsible for host range, differed. For example, TFP and RBP of AXO45B were only 19.9% and 30.3 % identical to their counterparts of AKFV33. To verify their function regarding host adsorption, their TFPs and RBP were expressed, purified, and added in adsorption assay to assess changes of adsorption rate as a result. The findings provide valuable insight into genomic and physiological features of Tequintavirus that may be used to prevent STEC from entering food supply chain. This study can also pave the way for molecular mechanism of host specificity of phages.

Optimization of a phage cocktail against *Salmonella enterica* from Kenyan chicken farms and their BIMs

K.M.Damitha Gunathilake, Stephanie Loignon, Angela Makumi, Josiah Odaba, Linda Guantai, Denise Tremblay, Nicholas Svitek, Sylvain Moineau

Université Laval

Here we present the in-vitro optimization of a phage cocktail against Salmonella enterica strains isolated from Kenyan chicken farms. Specifically, we tested different phage cocktails against six S. enterica strains belong to the serovars Enteritidis, Kentucky, Heidelberg and Crossness, which were isolated from chicken faeces and water samples obtained from chicken farms in Nairobi. The cocktails consisted of different combinations of three phages isolated from Kenyan chicken farms: K47 (Siphophage), K30 (Podophage), and K37 (Siphophage) and three phages stored at the Félix d'Hérelle Reference Center for Bacterial Viruses: S16 (Myophage/NC_020416), SE7 (Siphophage/MK972708), and SB3 (Siphophage/MK578530).

The cocktails were tested at 37oC and at 42oC which are the optimum temperature for Salmonella growth and the chicken body temperature, respectively. On day 1, we measured the OD600 of the phage-infected bacterial cultures during an overnight incubation. On day 2, the communities of BIMs obtained after day 1 were reinfected with a booster dose of the same phage cocktail. We observed that at 37oC, resistant BIMs were produced and the application of a booster dose was not effective. However, at 42oC the phage cocktails controlled the Salmonella strains and their BIMs better. A five-phage cocktail with K30, K37, K47, S16, and SB3 was found to be the most effective and at an initial ratio of 10:10:100:1:1. With this combination, the OD600 values kept hovering around 0.2 or below and the strains/BIMs were not observed entering an exponential growth phase for the tested incubation period of 14.5h. Animal studies will be performed shortly.

Synergistic interactions among Bcc phages reveal a novel therapeutic role for lysogenization-capable (LC) phages

Philip Lauman, Jonathan Dennis

University of Alberta

The increasing prevalence of multidrug resistant bacteria is an imminent danger to public health and threatens virtually all aspects of modern medicine. Particularly concerning are opportunistic, hospital-transmitted pathogens such as the members of the Burkholderia cepacia complex (Bcc), which cause life-threatening infections in immunocompromised patients and are notoriously resistant to antibiotics.

One potential solution to this crisis is phage therapy, the use of phages to treat bacterial infections. Phages are dichotomously categorized as either lytic or lysogenic, with the latter category considered therapeutically suboptimal since these phages, if they form stable lysogens, can potentially transfer antimicrobial resistance or virulence factors to their lysogens and do not always kill the cells they target. Recognizing that the tendency to form lysogens is not predicated solely on the ability to do so, we propose the term lysogenization-capable (LC) to describe phages which have the genetic capacity to form lysogens and occupy points on a spectrum in terms of their tendency to form stable lysogens, and we introduce lysogenization frequency (f(lys)) as a novel metric with which to quantify this variable.

We found that among Bcc phages, f(lys) varies substantially with environmental conditions such as host, temperature, and infection medium, and is inversely correlated with the Growth Reduction Coefficient (GRC), a novel metric used to quantify the antibacterial effects of phages. Moreover, many high-f(lys) Bcc phages interact synergistically with low-f(lys) phages to produce powerful antimicrobial effects. Taken together, these findings challenge our understanding of lysogenization, and reveal a novel therapeutic role for LC phages.

Control of Salmonella in pigs at the primary production level using spray dried pH-responsive encapsulated bacteriophage cocktail

Golam Islam, Keith Warriner, Qi Wang, Robert Friendship, Parviz Sabou
University of Guelph

Pigs are the second largest reservoir of non-typhoidal Salmonella and act as the major source of infections in humans. Use of antimicrobials in swine farming is linked to the emergence of antibiotic-resistant non-typhoidal Salmonella serovars. Mitigating Salmonella prevalence at the farm level can reduce Salmonella transmission to humans. Microencapsulated lytic bacteriophages have the ability to control Salmonella in pigs and potentially be used as antibiotic alternatives. We have developed a pH-responsive microencapsulated anti-Salmonella phage cocktail powder and tested it in newly weaned pigs challenged with Salmonella. Newly weaned pigs (n=18) were randomly selected and designated as Negative, Salmonella and Phage groups. Pigs in Phage groups received microencapsulated phage orally for 12 days while Negative group received placebo. Fecal samples were collected on 5th, 7th, 8th and 10th days of the experiment. On days 6 and 7 pigs in Salmonella group and Phage group were challenged with 2 mL of 5.5x106 CFU/mL Salmonella Typhimurium DT104NalR. All pigs were euthanized on the 12th day and intestinal contents were collected for analysis. Salmonella shedding was significantly reduced in Phage group (3.37 log cfu/g; ($P<0.05$)) on 10th day compared to Salmonella group (5.57 log cfu/g). Salmonella count (2.22 log cfu/g) in the jejunum and in the colon (3.75 log cfu/g) of the Phage group was significantly ($p<0.05$) lower than that in the Salmonella group (4.08 log cfu/g and 4.11 log cfu/g respectively). Spray-dried microencapsulated bacteriophage cocktail can be used as an economical antibiotic alternative to reduce Salmonella shedding and carriage in weaned pigs.

Understanding phage-phage interactions through population dynamics for the design of a more efficacious phage-based biopesticide

Lars Fieseler, **Steven Gayder**, Alan Castle, Antonet Svircev
Agriculture and Agri-Food Canada

Fire blight is a nectrotic disease of apples, pears, and other Roasceous plants caused by the bacterium Erwinia amylovora. Control of the pathogen has mainly involved the use of antibiotics, but as more countries ban the use of antibiotics in agriculture and antibiotic resistance continues to spread, there is a growing interest in the use of bacteriophages as biological control agents for the control of fire blight.

During both my PhD with Agriculture and Agri-Food Canada and my current postdoc position at the Zurich University of Applied Sciences in Switzerland, I have worked on the development of two different bacteriopage-based biopesticide products for the control of E. amylovora. Designing an effective phage-based therapeutic for the control of any bacterial pathogen requires an understanding of the interactions and dynamics between the phages of interest and their target host. In vitro study of this usually involves the measuring of bacterial growth control by plating or with optical density measurements, but one critical variable that these assays can't measure is the interactions of the phages themselves.

Using a quantitative real-time PCR approach, the populations of each indiviual phage in the cocktail as well as the pathogen can be measured over time. When the different combinations of the phages are tested in a modular fashion, the interactions and effects the phages exhibit on each other can be measured, which can be used to choose phages or varied phage concentrations to maximize synergistic potential and minimize antagonistic interactions between the phages.

Poster Session

The phage resistance mechanism of abortive infection k in *Lactococcus lactis*
Amy Du | University of Calgary

Adapt or die: investigating coevolution between the I-F CRISPR-Cas system and an anti-CRISPR protein
Beatrice Fung | University of Toronto

SMART design of a multi-receptor phage cocktail against *Salmonella* and impact of phage resistance on bacterial fitness
Carlos E. Martinez-Soto | University of Guelph

Systematic investigation of anti-phage systems in *Pseudomonas aeruginosa*
Charles Zhang | University of Toronto

Environmental DNA drives CRISPR adaptation in *S. thermophilus*
Felix Croteau | McMaster University

Quantitative analysis of the virulence of mycobacteriophages
Ilaria Rubino | University of Alberta

Genetic determinants for host range of Shiga toxin-producing *Escherichia coli* (STEC) targeting phages
Jieting Lin | University of Calgary

Investigation into scalable and efficient bacteriophage production for use against enterotoxigenic *Escherichia coli*
Katie Wiebe | University of Calgary

Tuning a machine-learning based classifier to predict phage-bacteria interactions in metagenomic derived data
Laura Camelo | McGill University

In vivo Evaluation of bacteriophage cocktail against colibacillosis in laying hens
Mawra Gohar | University of Calgary

First naturally-generated bacteriophage insensitive mutant of *Erwinia amylovora* and phage receptor identification
Nassereldin Ibrahim | Agriculture and Agri Foods Canada

Poster Session

A new piece in the phage cocktail puzzle to fight against furunculosis in Quebec
Nava Hosseini | Université Laval

A secondary receptor for the *Rhodobacter capsulatus* phage-like gene transfer agent RcGTA
Nawshin Tabassum Binte Alim | University of British Columbia

Investigating bacteriophage-bacteria dynamics in the mammalian gut
Nicola Pett | *University of British Columbia*

SEA-GENES: An approach to elucidate gene function of bacteriophages
Nicolas Toex | University of Ottawa

A dual specificity anti-CRISPR protein capable of blocking highly divergent Type I CRISPR-Cas systems
Robert Wilson | University of Toronto

Application of lytic bacteriophage cocktail to combat *Pseudomonas aeruginosa* biofilms
Sarah Kirst | University of Guelph

Legionella mobile element-1 forms phage-like particles and is an integrative mobile element exploiting Xer
Shayna Deecker | University of Toronto

Bacteriophage derived endolysins: A promising solution towards bovine respiratory diseases
Sidra Moqaddes | University of Calgary

Characterizing broad host range Listeria phages for biosanitation application
Steven Cucic | University of Guelph

Isolation and characterisation of eight novel bacteriophage against Mycobacterium avium subspecies paratuberculosis
Victoria Harman-McKenna | University of Calgary

DAY 3

Career Opportunities in Phage Research

12:00 - 13:30 Career Panel

1. Dr. Siyun Wang (Associate Professor UBC, Food Safety Engineering)
2. Dr. Greg German (Physician, Assistant Professor University of Toronto)
3. Dr. Nancy Tawil (CSO, Phagelux)
4. Dr. Steven Theriault (CEO and CSO, Cytophage)

13:30 - 14:15 Meet our career panelist

14:15 - 14:30 Awards ceremony & closing remarks

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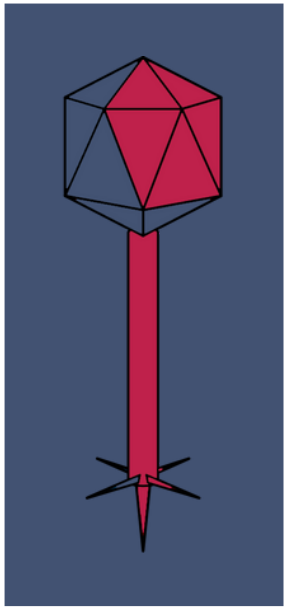

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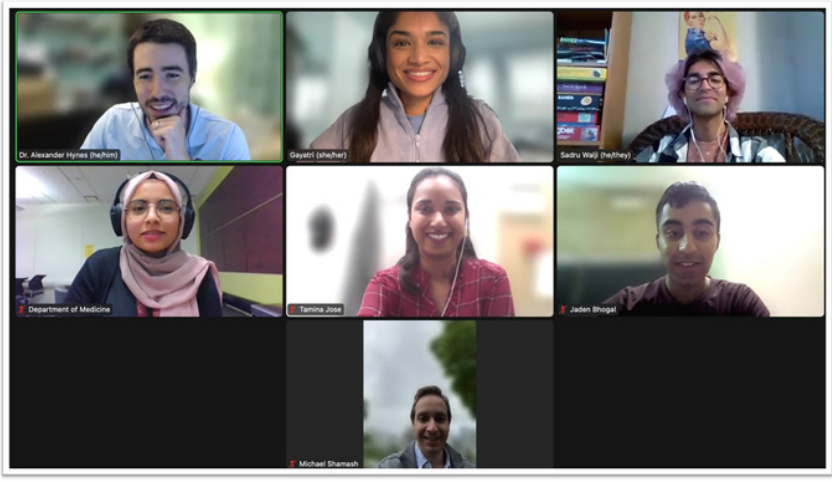

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